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Chancellor's Message

Prof. Dr. Khalil Ahmad Behsoodwal
Chancellor, Nangarhar University

Research is necessary in order to further knowledge and address challenging issues. In research projects, the synthesis and analysis of data are closely examined to the point where they frequently produce original and creative results. Accessing authorized domestic data and information within Afghanistan is now fairly difficult. Therefore, Nangarhar University has just started an online journal entitled “Nangarhar University International Journal of Biosciences (NUIJB)”, which shares the results of the researches within and outside Afghanistan; introduces the achievements of the new researches on domestic and international level; facilitates the exchange of views and knowledge among the research scholars; and paves the way to resolve complex academic problems. Indeed, the release of NUIJB's second volume second issue is a cause for celebration and pride. We make every effort to produce research articles that are accurate and standardized. Despite our best efforts, respected readers who encounter any academic or professional issues with the articles are kindly asked to notify the journal's administration in writing so that the appropriate modifications can be made.

With the hope of a better, peaceful, and advanced Afghanistan!

Regards,

Prof. Dr. Khalil Ahmad Behsoodwal
Chancellor, Nangarhar University

Message of Editor-in-Chief



Assoc. Prof. Ihsanullah Nasih
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I want to express my gratitude to the staff members and board members that helped Nangarhar University International Journal of Biosciences (NUIJB) get off the ground successfully. The journal welcomes submissions in the fields of Agriculture, Biology, Chemistry, Medical, Veterinary, and Animal sciences—a broad theme that should be interesting to as many readers as feasible. It is also important to note that the Ministry of Higher Education (MoHE) has approved NUIJB and that it has already earned an ISSN and indexes. The main aim and objective of NUIJB are to bridge the gap so that authors get a wider audience for their high quality scientific achievements. I believe we will be publishing a significant number of high quality original research article and scientific reviews from authors around the world. Additionally, we warmly appreciate any feedback and ideas that can help the journal become better.

Regards,

Assoc. Prof. Ihsanullah Nasih
Editor-In-Chief, NUIJB

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Effects of Fasciolosis on Haematological Parameters in Cattle

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ABSTRACT

Background: Bovine fascioliasis is a systemic infection of cattle caused by, *Fasciola spp.* The objective of this study was to investigate the effect of fasciolosis on hematological parameters in cattle.

Materials and Methods: In the present study, 40 local breeds (Watani) bulls and non-pregnant with ages ≥ 2 years heifers were randomly selected, of which 30 were infected with *Fasciola spp.* and 10 were not infected (control).

Findings: These results indicated that the infected group had significantly lower total red blood cell (RBC) count and Haemoglobin (Hb) levels than in the control group. In contrast, the infected group had a higher total white blood cell (WBC) count (9.3×10^3) compared to the not-infected group (7.7×10^3).

Conclusion: The hematological findings of the present study suggest that *Fasciola spp.* decreases the total RBC count and Hb levels, leading to anemia.

Keywords: Fasciolosis, RBC, WBS, Hb, and Cattle

INTRODUCTION

Fascioliasis is a systemic infection caused by *Fasciola spp.* in cattle, goats, and sheep that leads to economic losses by reducing production, production, and animal growth (Malone et al., 1998). According to a previous report, internal parasites annually affect the livestock industry by more than 2 billion USD by decreasing productivity (Axford et al., 2000). The liver fluke is among helminthic infections that decrease food intake and metabolism in infected cattle, leading to anemic conditions (Amarante, 2001; Keyyu et al., 2005; Muturi et al., 2005). *Fasciola spp.* has many negative effects on animal health, such as decreasing the total amount of blood in infected animals (Soun et al., 2006). Although the loss of blood is related to a load of mentioned parasites (Coop and Kryziakis, 2001; Wiedosari et al., 2006), both types of *Fasciola* (*F. hepatica* and *F. gigantica*) migrate through the liver tissues, leading to severe anemia (Wiedosari et al., 2006). Hematological parameters such as total RBC count, packed cell volume (PVC), Hb level, and differential count tests can provide a wide range of information for better diagnosis of diseases in cattle (Yokus and Cakir, 2006). Currently, little information is available on hematological changes induced by *Fasciola* through numerous hematological and biochemical changes associated with liver damage which had been indicated by a significant decline of total erythrocyte count (TEC), hemoglobin (Hb) level, packed cell volume (PCV) and mean corpuscular hemoglobin concentration (MCHC), the significant increase of erythrocyte sedimentation rate (ESR) and insignificant

alterations in the values of mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) content (Hammond and Sewell, 1974; Ogunrinade and Bomgboxe, 1980). None of the study has been conducted to determine the effects of *Fasciola* on the blood parameters of local breeds (Watani breed) in Afghanistan. Therefore, in this study, we investigated the effects of *Fasciola spp.* on the hematological parameters (RBCs, WBCs, and Hb) of local breeds. The results of the study may provide early warning signs in the detection of bovine *fasciolosis* and baseline data for future research in this local breed.

MATERIALS AND METHODS

Identification of animals and Study area:

This cross-sectional study was conducted on 40 local breed cattle, divided into two groups of males and females, each group divided into subgroups. Each subgroup contained 15 infected and 5 non-infected animals. The Female group animals were non-pregnant with ages ≥ 2 years. This study was conducted from the beginning of November 2016 to the end of June 2017 in the laboratory of the Pre-clinic department, Veterinary Science Faculty, Nangarhar University, and samples were collected from the cattle of the Bihsod district, Nangarhar province, Afghanistan.

The fecal samples collection for *Fasciola spp* determination:

Fecal samples were collected from the rectum of both male and female animals in sterilized plastic boxes and transported to the laboratory for microscopic examination using the sedimentation method, as previously described (Hansen and Perry, 1994).

Blood Samples collection:

Blood (5 ml) was collected from the Jugular vein of both infected and control animals using a 10 ml syringe with a 20-gauge needle and placed into the anticoagulant tube (EDTA) tubes. The samples were then mixed properly and analyzed within 12 hours to determine the hematological parameters, such as total RBC counts, and total WBC counts, using the hemocytometer (Neuberger's) counting method, as previously described (Cadena-Herrera et al., 2015). In addition, hemoglobin (Hb) values were estimated using the hemoglobinometer (Sahli, 1902).

Statistical Analysis

All the experimental data are shown as the mean \pm SEM and analyzed by ANOVA followed by Post-test. The statistical level of significance was considered at $P < 0.05$. The data were subjected to statistical analysis using SPSS version 17 software.

RESULTS

In the present study, we investigated the effects of *Fasciolosis spp.* on hematological parameters in cattle, the results show that the total red blood cell (RBC) count in heifers and the bull was decreased compared to the non-infected group. Particularly in infected heifers were significantly decreased (6.8×10^6) than the non-infected group (8.4×10^6) ($p < 0.05$). The total number of RBCs in heifers was lower than in bulls as shown in Figure 1. The levels of hemoglobin were also decreased in the infected groups of both bulls and heifers compared to the non-infected group (Figure 2). In contrast, the total of white blood cells (WBC) counts was significantly

increased in infected groups than non-infected groups. The bulls had greater total WBC count than the heifers as shown in Figure 3.

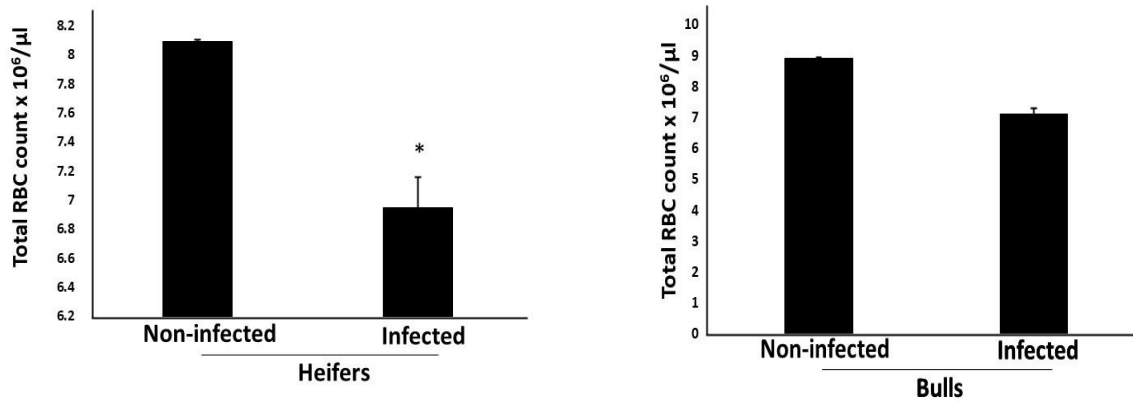


Figure 1. The total RBC count was decreased in infected cattle compared to the non-infected group, with a significant decrease observed in infected heifers compared to the non-infected group ($p < 0.05$), the total RBC count was also lower in heifers than in bull. The (*) indicates a significant decrease in infected cattle.

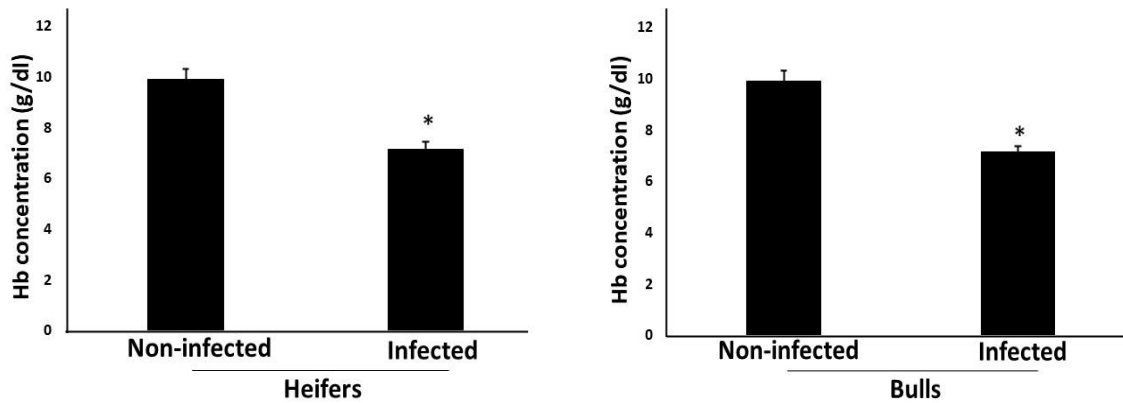


Figure 2. The levels of hemoglobin (Hb) in heifers and bulls were significantly decreased in the infected groups compared to the control groups ($p < 0.05$). The decrease was more pronounced in infected heifers than in infected Bulls. The (*) indicates a significant decrease in infected cattle.

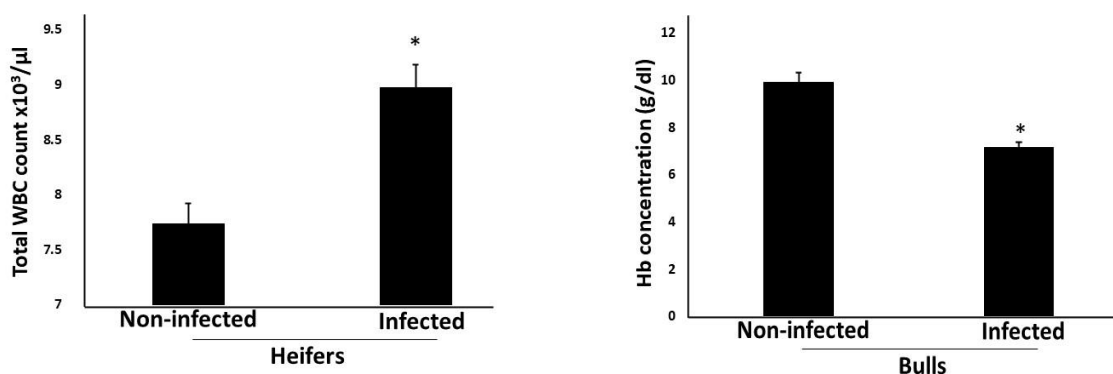


Figure 3. The total WBC count in heifers and bulls were significantly increased in infected heifers and bulls than the non-infected group. The (*) indicates a significant increase in infected cattle.

DISCUSSION

Fasciolosis is a worldwide disease in several species of mammalian, caused by *Fasciola spp.* (*F. hepatica* and *F. gigantica*) (Mas-Coma et al., 2019). It is generally accepted that in cattle only the chronic form of fascioliasis occurs (Soulsby, 1982). In the present study, we focused to identify the effect of fasciolosis on total RBCs, total WBCs counts, and Hb levels of cattle. This study showed that there was a significant decline in the total number of RBCs and Hb levels, whereas the total number of WBC significantly increased in the infected group compared to the non-infected group. Previous studies reported that the number of total RBCs significantly decreased in the infected group in cattle (Molina et al., 2006; Haroun and Hussein, 1975; Wahab et al., 2019 and Egbu et al., 2013) and in sheep (Doaa et al., 2007; Waweru et al., 1999; El-Aziz et al., 2002; Ahmed et al., 2006 and Matanović et al., 2007; Sykes et al., 1980). Our results of the present study are consistent with the above findings and support the concept that *Fasciola spp.* decreases the total number of RBCs in cattle.

In the present study, blood samples were collected from both *Fasciola spp.* infected and non-infected cattle to determine the effects of *Fasciola spp.* on Hb levels. Our study demonstrated that levels of Hb were significantly lower in the infected group compared to the control group. These results confirm and support the results of past conducted studies, which have shown a significant decline of Hb levels in cattle that were infected with *Fasciola spp.* (Leka et al., 2005; Doaa et al., 2007; Waweru et al., 1999; El-Aziz et al., 2002; Ahmed et al., 2006 and Matanović et al., 2007). Other studies that were conducted on sheep indicated that sheep infected with *Fasciola spp.* had lower Hb levels compared to those sheep which were free from *Fasciola spp.* (Sykes et al., 1980; Wahab et al., 2019 and Egbu et al., 2013). The reduction in the number of RBCs and levels of Hb may be attributed to acute anemia, which directly affects the production and reproduction of cattle.

Previous studies have been conducted to determine the effects of *Fasciola spp.* on the total number of WBCs in Holstein breed cattle. Their data demonstrated that there was a significantly higher total number of WBCs in *Fasciola spp.* The infected group compared to the control group (Egbu et al., 2013; Martinez-Valladares et al., 2010). In our current study, we examined the effects of *Fasciola spp.* in our local breed. Our data indicated that the total number of WBCs was significantly higher in the *Fasciola spp.* infected group. These results are in good agreement with the above-mentioned findings and strongly support their results. A previous study conducted in Lambs indicated that Females showed more significant increases in WBC counts than Males (Ljubičić et al., 2022). These findings suggest that *Fasciola spp.* causes deep changes in hematological parameters in cattle; decreasing the total number of RBCs and the levels of Hb in the blood, which lead the animal to an anemic condition. In contrast, increasing the total number of WBCs elevates the immune system to defend against infection.

CONCLUSION

The results of the present study indicate that *Fasciola spp.* has a significant impact on the hematological parameters of cattle, which can inhibit their growth and directly affect their production and reproduction. Further investigations are required to explore the effects of *Fasciola spp.* on remaining hematological parameters in cattle and to understand the underlying mechanisms involved. This will help the development of effective control strategies and management practices to minimize the impact of fasciolosis on the health and

productivity of cattle. The present study demonstrated that *Fasciola* affects the health of cattle and leads to anemic conditions; therefore we strongly suggest that farmers deworm their cattle against the parasites.

ACKNOWLEDGMENT

The authors express their grateful thanks to the head and all members of the Pre-clinic department, Veterinary Science Faculty, Nangarhar University for facilitating the environment to analyze the collected samples.

CONFLICT OF INTEREST

All authors express no conflict of interest in any part of the research.

REFERENCES

1. Ahmed MI, Ambali AG, Baba SS.(2006). Hematological and Biochemical Responses of Balami Sheep to Experimental *Fasciola Gigantica* Infection. *J Food, Agr Environ*, 4 (2), 71–74p.
2. Amarante AFT (2001). Controle de Endoparasitoses de Ovinos. Reuniao anual da sociedade brasileira de zootecnia. *Sociedade Brasileira de Zootecnia, Anais, Piracicaba*, 39, 461-471.
3. Axford RFE, Bishop SC, Nicholas FN, Owen JB (2000). Genetics of helminth resistance by breeding for disease resistance in farm animals. *2nd edition, CABI publishing, USA,1-413*.
4. Boray J.C. (1994). Disease of Domestic Animals Caused by Flukes. *Food and Agricultural Organisation of the United Nations; Rome, Italy*, 49.
5. Cadena-Herrera D, et al. (2015). Validation of three viable-cell counting methods: *Manual, semi-automated, and automated. Biotechnol. Rep*,7:9–16.
6. Coop RL, Kyriazakis I (2001). Influence of host nutrition on the development and consequences of nematode parasitism in ruminants. *Trends Parasitol.* 17:325-330.
7. Doaa FT, Soliman EK, Abd EL- Khalek TMM (2007). Effect of Fascioliasis on hematological, serum biochemical and histopathological changes in sheep. *Egyptian J. Sheep Goat Sci.* 2 (2):15-34.
8. Egbu, F.M.I., Ubachukwu, P.O. and Okoye, I.C., (2013). Hematological changes due to bovine fascioliasis. *Afr. J. Biotechnol.* 12: 1828-1835.
9. El-Aziz MZA, Emara SA, Salem FS (2002). Clinicopathological studies on fascioliasis among sheep in Giza province. *Egyptian J. Vet. Sci.* 36, 75-86.
10. Hansen J, Perry B. (1994). The epidemiology, diagnosis and control of helminth parasites of ruminants. *ILRI, Nairobi, Kenya*, 1, 1-10.
11. Hammond, J. A. and M. M. H. Sewell (1974). The pathogenic effect of experimental infections with *Fasciola gigantica* in cattle. *British Vet. Jour*, 130, 453.
12. Haroun EM, Hussein MF (1975). Clinico-pathological studies on naturally-occurring bovine fascioliasis in the Sudan. *J. Helminthol*, 49 (3),143-52.
13. Keyyu JD, Kyvsgaard NC, Monrad J, Kassuku AA (2005). Epidemiology of gastrointestinal nematodes in cattle on traditional, small-scale dairy and large-scale dairy farms in Iringa district, Tanzania. *Vet. Parasitol.* 127, 285-294.
14. Leka, O., Al-Quraishy, A. and Al-Moussawi, M. (2005). Effect of *Fasciola gigantica* infection on some blood physiological and biochemical aspects of infected cows in Babylon Governorate. *College of Medicine, University of Babylon*, 13, 1117-1123.
15. Ljubičić, I., S. Vince, A. Shek Vugrovečki, S. Milinković Tur, M. Šimpraga (2022). The effect of age and sex on selected haematological and biochemical parameters in Dalmatian Pramenka lambs. *Vet. Arhiv*, 92, 691-702.
16. Malone, J.B., Gommers, R., Hansen, J., Yilma, J.M., Slingenberg, J., Snijders, F., Nachet, O. and Ataman, F., (1998). A geographic information system on the potential distribution and abundance of *Fasciola hepatica* and *F. gigantica* in East Africa based on food and agriculture organization databases. *Vet. Parasitol.* 78, 87-101.

17. Martinez-Valladares, M., del Rosario, F.M., Fernandez- Pato, N., Castanon-Ordonez, L., Cordero-Perez, C. and Rojo-Vázquez, F.A. (2010). Efficacy of nitroxynil against *Fasciola hepatica* resistant to triclabendazole in a naturally infected sheep flock. *Parasitol. Res*, 107, 1205-1211.
18. Mas-Coma S., Valero M.A., Bargues M.D. (2019). Fascioliasis. *Adv. Exp. Med. Biol*, 1154:71–103.
19. Matanović K, Severin K, Martinković F, Šimpraga M, Janicki Z, Barišić J (2007). Hematological and biochemical changes in organically farmed sheep naturally infected with *Fasciola hepatica*. *J. Parasitol. Res.* 101(6), 1463-1731.
20. Molina EC, Lozano SP, Barraca AP (2006). The relationship between haematological indices, serum gamma-glutamyl transferase and glutamate dehydrogenase, visual hepatic damage and worm burden in cattle infected with *Fasciola gigantica*. *J. Helminthol*, 80 (3), 277-279.
21. Muturi KN, Scaife JR, Lomax MA, Jackson F, Huntley J, Coop RL (2005). The effect of dietary polyunsaturated fatty acids (PUFA) on infection with the nematodes *Ostertagia ostertagi* and *Cooperia oncophora* in calves. *Vet. Parasitol.* 129 (3-4), 273-283.
22. Ogunrinade, A.F. and Bamgboye, E.A. (1980). Bovine fascioliasis in Nigeria. Haematological indices and their correlation with worm burden in chronic fascioliasis. *Br. Vet. J.* 136, 457-462.
23. Sahli H (1902). An apparatus for the clinical estimation of haemoglobin. *Verh Dtsch Kongr Inn Med*, 20, 230–234.
24. Soulsby, E. J. L. (1982). Helminths, Arthropods and Protozoa of Domesticated Animals. *Baillier Tindall, London*, 6th edition, 1220.
25. Soun S, Hol D, Siek S, Mclean M (2006). Seasonal differences in the incidence of infection with *Fasciola gigantica* in Cambodian cattle. *Trop. Anim. Health Prod*, 38, 23-28.
26. Sykes AR, Coop RL, Rushton B. (1980). Chronic Subclinical Fascioliasis in Sheep: Effects on Food Intake, Food Utilisation and Blood Constituents. *Res Vet Sci*, 28, 63–70.
27. Wahaab A, Ijaz M, Ahmad SS, Iqbal U, Tawaab A, Khan, I (2019). Comparative Efficacy of Triclabendazole, Oxytoclozanide and Nitroxynil against Bovine Fasciolosis and its Effect on Various Blood Parameters. *Pakistan J. Zool*, 51(3), 843-847.
28. Waweru JG, Kanyari PWN, Mwangi DM, Ngatia TA, Nansen P (1999). Comparative parasitological and haematological changes in two breeds of sheep infected with *Fasciola gigantica*. *Trop. Anim. Health Prod*, 31, 363-372.
29. Wiedosari E, Hayakawa H, Copeman B (2006). Host differences in response to trickle infection with *Fasciola gigantica* in buffalo, Ongole and Bali calves. *Trop. Anim. Health Prod*, 38, 43-53.
30. Yokus B, Cakir UD (2006). Seasonal and physiological variations in serum chemistry and mineral concentrations in cattle. *Biol. Trace Elem. Res*, 109, 255-266.

Foliar Application of Multi-micronutrients Grade IV and Different Fruit Covering Materials: A Focus on Yield and Quality of Pomegranate (*Punica granatum* cv. Bhagwa)

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ABSTRACT

Background: Foliar fertilization as a highly effective approach to regulating plant nutrition has garnered significant attention in recent years. This study determined to elucidate the impact of foliar application of micronutrients grade IV and pre-harvest fruit covering bags on the quality and yield of pomegranate cv. Bhagwa.

Materials and Methods: Micronutrients grade IV was applied to foliage at the time of flowering and three weeks after first flowering with 0.5%, 1.0%, and 1.5% concentrations. Thereafter, the completely set fruits of pomegranates were covered in a newspaper bag, brown paper bag, white paper bag, non-woven bag, and non-covered fruits left as control treatment.

Findings: The results revealed that significantly ($P < 0.05$) higher amount of fruit yield (ton/hectare), fruit retention, total sugar (%), and reducing sugar (%) was observed with the application of multi-micronutrients at 1.0% level concentration. In addition, all the aforementioned parameters showed significantly ($P < 0.05$) higher values at 1.0% multi-micronutrients with the interaction of brown paper bags covering materials.

Conclusion: To conclude, fruit covering materials and the application of nutrients substantially influence the yield and quality of pomegranate.

Keywords: Chemical attributes, covering materials, foliar application, fruit quality, micronutrients, pomegranate

INTRODUCTION

Pomegranate is well adapted to Mediterranean climate conditions and is widely cultivated in Spain, India, Egypt, the USA, Afghanistan, etc. (Davarpanah et al., 2018). It is rich in polyphenols, antioxidants, and organic compounds consumed in either fresh fruit or processed forms (Zahedi et al., 2019). However, the quality and yield of pomegranates are substantially affected by soil conditions, tree nutrient management, and irrigation. The deficiency of micronutrients depends on soil type and available form of the nutrient (Dhillon et al., 2011). According to Davarpanah et al. (2016), essential elements are required for better plant growth, reproduction, fruit yield, and quality. Therefore, the supplemental addition of nutrients into the soil to improve soil fertility is necessary for sustainable agriculture production (Barker and Pilbeam, 2021). In pomegranates, the availability of microelements directly influences fruit set, retention, and quality. Zinc, in particular, plays a vital role in

activating various enzymes such as dehydrogenases, aldolases, isomerases, transphosphorylases, as well as RNA and DNA polymerases (Mirzapour and Khoshgoftarmanesh, 2012; Singh et al., 2020).

Boron is one of the essential constituents of the cell membrane and essential for cell division. It also acts as a regulator of the potassium/calcium ratio in plants, which helps in the absorption of nitrogen and sugar translocation increasing the fruit size and yield (Lindsay, 1972). In addition, copper, manganese, and potash are involved in physiological processes during fruit growth. Also, the individual foliar application of iron increased the yield of pomegranate by 20 – 31% (Davarpanah et al., 2020).

In pomegranates, fruit cracking, internal breakdown, and sunburn are major physiological disorders that catastrophically produce low and poor-quality production (Singh et al., 2020). Hence, physical protection through covering/bagging the fruits is required to mitigate the environmental stresses. The red-colored bagging of pomegranate fruits minimized the cracking and bacterial blight infection by 66% and 78%, respectively (Asrey et al., 2020a; Yuan et al., 2012), and substantially decreased the peel sunburn. During fruit development, bagging reduces the chance of physical damage while improving pomegranate's color, yield, and quality (Griñán et al., 2019).

The application of micronutrients as a supplemental additive for trees and bagging pomegranate fruits are rarely studied. In this study, we hypothesized that fruit bagging of pomegranates combined with the foliar application of micronutrients might improve pomegranate yield and quality.

MATERIALS AND METHODS

Plant materials, Multi-micronutrients, and Covering Materials

This research was conducted at Lal Baug, Fruit Research Station, Junagadh Agricultural University, Junagadh, India, in 2020. The experiment was based on a factorial randomized block design with three replications (each replication contained 5 trees). The foliar application of micronutrient grade IV (containing Zinc, Boron, Iron, Copper, and Manganese) in different concentrations as 0.5% (M1), 1.0% (M2), and 1.5% (M3) were applied on five years aged pomegranate trees of Bhagwa cultivar. The micronutrient grade IV was applied at the flowering stage and three weeks after flowering. After completion of the fruit set, the bagging materials included: non-covered (C1) fruits as control, newspaper bags (C2), brown paper bags (C3), white paper bags (C4), and non-woven bags (C5).

Fruit Chemical and Physical Properties

The number of male flowers per plant, number of hermaphrodite flowers per plant, number of intermediate flowers per plant, fruit yield per plant, total sugar, reducing sugar, non-reducing sugar, and total soluble solids (TSS) were measured as described by Davarpanah et al., (2016a).

Data Analysis

The data were analyzed based on two-way analysis of variance (ANOVA) using language R 3.6.2 statistical software. Differences among the treatments were separated using Tukey's test at $\alpha = 0.05$ significance level. The spacing between plants within a row and between rows was 5 m and 6 m, respectively. All fruits were harvested from the designated trees, and the yield was subsequently calculated in tons per hectare.

RESULTS

Effect of Micronutrient Foliar Application and Covering Materials on Fruit Yield, Fruit Retention, and Flowering Characteristics

There was a significant ($P < 0.05$) increase in pomegranate yield with the foliar application of micronutrients grade IV and using covering materials in all treatments as compared to control, except C5 treatment in all three concentrations (Figure 1). The highest fruit yield was observed in M2 concentration compared to M1 and M3. Moreover, the increase of fruit yield in M2 concentration with different covering materials was significantly higher with respect to its control (C1), and the difference was 47.4%, 20.8%, and 12.6% in C3, C4, and C2, respectively.

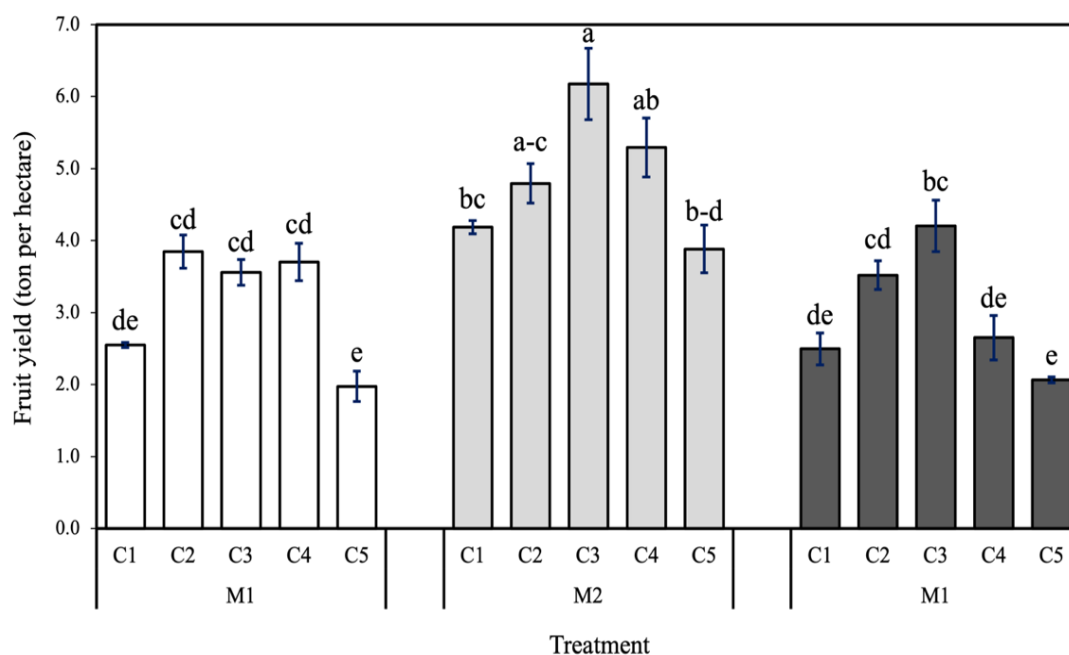


Figure 1. Integrated effects of foliar application of multi-micronutrient grade IV and different covering bags on fruit yield of pomegranate cv. Bhagwa. Vertical error bars denote the standard error. Different letters indicate significant differences among five treatments (covering bags) for each concentration at the $P < 0.05$ level. M and C abbreviation denotes micronutrient grade IV and covering bags, respectively.

The results revealed that foliar application of multi-micronutrient grade IV had diverse effects on fruit retention, which was substantially different based on concentration rate. At the same time, there was a highly significant difference ($P < 0.001$) with the covering materials. The highest fruit retention value was observed in M2 concentration with C3 covering bags, as shown in Table 1. In contrast, the lowest fruit retention was observed in C5 covering materials concerning all multi-micronutrient concentrations. In addition, the number of male flowers, hermaphrodite flowers, and intermediate flowers per tree didn't significantly differ under various covering materials and in the interaction between multi-micronutrients and covering materials. However, still, there were differences in the aforementioned parameters under the foliar application of micronutrients (Table 1).

Table 1. Effect of foliar application of multi micronutrient grade IV and different fruit covering bags on fruit retention and flower characteristics of pomegranate cv. Bhagwa.

Micronutrient grade IV (%)	Covering materials	Fruit retention	No. of male flowers/tree	No. of hermaphrodite flowers/tree	No. of intermediate flowers/tree
M1	C1	70.5 ± 1.3 b-d	33.1 ± 2.20 ab	120.4 ± 2.70 ab	8.4 ± 0.87 ab
	C2	62.7 ± 1.1 d-f	24.7 ± 1.89 b	87.5 ± 5.63 b	6.9 ± 0.99 b
	C3	82.0 ± 1.3 a-c	35.1 ± 2.42 ab	121.2 ± 2.21 ab	9.7 ± 0.53 ab
	C4	67.3 ± 3.5 c-e	38.6 ± 2.42 ab	119.3 ± 8.36 ab	9.2 ± 0.92 ab
	C5	48.3 ± 3.6 e	36.8 ± 3.37 ab	117.7 ± 7.14 ab	8.9 ± 0.45 ab
M2	C1	60.8 ± 0.7 d-f	37.7 ± 6.86 ab	94.2 ± 3.55 b	9.9 ± 0.14 ab
	C2	68.9 ± 2.5 b-e	38.4 ± 1.68 ab	107.4 ± 3.21 ab	10.0 ± 0.49 ab
	C3	85.5 ± 1.3 a	38.8 ± 2.34 ab	101.0 ± 5.17 ab	10.5 ± 0.70 ab
	C4	69.2 ± 1.30 b-e	39.4 ± 1.10 ab	106.1 ± 4.14 ab	10.5 ± 0.49 ab
	C5	64.6 ± 5.4 d-e	42.7 ± 2.03 a	104.3 ± 6.52 ab	10.3 ± 0.81 ab
M3	C1	65.2 ± 3.9 d-e	33.7 ± 1.29 ab	105.3 ± 5.13 ab	8.8 ± 1.20 ab
	C2	72.1 ± 4.0 a-d	35.9 ± 3.48 ab	123.8 ± 5.04 ab	10.1 ± 0.54 ab
	C3	82.3 ± 1.7 ab	35.4 ± 0.37 ab	125.7 ± 3.11 ab	10.6 ± 0.49 a
	C4	72.9 ± 1.8 a-d	36.8 ± 2.53 ab	126.4 ± 3.79 ab	10.6 ± 0.52 ab
	C5	55.1 ± 3.5 de	28.2 ± 3.17 ab	111.3 ± 5.62 a	7.3 ± 0.85 ab
Micronutrient		ns	**	**	**
Covering materials		***	ns	ns	*
Micronutrient × Covering		**	ns	ns	ns

The data are represented as mean and followed by the standard error. Different letters indicate significant differences among five treatments (covering bags) for each concentration at the $P < 0.05$ level. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, and ns: not significant. M and C abbreviation denotes micronutrient grade IV and covering bags, respectively.

The foliar application of micronutrient grade IV significantly ($P < 0.001$) affected the total sugar percentage with the various concentrations. However, the covering materials also showed significant ($P < 0.05$) differences under different covering bags. The M2 concentration increased total sugar as compared to M1 and M3 treatments. In the interaction of M2 and covering materials, C3 treatment increased the total sugar amount, followed by C2 and C1.

The statistical analysis of data showed that the foliar application of multi-micronutrient grade IV had a significant ($P < 0.001$) difference. At the same time, there was no difference between the utilization of covering materials and its interaction with micronutrients. Furthermore, the highest values reducing sugar were observed in the M2 which received C3 bagging material treatment compared to M1 and M3, as shown in Table 2. In contrast, non-reducing sugar increased in M1 concerning C2 covering materials (Table 2).

Table 2. Effect of foliar application of multi micronutrient grade IV and different fruit covering bags on biochemical attributes and taste of pomegranate cv. Bhagwa.

Micronutrient grade IV (%)	Covering materials	Total sugar (%)	Reducing sugar (%)	Non-reducing sugar (%)	TSS (°Brix)
M1	C1	12.0 ± 0.34 b	10.2 ± 0.15 ab	2.1 ± 0.10 ab	13.4 ± 0.26 b-d
	C2	12.2 ± 0.25 ab	10.7 ± 0.22 ab	2.1 ± 0.13 a	13.3 ± 0.09 b-d
	C3	12.0 ± 0.07 b	10.7 ± 0.15 ab	1.3 ± 0.10 a-c	14.1 ± 0.44 b
	C4	11.7 ± 0.21 b	10.6 ± 0.30 ab	1.1 ± 0.08 c	15.6 ± 0.30 a
	C5	12.1 ± 0.12 b	10.7 ± 0.17 ab	1.3 ± 0.26 a-c	13.4 ± 0.26 b-d
M2	C1	12.6 ± 0.12 ab	10.9 ± 0.23 ab	1.7 ± 0.13 a-c	14.1 ± 0.20 b
	C2	12.6 ± 0.19 ab	10.8 ± 0.25 ab	1.8 ± 0.08 a-c	13.9 ± 0.47 b
	C3	13.6 ± 0.20 a	11.6 ± 0.34 a	1.7 ± 0.32 a-c	12.4 ± 0.12 cd
	C4	12.5 ± 0.18 ab	10.8 ± 0.18 ab	1.6 ± 0.24 a-c	13.8 ± 0.15 b
	C5	12.6 ± 0.20 ab	10.8 ± 0.27 ab	1.8 ± 0.13 a-c	13.1 ± 0.06 b-d
M3	C1	12.1 ± 0.16 ab	10.5 ± 0.11 ab	1.6 ± 0.06 a-c	12.1 ± 0.06 d
	C2	11.5 ± 0.47 b	10.0 ± 0.51 b	1.6 ± 0.10 a-c	13.6 ± 0.10 bc
	C3	12.1 ± 0.55 b	10.1 ± 0.61 ab	1.9 ± 0.07 a-c	13.6 ± 0.50 bc
	C4	11.2 ± 0.41 b	10.0 ± 0.41 ab	1.2 ± 0.20 bc	13.5 ± 0.09 bc
	C5	11.4 ± 0.27 b	9.8 ± 0.25 b	1.6 ± 0.20 a-c	12.2 ± 0.12 d
Micronutrient		***	***	ns	***
Covering materials		*	ns	**	***
Micronutrient × Covering		ns	ns	*	***

The data are represented as mean and followed by the standard error. Different letters indicate significant differences among five treatments (covering bags) for each concentration at the $P < 0.05$ level. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, and ns: not significant. M and C abbreviation denotes micronutrient grade IV and covering bags, respectively.

DISCUSSION

The result of this study revealed that foliar application of multi-micronutrient grade IV increased the yield of pomegranate, as shown in Figure 1. In addition, a small amount of multi-micronutrient grade IV is needed to produce enough fruits. Therefore, the application of M2 (1%) showed higher fruit yield as compared to M1 (0.5%) and M3 (1.5%). These results were similar to those obtained by Obaid et al., (2013). Similarly, different fruit-covering materials increased the yield of pomegranate, as demonstrated in figure 1. Covering materials can protect fruits from different physical, mechanical, and chemical injuries; therefore, brown paper bags (C3) reported high fruit yield compared to other covering materials.

The result pertaining to variation in fruit retention was found non-significant as influenced by the application of multi-micronutrient grade IV. Whereas fruit-covering bags brought a significant effect on fruit retention, which is depicted in Table 1. Similarly, brown paper bags (C3) noticed maximum fruit retention, followed by white paper bags (C4) as compared to other covering materials. As we know that brown paper bags are strong against the sun, birds, and damaging environmental factors, compared to other covering bags, so it gives good results in fruit retention. The result of brown paper might be due to its effect of brown color for maintaining the inner temperature and acting as a repellent for birds, insects, etc. These results are also supported by (Ali et al., 2021; Feng et al., 2014) in apples, and (Asrey et al., 2020b) in pomegranates.

The variation due to micronutrient grade IV on the number of male flowers, number of hermaphrodite flowers, and intermediate flowers per tree were significant. A significantly higher number of male flowers, number of hermaphrodite flowers, and number of intermediate flowers per tree were recorded with foliar application of

micronutrient grade IV M2 (1%) compared to M1 (0.5%) and M3 (1.5%). The variation due to different fruit covering bags was observed non-significant effect on the number of male and hermaphrodite flowers per tree except intermediate flowers per tree. These findings are in agreement with Maity et al., (2021). The increased rate due to micronutrients might be due to Zn, which induces photosynthetic activity and produces more biomass. Zn aid in boosting the synthesis of auxin in the plant. Several other micronutrients activate the enzymes, which help in protein and carbohydrate metabolism, which increases the different flowers per tree. Micronutrient also contains boron which might have increased the fruit percentage. Metabolism of hormones such as auxin (IAA) and tryptophan increases with an increase in Zn concentration (Feng et al., 2014).

Variations in total sugar, reducing sugar, and TSS were significantly diverse by application of micronutrient grade IV. The maximum total sugar and reducing sugar were observed in micronutrient grade IV M2 (1%). However, TSS was higher in micronutrient grade IV M1 (0.5%) compared to M3 (1.5%), and non-reducing sugar was non-significant by application of micronutrient grade IV. In addition, the total sugar, non-reducing sugar, and TSS were significantly influenced by the fruit-covering materials except for reducing sugar which was non-significant in fruit-covering materials. Therefore, brown paper bags recorded higher total sugar, non-reducing sugar, and TSS than others. Zinc's involvement in various enzyme reactions, such as carbohydrate transformation, hexokinase activity, and cellulose formation, may contribute to variations in chemical parameters. Additionally, zinc's impact on zymohexose can influence changes in sugar levels. On the other hand, iron is associated with the development of flavor proteins, further highlighting its significance in this context. Furthermore, adequate zinc improves the auxin content, which catalyzes oxidation-reduction processes in plants (Singh et al., 2020). The increase in TSS is thought to be due to borate ion associated with the cell membrane, where it could be complex with sugar molecules and facilitate its passage across the membrane which might be the reason for increased total soluble solids (Ramegowda, 2019).

CONCLUSION

The results of this study indicated that foliar application of multi-micronutrient grade IV and covering bag prominently affected the yield and quality of pomegranate. Among different concentrations of multi-micronutrients, the 1% concentration showed the highest fruit yield, fruit retention, total sugar, and proper reducing sugar amount. In addition, the brown paper covering bags also showed the highest values of the parameters above concerning the interaction of a 1% concentration level of multi-micronutrients.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

1. Ali, M. M., Anwar, R., Yousef, A. F., Li, B., Luvisi, A., de Bellis, L., Aprile, A., & Chen, F. (2021). Influence of Bagging on the Development and Quality of Fruits. *Plants*, 10(2), 1–17. <https://doi.org/10.3390/PLANTS10020358>
2. Asrey, R., Kumar, K., Sharma, R. R., & Meena, N. K. (2020a). Fruit bagging and bag color affects physico-chemical, nutraceutical quality and consumer acceptability of pomegranate (*Punica granatum L.*) arils. *Journal of Food Science and Technology*, 57(4), 1469–1476. <https://doi.org/10.1007/S13197-019-04182-X/METRICS>

3. Asrey, R., Kumar, K., Sharma, R. R., & Meena, N. K. (2020b). Fruit bagging and bag color affects physico-chemical, nutraceutical quality and consumer acceptability of pomegranate (*Punica granatum L.*) arils. *Journal of Food Science and Technology*, 57(4), 1469–1476. <https://doi.org/10.1007/S13197-019-04182-X/METRICS>
4. Barker, A. v., & Pilbeam, D. J. (2021). *Handbook of plant nutrition*. <https://www.routledge.com/Handbook-of-Plant-Nutrition/Barker-Pilbeam/p/book/9781032098630>
5. Davarpanah, S., Tehranifar, A., Abadía, J., Val, J., Davarynejad, G., Aran, M., & Khorassani, R. (2018). Foliar calcium fertilization reduces fruit cracking in pomegranate (*Punica granatum cv. Ardestani*). *Scientia Horticulturae*, 230, 86–91. <https://doi.org/10.1016/J.SCIENTA.2017.11.023>
6. Davarpanah, S., Tehranifar, A., Davarynejad, G., Abadía, J., & Khorasani, R. (2016a). Effects of foliar applications of zinc and boron nano-fertilizers on pomegranate (*Punica granatum cv. Ardestani*) fruit yield and quality. *Scientia Horticulturae*, 210, 57–64. <https://doi.org/10.1016/j.scienta.2016.07.003>
7. Davarpanah, S., Tehranifar, A., Davarynejad, G., Abadía, J., & Khorasani, R. (2016b). Effects of foliar applications of zinc and boron nano-fertilizers on pomegranate (*Punica granatum cv. Ardestani*) fruit yield and quality. *Scientia Horticulturae*, 210, 57–64. <https://doi.org/10.1016/J.SCIENTA.2016.07.003>
8. Davarpanah, S., Tehranifar, A., Zarei, M., Aran, M., Davarynejad, G., & Abadía, J. (2020). Early Season Foliar Iron Fertilization Increases Fruit Yield and Quality in Pomegranate. *Agronomy 2020, Vol. 10, Page 832, 10(6)*, 832. <https://doi.org/10.3390/AGRONOMY10060832>
9. Dhillon, W. S., Gill, P.P.S., & Singh N.P. (2011). effect of nitrogen, phosphorus and potassium fertilization on growth, yield and quality of pomegranate “KANDHARI.” *Acta Horticulturae*, 890, 327–332. <https://doi.org/10.17660/ACTAHORTIC.2011.890.45>
10. Feng, F., Li, M., Ma, F., & Cheng, L. (2014). The effects of bagging and debagging on external fruit quality, metabolites, and the expression of anthocyanin biosynthetic genes in ‘Jonagold’ apple (*Malus domestica Borkh.*). *Scientia Horticulturae*, 165, 123–131. <https://doi.org/10.1016/J.SCIENTA.2013.11.008>
11. Griñán, I., Morales, D., Galindo, A., Torrecillas, A., Pérez-López, D., Moriana, A., Collado-González, J., Carbonell-Barrachina, Á. A., & Hernández, F. (2019). Effect of preharvest fruit bagging on fruit quality characteristics and incidence of fruit physiopathies in fully irrigated and water stressed pomegranate trees. *Journal of the Science of Food and Agriculture*, 99(3), 1425–1433. <https://doi.org/10.1002/JSFA.9324>
12. Lindsay, W. L. (1972). Zinc in Soils and Plant Nutrition. *Advances in Agronomy*, 24(C), 147–186. [https://doi.org/10.1016/S0065-2113\(08\)60635-5](https://doi.org/10.1016/S0065-2113(08)60635-5)
13. Maity, A., Gaikwad, N., Babu, K. D., Sarkar, A., & Patil, P. (2021). Impact of zinc and boron foliar application on fruit yield, nutritional quality and oil content of three pomegranate (*Punica granatum L.*) cultivars. <https://doi.org/10.1080/01904167.2021.1884711>, 44(13), 1841–1852. <https://doi.org/10.1080/01904167.2021.1884711>
14. Mirzapour, M. H., & Khoshgoftarmanesh, A. H. (2012). EFFECT OF SOIL AND FOLIAR APPLICATION OF IRON AND ZINC ON QUANTITATIVE AND QUALITATIVE YIELD OF POMEGRANATE. <http://dx.doi.org/10.1080/01904167.2012.733049>, 36(1), 55–66. <https://doi.org/10.1080/01904167.2012.733049>
15. Obaid, E. A., Eiada, M., & Al-Hadethi, A. (2013). Effect of Foliar Application with Manganese and Zinc on Pomegranate Growth, Yield and Fruit Quality. *Journal of Horticultural Science & Ornamental Plants*, 5(1), 41–45. <https://doi.org/10.5829/idosi.jhsop.2013.5.1.273>
16. Ramegowda, S. (2019). *Effect of foliar application of gibberellic acid (GA3) and nutrients on yield and quality of pomegranate (Punica granatum L.) cv. Bhagwa Nutrient management in grapes View project*. <https://www.researchgate.net/publication/336058509>
17. Singh, A., Shukla, A. K., & Meghwal, P. R. (2020). Fruit Cracking in Pomegranate: Extent, Cause, and Management – A Review. <https://doi.org/10.1080/15538362.2020.1784074>, 1–20. <https://doi.org/10.1080/15538362.2020.1784074>
18. Singh, T., Singh, D., Kumar, A., Patel, A., & Bose, U. (2020). Effect of micronutrients on growth, yield and fruit quality of mango (*Mangifera indica L.*) cv. Dashehari. *International Journal of Chemical Studies*, 8(6), 2055–2058. <https://doi.org/10.22271/CHEMI.2020.V8.I6AC.11075>

19. Yuan, Z. H., Yin, Y. L., Feng, L. J., Zhao, X. Q., Zhang, Y. X., & Hou, L. F. (2012). Evaluation of pomegranate bagging and fruit cracking in Shandong, China. *Acta Horticulturae*, 940, 125–129. <https://doi.org/10.17660/ACTAHORTIC.2012.940.15>
20. Zahedi, S. M., Hosseini, M. S., Daneshvar Hakimi Meybodi, N., & Teixeira da Silva, J. A. (2019). Foliar application of selenium and nano-selenium affects pomegranate (*Punica granatum* cv. Malase Saveh) fruit yield and quality. *South African Journal of Botany*, 124, 350–358. <https://doi.org/10.1016/J.SAJB.2019.05.019>

Improving Water Efficiency, Nutrients Utilization, and Maize Yield using Super Absorbent Polymers Combined with NPK during Water Deficit Conditions

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ABSTRACT

Background: The increasing global population poses a significant challenge, resulting in a scarcity of food resources on a global scale. Addressing this issue necessitates advancements in agricultural practices, particularly in drought-prone areas. Super absorbent polymers (SAPs) are crucial in improving crop production's water and nutrient utilization efficiency, making them highly relevant for drought-affected areas. Thus, this research aimed to assess the impact of SAPs, combined with manure and fertilizers, on the growth of maize (*Zea mays*) cv. Ts-1004.

Material and Methods: The experiment consisted of nine treatment groups, namely, T₁ (Nitrogen, N), T₂ (Potassium, K), T₃ (Phosphorus, P), T₄ (NPK), T₅ (Compost), T₆ (SAPs), T₇ (NPK + Compost), T₈ (NPK + SAPs), and T₉ (Control). These treatments were evaluated under two water level conditions: well-watered (W₁) and water-stressed (W₂) in a greenhouse environment. The plants were subjected to water stress by maintaining soil moisture content at 20 – 25% during the knee height and flowering stages for 8 days.

Results: The results revealed that significantly ($P < 0.05$) higher values in ear girth, ear length, number of seeds per ear, and ear weight were observed in the T₈ treatment compared to other treatments. Additionally, the T₈ treatment exhibited the highest yield under well-watered and water-stressed conditions (3,274.4 kg/ha). The application of SAPs improved soil moisture content, leading to enhanced water use efficiency (24.53 kg/ha/mm) and harvest index. Moreover, SAPs positively influenced the concentration percentage of N, P, K, Ca, and Mg in roots, stems, leaves, and seeds, with T₈ showing the highest values under water stress conditions.

Conclusion: These findings highlighted the effectiveness of SAPs in enhancing crop growth and productivity, particularly under water stress conditions. This approach will help farmers reduce water stress on crops.

Key words: WUE; NUE; Harvest index; Water deficit condition; NPK; Compost; SAPs

INTRODUCTION

Maize, a prominent cereal crop and the third most cultivated crop globally after wheat and rice, belongs to the Poaceae family (Kaul et al., 2011). Maize is extensively utilized for both direct and indirect human consumption, as well as for animal husbandry, and is even used in the production of traditional beverages (Asghar et al., 2010). Worldwide production of maize is estimated to be around 60 to 70 million tons (Kidist, 2013). The global population is steadily increasing and is projected to exceed 9.1 billion by 2025 (FAO, 2009). Consequently, the growing rate of population will require 70% more food by 2050, equating to an annual demand of 43 million metric tonnes of cereals (Bruinsma, 2009). However, despite only 18% of the world's arable land being irrigated, it is responsible for producing 40% of the world's food (FAO, 2006). Efforts are being made to expand irrigated areas, with an annual increase of nearly 1%, aiming for a target of 13.6% by 2025 (Rosegrant and Cai, 2002).

Enhancing nutrient use efficiency cannot solely rely on fertilizer management; it is influenced by the soil solution, where water stress can restrict the efficiency of soil nutrient utilization (Bossio et al., 2008). Balanced application of chemical and organic fertilizers in conjunction with proper soil moisture levels can improve nutrient and water use efficiency, leading to enhanced soil fertility, productivity, and ultimately increasing grain yield (Ryan et al., 2009). Nilson and Orcutt, (1996) stated that the effect of the drought stress one week before silking (R_2) and two weeks after silking stage (R_4) decreased the grain yield by 53%, compared to non-drought threaten plants. Maize crop needs 50–70% soil moisture level to maintain their normal physiological activities. In drought-affected areas of developing countries, cereal production often remains below 1.5 t/ha, even with the application of ample fertilizers. However, when sufficient irrigation water is available, the yield potential can exceed 8 t/ha (Thompson, 2012). Therefore, there is a strong positive correlation between water use efficiency (WUE), nutrient use efficiency (NUE), and crops yield production (Lucas et al., 2007). Maize has been found to have high WUE and NUE as compared with other crops, producing high biomass in linear response to nutrient availability without excessive evapotranspiration (Ogola et al., 2002).

The number of leaves, their size, and the expansion of leaf area in maize are influenced by turgor pressure and the availability of assimilates. However, under drought conditions, both turgor pressure and assimilation rates are reduced (Rucker et al., 1995). Water-limiting conditions severely impact plants' fresh and dry weight (Zhao et al., 2006). In maize, the stem girth and plant height experience significant reductions under water-limiting conditions (Khan et al., 2015). Similarly, both maize and sugarcane (*Saccharum officinarum* L.) exhibit reduced growth and net assimilation rate under heat and water stress conditions (Wahid and Close, 2007).

The grain-filling processes in cereal crops are regulated by four key enzymes: sucrose synthase, starch synthase, starch branching enzyme, and adenosine diphosphate glucose pyrophosphorylase (Taiz and Zeiger, 2006). Drought stress has been reported to decrease the activity of these enzymes, negatively impacting the yield of major cereals (Ahmadi and Baker, 2001). Maize yield is significantly reduced when exposed to drought conditions during tasseling stage (Anjum et al., 2011).

Drought conditions can cause an imbalance in assimilate distribution, leading to increased translocation of assimilates to the roots in order to enhance water uptake (Leport et al., 2006). Water stress disrupts the sink and source relationship, impairing the utilization of assimilates effectively (Kim et al., 2000). The acid invertase enzyme plays a crucial role in maintaining a balance between phloem loading and unloading pathways.

However, under water stress, the functionality of this enzyme is negatively affected, disturbing the mechanism of organ partitioning and adversely impacting dry matter distribution (Zinselmeier et al., 1999). Generally, drought and heat stress negatively impact nutrient cycling, uptake, and availability to plants, affecting various physiological functions within plants (Schimel et al., 2007). One approach to address this challenge is the utilization of superabsorbent polymers (SAPs). SAPs offer a way to increase fertilizer use efficiency while minimizing water application. These polymers can retain water and release it gradually, providing to crops available water and essential nutrients during their growth stages (Pawlowski et al., 2009).

SAPs can store water 400 times more than their dry weight and increase soil water retention capacity by 100–260%, which is recommended 2–8 g kg⁻¹ of soil (Rafiei and Noor mohammadi, 2013), while decreased nutrients percolation below the root zone and evaporation from the surface of the soil (Sarvas et al., 2007). The utilization of SAPs has been found to enhance soil productivity by optimizing the air-water ratio, an important aspect of soil productivity. Additionally, the presence of SAPs promotes improved soil microbe activities, which in turn strengthens crop growth (Orzeszyna et al., 2006). When SAPs are incorporated into the soil, they contribute to improved soil physical properties, leading to enhanced crop growth and increased yield. Furthermore, using SAPs reduces plants' irrigation requirements (Yazdani et al., 2007). However, it should be noted that including SAPs may also prolong the period before wilting occurs in plants (Karimi et al., 2009). Therefore, this study aimed to investigate the effectiveness of combining chemical fertilizers with SAPs to improve the soil's water-holding capacity, specifically focusing on enhancing water use efficiency (WUE), nutrient use efficiency (NUE), and crop yield.

MATERIALS AND METHODS

Experimental design and treatments

The greenhouse experiment was conducted at the Department of Agronomy, Faculty of Agriculture, Kasetsart University in Bangkok, Thailand, during the rainy season of 2017. A pot experiment with a lifespan of 105 days was conducted, following a randomized block design with a factorial treatment arrangement. The experiment consisted of nine different treatments such as T₁ (Nitrogen), T₂ (Potassium), T₃ (Phosphorus), T₄ (NPK), T₅ (Compost), T₆ (SAPs), T₇ (NPK + Compost), T₈ (NPK + SAPs), and T₉ (Control). These treatments underwent to well-water (W₁) and water-stressed (W₂) conditions. All the above treatments were applied to the pot surface area (0.42 sq m) in the experiment, including RDF of NPK fertilizer rate is 120:60:40 kg/ha, compost at a rate of 730.7 kg/ha, and SAPs at a rate of 312.5 kg/ha. These amounts were equivalent to 11 g of urea, 5.5 g of P₂O₅, 3.3 g of K₂O, 43 g of compost, and 13 g of SAPs per pot. Water deficit condition imposed 25 days after sowing (DAS) (at knee height stage) and 62 DAS at flowering stage.

Measurements and parameters

The soil moisture content (SMC), water use efficiency, nutrient use efficiency, yield and harvest index were tested according to the methods Wang et al. (2017), Singh et al. (2007), Lija (2014), Khaliq et al. (2006), and Nwachukwu and Ikeadigh, (2012), respectively, after harvesting. The daily water requirement was determined using the FAO's recommended method (Blaney-Cridle equation, 1950). A 20–25% soil moisture content was maintained for 8 days during the knee height and again during the flowering stages to ensure optimal soil moisture conditions. The nutrient uptake level and its utilization efficiency were assessed using the infrared

spectrometer model (Agri Quant) with the serial number QIN1384058-001. The grinded samples were utilized and passed through the lens tube, enabling the determination of element percentages in different plant parts. The procedure followed as described by Lija. (2014). The below formula is used for the calculation of nutrient use efficiency percentage:

$NUE\% = \frac{TNF - TNU}{RFA} * 100$; TNF: is the total nutrient uptake by fertilized treatment, TNU: is the total nutrient uptake by non-fertilized treatment, and RFA: is the rate of fertilizer applied.

Statistical analysis

The data was analyzed based on a one-way analysis of variance (ANOVA) using SPSS (version 16) statistical software. Differences among the treatments were separated using Tukey's test at $\alpha = 0.05$ significance level.

RESULTS

Ears girth, length, and weight

After harvesting, the ears' girth was studied in well-watered (W_1) and water-stressed (W_2) block treatments. The findings of the study indicated significant differences among the treatments, with the highest value recorded in T_8 (3.32 ± 0.07 cm), while the lowest value was observed in T_9 (2.12 ± 0.35 cm) across both blocks. Additionally, significant differences were observed between the water level and soil management factors among the treatments. However, the statistical analysis did not reveal any significant interactions between the water level and soil management factors in relation to the ear's girth, as shown in Table 1. A significant difference in ear length was observed in T_8 (13 ± 1.9 cm), whereas the minimum value among the treatments on both sides at T_2 (7.08 ± 1.5 cm) was observed. However, no significant differences were found among the treatments in relation to the effect of water levels: well-watered and water-stressed. Similar results were observed in the water level and soil management interaction (Table 1). The ear weights (gr) were extensively studied among the treatments after harvesting in both blocks. Significant differences were observed, with T_8 exhibiting the highest value (64.92 ± 22.1 g) in both blocks, as shown in Table 1. On the other hand, the lowest value was recorded in T_9 (13.9 ± 5.31 g) in both blocks among the treatments. However, no significant differences were found in the interaction between the two factors (water levels and soil management). However, significant results were noted in the two water levels among the treatments in both blocks, as indicated in Table 1.

Yield and its components

The average number of seed per ear was counted after the harvesting and threshing. It was found that highly significant differences among the treatments in both blocks at T_8 (255.83 ± 59.34). In contrast, the minimum number of grains per ear was recorded at T_9 (35.5 ± 72.5) in both blocks (Table 1). In the first step collected randomly three ears of grain from each treatment. After the seeds' moisture was decreased to 13–15%, the samples were randomly selected three times and weighed. The higher weight was noted in T_8 in both treatments (42.37 ± 4.2 g) (Table 1), while the lowest weight of the hundred (100) seeds within treatments was observed in both blocks at T_9 (16.9 ± 5.31 g) (Table 1). A significant difference in yield production among the treatments was observed in both blocks at T_8 treatment, with a value of $3,390.1 \pm 51.7$ kg/ha, as shown in Table 1. Conversely, the lowest expected yield was obtained in T_9 in both blocks, with a value of 694.4 ± 90.7 kg/ha. Significant differences were also observed among the treatments in the two water levels (W_1 and W_2). However,

no significant differences were found in the interaction between the two factors (water level and soil management) among the treatments, as presented in Table 2.

Soil moisture contents

The study investigated the soil moisture content (SMC), and it was observed that T₈ in W₁ block exhibited significantly higher SMC ($P \leq 0.05$), while T₉ had the lowest SMC, as shown in Figure 1. A similar trend was observed for T₈ and T₉ in W₂ blocks (Figure 1).

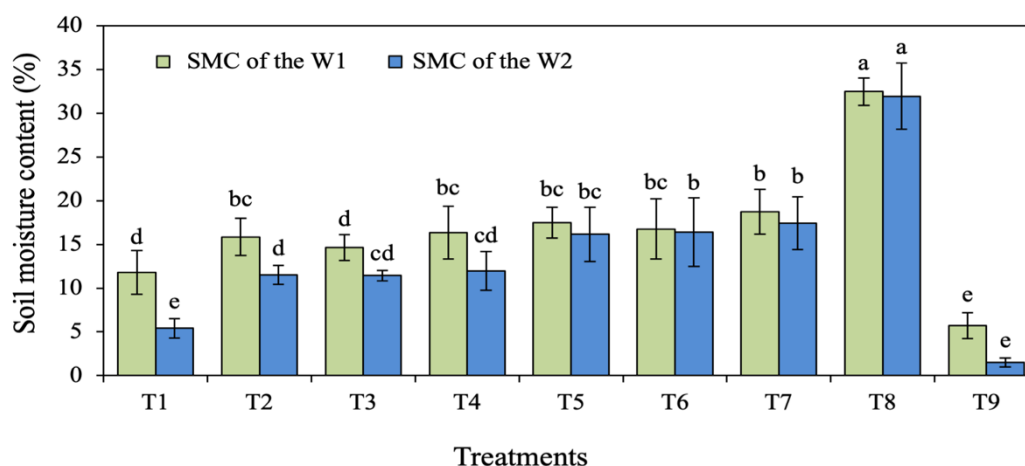


Figure 1. Effects of well-water and water-stressed condition on soil moisture content integrated with soil nutrients.

Harvest index

The economic yield of the harvested plant production over the biological yield of the plants considered the harvest index (HI) in the percentage of the reference plant. The harvest index was studied among the treatments of both blocks after the harvesting; no significant differences were observed when the plants were dried and weighed in both blocks. However, the highest value (HI %) was noted at T₈ (0.68 ± 0.3 %) in both blocks among the treatments (Table 3), whereas the minimum harvest index was observed in both blocks among the treatments in T₉ (0.33 ± 0.01 %). Similar significant results were noted in both blocks among the treatments in the interaction between the two water levels at well-watered and water-stressed blocks (Table 3).

Water use efficiency

The water use efficiency (WUE kg/ha/mm) is a measure of maximizing production while utilizing a minimal amount of water per unit area. WUE was measured in both blocks and observed the significant differences among the treatments in two water levels, the well-watered and water-stressed, and also about the soil management in T₈ (24.53 ± 5.5 kg/ha/mm) in both blocks. In contrast, the minimum water use efficiency was recorded in both blocks among the treatments again in T₉ (4.98 ± 2.5 kg/ha/mm) (Table 4). However, no significant differences were found between the interactions among the treatments for soil management in both blocks among the treatments (Table 4).

Table 1. Yield parameters within blocks among the treatments at different growth stages.

Water management	Ear girth (cm)	Ear length (cm)	No. of seeds/ear	Ear weight (g)	100 seeds weight (g)	Yield kg/ha
W ₁	3.266 ^a	9.593 ^a	166.85 ^a	38.801 ^a	31.141 ^a	2024.2 ^a
W ₂	2.712 ^b	9.648 ^a	78.48 ^b	29.187 ^b	25.715 ^b	1524.1 ^b
Mean	2.989	9.62	122.67	33.994	28.43	1774.2
CV (%)	13.69	24.74	43.03	41.15	12.4	41.29
LSD (0.05)	*	ns	*	*	*	*
Soil management	Ear girth (cm)	Ear length (cm)	No. of seeds/ear	Ear weight(g)	100 seeds weight (g)	Yield kg/ha
T1	2.9 ± 0.4 ^{bc}	8.33 ± 2.8 ^{cde}	65.7 ± 48.42 ^{ef}	26.2 ± 5.21 ^{cde}	28.8 ± 4.62 ^{cd}	1,149.7 ± 71.5 ^{de}
T2	3.01 ± 0.1 ^{bc}	7.08 ± 1.5 ^e	98.5 ± 66.7 ^{def}	31.14 ± 8.6 ^{bcd}	22.99 ± 3.23 ^e	1,627.5 ± 48.8 ^{bcd}
T3	2.98 ± 0.2 ^{bc}	11.3 ± 2.3 ^{ab}	13.7 ± 95.6 ^{cd}	37.16 ± 16.12 ^{bc}	29.1 ± 4.8 ^{bcd}	1,848.6 ± 41.9 ^{bcd}
T4	3.23 ± 0.14 ^{ab}	11.1 ± 3.5 ^{abc}	160.3 ± 95.7 ^{bc}	42.81 ± 8.7 ^b	30.2 ± 1.96 ^{bc}	2,074.8 ± 80.8 ^{bc}
T5	2.74 ± 0.5 ^c	7.67 ± 1 ^{de}	72.7 ± 27.2 ^{ef}	19.36 ± 13.7 ^{de}	27.76 ± 1.72 ^{cd}	1,562.56 ± 71.7 ^{bcd}
T6	3.06 ± 0.4 ^c	9.42 ± 1.1 ^{bcd}	91.5 ± 20.21 ^{def}	33.44 ± 5.8 ^{bcd}	25.14 ± 0.9 ^{de}	1,222.7 ± 30.3 ^{cde}
T7	3.06 ± 0.2 ^{ab}	10.1 ± 2.7 ^{bcd}	194.33 ± 48.8 ^b	37.16 ± 15.4 ^{bc}	32.63 ± 5.1 ^b	2,397.2 ± 45.3 ^b
T8	3.32 ± 0.07 ^a	13 ± 1.9 ^a	255.1 ± 59.4 ^a	64.92 ± 22.1 ^a	42.37 ± 4.2 ^a	3,390.1 ± 51.7 ^a
T9	2.12 ± 0.35 ^d	8.58 ± 1.9 ^{bcd}	35.5 ± 72.5 ^f	13.3 ± 7.5 ^e	16.9 ± 5.31 ^f	694.4 ± 90.7 ^e
Mean	2.989	9.62	122.67	33.994	28.43	1774.2
C.V (%)	13.69	24.74	43.03	41.15	12.4	41.29
LSD (0.05)	*	ns	*	*	*	*

W1: well-watered, W2: water-stressed, CV: coefficient of variation, LSD: least significant difference. The * indicates a significance level at P < 0.05 and ns: not significant. Different letters indicated significant difference among treatments.

Table 2. The interaction between the two water levels and soil management for yield and its components.

Water levels	Treatment	Ear girth (cm)	Ear length (cm)	No. of seeds/ear	Ears weight(g)	100 seeds weight(g)	Yield kg/ha
Well - watered	T1	3.1 ± 0.37 ^{abcdef}	8.83 ± 2.75 ^{bcdef}	98.3 ± 48.4 ^{efgh}	31.93 ± 5.2 ^{defg}	31.74 ± 4.62 ^b	1,230.83 ± 71.5 ^{efg}
	T2	3.4 ± 0.07 ^{defg}	7 ± 1.5 ^{cdef}	142.7 ± 66.7 ^{cde}	34.2 ± 8.6 ^{cdefg}	25.6 ± 3.23 ^{def}	1,785.8 ± 48.8 ^{efg}
	T3	3.5 ± 0.2 ^{abc}	11.7 ± 2.31 ^{abc}	188.7 ± 95.6 ^{bcd}	41.81 ± 16.1 ^{bcde}	32.92 ± 4.8 ^b	2,183.14 ± 41.9 ^{bcde}
	T4	3.6 ± 0.18 ^{ab}	10.83 ± 3.51 ^{abcde}	226.7 ± 95.7 ^{abc}	56.62 ± 8.7 ^{abc}	34.6 ± 1.96 ^b	2,972.62 ± 80.8 ^{abcd}
	T5	2.9 ± 0.52 ^{bcdefg}	6.5 ± 1 ^{bcde}	117.7 ± 27.3 ^{cdef}	23.57 ± 13.7 ^{efg}	30.5 ± 1.72 ^{bcd}	1,667.56 ± 71.7 ^{defg}
	T6	3.3 ± 0.4 ^{abcde}	8.83 ± 1.1 ^{bcdef}	92.7 ± 20.2 ^{efgh}	31.69 ± 5.8 ^{bcfg}	26.4 ± 0.93 ^{cde}	1,654.4 ± 30.3 ^{defg}
	T7	3.7 ± 0.19 ^{ab}	11 ± 2.65 ^{abcd}	264 ± 47.8 ^{ab}	47.4 ± 15.4 ^{abcd}	35.32 ± 5.1 ^b	2,986.7 ± 45.3 ^{abc}
	T8	3.67 ± 0.07 ^a	13.2 ± 1.9 ^a	312.7 ± 59.4 ^a	67.14 ± 22.1 ^a	43.6 ± 4.2 ^a	3,505.9 ± 51.7 ^a
	T9	2.3 ± 0.35 ^{gh}	8.2 ± 1.9 ^{cdef}	58.3 ± 72.5 ^{efgh}	14.97 ± 7.5 ^{fg}	19.94 ± 5.31 ^{gh}	7,81.6 ± 90.7 ^{fg}
Water stressed	T1	2.76 ± 0.4 ^{defg}	7.83 ± 1.5 ^{ef}	33 ± 11.5 ^{gh}	20.5 ± 4.55 ^{efg}	25.8 ± 4.2 ^{defg}	1,068.6 ± 37.5 ^{cdefg}
	T2	2.64 ± 0.99 ^{efg}	7.2 ± 0.8 ^{def}	54.3 ± 85.7 ^{fgh}	28.14 ± 8.9 ^{defg}	20.4 ± 5.9 ^{def}	1,469.3 ± 67.3 ^{defg}
	T3	2.46 ± 0.97 ^{fgh}	11 ± 4.4 ^{abcd}	70.7 ± 77.3 ^{efgh}	32.5 ± 37.96 ^{defg}	25.3 ± 0.86 ^{defg}	1,514.03 ± 28.2 ^{defg}
	T4	2.85 ± 0.4 ^{cdefg}	11.3 ± 2.6 ^{abc}	124 ± 53.1 ^{efgh}	28.99 ± 8.2 ^{defg}	25.84 ± 2.1 ^{def}	1,698.02 ± 82.5 ^{defg}
	T5	2.49 ± 0.62 ^{fgh}	8.83 ± 3.51 ^f	27.7 ± 27.14 ^h	15.14 ± 3.8 ^{cdef}	25.05 ± 2.8 ^{defg}	1,457.5 ± 21.2 ^{defg}
	T6	2.84 ± 0.4 ^{fgcdefg}	10 ± 3.5 ^{abcdef}	90.3 ± 67.9 ^{efgh}	35.19 ± 13.35 ^{abcd}	23.92 ± 1.41 ^{bcd}	1,290.61 ± 96.8 ^{fg}
	T7	2.98 ± 0.2 ^{bcdef}	9.2 ± 2.3 ^{bcdef}	154.7 ± 84.5 ^{def}	27.19 ± 4.1 ^{abc}	29.94 ± 2.13 ^{bcd}	2,237.62 ± 96.6 ^{cdef}
	T8	3.44 ± 0.13 ^{abc}	12.5 ± 1.32 ^{ab}	250 ± 48.1 ^{bcd}	62.7 ± 6.6 ^{ab}	41.2 ± 3.1 ^a	3,274.4 ± 43.5 ^{ab}
	T9	1.94 ± 0.3 ^h	9 ± 2.65 ^{bcdef}	12.7 ± 2.9 ^{fh}	11.63 ± 6.6 ^g	14 ± 2.73 ^h	6,07.32 ± 34.03 ^g
Mean	2.989	9.62	122.67	33.994	28.43	1,774.2	
CV (%)	13.69	24.74	43.03	41.15	12.4	41.29	
LSD (0.05)	ns	ns	ns	ns	ns	ns	

CV: coefficient of variation, LSD: least significant difference, and ns: not significant. Different letters indicated significant difference among treatments.

Table 3. The water use efficiency (kg/ha/mm) and HI % of maize under different treatments.

Water – management	HI%	WUE%
W ₁	0.54 ^a	13.14 ^a
W ₂	0.47 ^a	12.24 ^b
Mean	0.51	12.69
C.V	44.42	42.72
LSD	ns	*
Soil – Management	HI%	WUE%
T1	0.43 ± 0.4 ^{abc}	8.3 ± 4.7 ^{de}
T2	0.42 ± 0.15 ^{abc}	11.7 ± 2.9 ^{bcd}
T3	0.54 ± 0.2 ^{abc}	13.17 ± 5.5 ^{bcd}
T4	0.58 ± 0.12 ^{abc}	14.78 ± 5.4 ^{bc}
T5	0.55 ± 0.3 ^{abc}	11.26 ± 1.8 ^{bcd}
T6	0.41 ± 0.14 ^{bc}	8.54 ± 1.96 ^{cde}
T7	0.62 ± 0.1 ^{ab}	16.98 ± 2.95 ^b
T8	0.68 ± 0.3 ^a	24.53 ± 5.5 ^a
T9	0.33 ± 0.1 ^c	4.98 ± 2.5 ^e
Mean	0.51	12.69
CV (%)	44.42	42.72
LSD (0.05)	*	*

CV: coefficient of variation, LSD: least significant difference. * And ** indicates significance level at P < 0.05 and P < 0.01, respectively. Different letters indicated a significant difference among treatments.

Table 4. Interaction between the two water levels and soil management.

Water level	Treatment	HI%	WUE%
Well - watered	T1	0.51 ± 0.4 ^{ab}	7.992 ± 4.7 ^{cdef}
	T2	0.52 ± 0.2 ^{ab}	11.6 ± 2.92 ^{def}
	T3	0.56 ± 0.2 ^{ab}	14.2 ± 5.5 ^{bcde}
	T4	0.6 ± 0.12 ^{ab}	15.92 ± 5.43 ^{bcd}
	T5	0.59 ± 0.3 ^{ab}	10.83 ± 1.8c ^{def}
	T6	0.46 ± 0.2 ^{ab}	10.75 ± 1.9 ^{cdef}
	T7	0.66 ± 0.1 ^{ab}	19.2 ± 2.9 ^{abc}
	T8	0.69 ± 0.3 ^a	22.77 ± 5.5 ^{ab}
	T9	0.31 ± 0.1 ^b	5.1 ± 2.5 ^f
Water - stressed	T1	0.35 ± 0.2 ^{ab}	8.6 ± 1.9 ^{cdef}
	T2	0.36 ± 0.2 ^{ab}	11.8 ± 3.7 ^{cdef}
	T3	0.52 ± 0.1 ^{ab}	12.2 ± 3.44 ^{cdef}
	T4	0.56 ± 0.2 ^{ab}	13.64 ± 4.6 ^{cdefg}
	T5	0.51 ± 0.2 ^{ab}	11.71 ± 1.7 ^{cdef}
	T6	0.35 ± 0.4 ^{ab}	6.4 ± 1.6 ^{ef}
	T7	0.58 ± 0.1 ^{ab}	14.8 ± 5.6 ^{bcde}
	T8	0.66 ± 0.4 ^{ab}	26.3 ± 2.8 ^a
	T9	0.35 ± 0.2 ^{ab}	4.88 ± 2.7 ^f
	Mean	0.51	12.69
	CV (%)	44.42	42.72
	LSD (0.05)	*	**

CV: coefficient of variation, LSD: least significant difference. * and ** indicates significance level at P < 0.05 and P < 0.01, respectively. Different letters indicated a significant difference among treatments.

Percentage of elements concentration in plants and nutrients use efficiency

The grinded samples from roots, stems, leaves, and grains were analyzed using a near-infrared spectrometer (NIR) to determine the percentage of (N, P, K, Ca and Mg in both W_1 and W_2 blocks. The results indicated significant differences in root composition at T_8 treatment, with higher percentages of N (27.33%), K (20.9%), and Mg (4.6%) compared to other treatments. However, there were no significant differences in P% (1.03) and Ca% (5.98) among the treatments in the W_1 block (Table 5). The stem samples were also subjected to the same element concentration analysis, and similar patterns were observed. In T_8 , the stem composition showed significant differences for (2.05% P), (14.9% K), and (15.04% Ca), while no significant differences were found for (7.12% N) and (3.8% Mg). However, these values were still relatively higher compared to other treatments in the W_1 block (Table 5). Similar trends were observed in the analysis of leaf samples from the W_1 block. In T_8 , significant differences ($P \leq 0.05$) were found for (13.22% K), (9.36% Ca), and (3.51% Mg), while no significant differences were observed for (4.93% N) and (1.32% P). However, the values of N% and P% were higher compared to other treatments (Table 6).

Table 5. The percentage of the concentration of the root element within treatments of the well-watered block.

Treatment	Elements concentration in roots (%)					Elements concentration in stem (%)				
	N	P	K	Ca	mg	N	P	K	Ca	mg
T1	16.9 ^{cd}	0.9 ^{bc}	15.6 ^c	4.98 ^c	2.6 ^d	4.32 ^c	1.4 ^{bc}	11.99 ^{cd}	11.3 ^c	2.95 ^d
T2	9.98 ^e	0.9 ^c	10.1 ^d	3.6 ^e	2.7 ^d	1.74 ^d	1.8 ^a	6.01 ^f	9.9 ^d	2.72 ^{ef}
T3	14.6 ^d	1.03 ^a	15.6 ^c	5.8 ^{ab}	2.6 ^d	2.1 ^d	1.82 ^a	3.4 ^g	11.2 ^c	3.35 ^b
T4	16.2 ^{cd}	0.9 ^c	17.2 ^b	5.12 ^c	2.37 ^d	5.3 ^{bc}	1.7 ^{ab}	9.4 ^e	12.9 ^b	2.82 ^{de}
T5	16.5 ^{cd}	0.5 ^d	14.3 ^c	3.51 ^e	3.5 ^{bc}	2.2 ^d	1.1 ^c	2.9 ^g	9.03 ^{de}	2.62 ^f
T6	11.3 ^e	0.9 ^c	10.8 ^d	4.1 ^d	2.7 ^d	5.13 ^{bc}	0.7 ^d	12.3 ^c	7.3 ^f	3.2 ^c
T7	19.3 ^b	0.9 ^b	17.6 ^b	5.6 ^b	2.8 ^{cd}	6.1 ^{ab}	1.02 ^{cd}	13.5 ^b	9.7 ^d	3.7 ^a
T8	27.3 ^{a*}	1.0 ^a	20.9 ^{a*}	5.98 ^a	4.6 ^{a*}	7.12 ^a	2.1 ^{a*}	14.9 ^{a*}	15.1 ^{a*}	3.8 ^a
T9	18.4 ^{bc}	0.9 ^c	15.3 ^c	4.3 ^d	4.1 ^{ab}	2.93 ^d	1.03 ^{cd}	11.5 ^d	8.3 ^e	2.5 ^g
Mean	16.72	0.93	15.26	4.77	3.08	4.09	1.39	9.53	10.52	3.1
CV (%)	8.12	3.74	5.46	3.78	13.33	17.35	16.75	4.17	5.33	2.85
LSD (0.05)	*	ns	*	Ns	*	ns	**	*	*	ns

Table 6. The percentage of the concentration of the leaves element within treatments of the well-watered block.

Treatment	Elements concentration in leaves (%)					Elements concentration in seeds (%)				
	N	P	K	Ca	mg	N	P	K	Ca	mg
T1	-0.2 ^c	0.91 ^c	5.6 ^d	3.9 ^c	3.3 ^g	13.4 ^{cd}	3.3 ^{bc}	2.5 ^d	7.4 ^c	-1.9 ^b
T2	-0.95 ^d	0.97 ^c	3.7 ^f	3.4 ^{cd}	3.3 ^{fg}	11.3 ^e	3.2 ^{bc}	5.6 ^d	1.54 ^e	-0.7 ^c
T3	3.62 ^b	1.2 ^b	12.5 ^b	8.1 ^b	3.5 ^b	13.2 ^d	3.2 ^a	3.6 ^c	7.1 ^{ab}	-0.8 ^d
T4	4.7 ^a	1.21 ^b	12.2 ^b	8.4 ^b	3.3 ^f	13.7 ^{cd}	3.4 ^c	2.1 ^c	6.4 ^c	-0.5 ^e
T5	-0.5 ^e	0.95 ^c	4.8 ^e	3.6 ^c	3.4 ^c	12.4 ^{cd}	3.4 ^d	0.2 ^{bc}	2.9 ^e	-1.4 ^{bc}
T6	4.84 ^a	1.3 ^{ab}	11.1 ^b	8.5 ^b	3.4 ^e	13.7 ^e	3.5 ^c	1.2 ^d	4.1 ^d	-0.8 ^{bc}
T7	4.7 ^a	1.3 ^{ab}	12.6 ^b	9.1 ^a	3.4 ^d	9.4 ^b	3.5 ^b	-8.6 ^d	8.5 ^b	-1.9 ^b
T8	4.93 ^a	1.32 ^a	13.22 ^a	9.3 ^{a*}	3.5 ^{a*}	14.4 ^{a*}	3.6 ^a	3.4 ^{a*}	9.9 ^{a*}	-0.4 ^a
T9	-0.98 ^d	0.8 ^d	3.9 ^f	3.01 ^d	3.5 ^a	8.6 ^{bc}	2.9 ^c	-2.1 ^d	6.9 ^d	-3.3 ^c
Mean	2.24	1.1	8.85	6.37	3.4	16.72	0.93	15.26	4.77	3.08
CV (%)	8.66	4.28	3.23	5.49	0.5	8.12	3.74	5.46	3.78	13.33
LSD (0.05)	ns	ns	*	*	**	*	ns	*	*	*

CV: coefficient of variation, LSD: least significant difference. * And ** indicates significance level at $P < 0.05$ and $P < 0.01$, respectively. Ns: not significant. Different letters indicated a significant difference among treatments.

The grain grinded samples were analyzed using NIR, revealing significant differences in element concentration among the treatments. However, in T₈, no significant difference was observed for P% (3.61%), although it had a higher value than other treatments. A negative value of -0.4% was also observed for Mg% in T₈, the lowest among the treatments (Table 6). Further statistical analysis showed highly significant differences in root diagnoses for K% (24.44%) and Mg% (8.2%) (Table 7). Similarly, in stem diagnoses, highly significant differences were observed among all treatments of the W₂ block, particularly in T₈. Notably, N% (10.04%), P% (2.307%), K% (19.21%), Ca% (14.86%), and Mg% (3.803%) exhibited the highest values compared to other treatments (Table 7).

A similar trend was also used in leaf diagnoses. The highly significant differences tested in T₈ of the W₂ block, the percentage of the N, K, Ca, and Mg were (4.51%), (12.3 %), (8.813 %) and (3.59 %), respectively, while in P% did not find significant differences among the treatments. In contrast, in T₈, the percentage of the P% still was high (1.33%) than in the rest of the treatments in the same block (Table 7). The grain diagnoses results showed significant differences simultaneously in T₈, the result noted in the percentage of N %, P %, K%, and Ca %, (15.64), (4.5), (2.12), and (18.81). In contrast, in part of the Mg % did not find significant differences among the treatments in w₂ blocks; it was found in negative status (-1.52 Mg %) at all treatments, especially in T₈, found the least one (Table 8).

Table 7. The percentage of the concentration of the root element within treatments of the stress-watered block.

Treatment	Elements concentration in roots (%)					Elements concentration in stem (%)				
	N	P	K	Ca	mg	N	P	K	Ca	mg
T1	19.4 ^c	0.91 ^b	18.1 ^d	4.5 ^e	3.03 ^{ef}	6.1 ^c	0.96 ^f	5.4 ^f	9.9 ^e	2.8 ^e
T2	10.45 ^e	0.74 ^e	12.6 ^f	3.3 ^f	2.6 ^{ef}	2.4 ^f	1.1 ^d	14.8 ^b	9.97 ^c	3.8 ^a
T3	26.4 ^b	0.86 ^{bc}	20.2 ^c	4.9 ^{cd}	5.6 ^b	4.6 ^d	1.13 ^d	12.7 ^d	10.96 ^d	2.8 ^d
T4	30.7 ^a	0.98 ^a	22.7 ^b	5.53 ^b	4.5 ^c	3.12 ^e	1.1 ^{de}	11.13 ^e	10.4 ^e	2.38 ^h
T5	16.4 ^d	0.81 ^{cd}	18.2 ^{de}	5.02 ^c	3.43 ^{de}	2.6 ^f	1.05 ^e	11.54 ^e	7.6 ^f	3.11 ^b
T6	18.7 ^{cd}	0.77 ^{de}	16.6 ^e	5.4 ^b	4.1 ^{cd}	6.2 ^c	1.95 ^b	13.98 ^c	13.2 ^b	2.9 ^c
T7	19.91 ^c	0.91 ^b	19.01 ^{cd}	5.83 ^a	3.4 ^{de}	8.82 ^b	1.4 ^c	14.6 ^{bc}	12.3 ^c	2.6 ^g
T8	31.12 ^a	1.03 ^a	24.4 ^{a*}	6.01 ^a	8.2 ^{a*}	10.1 ^{a*}	2.3 ^{a*}	19.2 ^{a*}	14.9 ^{a*}	3.8 ^{a*}
T9	12.96 ^e	0.8 ^d	13.8 ^f	4.7 ^{de}	2.4 ^f	2.96 ^e	0.9 ^f	4.9 ^f	6.97 ^f	2.7 ^f
Mean	20.7	0.87	18.4	5.01	4.13	5.20	1.32	12.03	10.67	2.99
CV (%)	8.01	3.8	5.42	2.73	14.47	2.59	2.93	3.19	3.25	0.51
LSD (0.05)	ns	ns	*	ns	*	*	ns	*	**	*

CV: coefficient of variation, LSD: least significant difference. * And ** indicates significance level at P < 0.05 and P < 0.01, respectively. Ns: not significant. Different letters indicated a significant difference among treatments.

Table 8. The percentage of the concentration of the leaf element within treatments of the stress-watered block.

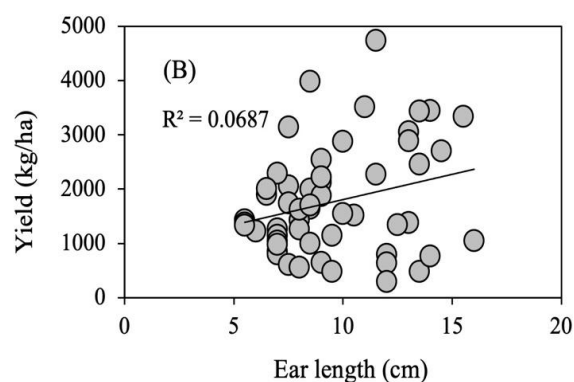
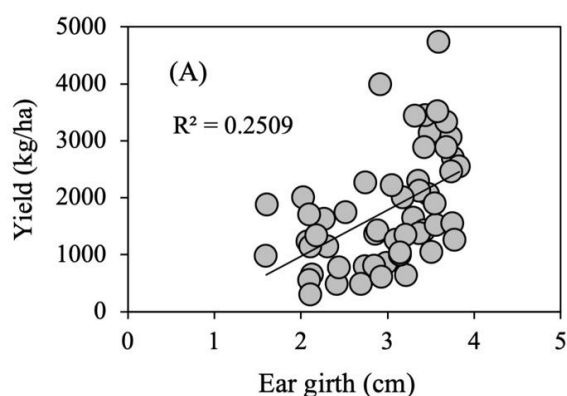
Treatment	Elements concentration in leaves (%)					Elements concentration in seeds (%)				
	N	P	K	Ca	mg	N	P	K	Ca	mg
T1	2.8 ^d	1.2 ^c	9.7 ^d	7.9 ^c	3.5 ^c	11.3 ^d	3.5 ^f	-12.2	9.75 ^c	-6.5
T2	0.18 ^e	0.9 ^e	5.9 ^e	4.4 ^d	3.4 ^d	10.5 ^e	3.5 ^{ef}	0.98	7.3 ^g	-2.2
T3	3.64 ^c	1.03 ^d	11.2 ^b	8.3 ^b	3.4 ^e	9.5 ^f	3.8 ^d	-15.3	12.9 ^b	-3.3
T4	3.6 ^c	1.2 ^b	10.07 ^c _d	8.2 ^b	3.5 ^b	8.9 ^g	4.2 ^b	-20.7	12.5 ^b	-2.1
T5	-0.7	0.8 ^g	4.8 ^g	3.1 ^e	3.4 ^d	13.01 ^b	3.4 ^g	-4.3	9.1 ^e	-1.65
T6	-0.9	0.9 ^f	3.98 ^h	3.03 ^e	3.5 ^b	13.05 ^b	3.9 ^c	-10.7	12.5 ^b	-2.5
T7	3.9b	1.3 ^a	10.3 ^c	8.5 ^b	3.3 ^f	11.3 ^d	3.6 ^e	1.13	10.9 ^c	-2.5
T8	4.51 ^{a*}	1.3 ^a	12.3 ^{a*}	8.8 ^{a*}	3.6 ^{a*}	15.6 ^{a*}	4.5 ^{a*}	2.12 ^{a*}	18.8 ^{a*}	-1.52
T9	-0.23	0.8 ^g	5.24 ^f	2.9 ^e	3.2 ^g	12.6 ^c	3.2 ^h	-6.9	8.14 ^f	-2.1
Mean	1.87	1.05	8.2	6.13	3.42	11.74	3.73	-8.63	11.32	-2.7
CV (%)	3.64	2.18	2.67	2.78	0.5	0.7	1.48	-5.64	2.33	-3.7
LSD	*	ns	*	*	*	*	**	*	*	ns

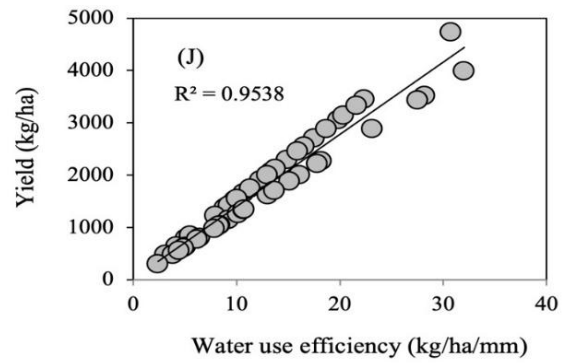
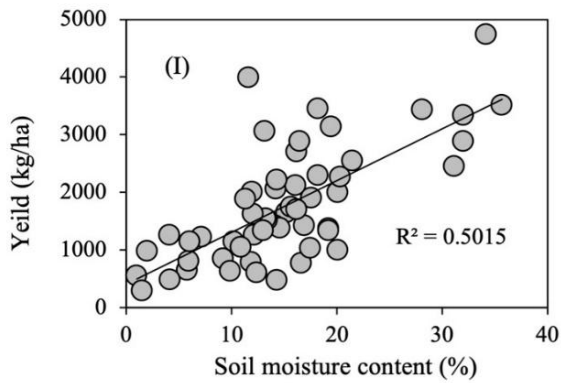
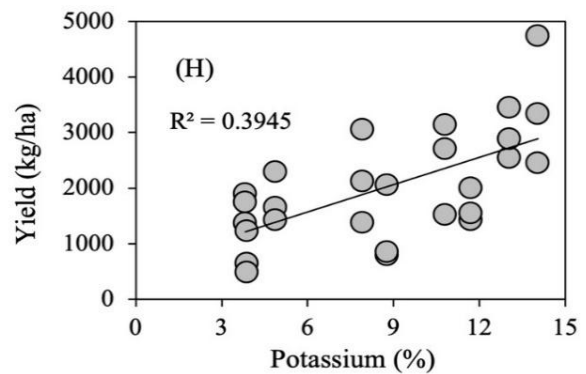
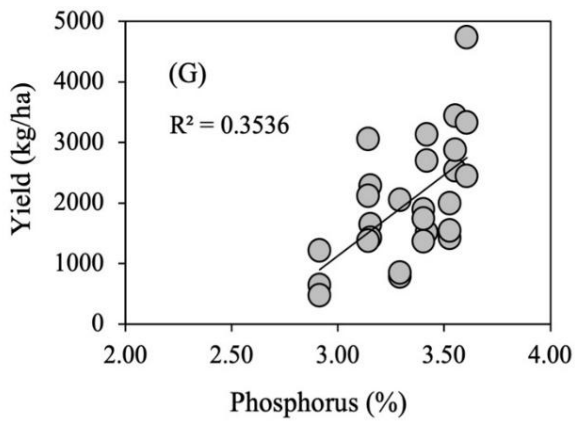
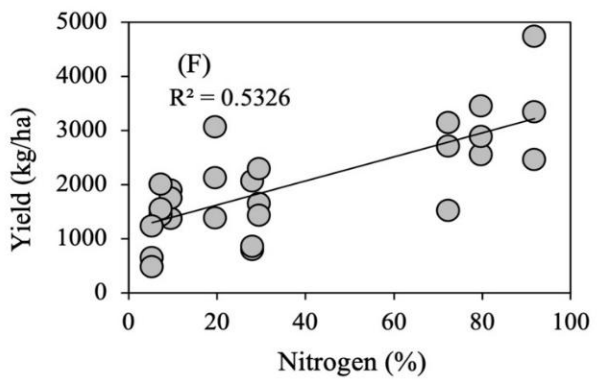
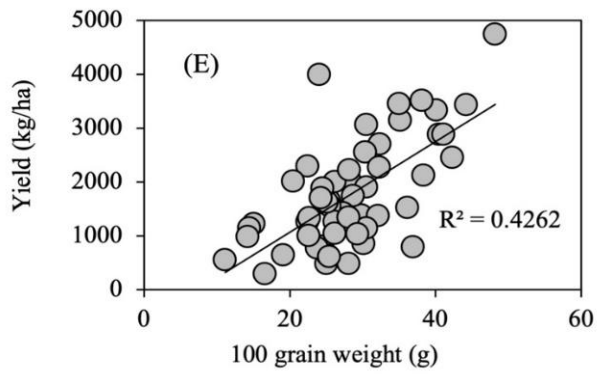
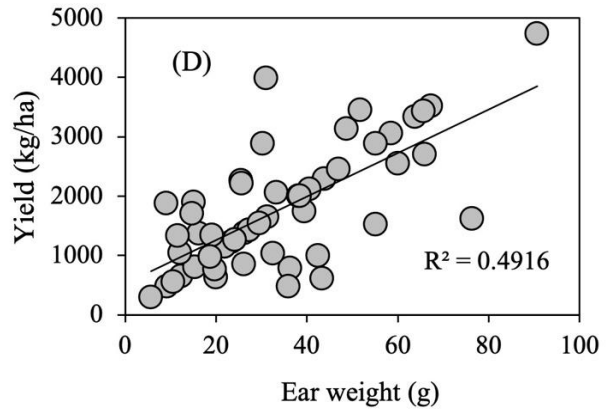
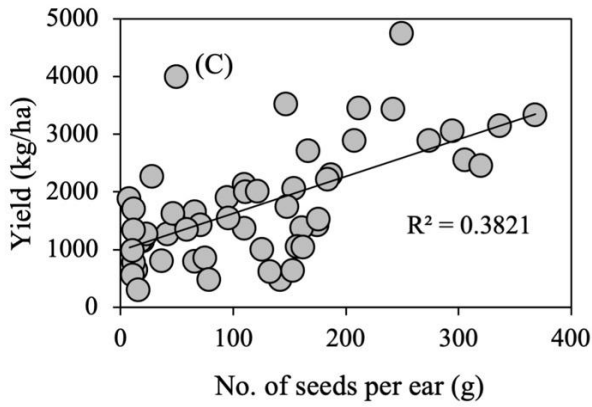
CV: coefficient of variation, LSD: least significant difference. * And ** indicates significance level at $P < 0.05$ and $P < 0.01$, respectively. Ns: not significant. Different letters indicated a significant difference among treatments.

Relationship of the Ears, WUE, NUE, SMC with yield

The relationship of phenological parameters such WUE, NUE, SMC, and ear related parameters measured. The results revealed that ear girth had positive relations ($r^2=0.251$) with yield as shown in Figure 2A, while ear length showed weak relationship ($r^2=0.069$) with yield under water-stressed conditions (Figure 2B).

A positive relationship observed between number of seeds per ear and ear weight with yield, while the r^2 value were 0.382 and 0.492, respectively (Figure 2C and D). Similar result observed for the correlation between the 100 grains weight with yield, which had strong positive correlation ($r^2=0.426$) as shown in Figure 2E. In term of phenological parameters such as soil moisture content, water use efficiency, and harvest index % have shown strong positive correlation with yield, while the relation strength were ($r^2=0.865$), ($r^2=0.954$), and ($r^2=0.502$), respectively, as shown in Figure 2I, 2J, and 2K. In addition, there was positive relationship between NPK percentage and yield of maize under water-stressed conditions, as shown in Figure 2F, 2G, and 2I.





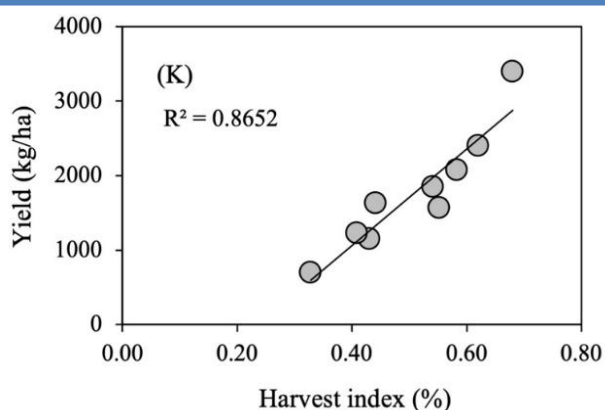


Figure 2. These collections of sub-figures shows the relationship of different parameters with the yield of maize under stress-water conditions. **A:** relationship of ear girth (cm) with yield, **B:** relationship of ear length (cm) with yield, **C:** relationship of the number of seeds per ear (g) with yield, **D:** relationship of ear length (cm) with yield, **E:** relationship of 100-grain weight (g) with yield, **F:** relationship of nitrogen % with yield, **G:** relationship of phosphorous % with yield, **H:** relationship of potassium with yield, **I:** relationship of soil moisture content % with yield, **J:** relationship of water use efficiency (kg/ha/mm), and **K:** relationship of harvest index % with yield.

DISCUSSION

Improvement in nutrient use efficiency cannot possibly be viewed only by fertilizers management. It will be governed due to the soil moisture contents and soil solution, while water stress limits soil nutrient use efficiency (Bossio et al., 2008). However, water cannot fulfill the plant's requirements alone; balancing applying the chemical and organic fertilizers with proper soil moisture contents increases nutrient and water use efficiency in terms of soil fertility and productivity, ultimately increasing grain yield (Ryan et al., 2009). In maize, vegetative and reproductive growth stages are the sensible stages. Drought stress at the flowering stage caused the ear and silk reduction, resultantly extending the gap between silking and anthesis (Jaleel et al., 2009; Sarobol.E.et al., 2004).

Nilson and Orcutt, (1996) stated that the effect of the drought stress one week before silking ($R_2 \dots R_4$) decreased the grain yield by 53%, compared to non-drought threatened plants. Maize crop needs 50%–70% soil moisture to maintain their normal physiological activities. The cereal productions rarely exceed 1.5 t/ha even when an ample number of fertilizers are used in drought-affected areas in developing countries, while in case of sufficient water of irrigation leads, the yield amount exceeds 8 t/ha (Thompson, 2012). So a strong positive correlation exists between WUE, NUE, and crop yield production (Lucas et al., 2007). Therefore, this study revealed that the use of chemical fertilizers within the combination of SAPs enhanced the water holding capacity, particularly water, nutrients use efficiencies, and maize yield. The current study elucidates that applying SAPs regularly increased water holding capacity and soil moisture content by 40 to 60% at water deficit conditions, prolonging the wilting period from 6 to 12 days and water holding capacity from 171 to 402%.

During the reproductive growth stage (silking & pollen shed), drought reduced corn yield from 3 to 8% per day (Lauer, 2007). However, after two weeks, the mentioned stages of severe drought downed the yield by 6% per day (khalili et al., 2013). Drought stress negatively affected corn growth and yield, including reduced assimilate

partitioning, decreased photosynthesis, and changes in protein abundance (Fahad et al., 2017). Bahamin et al., (2021) stated that drought could decrease nitrogen uptake from soil and reduce the N concentration in corn crop tissues. Drought reduced N concentration by 44-51% and P by 39-48% in drought-sensitive stages revealed by (Bista et al., 2018). However, drought at initial and severe times reduced N & P uptake rates by 46-72% and 54-80%, respectively (Tarighaleslami et al., 2012). (Alam, 1999) Investigated that drought reduced root development which caused limited nutrient uptake, especially N, P, and K. Dry and irregular soil moisture caused insufficient nutrient uptake. Based on the experiment result, nutrients uptake levels improved in the root, stem, leaf, and seeds for N (27.3, 7.12, 4.93, and 14.4%), P (1, 2.1, 1.32, and 3.6%), and K (9, 14.6, 13.22, and 3.4%) due to the application of SAPs in T₈ incorporated of the recommended dose of fertilizer of NPK. SAPs improved the soil moisture content, root better uptake level, and enough soil moisture and nutrient availability. Applying SAPs increased water productivity by 12.8 to 17.2% (Abdullah et al., 2021). SAPs also increased relative water content, leaf water potential recorded higher in corn treated by SAPs, and increased biomass accumulation by 11.1, 39.0, and 98.7% at proper, moderate, and deficit irrigation, respectively (Zheng et al., 2023). Combine application of SAPs with cow manure increased N-uptake, CEC, and SMC in corn fields stated by (Khadem et al., 2010). (Singh et al., 2018) suggested that 15% of SAPs concentration increased 26.5% yield of corn over the control. A similar study (Krasnopeevea et al., 2022) revealed that 100 kg of SAPs ha⁻¹ was the most appropriate rate, increasing corn yield and dry matter yield. The results of the current study revealed the water use efficiency of 24.53 kg ha⁻¹ mm⁻¹ and HI% (0.68) again in T₈ to declare that using SAPs in crops field during drought conditions could enhance vegetative and reproductive growth. Lastly, the corn crop yield applied 13 gr per pot based on the calculation 325 kg ha⁻¹, as literature revealed SAPs resulted based on their concentration rather than their types.

Asiimve et al., (2023) reported that SAPs incorporated with organic and inorganic fertilizers increased soil fertility and productivity. While 70% evaporated stress reduced 22% and 7% corn yield and soil moisture content (Wei et al., 2018). The cereal productions rarely exceed 1.5 t/ha even when ample amount of fertilizers are used in drought-affected areas in developing countries. In contrast, in case of sufficient water from irrigation leads, the yield amount exceeds 5 t/ha (Thompson, 2012). However, the number of rows, kernels row⁻¹, and overall seed per ear were recorded at 14, 20, and 250-300 without SAPs plots or stressed corn fields (Strachan, 2004).

This experiment's findings elucidated that applying SAPs enhanced the soil moisture content from 25% to 50%, while in control, recorded less than 20%. The proper soil moisture content with other extreme parameters improved by SAPs under the water deficit condition maximized the ear length (13 cm), the number of rows in one ear or cob was recorded from 16 to 18, number of kernels in one row was recorded from 25 to 30, and overall seeds per ear were 350 to 400, 100 seed weight 42.1 gr and finally yield per ha recorded 3390 kg in T₈ (SAPs + NPK). However, only NPK treatment in T₄ produced 2074 kg ha⁻¹. Several experiments were conducted to investigate the impact of Super Absorbent Polymers only and in combination with organic and inorganic fertilizers on soil physical and chemical properties. The results demonstrated significant enhancements in soil moisture content, water holding capacity, water retention power, leaching, percolation, and reduced surface runoff. These practices collectively stimulated corn root growth and improved nutrient uptake levels, leading to improved corn production parameters and ultimately increasing the yield of corn under drought conditions.

CONCLUSION

Using super absorbent polymers (SAPs) combined with manure and fertilizers showed significant improvements in maize growth and productivity. The integrated application of NPK fertilizers and SAPs resulted in higher values for ear-related parameters compared to other treatments. Additionally, it is exhibited the highest yield under both well-watered and water-stressed conditions. The application of SAPs contributed to improved soil moisture content, water use efficiency, and harvest index. Furthermore, SAPs positively influenced the concentration of essential elements in different plant parts. These findings emphasize the potential of SAPs in enhancing crop performance, particularly in water-limited environments.

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REFERENCES

1. Ahmadi, A., and Baker, D. A. (2001). The effect of water stress on the activities of key regulatory enzymes of the sucrose to starch pathway in wheat. *Plant Growth Regul.* 35, 81–91.
2. Anjum, S. A., Wang, L. C., Farooq, M., Hussain, M., Xue, L. L., and Zou, C. M. (2011). Brassinolide application improves the drought tolerance in maize through modulation of enzymatic antioxidants and leaf gas exchange. *J. Agron. Crop Sci.* 197, 177–185.
3. Asghar, A., A. Ali, W. Syed, M. Asif, T. Khaliq and A. Abid. (2010). Growth and Yield of Maize (*Zea Mays L.*) Cultivars Affected by NPK Application in Different Proportion. *Pakistan Journal of Science.* 62(4): 211-216.
4. Asiimwe, G., Jaafar, H., Haidar, M., & Mourad, R. (2022). Soil Moisture or ET-Based Smart Irrigation Scheduling: A Comparison for Sweet Corn with Sap Flow Measurements. *Journal of Irrigation and Drainage Engineering*, 148(6), 04022017.
5. Alam, S. M. (1999). Nutrient uptake by plants under stress conditions. *Handbook of plant and crop stress*, 2, 285-313.
6. AbdAllah, A. M., Mashheet, A. M., & Burkey, K. O. (2021). Super absorbent polymers mitigate drought stress in corn (*Zea mays L.*) grown under rainfed conditions. *Agricultural Water Management*, 254, 106946.
7. Bossio, D.; Noble, A, Molden, D, Nangia, V. (2008). Land degradation and water productivity in agricultural landscapes. In *Conserving land, protecting water*, ed., Bossio, D, Geheb, K. Wallingford, UK: CABI; Colombo, Sri Lanka: International Water Management Institute (IWMI); Colombo, Sri Lanka: CGIAR Challenge Program on Water and Food, pp.20-32.
8. Bahamin, S., Koocheki, A., Mahallati, M. N., & Behashti, S. (2021). Effect of nitrogen and phosphorus fertilizers on yield and nutrient efficiency indices in maize under drought stress. *Environmental Stresses in Crop Sciences*, 14(3), 675-690.
9. Bista, D. R., Heckathorn, S. A., Jayawardena, D. M., Mishra, S., & Boldt, J. K. (2018). Effects of drought on nutrient uptake and the levels of nutrient-uptake proteins in roots of drought-sensitive and-tolerant grasses. *Plants*, 7(2), 28.
10. Bruinsma, J. (2009). *The resource outlook to 2050: by how much do land, water, and crop yields need to increase by 2050*, FAO, Rome, Italy.
11. Fahad, S., Bajwa, A. A., Nazir, U., Anjum, S. A., Farooq, A., Zohaib, A. & Huang, J. (2017). Crop production under drought and heat stress: plant responses and management options. *Frontiers in plant science*, 1147.
12. Food and Agriculture Organization. (FAO) World Summit on Food Security, Rome, 16–18 November, (2009).

13. He, Y., Tian, Z., Ma, R., Liang, Y., Zhu, X., & Qu, L. (2023). Effects of superabsorbent polymers (SAPs) incorporated with organic and inorganic fertilizer on the water and nutrient retention of soil in rare earth mine tailing areas. *Journal of Soils and Sediments*, 1-12.
14. Jaleel C.A., P. Manivannan, A. Wahid, M. Farooq, R. Somasundaram, R. Paneerselvam, (2009). Drought stress in plants: a review on morphological characteristics and pigments composition. *Int. J. Agric. Biol.*, 11: 100-105.
15. Jensen M.E. (1993). The impacts of irrigation and drainage on the environment. 5th Gulhati Memorial Lecture. 15th ICID Congress, The Hague, ICID, New Delhi.
16. Karimi A, Noshadi M, Ahmadzadeh M. (2009). Effects of superabsorbent polymer (SAPs) on crop, soil water and irrigation interval. *Journal of Science and Technology of Agriculture and Natural Resources* 12: 415-420.
17. Kaul J, kumar RS, Dass S. (2011). Varietal improvement in maize: development of single cross hybrids in India.
18. Kim, J. Y., Mahe, A., Brangeon, J., and Prioul, J. L. (2000). A maize vacuolar Invertase, IVR2, is induced by water stress. Organ/tissue specificity and diurnal modulation of expression. *Plant Physiol.* 124, 71–84. Doi: 10.1104/pp.124.1.71.
19. Kidist. (2013). Growth and productivity of maize as influenced by rate and time of nitrogen. *Journal of Applied Sciences Research*, 9(4): 1920-1928.
20. Khan, M. B., Hussain, M., Raza, A., Farooq, S., and Jabran, K. (2015). Seed priming with CaCl₂ and ridge planting for improved drought resistance in maize. *Turk. J. Agric. For.* 39, 193–203.
21. Khaliq, Abbasi and T. Hussain. (2006). Effects of Integrated Use of Organic and Inorganic Nutrient Sources with Effective Microorganisms (Em) on Seed Cotton Yield in Pakistan.
22. Khalili, M., Naghavi, M. R., Aboughadareh, A. P., & Rad, H. N. (2013). Effects of drought stress on yield and yield components in maize cultivars (*Zea mays* L.). *International Journal of Agronomy and Plant Production*, 4(4), 809-812.
23. Khadem, S. H., Roustia, M. J., Chorom, M., Khadem, S. A., & Kasraeyan, A. (2010). The effects of different rates of super absorbent polymers and manure on corn nutrient uptake. In *Proceedings of the 19th world congress of soil science: soil solutions for a changing world*, Brisbane, Australia (Vol. 2, pp. 1-6).
24. Krasnopeevea, E. L., Panova, G. G., & Yakimansky, A. V. (2022). Agricultural Applications of Superabsorbent Polymer Hydrogels. *International Journal of Molecular Sciences*, 23(23), 15134.
25. Leport, L., Turner, N. C., French, R. J., Barr, M. D., Duda, R., and Davies, S. L. (2006). Physiological responses of chickpea genotypes to terminal drought in a Mediterranean-type environment. *Eur. J. Agron.* 11, 279–291.
26. Lija, M., A.O. Haruna and S. Kasim. (2014). Maize (*Zea Mays* L.) Nutrient Use Efficiency as Affected by Formulated Fertilizer with Clinoptilolite Zeolite.
27. Lucas, A.C., Jorge, A., John, D.M. and Klaus, W. (2007). Large variation in whole-plant water-use efficiency among tropical tree species. *New phytologist*, 173: 294-305.
28. Lauer, J. (2007). How do you manage a corn crop after stress? *Field Crop. Res.* 28, 28-46.
29. Nurlaeny, N., Herdiyantoro, D., Putra, R. M., Ratuliami, N., Pratiwi, W. N., & Nurfadilah, F. S. (2021). Drought stress and K effects on contents of soil water and organic matter, CEC, exchangeable-K, yield and water productivity of sweet corn on Inceptisols. In *IOP Conference Series: Earth and Environmental Science*.
30. Niu, L., Wang, Z., Zhu, G., Yu, K., Li, G., & Long, H. (2022). Stable Soil Moisture Improves the Water Use Efficiency of Maize by Alleviating Short-Term Soil Water Stress. *Frontiers in Plant Science*, 13.
31. Nielson, K.A., S.A. Langenecker and H. Garavan. (2002). Differences in the Functional Neuroanatomy of Inhibitory Control across the Adult Life Span. *Psychol. Aging*.
32. Nwachukwu, O. And M. Ikeadigh. (2012). Water Use Efficiency and Nutrient Uptake of Maize as Affected by Organic and Inorganic Fertilizer. *PAT.* 8(1): 199-208.
33. Orzeszyna H, Garlikowski D, Pawlowski A. (2006). Using of geocomposite with superabsorbent synthetic polymers as water retention element in vegetative layers. *Institute of Agrophysics, Polish Academy of Science* 20: 201-206.

34. Pawlowski A, Lejcus K, Garlikowski D, Orezesyna H. (2009). Geocomposite with superabsorbent as an element improving water availability for plants on slopes. *Geophys Res Lett* 11: 1-2.
35. Pimentel D, Berger B, Filiberto D, Newton M, Wolfe B, Karabinakis E, Clark S, Poon E, Abbett E, Nandaopal S. (2004). *Water Resources, Agriculture, and the Environment*. Ithaca (NY): New York State College of Agriculture and Life Sciences, Cornell University. Environmental Biology Report 04-1.
36. Rafiei F, Nourmohammadi G, Chokan R, Kashani A, Haidari H. (2013). Investigation of superabsorbent polymer usage on maize under water stress. *Global Journal of Medicinal Plant Research* 1: 82-87.
37. Rosegrant M.W. and CAI. (2002). Global water demand and supply projections: Results and prospects to 2025. *Water Intern*, 27: 170-182.
38. Rucker, K. S, Kvien, C. K., Holbrook, C. C., and Hook, J. E. (1995). Identification of peanut genotypes with improved drought avoidance traits. *Peanut Sci.* 24, 14–18.
39. Sarobol, E., T. Pakokton and S. Chowchong. (2003). The anaerobic protein of maize. *Cell* 20: 761-767 under the drought conditions. pp.165-172. In proceeding of the 31st National corn and sorghum Research Conference 2003, May 11-15, 2003, Nakhon Pathom, Thailand.
40. Sarvas, M., P. Pavlenda and E. Takacova. (2007). Effect of hydrogel application on survival and growth of pine grainling in reclamations.
41. Singh, J.B., Pradeep, B. and Yadava, R.B. (2007). *Water Relations Current Science*. *Current Science*, 93(1).
42. Schimel, J., Balsler, T. C., and Wallenstein, M. (2007). Microbial stress response physiology and its implications for ecosystem function. *Ecology* 88, 1386–1394.
43. Singh, S. K., Suman, S. N., & Kumari, A. (2018). Performance of autumn maize crop as influenced by seaweed saps. *IJCS*, 6(2), 2341-2345.
44. Tarighaleslami, M., Zarghami, R., Boojar, M. M. A., & Oveysi, M. (2012). Effects of drought stress and different nitrogen levels on morphological traits of proline in leaf and protein of corn seed (*Zea mays* L.). *American-Eurasian Journal of Agricultural and Environmental Sciences*, 12, 49-56.
45. Taiz, L., and Zeiger, E. (2006). *Plant Physiology*, 4th Edn. Sunderland, MA, Sinauer Associates Inc Publishers.
46. Thompson, Helen. (2012). Food science deserves a place at the table – US agricultural research chief aims to raise the profile of farming and nutrition science. *Nature*, July 12.
47. Wahid, A., and Close, T. J. (2007). Expression of dehydrins under heat stress and their relationship with water relations of sugarcane leaves. *Biol. Plant.* 51, 104–109.
48. Wang, J. G.H., et al, Effect of soil moisture-based furrow irrigation scheduling on melon (*Cucumis melo* L.) yield and quality in an arid region of Northwest China *Agric. Water Manag.* 179 (2017), pp. 167-176.
49. Woldesenbet, M. And A. Haileyesus. Effect of Nitrogen Fertilizer on Growth, Yield and Yield Components of Maize (*Zea Mays* L.) in Decha District, Southwestern Etiopia.
50. Wei, L. C., Zhang, H. P., Wang, X. L., & Zhang, S. Q. (2023). Improved Water Use of the Maize Soil–Root–Shoot System under the Integrated Effects of Organic Manure and Plant Density. *Agronomy*, 13(4), 1172.
51. Yazdani F, Allahdadi I, Akbari GA. (2007). Impact of superabsorbent polymer on yield and growth analysis of Soybean (*Glycine max* L.) under drought stress condition. *Pakistan Journal of Biological Sciences* 10: 4190-4196.
52. Zhao, T. J., et al. (2006). Regulating the drought-responsive element (DRE)-mediated signaling pathway by synergic functions of trans-active and Trans in active DRE binding factors in *Brassica napus*. *J. Biol. Chem.* 281, 10752–10759.
53. Zinselmeier, C., Jeong, B. R., and Boyer, J. S. (1999). Starch and the control of kernel number in Maize at low water potentials. *Plant Physiol.* 121, 25–35.
54. Zheng, H., Mei, P., Wang, W., Yin, Y., Li, H., Zheng, M., & Cui, Z. (2023). Effects of super absorbent polymer on crop yield, water productivity and soil properties: A global meta-analysis. *Agricultural Water Management*, 282, 108290.

Diabetic Ketoacidosis Prevalence and Clinical Presentation in Diabetic-Covid Comorbidity in Nangarhar Afghanistan

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ABSTRACT

Background: Diabetic ketoacidosis (DKA) as an acute complication of diabetes mellitus is a life threatening medical emergency causing mortality and morbidity in patients. The aim of the study was to find out DKA prevalence and existing clinical presentation in diabetic-covid comorbidity.

Materials and Methods: The retrospective cross sectional study included 791 both male and female diabetic patients with a confirmed diagnosis of covid 19 based on purposive sampling for a period of one year from Aug 2021 to Aug 2022 from two centers (Corona Center and Nangarhar Regional Hospital) in Nangarhar, Afghanistan.

Findings: The study included 300 (37.9%) male and 491 (62.1%) female patients, 45 (5.7%) Type 1, 746 (94.3%) Type 2 diabetic patients, 511 (64.6%) old diabetes, and 280 (35.4%) new onset diabetes patients. Mean age at the study was 58.7 ± 13 , BMI was 31.2 ± 4 , Systolic BP 128.3 ± 24.4 , oxygen was 79.1 ± 15.4 , glycaemia was 297.5 ± 8 , and mean hospital stay was 8.8 ± 8.1 days. In fact, 149 out of 791 patients (19%) had diabetic ketoacidosis (DKA) of which 140 patients out of 149 (93.96%) were hyperglycemic and 9 out of 149 patients (6%) were euglycemic DKA. Furthermore, DKA was more prevalent in males 58.4% vs 41.6% in females, young age (20-39 years) 31.2%, Type 1 diabetes 33.3% vs 21.9% type 2 diabetes, and old diabetes 19.5% vs 17.6% new onset diabetes. Moreover, clinical presentation included dyspnea 143 (96%), abdominal pain 124 (83.2%), nausea/vomiting 121 (81.2%), tachycardia 105 (70.5%), polydipsia of diabetic classic symptoms 70 (47%), and crepitation in chest auscultation though not significant 77 (51.7%). Pneumonia 92 (61.7%), and ARDS 54 (36.2%) were respectively the most prevalent clinical and X-ray findings in DKA patients. In addition, hospitalization duration was comparatively higher for females (10 vs 9), T2DM (10 vs 6), and new onset DM (14 vs 7) and it increased with advancing age (most for patients of ≥ 80 years) in DKA. Death and referral measures were significantly different across DKA positive and DKA negative patients i.e. 37.6% vs 14.8% and 16.1% vs 8.1% respectively. While, discharge status with home rest was more prevalent in DKA negative patients i.e. 46.3% vs 77.1%.

Conclusion: We concluded that DKA prevalence has increased almost two fold in diabetic patients suffering from corona virus affecting in-hospital mortality, hospital stay, morbidity and the preexisting clinical picture. In fact, obesity, hypertension, young age and male gender were significant factors contributing to the prevalence. In addition, mortality and referral rates to specialty specific centers were significantly higher in DKA positive patients with the aforementioned factors being the leading contributors.

Keywords: Diabetes, Diabetic Ketoacidosis, DKA, Covid 19, Afghanistan

INTRODUCTION

The novel corona virus infected pneumonia (NCIP) first appeared in Wuhan, China, with the number of cases increasing over periods of time causing lockdowns in the city then in the whole country leaving great impact on social life (Wang et al., 2020). In Afghanistan, approximately ten million people (31.5% of the whole country) were reported to suffer from either a pre-existing or a new Covid disease up to July 2020 (Saeedzai et al., 2022). In fact, 7321 deaths were reported on 10th Dec 2021 in association with Covid (Shah et al., 2022) which may be an underestimated figure representing the whole country since there were numerous rumors about the virus and it is still an under-looked topic. Shutdown of healthcare centers due to lack of financial aid and lack of in-hospital beds, oxygen supply, ventilators and professional staff were the leading contributors to the increased number of mortality in a small population residing country (Shah et al., 2022).

Diabetes Mellitus is an independent risk factor for in-hospital complications in Covid patients, which include hyperglycemic medical emergencies as diabetic ketoacidosis (DKA) and others as well as increased hospitalization and hospital stays in comparison with controls (Erratum, 2022). Increased ACE2 (Angiotensin converting enzyme 2) expression and later downregulation specifically after the virus entry (Roca et al., 2017), increased Furin expression (Fernandez et al., 2018), impaired T cell function (Kulcsar et al., 2019) and increased interleukin 6 appearance (Maddaloni et al., 2020) in diabetic patients are the known pathophysiologic mechanisms predisposing to Covid 19 and the subsequent lung injury and ARDS. On the other hand, binding of SARS Corona virus to the receptors (ACE2 and Furin), causes pancreatic damage which in turn results in acute diabetes calling it a bidirectional relationship (Yang et al., 2010).

Despite the recent explosion of articles on the topic, Covid, still little is known about Covid-DM comorbidity and its sequences and local data on the prevalence of diabetic ketoacidosis and its associated characteristics during the comorbidity lacks, so we aimed to conduct the study to find out the prevalence of diabetic ketoacidosis in Covid further elaborating its associated factors, clinical trends, types of diabetes, hospital stay and mortality.

MATERIALS AND METHODS

It is a retrospective cross-sectional multicenter (Nangarhar Regional Hospital, Nangarhar Corona Center) study, which is based on purposive sampling including 791 patients for a period of one year from Aug 2021 to Aug 2022. Patients of either gender with either type of diabetes regardless of age but with a positive Covid test (real time PCR on a nasopharyngeal swab) were included in the study and patients with diagnosis other than Covid and other known KDA predisposing factors such as insulin withdrawal, infection/stress, infarction and etc were excluded from the study. Patients' identity was secured for ethical purpose and the study was registered at the ethical committee as IRB-3121. In fact, Diabetes mellitus was diagnosed under the criteria, two positive tests or a single positive tests plus classic clinic. And DKA was defined according to the American Diabetic Association as PH <7.35, bicarbonate <18 mEq/L, positive serum or urine ketones and serum glucose >250 mg/dl (Dhatariya, 2007). Patients' demographics, baseline characteristics and other variables of interest achieved via thorough approach including history, physical exam and labs were analyzed using both qualitative and quantitative measures after verifying the data normality via Kolmogorov test by SPSS version 26. Mean \pm standard deviation was used for continuous variables while frequencies percentages for categorical variables.

RESULTS

The study included 791 both female and male (300 males & 491 females) patients with known Diabetes Mellitus and Covid 19 with most cases coming from Nangarhar province where Corona health centers are located. 407 (51.45%) of the cases were obese, 253 (31.98%) were overweight and 131 (16.56%) were of normal weight. Further demographic characteristics of the study participants are described in Table 1.

Table 1. Demographics

Parameter		Frequency (%)
Gender	Male	300 (37.9%)
	Female	491 (62.1%)
Age	20-39	48 (6.1%)
	40-59	304 (38.4%)
	60-79	376(47.5%)
	>=80	63(8%)
	Single	6(0.8%)
Marital Status	Married	785(99.2%)
	Normal weight	131(16.7%)
BMI	Overweight	253(32%)
	Obese	407(51%)
	Poor	369(46.6%)
Economic Status	Fair	234(29.6%)
	Good	188(23.8%)
DM Type	Type1	45(5.7%)
	Type2	746(94.3%)
Residence	Nangarhar	659(83.3%)
	Laghman	59(7.5%)
	Kunar	26(3.3%)
	Other Provinces	47(5.9%)
Diabetes Onset	Old onset Diabetes	511(64.6%)
	New onset Diabetes	280(35.4%)

Mean age of the cases during study was 58.7 ± 13 years, mean glycaemia 297.48 ± 8 mg/dl and mean hospital stay was 8.8 ± 8.1 days. Table 2 describes the baseline characteristics of the study participants (patient values at the time of admission under diabetic ketoacidosis like age, blood pressure, BMI and etc).

Table 2. Baseline Characteristics

Parameter	Mean	Minimum	Maximum
Age	58.7 ± 13	20	100
BMI	31.2 ± 4	24.7	48.5
Heart Rate	111.8 ± 26	65	180
Respiratory Rate	25.1 ± 4	16	36
Temperature	37.7 ± 0.6	35.6	40
Systolic BP	128.3 ± 24.4	70.0	190
Oxygen saturation	79.1 ± 15.4	30	96
Glycaemia	297.5 ± 8	69	610
ALT	79.4 ± 6	6	328
AST	51 ± 34	10	230
Hemoglobin	12 ± 1.3	7	16
Total Leukocytes	13298 ± 5660	3500	42300
Creatinine	1.63 ± 1	0.1	5.50
Potassium	3.8 ± 0.4	2.2	5.80

pH	7.3±0.4	6.7	7.90
Hospital stay duration	8.8±8.1	0	75

BMI= Body mass index, BP= Blood pressure, ALT= Alanine transaminase, AST=Aspartate transaminase, pH= Power of hydrogen

In fact, 149 cases (19%) out of 791 diabetic-covid 19 patients were complicated to diabetic ketoacidosis as shown in Figure 1.

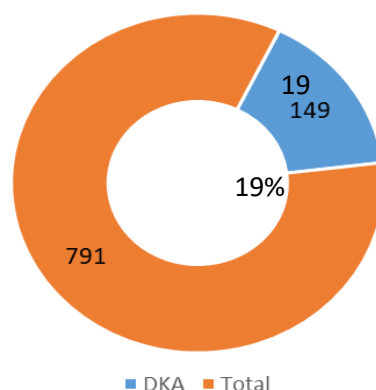


Figure 1. Ketoacidosis Prevalence

DKA was significantly more prevalent in males i.e. 87 out of 300 (29%) compared to females, 62 out of 491 (12.6%). In addition, 33.3% of Type 1 DM and 21.9% of Type 2 DM were complicated to DKA during the comorbidity. However, DKA prevalence was not significantly different across different age groups as shown in Table 2. In fact, 67.1% cases of DKA were diagnosed as having old DM while the rest 32.9% had new onset DM. 58.3% of the DKA positive patients were males and the rest 41.7% were females despite knowing that majority of the cases included in the study were females. 140 patients out of 149 (93.96%) were diagnosed with hyperglycemic DKA while 9 out of 149 patients (6%) were diagnosed with euglycemic DKA.

Table 3. DKA Prevalence across gender, age groups, and diabetes types

Parameters		Total Count	DKA Positive
Gender	Male	300	87 (29%)
	Female	491	62 (12.6%)
Age Groups	20-39	48	15 (31.2%)
	40-59	304	62 (20.4%)
	60-79	376	63 (16.7%)
	>=80	63	9 (14.3%)
Diabetes Mellitus	Type 1	45	15 (33.3%)
	Type 2	612	134 (21.9%)
Old/New Diabetes	Old	511	100 (19.6%)
	New	280	49 (17.5%)
Glycemic status	Hyperglycemic DKA	444	140 (24%)
	Euglycemic DKA	198	9 (4.34%)

Moreover, DKA was analyzed over glycemic status as whether DKA was euglycemic or hyperglycemic; euglycemic DKA was defined as having blood glycemic level less than 250 mg/dl at the time of diagnosis. Nine out of 149 (6.04%) cases were euglycemic while 140 out of 149 (93.96%) were hyperglycemic were diagnosed with hyperglycemic DKA. Surprisingly, we did not have any euglycemic DKA event in males and Type 1 DM while we had 9 (6.04%) euglycemic DKA events in females and Type 2 DM. However, 4 out of 9 euglycemic DKA events were found in patients with old DM and the rest in new onset DM.

Dyspnea and tachypnea were respectively the most frequent clinical findings followed by abdominal pain and up to some range, the classic clinic of diabetes mellitus such as polydipsia, polyuria and weight loss as described in Table 4. Almost half of the patients revealed crepitation during their chest auscultation followed by rhonchi.

Table 4. Frequency of signs and symptoms across DKA positive Covid patients

Parameter	Frequency	
Tachycardia	105 (70.5%)	
Tachypnea	133 (89.3%)	
Dyspnea	143 (96%)	
Kussmaul Breathing	102 (68.5%)	
Abdominal pain	124 (83.2%)	
Nausea/Vomiting	121 (81.2%)	
Dehydration	97 (65.1%)	
DM Classic symptoms	No Symptoms	27 (18.1%)
	Polydipsia	70(47%)
	Polyuria	25(16.8%)
	Weight loss	3(2%)
	Multiple Symptoms	24(16.1%)
	NAF	10(6.7%)
Chest Auscultation	Crepitation	77(51.7%)
	Rhonchi	16(10.7%)
	Wheezing	15(10.1%)
	Combined	31(20.8%)

Mean serum potassium level, pH, Creatinine, Total Leukocytes, ALT, AST, Glycaemia, systolic BP, and Oxygen saturation on a pulse oximeter were all comparatively high in DKA positive Covid 19 patients (3.6507±0.43, 7.2914±0.3, 1.9643±1.13, 26300±13457, 110±83, 73.14±47.7, 384±82, 130±30, 72%±20 respectively).

Pneumonia (Table 5) was the most prevalent x ray finding in both DKA negative and DKA positive patients as seen in 500 patients (63.2.8%) vs 92 (11.6%) followed by the findings of ARDS 120 (15.2%) vs 54 (6.8%), pleural effusion 6 (0.8%) vs 3 (0.4%), fibrosis 7 (0.9%) vs 0 (0.0%) and hyperinflation 6 (0.8%) vs 0 (0.0%).

Table 5. Chest X-ray (PA) changes across DKA

X ray findings	DKA negative	DKA positive
Normal	3 (0.5%)	0 (0%)
Pneumonia	500 (77.9%)	92 (61.7%)
ARDS	120 (18.7%)	54 (36.2%)
Hyperinflation	6 (0.9%)	0 (0%)
Fibrosis	7 (1.1%)	0 (0%)
Pleural Effusion	6 (0.9%)	3 (2%)
Total	642 (100%)	149 (100%)

The following medicines were used in patients: Tocilizumab 24 (3%), Remdesivir 230(29.1%), steroid 105 (13.3%) and anticoagulant 212 (26.8%). Mortality is calculated out of 100% for each medicine group across DKA positive and DKA negative patients; being the least in both DKA positive and negative patients who received tocilizumab as shown in Table 6.

Table 6. Mortality across different drugs

Medicine		Mortality	
		DKA negative	DKA positive
Tocilizumab	No	92 (96.8%)	47 (83.9%)
	Yes	3 (3.2%)	9 (16.1%)
Remdesivir	No	77 (81.1%)	40 (71.4%)
	Yes	18 (18.9%)	16 (28.6%)
Steroid	No	77 (81.1%)	47 (83.9%)
	Yes	18 (18.9%)	9 (16.1%)
Anticoagulant	No	62 (65.3%)	44 (78.6%)
	Yes	33 (34.7%)	12 (21.4%)

DKA= Diabetic Ketoacidosis

Ventilator was used in 35% of DKA positive patients in comparison to 15% of DKA negative patients.

Mean hospital stay in days was comparatively high though not significant in DKA positive Covid patients (10 vs 9). Hospitalization duration in Covid-DKA patients across gender, age groups and diabetes type is shown in Figure2.

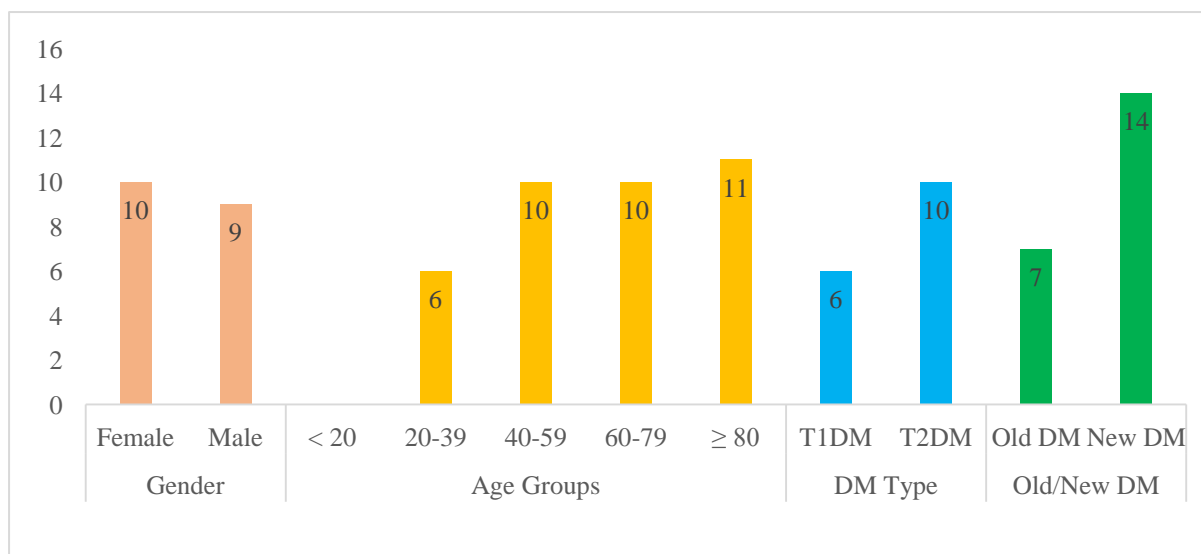
**Figure 2.** Mean Hospital Stay (Days) in DKA Positive Patients

Figure 2 shows that hospitalization duration was comparatively high for females (10 vs 9), T2DM (10 vs 6), and new onset DM (14 vs 7) and it increased with advancing age (most for patients of ≥ 80 years).

76 patients out of 791 (9.6%) died regardless of diabetic ketoacidosis in which mortality almost doubled. Discharge status across diabetic ketoacidosis patients is shown in Figure 3.

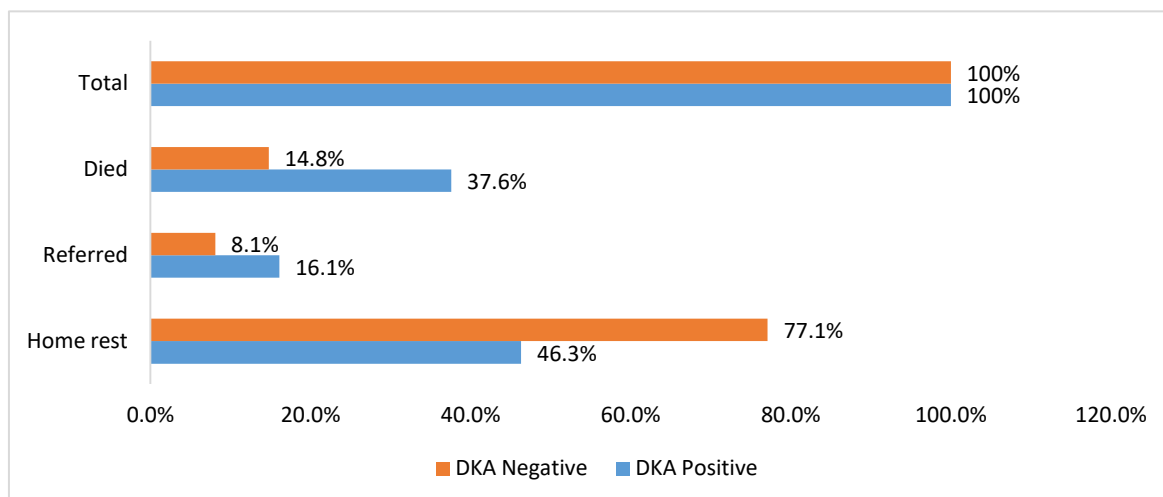


Figure 3. Discharge Status in Covid-DKA Patients

Death and referral measures were significantly different across DKA positive and DKA negative patients i.e. 37.6% vs 14.8% and 16.1% vs 8.1% respectively. While discharge status with home rest was more prevalent in DKA negative patients i.e. 46.3% vs 77.1%. In fact, referral was made to tertiary care or specialty specific care centers for kidney disease, heart diseases such as infarction and heart failure, cerebrovascular diseases such as stroke, etc. Mortality rate was higher in obese diabetic ketoacidosis patients suffering from Corona viral disease i.e. mortality rate almost doubled in obese DKA patients compared with non DKA patients 29 (35.8%) vs 49 (14.4%) as in Table 7. However, mortality was not significantly different across overweight and normal weight patients despite being higher in the respective groups across DKA.

Table 7. DKA mortality across BMI groups

Discharge Status	DKA negative			DKA positive		
	Normal Weight	Overweight	Obese	Normal Weight	Overweight	Obese
Home rest	94 (83.9%)	148 (74.4%)	240 (73.6%)	7 (36.8%)	22 (44.9%)	40 (49.3%)
Referred	11 (9.8%)	34 (17.1%)	39 (12%)	3 (15.8%)	9 (18.1%)	12 (14.8%)
Died	7 (6.3%)	17 (8.5%)	47 (14.4%)	9 (47.4%)	18 (36.7%)	29 (35.8%)
Total	112 (100%)	199 (100%)	326 (100%)	19 (100%)	49 (100%)	81 (100%)

DISCUSSION

The major findings of the study include diabetic ketoacidosis prevalence in diabetic patients suffering from recent covid, old vs new onset diabetes mellitus, euglycemic vs hyperglycemic diabetic ketoacidosis, and clinical presentation in the comorbidity complicating to diabetic ketoacidosis.

New onset diabetes prevalence was higher in hypertensive, young age, and male patients as comparable with a study by Heaney A. et al in the United States of America (Heaney et al., 2020).

DKA was more prevalent in diabetic patients suffering from Covid; this is consistent with the findings of a study by Faraz khan et al. in USA revealing that DKA prevalence doubled during the pandemic (Khan et al., 2022).

DKA was significantly more prevalent in males despite the large number of the female patients in the study, hypertensive, obese and young age and it is comparable with the studies by Rimesh Pal et al. in India (Pal et al., 2020) and Heaney A. et al in USA (Heaney et al., 2020).

Most of the DKA positive covid patients were diagnosed as having preexisting diabetes while a minority of patients had new onset DM which is compatible with the findings of a study by Rimesh Pal et al. (Pal et al., 2020) stating that 77% of DKA positive covid patients had preexisting diabetes.

A minority of the cases in our study had euglycemic DKA; in fact, euglycemic DKA in a study by Rebecca et al. in Massachusetts was shown to be due to sodium glucose cotransporter 2 inhibitors and they prosed the medicine to be counselled for discontinuation during Covid infection (Vitale et al., 2021).

Mortality was significantly higher in DKA positive arm compared with DKA negative arm such that it doubled in diabetic covid patients complicated to DKA which is comparable with numerous studies in United Kingdom, India, and America (Dhatariya et al., 2007, Pal et al., 2020, Chamorro et al., 2020). Furthermore, DKA mortality was higher in elderly males and obese patients i.e. almost half of the obese DKA patients died which is consistent with a study by Francisco et al. in 17 different states of America (Pasquel et al., 2021). Lack of hospital beds, Corona specific hospitals, political instability, and lack of ventilators and experienced technicians were the leading factors contributing to the high number of mortality in the eastern Afghanistan due to corona-diabetes comorbidity complicated to DKA. However, mortality has recently decreased. In addition, referral rate was also double in the DKA patients who were referred to tertiary care or specialty specific care centers due to kidney disease, heart diseases such as infarction and heart failure, cerebrovascular disease such as stroke, etc.

Remdesivir, Tocilizumab and steroid were used for severe cases in the study and mortality was significantly lower in patients receiving Tocilizumab and Remdesivir. Tocilizumab, an IL 6 antagonist, expressed in a study by Zhang et al. in China, (Zhang et al., 2020) and steroids are effective in proinflammatory status (cytokine storm) which is a known key element in the pathogenesis of Covid 19 in a study by Maddaloni et al. in Italy (Maddaloni et al., 2020). The combination of Tocilizumab with steroid showed efficacy in decreasing mortality compared with Tocilizumab alone and the control group in a study by Mahmood Mousazadeh et al. in France (Mossazadeh et al., 2022). On the other hand, Remdesivir in a study by Tchesnokov et al. in Canada is known to inhibit viral replication of SARS COV2 (Tchesnokov et al., 2020).

There were a few limitations in the study despite the interesting results and the realistic topic but the contributors in the study have made their outmost efforts to collect clean data and to reduce bias. First, confounders in the retrospective descriptive study may have affected the results; for example, stress, hypertension, ischemic heart disease and etc. all alleviate glycemic level and may predispose diabetic patients to diabetic ketoacidosis, on the other hand we know that all the aforementioned factors could be due to either diabetes or covid, so we were hesitant to exclude them from the study. Second, Absence of database resulted in loss of a few variables of interest we planned to include in the study such as HBA1C levels, Ketones quantitative measures and etc.; and loss of follow up being the last one which may have increased the prevalence of diabetic ketoacidosis in diabetes-covid comorbidity in the future.

We recommend that clinicians should screen diabetic or diabetic ketoacidosis patients for covid 19, isolate them in case being positive to avoid transmission to other patients or healthy individuals, properly educate their patients, and provide them with prompt and proper management to avoid or decrease morbidity and mortality. In addition, policy makers and program managers to provide free insulin, ventilators, and the medicines shown in

literature to decrease mortality in such patients. They should also design and run awareness programs on social media, hire new staff, design special capacity building programs for them, and provide grants for large analytical studies on the topic on the country level.

CONCLUSION

We concluded that DKA prevalence has increased almost two fold in diabetic patients suffering from Corona virus affecting in-hospital mortality, hospital stay, morbidity and the preexisting clinical picture. In fact, obesity, hypertension, young age and male gender were significant factors contributing to the prevalence. In addition, mortality and referral rates to specialty specific centers were significantly higher in DKA positive patients with the aforementioned factors being the leading contributors.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Wang, D., Hu, B., Hu, C., Zhu, F., Liu, X., Zhang, J., Wang, B., Xiang, H., Cheng, Z., Xiong, Y., Zhao, Y., Li, Y., Wang, X., & Peng, Z. (2020). Clinical Characteristics of 138 Hospitalized Patients with 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA - Journal of the American Medical Association*, 323(11), 1061–1069. <https://doi.org/10.1001/jama.2020.1585>
2. Saeedzai, S. A., Sahak, M. N., Arifi, F., Aly, E. A., Gulp, M. Van, White, L. J., Chen, S., Barakat, A., Azim, G., Rasoly, B., Safi, S., Flegg, J. A., Ahmed, N., Ahadi, M. J., Achakzai, N. M., & Abouzeid, A. (2022). COVID-19 morbidity in Afghanistan: a nationwide, population-based seroepidemiological study. *BMJ Open*, 12(7). <https://doi.org/10.1136/bmjopen-2021-060739>
3. Shah, J., Essar, M. Y., Qaderi, S., Rackimuthu, S., Nawaz, F. A., Qaderi, F., & Shah, A. (2022). Respiratory health and critical care concerns in Afghanistan. *The Lancet Respiratory Medicine*, 10(3), 229–231. [https://doi.org/10.1016/S2213-2600\(21\)00583-X](https://doi.org/10.1016/S2213-2600(21)00583-X)
4. Erratum regarding missing Declaration of Competing Interest statements in previously published articles (Diabetes & Metabolic Syndrome: Clinical Research & Reviews (2020) 14(5) (881–885), (S1871402120301508), (10.1016/j.dsx.2020.05.031)). (2022). *Diabetes and Metabolic Syndrome: Clinical Research and Reviews*, 16(5), 102505. <https://doi.org/10.1016/j.dsx.2022.102505>
5. Roca-Ho, H., Riera, M., Palau, V., Pascual, J., & Soler, M. J. (2017). Characterization of ACE and ACE2 expression within different organs of the NOD mouse. *International Journal of Molecular Sciences*, 18(3). <https://doi.org/10.3390/ijms18030563>
6. Fernandez, C., Rysä, J., Almgren, P., Nilsson, J., Engström, G., Orho-Melander, M., Ruskoaho, H., & Melander, O. (2018). Plasma levels of the proprotein convertase furin and incidence of diabetes and mortality. *Journal of Internal Medicine*, 284(4), 377–387. <https://doi.org/10.1111/joim.12783>
7. Kulcsar, K. A., Coleman, C. M., Beck, S. E., & Frieman, M. B. (2019). Comorbid diabetes results in immune dysregulation and enhanced disease severity following MERS-CoV infection. *JCI Insight*, 4(20). <https://doi.org/10.1172/jci.insight.131774>
8. Maddaloni, E., & Buzzetti, R. (2020). Covid-19 and diabetes mellitus: unveiling the interaction of two

- pandemics. *Diabetes/Metabolism Research and Reviews*, 36(7), 19–20. <https://doi.org/10.1002/dmrr.3321>
9. Yang, J. K., Lin, S. S., Ji, X. J., & Guo, L. M. (2010). Binding of SARS coronavirus to its receptor damages islets and causes acute diabetes. *Acta Diabetologica*, 47(3), 193–199. <https://doi.org/10.1007/s00592-009-0109-4>
 10. Dhatariya, K. K. (2007). Diabetic ketoacidosis. *British Medical Journal*, 334(7607), 1284–1285. <https://doi.org/10.1136/bmj.39237.661111.80>
 11. Heaney, A. I., Griffin, G. D., & Simon, E. L. (2020). Newly diagnosed diabetes and diabetic ketoacidosis precipitated by COVID-19 infection. *American Journal of Emergency Medicine*, 38(11), 2491.e3-2491.e4. <https://doi.org/10.1016/j.ajem.2020.05.114>
 12. Khan, F., Paladino, L., & Sinert, R. (2022). The impact of COVID-19 on Diabetic Ketoacidosis patients. *Diabetes and Metabolic Syndrome: Clinical Research and Reviews*, 16(1), 102389. <https://doi.org/10.1016/j.dsx.2022.102389>
 13. Pal, R., Banerjee, M., Yadav, U., & Bhattacharjee, S. (2020). Clinical profile and outcomes in COVID-19 patients with diabetic ketoacidosis: A systematic review of literature. *Diabetes and Metabolic Syndrome: Clinical Research and Reviews*, 14(6), 1563–1569. <https://doi.org/10.1016/j.dsx.2020.08.015>
 14. Vitale, R. J., Valtis, Y. K., McDonnell, M. E., Palermo, N. E., & Fisher, N. D. L. (2021). Euglycemic Diabetic Ketoacidosis With COVID-19 Infection in Patients With Type 2 Diabetes Taking SGLT2 Inhibitors. *AACE Clinical Case Reports*, 7(1), 10–13. <https://doi.org/10.1016/j.aace.2020.11.019>
 15. Chamorro-Pareja, N., Parthasarathy, S., Annam, J., Hoffman, J., Coyle, C., & Kishore, P. (2020). Letter to the editor: Unexpected high mortality in COVID-19 and diabetic ketoacidosis. *Metabolism: Clinical and Experimental*, 110, 154301. <https://doi.org/10.1016/j.metabol.2020.154301>
 16. Pasquel, F. J., Messler, J., Booth, R., Kubacka, B., Mumpower, A., Umpierrez, G., & Aloji, J. (2021). Characteristics of and Mortality Associated With Diabetic Ketoacidosis Among US Patients Hospitalized With or Without COVID-19. *4(3)*, 28–31. <https://doi.org/10.1001/jamanetworkopen.2021.1091>
 17. Zhang, S., Li, L., Shen, A., Chen, Y., & Qi, Z. (2020). Rational Use of Tocilizumab in the Treatment of Novel Coronavirus Pneumonia. *Clinical Drug Investigation*, 40(6), 511–518. <https://doi.org/10.1007/s40261-020-00917-3>
 18. Moosazadeh, M., & Mousavi, T. (2022). Combination therapy of tocilizumab and steroid for COVID-19 patients: A meta-analysis. *Journal of Medical Virology*, 94(4), 1350–1356. <https://doi.org/10.1002/jmv.27489>
 19. Tchesnokov, E. P., Gordon, C. J., Woolner, E., Kocinkova, D., Perry, J. K., Feng, J. Y., Porter, D. P., & Götte, M. (2020). Template-dependent inhibition of coronavirus RNA-dependent RNA polymerase by remdesivir reveals a second mechanism of action. *Journal of Biological Chemistry*, 295(47), 16156–16165. <https://doi.org/10.1074/jbc.AC120.015720>

The Effect of Resistance Training on Physical Performance in Obese Women after Bariatric Surgery

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ABSTRACT

Background: Bariatric surgery is one of the most common methods for weight loss. This study, explores the effects and differences of control and exercise on obese patients after surgery and observed the effects of a 4-week exercise program combined with standard postoperative treatment on the physical function and glycolipid metabolism of obese individuals following bariatric surgery.

Materials and Methods: Eight of the seventeen obese female individuals who satisfied the inclusion criteria were placed in the group of exercise, and nine were placed in the group of control. During four weeks, the exercise group got a 60-minute exercise intervention three times per week (training every other day). The Control group did not engage in any athletic activity. Before and after the intervention, data were gathered. Software SPSS26.0 was employed. Since the data had a normal distribution, the groups individually were compared using the independent sample T test, whereas the groups before and after the group were contrasted using the paired sample T test. Use a non-normal distribution with a nonparametric Mann-Whitney U test.

Findings: Weight, BMI, waist and neck circumferences, and hip circumferences in the exercise and control groups were substantially decreased ($P < 0.01$) compared to the pre-test, as well as the waist-to-hip ratio (WHR) in the group of the exercise was statistically significantly reduced ($P < 0.05$), while WHR in the Control group was not statistically different ($P > 0.05$). The decrease in basal metabolic rate (BMR) was better in than in the Control group ($P < 0.05$) in the exercise group. When compared to the pre-test, both groups' total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels considerably decreased ($P < 0.01$).

Conclusion: Both groups had improvements in the composition of the body; however, exercise had a more significant effect on subjects' WHR and TF, as well as delaying the loss of skeletal muscle and BMR, than the Control group did on subjects' WC decline.

Keywords: Exercise; Bariatric surgery; Obesity; physical function

INTRODUCTION

Between 1975 and 2016, the prevalence of overweight and obesity quadrupled worldwide (Nam et al., 2020). According to studies, obesity affects about 30% of the world's population, and its prevalence has considerably grown (Li et al., 2015). China, formerly thought to have one of the world's leanest populations, has experienced a sharp increase in overweight and obesity (Wang & Lobstein, 2006). In 2016, China's population of obese people quickly surpassed that of the United States to take the top spot globally. Studies have shown that as the obese population increases year by year, it further increases the economic burden on the medical system (Shen et al., 2012). From 2000 to 2025, the indirect loss caused by obesity in China accounted for 3.6%-8.7% of the gross national product (Zhang & Chaaban, 2013). There are several diseases associated with obesity, including: type 2 diabetes; high blood pressure; heart disease and stroke, sleep apnea and other breathing problems (Wilcox, 1998). Osteoarthritis, fatty liver disease; kidney disease; certain types of cancer, such as breast, colon, and prostate cancer, depression and anxiety disorders (Ilie et al., 2020). Infertility and menstrual irregularities in women (Hollmann et al., 1996). Obesity can also increase the risk of developing other health problems such as gallstones, gastroesophageal reflux disease (GERD), and skin infections (Neff et al., 2015).

Resistance training is a type of physical exercise that involves using weights or other forms of resistance to build strength and endurance. It typically involves exercises such as weightlifting, bodyweight exercises, and resistance band training. Obesity is generally defined as having a body mass index (BMI) of 30 or higher. BMI is calculated by dividing a person's weight in kilograms by their height in meters squared (Racette et al., 2005).

However, it's important to note that BMI is not always a perfect measure of health, as it doesn't take into account factors such as muscle mass and body composition. Bariatric surgery is a type of weight loss surgery that involves modifying the digestive system to limit the amount of food a person can eat and/or absorb. There are several different types of bariatric surgery, including gastric bypass, sleeve gastrectomy, and adjustable gastric banding (Freeman et al., 2014). These procedures are typically reserved for people who have severe obesity and have been unable to lose weight through other methods such as diet and exercise.

Obesity is a major health concern worldwide and can lead to various health problems such as cardiovascular diseases, diabetes, and musculoskeletal disorders (Montenegro, 2011). Bariatric surgery has been shown to be an effective treatment for obesity by reducing weight and improving metabolic and cardiovascular function. However, weight loss alone may not result in significant improvements in physical performance. Resistance training is a type of exercise that has been found to improve physical performance in various populations, including obese individuals. Therefore, this study aims to investigate the effect of resistance training on physical performance in obese women after bariatric surgery. In addition to measuring physical performance, blood pressure and heart rate variations are also important to monitor in this study. Obese individuals are at risk of developing hypertension and cardiovascular diseases, and bariatric surgery can have an impact on blood pressure regulation. By monitoring blood pressure and heart rate, we can evaluate any changes that may occur as a result of resistance training and assess the overall cardiovascular health of the participants (Genelhu et al., 2009).

This information can help to better understand the potential benefits of resistance training on both physical performance and cardiovascular health in this population. Millions of people worldwide are impacted by obesity, which is a major public health issue (Nguyen & Lau, 2012). Since bariatric surgery may result in considerable weight

reduction and better health outcomes, it has become a more frequently used strategy for those with extreme obesity. Bariatric surgery, however, can potentially have unfavorable side effects, such as a loss of muscle mass and a decline in athletic ability.

Exercise, known as "resistance training," includes using weights or other types of resistance in order to increase physical function and build strength (Kraemer et al., 2002). In several groups, it has been demonstrated to be useful in enhancing physical performance and reducing muscle loss, but its effects on obese women following bariatric surgery are little known. It may be crucial for resistance training to understand how resistance training affects physical performance in obese women following bariatric surgery. Healthcare professionals can better support patients who have bariatric surgery and enhance their quality of life by discovering effective therapies to stop muscle loss and enhance physical function in this group. Overall, the impact of resistance training on physical performance in obese women following bariatric surgery is a significant field of research with the potential to guide resistance training and enhance the health outcomes of people who are fat.

MATERIALS AND METHODS

Research Design and Ethics

According to the procedure of the ethical committee of Beijing Sports University (approved by Beijing Sport University's Sports Science Experimental Ethics Committee, effective date: October 2020 to In October 2021, it will be implemented at the China-Japan Friendship Hospital and Beijing Sport University), the purpose of the research and potential risks and benefits were shared with the responsible person of the participants' families, permission was taken from him, and public awareness was also given. Any personal information about the participants in this study will be kept confidential. The Ethics Committee of Beijing Sports University re-evaluated the research proposals and ethical standards, and the Ethics Committee of Beijing Sports University took responsibility for the ethical results of the overall research and the well-being of the participants in this research. That this practice is considered appropriate for research. The approval number of this study is: 2020131H.

Documentation analysis

In this paper, through search websites such as CNKI, PubMed, Google scholar and other search sites. The research results related to bariatric surgery and exercise at home and abroad were consulted, which provided a theoretical basis for the later experimental research of this paper. Search terms: exercise, aerobic exercise, resistance training, glucose metabolism, lipid metabolism, glucose and lipid metabolism, insulin resistance, etc. Primary data in this study were collected through form and clinical examination. In terms of form, the researchers developed a special questionnaire to collect information from the participants about their physical activity and other relevant factors such as medical history, current medications, and lifestyle habits. The questionnaire will be administered by the researchers, and the responses will be recorded and entered into a database for analysis. A clinical examination refers to a physical assessment of participants performed by a trained health care professional. This includes measurements such as body weight, height, and waist circumference, as well as assessments of muscle strength, balance, and mobility. The results of these assessments were also recorded and included in the data set for analysis. Regarding the similarities and differences between the two groups, researchers are likely to compare various characteristics of the participants at baseline to ensure that they are comparable. For example, they may have looked at age, BMI, and other demographic factors. They will then compare the results between the two groups after the intervention to determine if there are any significant differences in physical activity, as

well as other measures of interest such as quality of life, medication use, and health care use. The research results related to bariatric surgery and exercise at home and abroad were consulted (Table 1).

Table 1. Basic characteristics of subjects

Item	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m ²)
control group=9	30.44±5.10	166.89±4.31	95.96±13.37	34.42±4.33
exercise group=8	31.88±6.49	166.50±5.10	88.50±6.16	31.93±1.90
p-value	<i>P</i> > 0.05	<i>P</i> > 0.05	<i>P</i> > 0.05	<i>P</i> > 0.05

Stage of preparation prior to the experiment

Prior to the experiment's start, the subjects were screened for medical aspects, and the subjects with higher risk were excluded. The subjects were given action instructions and told to sign the informed consent form. At the same time, the body Baseline tests such as scores and blood indicators.

Exercise program

Blackburn and other specialist organizations in bariatric surgery advise patients to enhance their physical activity from preoperative to postoperative times, focusing on low- to moderate-intensity activities (Blackburn et al., 2009). The study's exercise intervention is as follows: Each workout session lasts 70 minutes and consists of a 5-minute pre-workout warm-up, 30 minutes of strength training, 30 minutes of aerobic training, and 5 minutes of stretching and relaxation. The exercise is conducted online, lasts for 4 weeks, three times per week, and subjects are required to practice every other day. Strength training is done at a 40% 1RM and 50% HRmax intensity. The 1RM during strength training is determined by having the subject warm up and attempting to lift a weight estimated by the tester for no more than 10 repetitions (Robergs & Landwehr, 2002). Do total attempts, resting 1-2 minutes between each attempt, then note the most repeats possible. $1RM = (0.33 \times \text{number of repetitions}) \text{ weight used} + \text{weight used}$ is the outcome of the calculation (Rieckmann, 2011).

Analysis method

The data of obese women before and after 4 weeks were statistically processed using SPSS 26.0 statistical analysis program in this study. The mean and standard deviation (mean ±SD) are used to represent the test results for each topic index. The independent sample T test is used to compare groups when the experimental data are within the normal distribution. Instead, use the nonparametric Mann-Whitney U test. A paired-samples t-test was used to assess differences between before and after within groups. $P < 0.05$ was chosen as the significance threshold, and $P < 0.01$ as the highly significant level.

Selection of topics

In this study, seventeen obese female individuals who had undergone bariatric surgery underwent a 4-week experimental intervention. 1. Assess any modifications in physical performance as well as the metabolism of glucose and lipids in the exercise group and the control group before and after the trial. 2. Examine the alterations and variations in each indicator between the exercise group and the control group. 3. To offer resources for creating scientifically sound and practical exercise regimens for those who have undergone weight-loss surgery.

RESULTS

Changes in body shape before and after exercise intervention

Weight, Body Mass Index(BMI), a measure of body fat based on height and weight, Neck Circumference, the measurement around the neck(NC), Waist Circumference, the measurement around the waist(WC), Hip Circumference, the measurement around the hips(HC), and Waist-to-Hip Ratio, a ratio of waist circumference to hip circumference(WHR), that is used as an indicator of health risks associated with excess abdominal fat, of control and exercise groups by paired-sample t-test. The changes before and after the 4-week experiment are shown in Table 2. The data from the exercise group and the Control group were analyzed using SPSS.26, and the body weight, BMI, NC, WC, HC, and WHR all followed the normal distribution. The independent sample T test revealed no significant difference between the Control group and the exercise group in any of the six baseline variables of body weight, WHR, WC, NC, HC, and BMI ($P>0.05$). It can be seen from Table 2 that the weight loss before and after the exercise group was 9.95 ± 2.53 kg, and the weight loss before and after the group of Control was 10 ± 2.31 kg, both groups were statistically significant ($P<0.01$). Between the population and the standard control group, there was no difference in body weight ($P>0.05$).

Table 2. shows the difference in body composition between the two time periods

Norm	Pretest	Control group	Variation	Pretest	exercise group	Variation
Weight(kg)	95.96 \pm 13.37	85.96 \pm 12.80**	-10 \pm 2.31	88.50 \pm 6.16	78.55 \pm 6.54**	-3.71 \pm 1.06
BMI(kg/m ²)	34.42 \pm 4.33	30.84 \pm 4.28**	-3.57 \pm 0.75	31.93 \pm 1.90	28.34 \pm 2.21**	-2.19 \pm 0.92
NC (cm)	37.79 \pm 2.41	35.89 \pm 2.46**	-1.9 \pm 1.39	38.00 \pm 2.27	35.81 \pm 2.07**	-2.19 \pm 0.92
WC(cm)	109.28 \pm 13.94	100.61 \pm 13.39**	-8.67 \pm 4.9	101.63 \pm 3.34	92.38 \pm 4.03**	-9.25 \pm 5.82
HC (cm)	120.06 \pm 8.57	110.17 \pm 5.41**	-9.89 \pm 4.62	109.75 \pm 5.09	105.31 \pm 5.12**	-4.44 \pm 3.54#
WHR	0.91 \pm 0.08	0.91 \pm 0.09	0.00 \pm 0.04	0.93 \pm 0.06	0.88 \pm 0.04*	-9.25 \pm 5.82##

Note: * $p < 0.05$; ** $p < 0.01$. BMI stands for Body Mass Index, which is a measure of body fat based on height and weight. NC stands for Neck Circumference, which is the measurement around the neck just below the Adam's apple. WC: Waist Circumference, the measurement around the waist HC: Hip Circumference, the measurement around the hips. HC stands for Hip Circumference, which is the measurement around the widest part of the hips. WHR stands for Waist-to-Hip Ratio, which is the ratio of the circumference of the waist to that of the hips. These measurements are commonly used in healthcare to assess a person's overall health and risk for certain diseases.

Blood pressure and heart rate variations before and after exercise intervention

Prior to and following the 4-week trial, paired T-tests were performed on the HR rest, SBP, and DBP of the group of Control and the exercise group. For the variation difference, see Table 3. It can be seen from Table 4. that the HR rest pre-test and post-test changes of the two groups of subjects are different, and the HR rest index of the group of Control has no significant change after 4 weeks ($P>0.05$), and the HR rest index has increased by 3.67 compared with the pre-test ± 16.02 times/min, 4 weeks of exercise significantly decreased the HR rest index of the subjects ($P<0.01$).

Table 3. shows the variations in heart rate and blood pressure prior to and during the four-week intervention.

Norm	Pretest	Control group	Variation	Pretest	Exercise group	Variation
HRrest (min)	77.56±9.58	81.22±15.54	3.67±16.02	84.50±10.61	76.13±9.83**	-8.38±6.48
SBP (mmHg)	123.00±8.44	119.56±12.77	-3.44±12.91	131.50±11.60	123.75±7.74	-7.75±11.18
DBP (mmHg)	79.22±13.05	79.33±10.54	0.11±12.47	78.88±7.16	73.88±6.31	-5.00±6.44

Note: * $p < 0.05$; ** $p < 0.01$. Variations are shown within groups. HR: Heart Rate, the number of heart beats per minute, SBP: Systolic Blood Pressure, the highest pressure exerted by the blood against the walls of arteries when the heart beats and DBP: Diastolic Blood Pressure, the lowest pressure exerted by the blood against the walls of arteries when the heart is at rest between beats.

Changes in exercise capacity before and after exercise intervention

The 6MWT, STS and ACT of the group of Control and the exercise group before and after the 4-week experiment were compared by paired T test See Table 4. The normal distribution test was carried out on the pretest data of the two groups through SPSS. The 6MWT, STS and ACT all conformed to the normal distribution. Statistical difference ($P > 0.05$).

Table 4. Changes in exercise capacity before and after the four-week intervention.

Norm	Pretest	Control group	Variation	Pretest	Exercise group	Pretest
6MWT	501.11±42.27	524.67±30.00**	23.56±16.64	522.38±31.49	547.50±15.65**	25.13±16.61
STS	12.78±2.64	14.00±2.00**	1.22±0.97	12.25±1.67	14.13±0.99**	1.88±1.25
ACT	13.22±1.86	15.00±1.22**	1.78±1.39	13.88±1.55	15.00±1.07**	1.13±0.83

Note: * $p < 0.05$; ** $p < 0.01$. 6MWT: Six-minute Waking Test, STS: Sit- To Stand, and ACT: Aram Coral Test.

Changes of glucose metabolism before and after exercise intervention

Through the paired T test, before and after the 4-week experiment, the differences in FBG, FINS, CP, HbA1c, Table 5 displays the HOMA-IR and ISI results for the exercise group and the control group. The normal distribution test of the two groups of pretest data was carried out by SPSS, and FBG, FINS and CP all conformed to the normal distribution. Through the independent sample T test, there was no difference between the group of Control and the exercise group in FBG, FINS and CP ($P > 0.05$). HbA1c, HOMA-IR and ISI tests were non-normal, using non-parametric Mann-Whitney U test, there was no difference in the three indicators between the exercise group and the group of Control ($p > 0.05$).

Table 5. Differences in the metabolism of glucose before and after four weeks of treatment

Norm	Pretest	Control group	Variation	Pretest	Exercise group	Pretest
FBG (mmol/L)	6.21±2.41	3.95±0.81**	-2.26±1.66	5.34±0.65	4.93±0.67	-0.41±0.49
FINS (uIU/ml)	22.46±12.06	8.20±6.91**	-14.27±10.12	20.33±8.31	7.86±3.00**	-12.47±9.68
CP (ng/ml)	3.63±0.84	1.92±1.15**	-1.71±0.94	3.33±0.64	2.08±0.49**	-1.25±0.93
HbA1c (%)	6.28±1.51	5.42±1.48**	-0.83±0.60	6.30±1.50	5.55±0.56	-0.75±0.97
HOMA-IR	6.39±4.70	1.61±1.62**	-4.81±3.42	4.81±1.89	1.78±0.86**	-3.03±2.18
ISI	0.01±0.01	0.09±0.11*	0.09±0.11	0.01±0.00	0.03±0.02*	0.02±0.02

Note: * $p < 0.05$; ** $p < 0.01$. FBG: Fasting blood glucose, FINS: Fasting insulin, CP: Cerebral palsy, HbA1c: Glycated hemoglobin, HOMA-IR: Homeostatic model assessment of insulin resistance, and ISI: Insulin sensitivity index.

Changes of lipid metabolism before and after exercise intervention

Through the paired T test, the differences in TC, TG, HDL-C and LDL-C between the group of Control and the exercise group before and after the 4-week experimental intervention are shown in Table 6. Through the analysis of the data, TC and LDL-C conformed to the normal distribution, and the data were analyzed by independent sample T test, and there were differences in TC and LDL-C between the Control group and the exercise group ($P < 0.05$). Exercise has been shown to have a significant impact on lipid metabolism, leading to improvements in lipid profiles and reducing the risk of cardiovascular disease. Here are some of the changes that occur (Haskell et al., 1994). 1. Increased lipolysis: Exercise stimulates lipolysis, which is the breakdown of stored fat into fatty acids that can be used for energy. 2. Increased oxidation of fatty acids: The fatty acids released during lipolysis are then transported to the mitochondria where they are oxidized to produce energy (Wolfe, 1998).

Table 6. Changes of lipid metabolism before and after four weeks of intervention

Norm	control group			exercise group		
	Pretest	Posttest	Variation within groups	Pretest	Posttest	Variation within groups
TC(mmol/L)	4.87±0.43	3.60±0.5**	-1.27±0.38	5.60±0.80a	4.30±0.74**	-1.30±0.87
TG(mmol/L)	1.58±1.11	0.99±0.37	-0.60±1.12	1.79±0.63	1.12±0.16*	-0.67±0.63
HDL-C(mmol/L)	0.96±0.21	0.64±0.11**	-0.32±0.13	0.98±0.22	1.02±0.25	0.03±0.16##
LDL-C(mmol/L)	3.03±0.43	2.06±0.40**	-0.97±0.20	3.66±0.69a	2.68±0.59**	-0.98±0.72

Note: A means $P < 0.05$, which indicates that there was a difference between the two groups prior to the test; * means $P < 0.05$, which indicates that the intervention was significant; ** means $P < 0.01$, which indicates that the difference was highly significant; and # means $P < 0.05$, which indicates that the two groups differed after the intervention. Significant change within the group; ## denotes $P < 0.01$, indicating that the disparity between the two groups' increases is extremely significant; TC stands for Total Cholesterol, which is the sum of all types of cholesterol in the blood including low-density lipoprotein (LDL), high-density lipoprotein (HDL), and very-low-density lipoprotein (VLDL). TG stands for Triglycerides, which are a type of fat found in the blood. HDL-C stands for High-Density Lipoprotein Cholesterol. HDL is often referred to as "good" cholesterol because it helps remove excess cholesterol from the blood vessels and reduces the risk of heart disease. LDL-C stands for Low-Density Lipoprotein Cholesterol. LDL is often referred to as "bad" cholesterol because it can build up in the walls of arteries, leading to blockages that increase the risk of heart disease and stroke.

DISCUSSION

Exercise's effects on the physique

Exercise increases the body's extra energy consumption, which has been proven to be one of the more effective ways to improve body shape. In this study, 4 weeks of postoperative exercise was compared with postoperative routine management of the control group. Both groups effectively reduced and improved body weight, BMI, NC, WC, and HC indicators (Marcon et al., 2011). evaluated the changes in body weight and metabolic indicators of a group of obese patients undergoing bariatric surgery, and found that postoperative weight and BMI were significantly lower than those before surgery, metabolic parameters FBG decreased, TC, TG, LDL- The three

indicators of C also decreased after the operation, and the value of HDL-C, which is beneficial to the body, showed an upward trend. According to some studies, postoperative weight changes typically begin with rapid weight loss in the first three months following bariatric surgery before slowing down and finally stabilizing. The primary cause of the initial rapid weight loss is reduced energy intake (King et al., 2012). (1) Via a 4-week workout program, the body index of obese female patients after weight loss after exercise was clarified. (2) Compared with obese female patients who do not participate in exercise after bariatric surgery, it is clear that exercise can improve the physical function and glucose and lipid metabolism of obese patients.

Exercise's effects on blood pressure and heart rate

The foundation of healthy blood pressure is a proper metabolism and blood flow. Both too high and too low blood pressure can have serious consequences for the body. In this study, after 4 weeks of experimental intervention, after four weeks after the pre-test, there was no significant change in the control group's HR ($P > 0.05$), SBP, or DBP ($P > 0.05$). Three months following bariatric surgery, 17 patients were assessed by (Marcon et al., 2011), who discovered that postoperative SBP was considerably lower than pre-bariatric surgery, whereas postoperative HR rest values were significantly lower than pre-bariatric surgery ($P < 0.01$). There was no difference in DBP from pre-bariatric surgery ($P = 0.7$), nor was there a significant decrease ($P = 0.2$). Because of the shorter cycle period in this investigation, there was not a substantial drop in HR rest in the control group, and changes in SBP and DBP were mostly in line with the aforementioned experimental findings. After a 4-week exercise intervention, the HR rest in the exercise group in this research was considerably lower ($P < 0.01$).

Exercise's impact on exercise capacity

Obesity is strongly associated with impaired movement, lacking aerobic ability and having limited muscular power. According to this study, both the control groups and the exercise group's exercise capacity post-test values (6MWT, STS, and ACT) were considerably higher than they were pre-test. In a study by (Baillot et al., 2018), it was shown that physical activity can be significantly improved by bariatric surgery. (Hassannejad et al., 2017). showed that the improved exercise capacity after bariatric surgery may be related to postoperative weight loss and increased physical activity. At the same time (Marcon et al., 2011). believed that postoperative weight loss may reduce the burden on the body during activities, which may be an important factor in improving exercise capacity (Hassannejad et al., 2017). The idea that physical activity improves postoperative exercise capacity has also been confirmed by other researchers. A meta-analysis showed (King et al., 2012) that the 6MWT distance increased to 75 m in patients 3 to 6 months after surgery, and 6 to 12 m after surgery.

Comparing the Effects of Exercise and Bariatric Surgery on Glucose Metabolism Indicators

In this study, both the Control group and the exercise group had improved glucose metabolism indicators. Compared with the pretest, FBG ($P < 0.01$), FINS ($P < 0.01$), CP ($P < 0.01$), HbA1c ($P < 0.01$) and HOMA-IR ($P < 0.01$) in the Control group were significantly decreased, and ISI ($P < 0.05$) significantly improved. At the same time, in the exercise group, FINS ($P < 0.01$), CP ($P < 0.01$) and HOMA-IR ($P < 0.01$) decreased significantly, and ISI ($P < 0.05$) significantly improved. There was no significant difference in FINS, CP, HbA1c, HOMA-IR and ISI between the two groups ($P > 0.05$), while the Control group had an advantage in improving FBG indicators ($P < 0.01$). A study showed that (Zakas et al., 2006), Indicators were gathered prior to surgery as well as 6, 12, and 24 months following surgery, and it was discovered that the combined exercise group of 60

patients who had undergone bariatric surgery performed better than the control group. Both the control group and the group of controls had considerably lower glucose metabolism scores. Between the exercise intervention group and the control group, there was no discernible difference in the effects of FBG, FINS, HbA1c, and HOMA-IR ($P>0.05$). Studies like Campos (King et al., 2012) and Lin (Rieckmann, 2011) found that bariatric surgery not only resulted in considerable weight reduction but also quickly enhanced FBG and ISI.

CONCLUSION

Both groups improved body composition; however, the Control group was more effective at lowering subjects' WC, while exercise was more effective at lowering subjects' WHR and TF, postponing the loss of skeletal muscle, and lowering BMR. The glucose and lipid metabolism in both groups improved, but the Control group had a stronger impact on lowering FBG levels. But exercise might be able to influence how stable FBG levels are. Exercise significantly increases HDL-C. At present, there are few domestic studies on exercise intervention after bariatric surgery, and the specific exercise program is not yet mature. This study clarified the feasibility of exercise for postoperative obese patients through exercise intervention after weight loss. Finding a scientifically sound and efficient workout program may also serve as a guide for patients' post-weight reduction surgery rehabilitation training, so as to achieve better postoperative training management. Secondly, the exercise intervention in this study adopts the method of online supervision and guidance, and conducts 4 weeks of "face-to-face" intervention training for postoperative patients to explore the effect and feasibility of online exercise. Finally, this study is also combined with resistance training to better respond to the slogan of "integration of sports and medicine".

CONFLICT OF INTEREST: The authors declared no conflicts of interest.

REFERENCES

1. Baillot, A., Vallée, C.-A., Mampuya, W. M., Dionne, I. J., Comeau, E., Méziat-Burdin, A., & Langlois, M.-F. (2018). Effects of a Pre-surgery Supervised Exercise Training 1 Year After Bariatric Surgery: a Randomized Controlled Study. *Obesity Surgery*, 28(4), 955–962. <https://doi.org/10.1007/s11695-017-2943-8>
2. Blackburn, G. L., Hutter, M. M., Harvey, A. M., Apovian, C. M., Boulton, H. R. W., Cummings, S., Fallon, J. A., Greenberg, I., Jiser, M. E., Jones, D. B., Jones, S. B., Kaplan, L. M., Kelly, J. J., Kruger, R. S., Lautz, D. B., Lenders, C. M., LoNigro, R., Luce, H., McNamara, A., ... Annas, C. L. (2009). Expert panel on weight loss surgery: Executive report update. *Obesity*, 17(5), 842–862. <https://doi.org/10.1038/oby.2008.578>
3. Freeman, R. A., Overs, S. E., Zarshenas, N., Walton, K. L., & Jorgensen, J. O. (2014). Food tolerance and diet quality following adjustable gastric banding, sleeve gastrectomy and Roux-en-Y gastric bypass. *Obesity Research and Clinical Practice*, 8(2). <https://doi.org/10.1016/j.orcp.2013.02.002>
4. Genelhu, V. A., Celoria, B. M. J., Duarte, S. F. P., Cabello, P. H., & Francischetti, E. A. (2009). Not all obese subjects of multiethnic origin are at similar risk for developing hypertension and type 2 diabetes. *European Journal of Internal Medicine*, 20(3), 289–295. <https://doi.org/10.1016/j.ejim.2008.09.009>
5. Haskell, W. L., Alderman, E. L., Fair, J. M., Maron, D. J., Mackey, S. F., Superko, H. R., Williams, P. T., Johnstone, I. M., Champagne, M. A., Krauss, R. M., & Farquhar, J. W. (1994). Effects of intensive multiple risk factor reduction on coronary atherosclerosis and clinical cardiac events in men and women with coronary artery disease: The Stanford Coronary Risk Intervention Project (SCRIP). *Circulation*, 89(3), 975–

990. <https://doi.org/10.1161/01.CIR.89.3.975>
6. Hassannejad, A., Khalaj, A., Mansournia, M. A., Rajabian Tabesh, M., & Alizadeh, Z. (2017). The Effect of Aerobic or Aerobic-Strength Exercise on Body Composition and Functional Capacity in Patients with BMI ≥ 35 after Bariatric Surgery: a Randomized Control Trial. *Obesity Surgery*, 27(11), 2792–2801. <https://doi.org/10.1007/s11695-017-2717-3>
 7. Hollmann, M., Runnebaum, B., & Gerhard, I. (1996). Effects of weight loss on the hormonal profile in obese, infertile women. *Human Reproduction*, 11(9), 1884–1891. <https://doi.org/10.1093/oxfordjournals.humrep.a019512>
 8. Ilie, G., Rutledge, R., & Sweeney, E. (2020). Anxiety and depression symptoms in adult males in Atlantic Canada with or without a lifetime history of prostate cancer. *Psycho-Oncology*, 29(2), 280–286. <https://doi.org/10.1002/pon.5244>
 9. King, W. C., Hsu, J. Y., Belle, S. H., Courcoulas, A. P., Eid, G. M., Flum, D. R., Mitchell, J. E., Pender, J. R., Smith, M. D., Steffen, K. J., & Wolfe, B. M. (2012). Pre- to postoperative changes in physical activity: report from the Longitudinal Assessment of Bariatric Surgery-2 (LABS-2). *Surgery for Obesity and Related Diseases*, 8(5), 522–532. <https://doi.org/10.1016/j.soard.2011.07.018>
 10. Kraemer, W. J., Ratamess, N. A., & French, D. N. (2002). Resistance training for health and performance. In *Current sports medicine reports* (Vol. 1, Issue 3, pp. 165–171). <https://doi.org/10.1249/00149619-200206000-00007>
 11. Li, P., Chen, K., Nie, Y., Guo, L. L., Wang, H. Bin, Wang, S. S., Wang, A. P., Hu, D. Y., Mu, Y. M., & Li, J. H. (2015). Association of obesity with glucose, blood pressure, and lipid goals attainment in patients with concomitant diabetes and hypertension. *Current Medical Research and Opinion*, 31(9), 1623–1631. <https://doi.org/10.1185/03007995.2015.1058770>
 12. Marcon, E. R., Gus, I., & Neumann, C. R. (2011). Impacto de um programa mínimo de exercícios físicos supervisionados no risco cardiometabólico de pacientes com obesidade mórbida. *Arquivos Brasileiros de Endocrinologia & Metabologia*, 55(5), 331–338. <https://doi.org/10.1590/S0004-27302011000500006>
 13. Montenegro, R. (2011). Advances and perspectives in mental health: is psychiatry being stigmatized? In *Psychiatrikē = Psychiatriki* (Vol. 22, Issue 4, pp. 283–289).
 14. Nam, G. E., Kim, Y.-H., Han, K., Jung, J.-H., Rhee, E.-J., Lee, S.-S., Kim, D. J., Lee, K.-W., & Lee, W.-Y. (2020). Obesity Fact Sheet in Korea, 2019: Prevalence of Obesity and Abdominal Obesity from 2009 to 2018 and Social Factors. *Journal of Obesity & Metabolic Syndrome*, 29(2), 124–132. <https://doi.org/10.7570/jomes20058>
 15. Neff, K. J., Ferrannini, E., & le Roux, C. W. (2015). Treatment of obesity: bariatric surgery. In *International Textbook of Diabetes Mellitus* (Vol. 74, Issue 4, pp. 505–518). John Wiley & Sons, Ltd. <https://doi.org/10.1007/s11695-017-2717-3>
 16. Nguyen, T., & Lau, D. C. W. (2012). The Obesity Epidemic and Its Impact on Hypertension. In *Canadian Journal of Cardiology* (Vol. 28, Issue 3, pp. 326–333). Elsevier Inc. <https://doi.org/10.1016/j.cjca.2012.01.001>
 17. Racette, S. B., Deusinger, S. S., Strube, M. J., Highstein, G. R., & Deusinger, R. H. (2005). Weight changes, exercise, and dietary patterns during freshman and sophomore years of college. *Journal of American College Health*, 53(6), 245–251. <https://doi.org/10.3200/JACH.53.6.245-251>

18. Rieckmann, M. (2011). Key Competencies for a Sustainable Development of the World Society. Results of a Delphi Study in Europe and Latin America Schlüsselkompetenzen für eine nachhaltige Entwicklung der Weltgesellschaft Ergebnisse einer europäisch-lateinamerikanischen Delphi-. *GAIA - Ecological Perspectives for Science and Society*, 20(1), 48–56. <https://doi.org/10.14512/gaia.20.1.10>
19. Robergs, R. A., & Landwehr, R. (2002). JEP online Journal of Exercise Physiology online. *Journal of Exercise Physiology Online*, 1971(1), 1–10.
20. Shen, J., Goyal, A., & Sperling, L. (2012). The Emerging epidemic of obesity, diabetes, and the metabolic syndrome in China. *Cardiology Research and Practice*, 1(1), 16–18. <https://doi.org/10.1155/2012/178675>
21. Wang, Y., & Lobstein, T. (2006). Worldwide trends in childhood overweight and obesity. *International Journal of Pediatric Obesity*, 1(1), 11–25. <https://doi.org/10.1080/17477160600586747>
22. Wilcox, I. (1998). “Syndrome Z”: The interaction of sleep apnoea, vascular risk factors and heart disease. *Thorax*, 53(SUPPL. 3), 53–56.
23. Wolfe, R. R. (1998). Metabolic interactions between glucose and fatty acids in humans. *American Journal of Clinical Nutrition*, 67(3 SUPPL.). <https://doi.org/10.1093/ajcn/67.3.519S>
24. Zakas, A., Galazoulas, C., Doganis, G., & Zakas, N. (2006). Effect of two acute static stretching durations of the rectus femoris muscle on quadriceps isokinetic peak torque in professional soccer players. *Isokinetics and Exercise Science*, 14(4), 357–362. <https://doi.org/10.3233/ies-2006-0249>
25. Zhang, J., & Chaaban, J. (2013). The economic cost of physical inactivity in China. *Preventive Medicine*, 56(1), 75–78. <https://doi.org/10.1016/j.ypmed.2012.11.010>

Simulation of Diapause Induction in Spider Mites (*Tetranychus urticae* and *T. kanzawai*) by Reproducing Field Environments in the Laboratory

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ABSTRACT

Background: Diapause as the basic mechanism, helps insects and mites to synchronize their life cycles with local seasonal changes. Field tests are desirable to determine the timing of diapause accurately, but the environments are variable, making it challenging to validate the reproducibility of results.

Materials and Methods: We designed an environmental simulator that reproduces the field variation in the laboratory based on the installation of 20 years of climate data in the Hokkaido region. We computed a regression equation to predict the light intensity and temperature between the laboratory simulator and field data and its accuracy was clearly demonstrated.

Findings: Photoperiodic reaction curves of *T. urticae* and *T. kanzawai* indicated a generic short-day reaction type with critical photoperiods at 18.0°C of approximately 13.5 h for *T. urticae* and 12.5 h for *T. kanzawai*. Diapause of *T. urticae* and *T. kanzawai* in the field was induced on 22 and 23 September, respectively, which is close to the dates of diapause induction estimated by using critical photoperiods (19 and 30 September, respectively). Little variation between observed and estimated critical photoperiods of the mentioned species may be due to minor deviations in temperature in the simulator.

Conclusion: The present study demonstrates the importance of factoring in variable temperatures in the field for accurate prediction of the timing of diapause induction in spider mites. Our experimental system may also be useful in pest mite forecasting by predicting its diapause termination and spring emergence in any geographic area where environmental data are available.

Keywords: Tetranychidae, diapause induction, light intensity, simulator, photoperiod

INTRODUCTION

An understanding of the pattern of diapause is fundamental to an understanding of population growth, migration, speciation, species distribution, and species interaction (Butler et al. 1978). Detailed knowledge of insect seasonality is required for accurate forecasts of seasonal activity which is essential for population management. With facultative diapause, day length (photoperiod), temperature, diet, and moisture have all been

implicated as being involved in the induction of diapause, but of these several factors, day length and temperature have proved to be of the highest importance (Beck 1980; Tauber et al. 1986; Danks 1987). It is well known that a broadly latitudinally distributed insect species with winter diapause will tend to develop into geographical populations exhibiting adaptive photoperiodic response characteristics. It is not astonishing that diapause may have arisen independently many times during natural selection since the components of diapause have a different genetic basis in various insects (Hoy 1978).

Several spider mite species can enter reproductive diapause in reaction to the physical environment, i.e., short light periods and low air temperature (Veerman 1985; Takafuji et al. 2003). Photoperiodic diapause possibly progressed on numerous instances as insects and mites extended their distributions into higher latitudes with a prominent winter season (Saunders 2009). As all insect photoperiodic mechanisms are probably circadian-based, their development from a prior homodynamic life cycle in the tropics must have employed the pre-existing (and ubiquitous) circadian system.

Tetranychus urticae Koch (Acari: Tetranychidae) is one of the most important and economic polyphagous herbivorous arthropods, infesting on more than 1,100 plant species in over 140 plant families (Grbić et al. 2011; Migeon & Dorkeld 2014). In addition, *Tetranychus kanzawai* Kishida is distributed all over Asia, Oceania, North America and Mexico, where it is of considerable economic importance (Bolland et al. 1998) and also a serious pest of a variety of crops such as solanaceous and rosaceous plants in Japan (Ehara & Gotoh 2009). Both species were collected from the northern areas of Japan, Hokkaido, and the extension of the distribution into southern areas suggests that the diapause attributes of the northern populations may have been modified as compared with those in the southern ones. *T. urticae* did not enter diapause in a photophase ranging from 14 to 16 h in Hokkaido and the diapausing females reached 96-100% in a photophase of 10 h (Gotoh 1986). However, diapause in females of *T. urticae* reached 50% in late September in 1981 and 1983, but it reached 100% in early September in 1982. Since a daylength at the same time of year in the same region does not vary from year to year, the different timing of diapause induction may have been influenced by other factors, most likely different temperatures. However, this difference cannot be verified by a conventional experimental system that relies on photoperiod control and is maintained at a constant temperature in the laboratory.

An environmental simulation system (ESS) developed by our group can reproduce the fluctuating temperatures and photoperiods in any region where environmental data are available, making it possible to experimentally verify the effects of the field environments on organisms in the laboratory (Nishide et al. 2017a,b; Ghazy et al. 2018, 2019). ESS offers the possibility of accurately predicting the timing of diapause induction in spider mites. In this study, we installed 20 years' environmental data from the Hokkaido region in ESS to evaluate the timing of diapause induction of *T. urticae* and *T. kanzawai* under simulated field environments.

MATERIALS AND METHODS

To determine the timing of diapause induced by the field environments, we used ESS that reproduces the outdoor photoperiods formed by changes in light intensity and temperatures over time in the laboratory (Nishide et al. 2017a,b; Ghazy et al. 2018, 2019). ESS consisted of a computer (PC, XC cube EZ 65, Aopen Inc., Tokyo, Japan), a refrigerator (JF-NU40B, Haier Japan Inc., Osaka, Japan), an hair dryer (Tescom Denki Inc., Tokyo, Japan), white LEDs (GW5BTF50k00, Sharp Inc., Osaka, Japan), a temperature-humidity sensor (THA-3151, T & D Co., Nagano, Japan), an illumination sensor (ISA-3151, T & D, Co., Nagano, Japan), a data logger (TR-74Ui, T & D Co., Nagano, Japan), and a control unit (Figure 1). Temperatures were regulated by on/off controls

of the refrigerator and the hair dryer. Light intensity was regulated by pulse-width modulation of LEDs (Suzuki et al. 2011). Temperature and light intensity were recorded every 10 seconds. Setting data of temperatures and photoperiods in ESS was based on the mean hourly values of 20 years from 1981 to 2000 in Sapporo, Hokkaido (Meteorological Data System Co., Kagoshima, Japan). We set ESS to reproduce the temperatures and photoperiods precisely and continuously from the beginning till the end of experiment. As the sunlight is strong and the LEDs used in this study cannot reproduce the same output of light, the light intensity during day time was set at a constant value. To get the exact period for diapause induction of *T. urticae* and *T. kanzawai* in field, the environment was reproduced at five dates (21 August, 1 September, 6 September, 11 September, and 16 September).

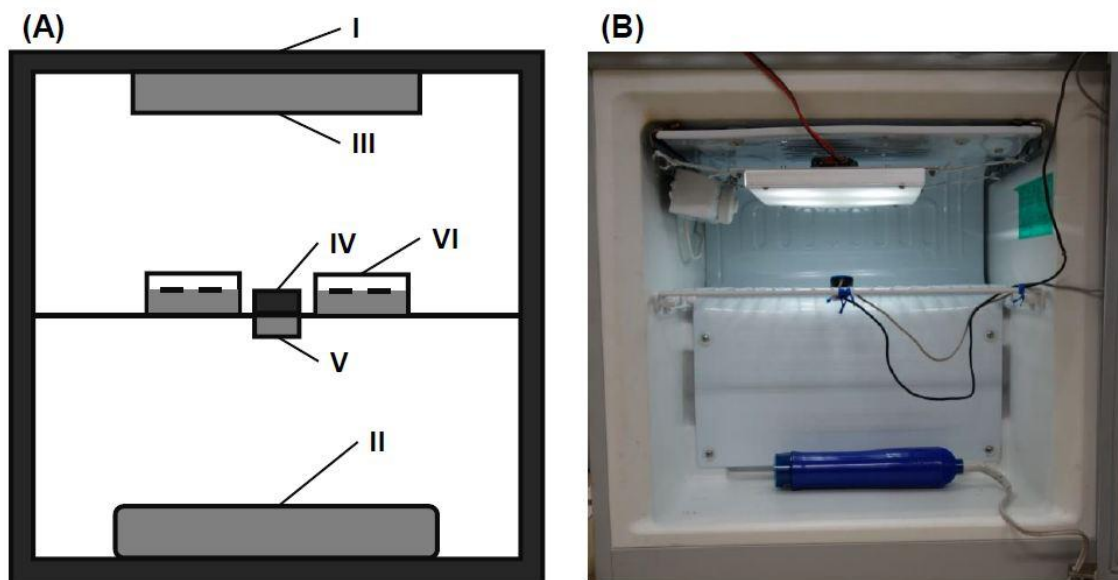


Figure 1. Schematic diagram (A) and photograph of inner structure (B) of the simulator. (I) mini refrigerator, (II) hairdryer, (III) white LED, (IV) illumination sensor, (V) temperature-humidity sensor and (VI) leaf disk on wetted cotton in Petri dish (9 cm diameter).

Statistical Analysis

The critical photoperiod was generated based on logistic analysis consisting of a generalized linear model. To better understand the significance of correlations between the field and laboratory data, relative light intensity and temperature on each date were subjected to correlation analysis. All analyses were carried out using R version 3.1.2 (R Core Team 2014).

RESULTS

Validation of ESS data

Expectations concerning the ability to match the data collected from field and laboratory can be developed by attempting to fit regression equations to validate the data. We computed a regression equation to predict temperature between ESS-in-laboratory and field data (Fig. 3). The regression analysis showed the positive relationship in temperatures between field and ESS with the R^2 value of 0.795.

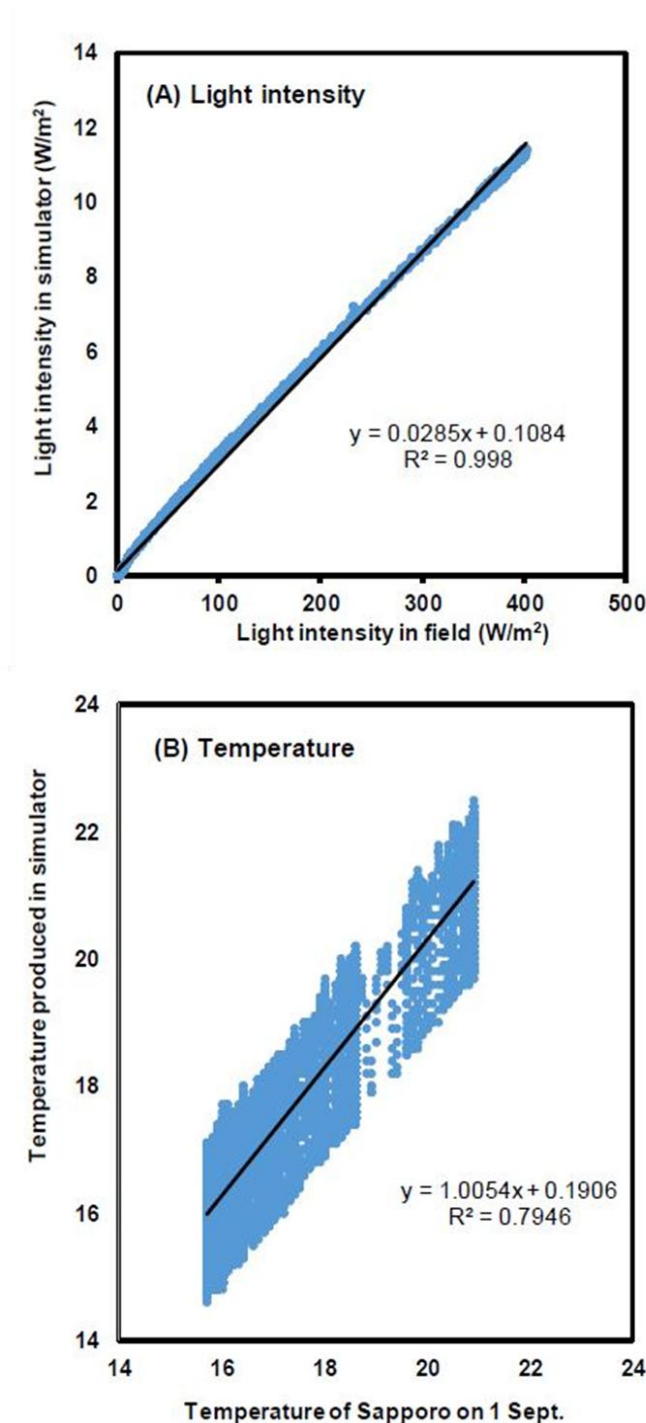


Figure 2. Correlation of light intensity (A) and temperature (B) produced inside the simulator to validate the data with field in Sapporo on 1st September.

Diapause induction

Diapause induction of *T. urticae* and *T. kanzawai* collected in Hokkaido was observed in recreated environmental condition by simulator in laboratory (Fig. 4). No diapause induction was observed in either species in the beginning of September. In the middle of September diapause was 21.7 and 12.8% in *T. urticae* and *T. kanzawai*, respectively. On September 23, the diapause induction of *T. urticae* was slightly lower than that of *T. kanzawai*, but at the end of September both species showed a similar response. Finally, in the early week of October 100% females had entered diapause in both species. The photoperiodic response curve of *T.*

urticae and *T. kanzawai* indicated that a typical short-day response type with a critical photoperiod (at 18.0°C) of approximately 13.5 h for *T. urticae* and 12.5 h for *T. kanzawai*. Diapause induction of *T. urticae* and *T. kanzawai* was observed on 22 and 23 September, whereas the estimated dates of diapause induction were on 19 and 30 September, respectively, based on the photoperiodic response curves. So, the observed date of diapause induction was 4 days later in *T. urticae* and 7 days earlier in *T. kanzawai*.

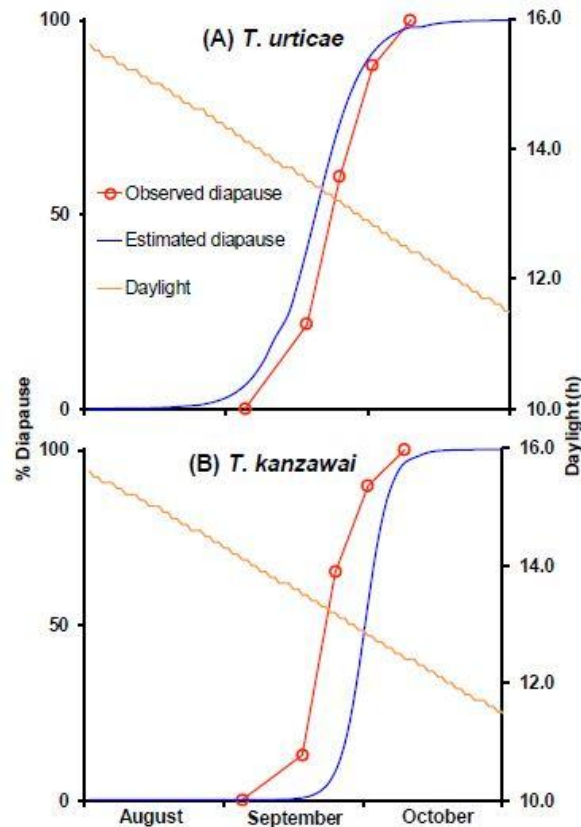


Figure 3. Photoperiodic response curves of diapause induction of *Tetranychus urticae* and *T. kanzawai* inside the simulator experienced with the environment set as field from August to October.

DISCUSSION

The results with the laboratory condition which could be recreated using the ESS are valid and similar with field data indicated that twilight, light intensity and temperatures. Both the temperatures and photoperiods are important in predicting the timing of diapause induction of *T. urticae* and *T. kanzawai*. The estimated critical daylengths for the photoperiodic induction of diapause was slightly different from the observed diapause induction.

The scientific significance of ESS can only be substantiated by rigorous validation with respect to field data. It can be seen from the comparison of temperatures in ESS and field data that the ESS is well suited. This result is possibly presented by the static diapause parameters in ESS that could fully capture the climatic-driven mechanism of diapause. When the diapause effect is included, the ESS demonstrates a substantial improvement in cases of Hokkaido, where diapause shows an established seasonal pattern. Adjusting the ESS with light intensity and temperature-related parameters yields moderate improvement in this study areas and the best performance is achieved with both facets of adjustments applied.

A few cautionary notes should be highlighted. First, the ESS did not account for the accurate light intensity occurring in the field. Generally, the high light intensity observed in the field cannot be produced by the ESS. ESS can create a proportionate light intensity that is fitted with the field light intensity. A change in critical photoperiods for diapause induction with ongoing climate change was already observed in nature (Stoekli et al. 2012). Several studies on photoperiod-induced diapause induction have concentrated on the operation of the 'circadian clock' that identifies the length of day and night, and the 'photoperiodic calendar' for seasonal changes in photoperiod (Saunders 1981). Outcomes of these and photoperiod change experiments have been related to the operation of the circadian clock and calendar (Košťál 2011; Saunders 2009, 2010a,b, 2013). Although several previous studies have documented seasonal monitoring data, suggesting that *T. urticae* populations from red currant and red clover gradually underwent a change from 10 to 16 h (Gotoh 1986). The diapause induction of field *T. urticae* population from these two food plants corresponded to the timing predicted by the critical photoperiod, when considered the twilight light hours (Beck & Hance 1960). It was also reported that the proportion of diapausing females of *T. urticae* was high in early September and it was low in October, as compared with 1981 and 1983 for red currant populations and with the 1982 for the red clover population (Gotoh 1986). The critical photoperiods of two populations of *T. urticae* appeared to be 13 h, which appeared in mid-September in Sapporo (Gotoh 1986). Our study, using the ESS, also showed that the critical photoperiod was on 22-23 September, which is very close to the critical photoperiod observed in field. The little variation between these two studies was likely due to the slight deviation in temperature in the ESS. The present study accurately simulated the relationship between temperature and relative light intensity in field and in laboratory, and may be used to predict its post-diapause development or spring emergence in future.

Tetranychus urticae and *T. kanzawai* is a significant and frequently infesting agricultural pest in Japan (Gotoh 1986; Shah et al. 2011a,b). Understanding photoperiodic as well as other factors involved in diapause induction in these species is important to improve simulation models of developmental timing and therefore for monitoring and management program. In an attempt to approximate natural photoperiod and temperature change experienced during the life of spider mites in ESS could help us to predict diapause characteristics. Therefore, this study concentrated on temperature, photoperiod and relative light intensity could be recreated in laboratory using ESS similar to field conditions, which are the major factors regulating diapause induction and provided the fundamental information necessary to predict spring emergence.

CONCLUSION

The prediction of diapause induction in the laboratory using the simulator indicated effectiveness and was consistent with the field observations. Temperature, photoperiod, and relative light intensity could be recreated in the laboratory using a simulator similar to field conditions, which are the major factors regulating diapause induction and provided the fundamental information necessary to predict spring emergence. The photoperiodic reaction of diapause induction in both species using the simulator in the laboratory was accurate. It may be a useful instrument for monitoring and management procedures.

CONFLICT OF INTEREST

All authors express no conflict of interest in any part of research, manuscript and submission to the journal.

REFERENCES

1. Beck, S.D. & Hanec, J.W. (1960) Diapause in the European com borer, *Pyrausta nubilalis* (Hubn.). *Journal of Insect Physiology*, 4, 304-318.
2. Beck, S.D. (1980) Insect photoperiodism. Second edition, Academic Press, New York. pp. 387.
3. Bolland, H.R., Gutierrez, J. & Flechtman, C.H.W. (1998) World catalogue of the spider mite family (Acari: Tetranychidae). Brill Academic Publisher, Leiden, pp 392.
4. Butler, G.D., Hamilton, Jr., A.G. & Gutierrez, A.P. (1978) Pink bollworm: Diapause induction in relation to temperature and photoperiod. *Annals of Entomological Society of America*, 71, 202-304.
5. Danks, H.V. (1987) Insect Dormancy: An Ecological Perspective. Biological Survey of Canada, Ottawa, Canada. pp. 639.
6. Ehara, S. & Gotoh, T. (2009) Colored guide to the plant mites of Japan (eds). Zenkoku-Noson-Kyoiku-Kyokai, Tokyo, pp 349 (in Japanese).
7. Ghazy, N.A., Suzuki, T. & Amano, H. (2018) Development and reproduction of *Neoseiulus californicus* (Acari: Phytoseiidae) and *Tetranychus urticae* (Acari: Tetranychidae) under simulated natural temperature. *Environmental Entomology*, 47, 1005–12.
8. Ghazy, N.A., Gotoh, T. & Suzuki, T. (2019) Impact of global warming scenarios on life-history traits of *Tetranychus evansi* (Acari: Tetranychidae). *BMC Ecology*, 19, 48.
9. Gotoh, T. (1986) Annual life cycle of the two-spotted spider mite, *Tetranychus urticae* Koch (Acarina: Tetranychidae), on *Ribes rubrum* L. in Sapporo: the presence of non-diapausing individuals. *Applied Entomology and Zoology*, 21, 454–460.
10. Grbić, M., Van Leeuwen, T. et al. (2011) The genome of *Tetranychus urticae* reveals herbivorous pest adaptations. *Nature*, 479, 487–492.
11. Hoy, M.A. (1978) Variability in diapause attributes of insects and mites: some evolutionary and practical implications. In: Dingle, H. (ed.) *Evolution of Insect Migration and Diapause*, Springer-Verlag, New York, pp. 101-126.
12. Košťál, V. (2011) Insect photoperiodic calendar and circadian clock: independence, cooperation, or unity? *Journal of Insect Physiology*, 57, 538–56.
13. Migeon, A. & Dorkeld, F. (2014) Spider mites web: a comprehensive database for the Tetranychidae. <http://www.montpellier.inra.fr/CBGP/spmweb>. Accessed 01 July 2014.
14. Nishide, Y., Suzuki, T. & Tanaka S. (2017a) Synchrony in the hatching of eggs in the desert locust *Schistocerca gregaria* (Orthoptera: Acrididae): egg condition influences hatching time in the laboratory and under simulated field temperatures. *Applied Entomology and Zoology*, 52, 599–604.
15. Nishide, Y., Suzuki, T. & Tanaka S. (2017b) The hatching time of *Locusta migratoria* under outdoor conditions: role of temperature and adaptive significance. *Physiological Entomology*, 42, 146–155.
16. Saunders, D.S. (1981) Insect photoperiodism—the clock and the counter: a review. *Physiological Entomology*, 6, 99–116.
17. Saunders, D.S. (2009) Circadian rhythms and the evolution of photoperiodic timing in insects. *Physiological Entomology*, 34, 301–308.
18. Saunders, D.S. (2010a) Controversial aspects of photoperiodism in insects and mites. *Journal of Insect Physiology*, 56, 1491–1502.

19. Saunders, D.S. (2010b) Photoperiodism in insects: migration and diapause responses. In: Nelson RJ, Denlinger DL, Somers DE, editors. *Photoperiodism: the biological calendar*. Oxford: Oxford University Press. p. 258–286.
20. Saunders, D.S. (2013) Insect photoperiodism: measuring the night. *Journal of Insect Physiology*, 59, 1–10.
21. Shah, M., Suzuki, T., Ghazy, N. A., Amano, H. & Ohyama, K. (2011a) Effect of photoperiod on immature development and diapause induction in the Kanzawa spider mite, *Tetranychus kanzawai* (Acari: Tetranychidae). *Experimental and Applied Acarology*, 55, 183–190.
22. Shah, M., Suzuki, T., Ghazy, N.A., Amano, H. & Ohyama, K. (2011b) Night-interrupting light inhibits diapause induction in the Kanzawa spider mite, *Tetranychus kanzawai* Kishida (Acari: Tetranychidae). *Journal of Insect Physiology*, 57, 1185–1189.
23. Stoeckli, S., Hirschi, M., Spirig, C., Calanca, P., Rotach, M.W., et al. (2012) Impact of Climate Change on Voltinism and Prospective Diapause Induction of a Global Pest Insect –*Cydia pomonella* (L.). *PLoS ONE*, 7, e35723.
24. Suzuki, T., Shah, M., Ghazy, N.A., Takeda, M., Amano, H. & Ohyama, K. (2011) An improved space-saving system for testing photoperiodic responses of insects and mites: its use in diapause experiments for the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae). *Applied Entomology and Zoology*, 46, 449–454.
25. Takafuji, A., Santoso, S., Hinomoto, N., Chain-Ing, T.S., Chyi-Chen, H. & Gotoh, T. (2003) Diapause characteristics of two species of tetranychids mites (Acari: Tetranychidae) in southern Japan and Taiwan. *Applied Entomology and Zoology*, 38, 225–232.
26. Tauber, M.J., Tauber, C.A. & Masaki, S. (1986) *Seasonal adaptations of insects*. Oxford University Press, New York, pp. 414.
27. Veerman, A. (1985) Diapause. In *Spider Mites Their Biology, Natural Enemies and Control*, Vol. 1A (W. Helle and M.W. Sabelis eds.). Elsevier, Amsterdam, pp 279-316.

Supply and Consumption Methods of Medicinal Plants in Mazar-e-Sharif City, Afghanistan

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ABSTRACT

Background: Balkh province is located in the northern part of Afghanistan, which is rich in medicine plant diversity. The large number of medicinal plant shops (Attari) in the center of Mazar-e-Sharif city indicates that the citizens of Balkh province are familiar with medicinal plants and their uses. In this study, an attempt has been made to investigate the socioeconomic characteristics, medicinal plant distribution patterns, consumption methods, and types of medicinal plants in the Mazar-e-Sharif city Attaris.

Materials and Methods: The study focused on 102 Attaris in the research area, research factors such as the age of medicinal plant sellers, monthly income, education level, marital status, number of people working in Attaris, and work experience of Attaris were studied.

Findings: The results revealed that the average age of people in Attaris is 49 years with a work experience of about 15 years, and only 3% have an education related to medicinal plants. About 20 % of medicinal plant sellers had a university education but not related to medicinal plants science, about 17 % had a diploma, 30 % had a high school education and the remaining 12 % had primary education. Unbelievably, about 50 % of consumers were rich people and the remaining 50 % of the clients were low-income. People visited Attari's to heal their digestive system disorders are the most common health issues, followed by issues with the kidneys, bladder, and urinary system, respiratory conditions, skin and wound issues, hypertension and cardiovascular conditions, liver issues, diabetes, typhoid, pain, helminthiasis, anorexia, gynecological complaints, sexual dysfunction, weakness, fever, and obesity. Lemon balm, Common jujube, Starflower, Black pepper, Cumin, and Turmeric were the high-selling medicinal plants offered in Mazar-e-Sharif Attaris'.

Conclusion: A large number of medicinal plant shops (Attaris) in the center of Mazar-e-Sharif city indicates that the citizens of Balkh province are familiar with medicinal plants and their uses. However, unfavorable economic situation of some people who referred Attaris showed that the existence of traditional medicine systems (Attaris) is considered to be one of the urgent needs of the Mazar-e-Sharif.

Keywords: Medicinal plant, Mazar-e-Sharif, Poverty; Traditional medicine; Side effects, Attari.

INTRODUCTION

The use of medicinal plants is as old as human life and like diseases, they have been born with the emergence of mankind amazingly the available several thousand-year-old documents of medicine and pharmacy contain valuable medicinal plant experiences and information. These easily accessible, culturally significant traditional medicines serve as the cornerstone of an affordable, accessible healthcare system and provide a crucial source of income for rural and indigenous populations. With the rapid progress of science on the one hand and economic issues on the other, consumption of medicinal plants has been reduced compared to the past. In recent years, there has been an increase in scientific and economic interest in the medicinal species that inhabit natural environments. Up to 80,000 blooming plants are utilized medicinally worldwide (Marinelli, 2005).

The use of medicinal plants has been done since ancient times and may even be considered the origin of modern medicine. Compounds of plant origin have been and still are an important source of compounds for drugs. New compounds, such as one recently discovered in a plant in Madagascar, are likely to provide novel antibiotics and help curb the epidemic of antibiotic-resistant diseases (Wang et al., 2006). Pharmacologic testing of herbs on a broad scale is being done by pharmaceutical corporations as the development of medications from plants develops (Vickers et al., 2001). Today, ethnobotanical research can be a trusted and favored method for finding new drugs and is crucial for biodiversity preservation (Shah and Hussain 2021).

According to the National Cancer Institute, at least 70 percent of new drugs introduced in the United States in the last 25 years are derived from natural sources (Steenhuysen, 2007). As of 2015, few products made from medicinal plants had undergone safety and efficacy testing, and those that had been sold in rich economies and given by traditional medicines to people in underdeveloped nations were of variable quality and occasionally contained harmful pollutants plants (Chan, 2015). Along with other substances and methods, a number of plants are used in traditional Chinese medicine. Ten percent of all vascular plants are used as medicinal plants (Fonnegra et al., 2007) since ancient times, plants have been used in medicine and are still used today (Grover et al., 2002). To create herbal medicines, herbal materials are primarily treated to extraction, fractionation, purification, and concentration; they may be consumed directly or after processing. In addition to their active constituents, herbal remedies may also contain excipients or inactive substances (Shafique et al., 1987).

Since much of Afghanistan's flora is rich in volatile oils but isn't fully utilized, it has a large number of plant species that are used in traditional medicines and should be mentioned that Afghanistan is a mountainous country (National Statistics and Information authority, 2021). The use of traditional remedies predates the introduction of modern medications and is among the world's oldest forms of medicine. A large variety of plant species are utilized in traditional medicines in Afghanistan due to its diverse flora (Pelt, 1967). These traditional medicines can be split into two categories: (i) Greek, Indian, and Islamic medicines are examples of traditional medicines that are based on ancient systems that produced written literature and theories; (ii) folk medicines are based on oral transmission and are used by medicines of all kinds. In Afghanistan, several medicinal plants plant uses are widespread. The most typical conventional method is boiling herbs or creating herbal tea by decocting plant blossoms, leaves, or stems in water, and then the extract is filtered, whereas many people cure their health issues with plants in an unscientific approach. According to estimates from the World Health Organization (WHO), several nations in Asia and Africa have 80% of their people use herbal remedies for some of their fundamental medical needs (Traditional medicine, 2003).

According to the Afghanistan National Statistics and Information Authority, Mazar-e-Sharif is the fourth largest city in Afghanistan in terms of population in 2021. Highways connect it to Kunduz in the east, Kabul in the

southeast, Herat in the southwest, and Termez, Uzbekistan in the north. At Mazar-i-Sharif, the summers are sweltering, arid, and clear and the winters are very cold, snowy, and partly cloudy. The hot season lasts for 3.6 months, from May 22 to September 11, with an average daily high temperature above 33 C°. The hottest month of the year at Mazar-i-Sharif is July, with an average high of 102°F and low of 29 C°. The cool season lasts for 3.5 months, from November 22 to March 7, with an average daily high temperature below 15 C°. The coldest month of the year at Mazar-i-Sharif is January, with an average low of 0 C° and high of 8 C°. Low levels of precipitation typically occur between December and April. It has a fairly hot climate. (The United Nations Human Settlements Programme, 2015).

Local hakim (doctors) use a variety of regional plants and herbs for medicinal plants purposes. Some are gathered for export, particularly artemesia and asafetida. There are numerous pharmacies in every bazaar. A lot of these traditional medicines are used as poultices to treat infections or as purgatives for diarrhea, as well as to treat headaches and cold discomfort. Numerous nostrums with undeniable medicinal efficacy have been developed over generations of unsuccessful experimentation. The hakim refer to the usage of medicinal plants as dawa-yunani, or "Greek medicine," and they assert to be the descendants of the doctors who accompanied Alexander the Great on his travels. But the official authorities in the health sector of Afghanistan did not agree with the common Attars. In May 2013, a research on herbal medication use among children in Taif, Saudi Arabia, found that 70% of parents had previously given their kids herbs. Anise (24.7%) and fenugreek (14.7%) were the herbs most frequently utilized (El-Mawla et al., 2013).

People in Mazar-e-Sharif are traditionally quite willing to employ herbal medicines to treat common ills including gastrointestinal illnesses, urinary tract infections, respiratory issues, skin conditions, cardiovascular diseases, etc. People believe that chemical drugs have replaced medicinal plants in many cases. The experience of the last few decades shows that despite chemical drugs full efficiency, they have many unpleasant and unfortunate side effects. However, Attars are one of the significant traditional medicines offering traditional medical care in Mazar-e-Sharif. They do not have license or formal medical education only they can obtain trading license that is provided under supervision of Afghanistan Ministry of Commerce.

Current research was to compile a list of the plants employed in the 12 principal towns of Mazar - e -Sharif. In this research, it is the first time that the supply and consumption of medicinal plants in Mazar - e -Sharif have been reported on. Similar studies are encouraged to be conducted, though, for local health records and for compiling a list of the medicinal plants utilized throughout Afghanistan. It can be proudly noted that Balkh province is the birthplace of Abu Ali Sina, the founder of traditional medicine and a great Afghan scientist, unfortunately, no significant work has been done on traditional medicine in Mazar-e-Sharif.

MATERIAL AND METHODS

To study the economic features as well as the supply and consumption methods of medicinal plants in the Attaris of Mazar - e -Sharif in summer 2022, a survey was conducted in the Attaris of study area (Figure 1). Information was obtained from traditional medicines in 12 principal towns of Mazar - e -Sharif by distributing of relevant questionnaires with specifications such as, name of the medicinal plant, place of origin (local or imported), and plant parts used among the 102 eligible Attaris. In order to increase the information accuracy, the Attaris that had been established at least in the last three years were studied.

tried to provide more services to their customers to improve the level of customer satisfaction and create mutual trust. More than 90% of the shops were rented, which had a direct negative impact on Attaris income (Table 1).

Table 1. Major traits of Attaris in Mazar - e -Sharif city

Age	48.8 ± 18.2(year)
Work experience	15.3 ± 6.3(year)
Monthly income	27466.6 ± 15673.3 (Afg.)
Marital status	Married 72 and single 50 person
Special training course taken	Attended course 12 person, not attended 90 person
Shop status	93 rented shops and 9 owned shops
As main occupation	84 person as main occupation and 8 as second job

*Afg. Refers to Afghanistan currency.

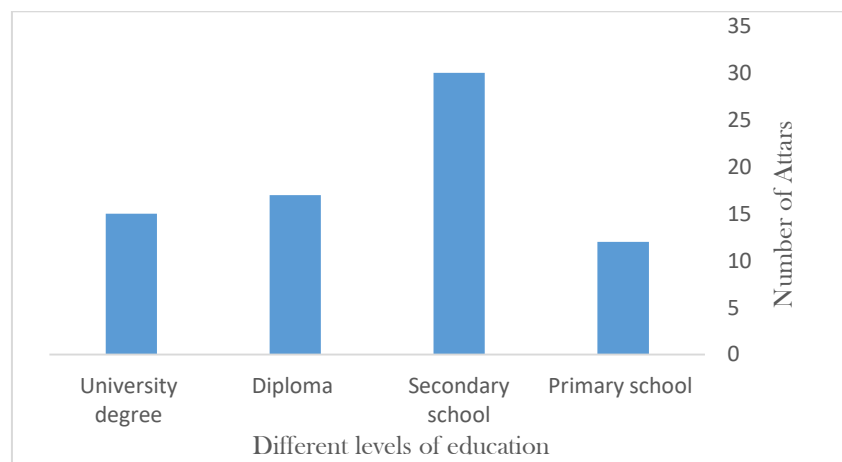


Figure 2. Education level of the Attaris of Mazar - e -Sharif city

The results showed that 41% of studied Attaris, could provide only one job opportunity, in 38% of the Attaris two people were worked and in the remaining 21% of Attaris more than two people were worked (Figure 3). Regarding the length of work experience, a significant correlation between the work experience and monthly income was noticed. Unlike the length of work experience, no significant correlation was observed between education level and monthly income. It showed that work experience in medicinal plants can be more profitable than attending a training course on medicinal plants.

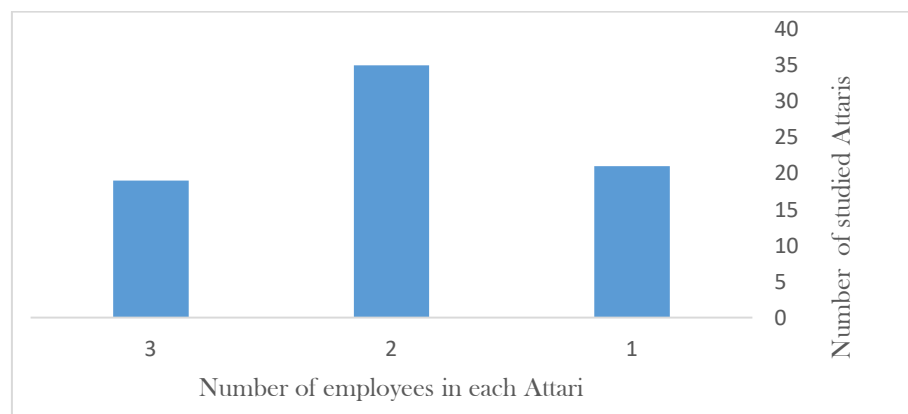


Figure 3. The number of employees who were engaged in Attaris

Almost 50% of the Attaris' customers were rich people, while others were poor. Based on use of medicinal plants by rich and poor people, it was concluded that poor people depend on traditional medicine due to the poor economic situation, while wealthy people have become dependent on traditional medicine in order to avoid the side effects of chemically synthesis modern drugs. Medicinal plant sellers admitted that some of their customers visited them many times to buy medicinal plants. The study showed that those who were interested in traditional medicine have visited medicinal plant sellers at least twice in a year.

Regarding seasonal plants sales, the bestselling medicinal plants from March to September were Flixweed, Lemon balm, Mint. Subsequently Sweet fennel, Starflower, Ispaghula husk, Common jujube, Common mugwort, Garden heliotrope, English lavender, Indian laburnum, Cardamom, Turmeric, Myrobalan, Opium poppy, Black seed, Black pepper, Common ginger, Cinnamon, and Cumin were the bestselling medicinal plants from October to February (Table 2). In terms of volume, Cinnamon, Common ginger, Flixweed, Common jujube, Black seed, and Chebulic myrobalan respectively were the bestselling plants throughout the year. The most valuable plants available in the Mazar - e -Sharif Attaris respectively were *Ferula assa-foetida L*, *Crocus sativus*, *Orchis mascula L.*, *Nigella sativa L*, *Cuminum cyminum. L*. (Figure 4).

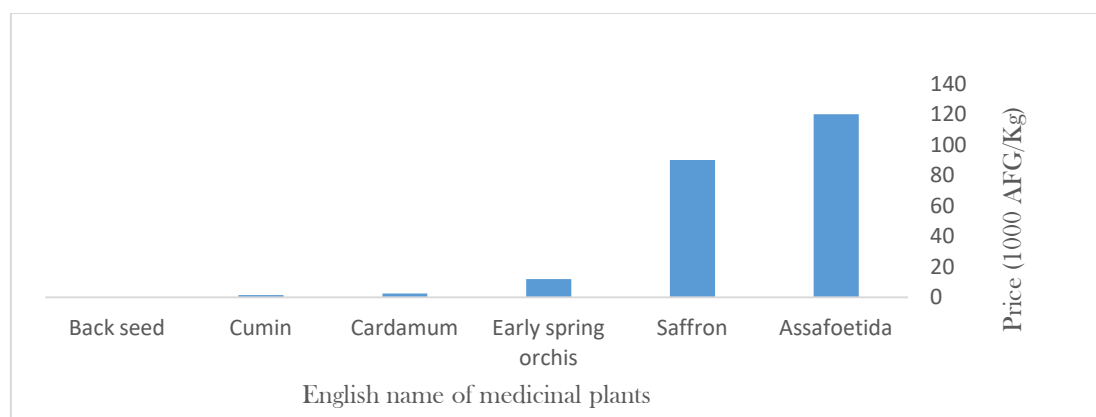


Figure 4. The most precious medicinal plants available in Mazar - e –Sharif's Attaris based on local market.

The research results showed that some special medicinal plants are imported from foreign countries (*Sisymbrium sophia L* from Iran, *Curcuma longa* from Pakistan and *Zingiber officinale* from India and China) and some medicinal plants are provided from some specific provinces in Afghanistan and the remaining medicinal plants could be boating from entire provinces of Afghanistan (Figure 5).

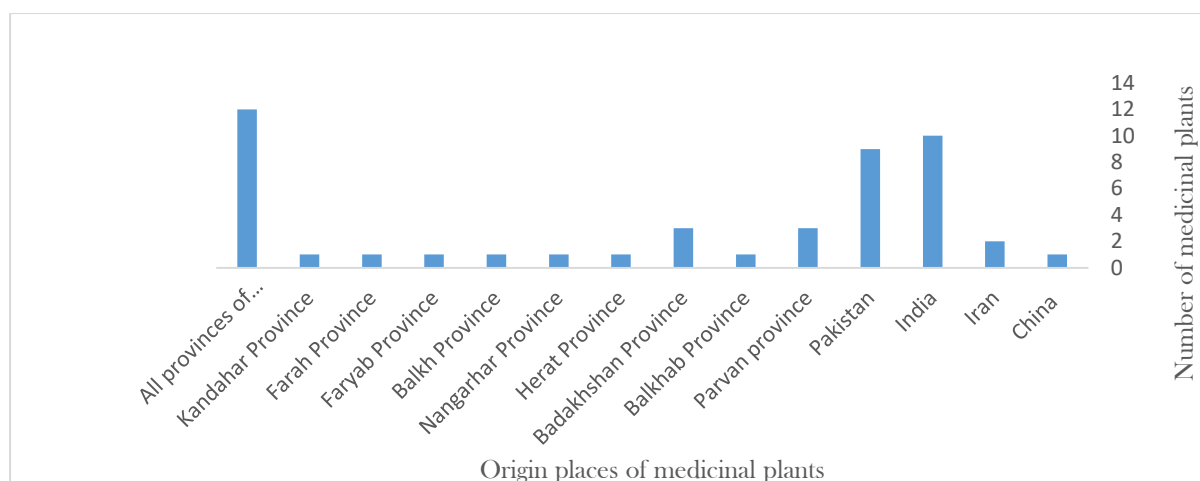


Figure 5. Different Supply places of medicinal plants used in Mazar-e-Sharif's Attaris

Table 2. Seasonal bestselling medicinal plant in Mazar - e -Sharif city.

S/N	Hot season in Mazar - e -Sharif (March to September)	English names	Local names (Dari)	S/N	Cold season in Mazar - e Sharif (October to February)	English names	Local names (Dari)
1	<i>Sisymbrium sophia L</i>	Flixweed	خاکشیر	1	<i>Cassia fistula</i>	Indian laburnum	فلوس
2	<i>Melissa officinalis L</i>	Lemon balm	بادرنجبویه	2	<i>Elettaria cardamomum (L.) Maton</i>	Cardamom	هل
3	<i>Mentha piperata L.</i>	Mint	نعناء	3	<i>Curcuma longa</i>	Turmeric	زرد چوبه
4	<i>Foeniculum vulgare Mill.</i>	Sweet fennel	بادیان	4	<i>Terminalia chebula</i>	Chebolic myrobalan	هليله سیاه
5	<i>Barago officinalis L.</i>	Starflower	گل گاوزبان	5	<i>Papaver somniferum</i>	Opium poppy	کونکار
6	<i>Plantago ovate</i>	Ispaghula husk	اسپرزه	6	<i>Nigella sativa L.</i>	Black seed	سیاه دانه
7	<i>Ziziphus jujuba</i>	Common jujube	عناب	7	<i>Piper nigrum</i>	Black pepper	مرچ سیاه
8	<i>Artemisia vulgaris L.</i>	Common mugwort	بوی مادران	8	<i>Zingiber officinale</i>	Common ginger	زنجبیل
9	<i>Valeriana officinalis L.</i>	Garden heliotrope	سنبل الطیب	9	<i>Cinnamomum zeylanicum</i>	Cinnamon	دارچین
10	<i>Lavandula angustifolia</i>	English lavender	خیری دشتی	10	<i>Cuminum cyminum. L</i>	Cumin	زیره خوشبو

It was also found that leaves were the most common consumption parts of medicinal plants in Mazar - e -Sharif city, followed by seeds, herb, fruits, roots, bark, stigma, and flower, as shown in Figure 6. In terms of consumption methods, out of the 66 medicinal plants, 54 plant species were consumed orally, 7 plant species applied topically, and 5 plant species used for gargle.

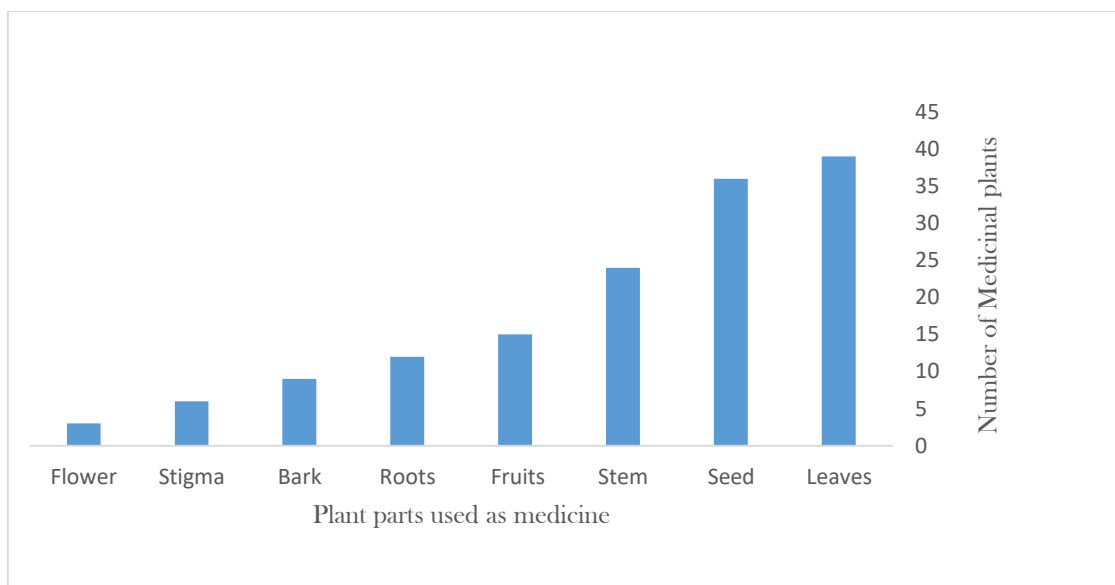


Figure 6. Plant parts used as medicine by Attaris in Mazar - e -Sharif city.

DISCUSSION

The present study aimed to investigate the supply and consumption methods of medicinal plants in Mazar - e - Sharif, the results of the study revealed that in different seasons (hot season from March to September and cold season from October to February), and different medicinal plants are used, that is also reported by Hossaini et al., (2008). Most of the people who visited Attaris in Mazar-i- Sharif, expected to treat their illness such as colds, kidney, arthritis, diabetes, Stomach upset and high blood cholesterol by consuming advised medicinal plants by Attars that a study by Mojab et al., (2003) indicated the same result. Except few medicinal plants such as Cardamom, Cinnamon, Common ginger, Flixweed, and Starflower that are imported from Iran, Pakistan, India and china, the majority of medicinal plants can be supplied from the other provinces of Afghanistan. Hosseini et al (2017) stated that proper planning by officials can accelerate the production and supply of commonly used medicinal plants in the natural habitats inside the Shiraz province, Iran. Although 20% of Attars in Mazar - e -Sharif had a university education but their education was not linked to their job (Attari) and it is indicated that most of the Attars gained their ethno botanical Knowledge from their ancestors or working in Attaris as medicinal plants sellers or trainee. It is concluded that local traditional knowledge of medicinal plants is the main platform for providing services in Mazar - e -Sharif Attaris'.

CONCLUSION

The large number of medicinal plant shops (Attaris) in the center of Mazar-e-Sharif city indicates that the citizens of Balkh province are familiar with medicinal plants and their uses. It is estimated that the people who sell medicinal plants in Mazar-e-Sharif city have sufficient knowledge about the use and consumption methods of medicinal plants. On one hand the unfavorable economic situation of some people who referred Attaris in Mazar-i- Sharif showed that the existence of traditional medicine systems (Attaris) is considered to be one of the urgent needs of the Mazar - e -Sharif society. On the other hand, the study showed that equally beside the poor people the rich people also visited Attaris and it is confirmed that the modern medical service/system in Mazar - e -Sharif is not very reliable. Analyzing the level of people's interest in the demand for medicinal plants and clarifying the supply capacity led to the design of long-term programs with the aim of

providing and facilitating cheap and accessible health services. It is inferred that due to the limited services of the modern medical system in Mazar - e -Sharif, officials, and planners must focus on cultivating medicinal plants and increasing awareness of the traditional medicine system to provide effective and cheap health services in Mazar - e -Sharif.

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CONFLICTS OF INTEREST

The authors declared no conflicts of interest.

REFERENCES

1. Ajazuddin, & Saraf, S. (2012). Legal regulations of complementary and alternative medicines in different countries. *Pharmacognosy Reviews*, 6 (12), 154-160.
2. Chan, M. (2015, August 19). WHO Director-General addresses traditional medicine forum. WHO. Archived from the original on August 22, 2015.
3. El-Mawla, A.M.A.A., Albarrag, A.A., & Abdallah, M.A. (2013). Herbal medicine use in a group Taif children, *Saudi Arabia -ScopeMed.org - index for medical articles*. Spatula DD, 3(2), 41-44.
4. Fonnegra F.G. (2007). *Plantas Medicinales Aprobadas en Colombia*. University of Antioquia; Antioquia, Colombia.
5. Grover J.K., Yadav S., & Vats V. (2002). Medicinal plants of India with anti-diabetic potential. *J. Ethnopharmacol.*, 81, 81–100. doi: 10.1016/S0378-8741(02)00059-4.
6. Hossaini, S.A., Abrasji, Gh., & Hossaini, S.A. (2008). Medicinal plants of Golestan province. *Iranian Journal of Medicinal and Aromatic Plants*, 24:472-498.
7. Hosseini, Z., Feizi, H., & Moradi R. (2017). Assessing supply and demand of medicinal plants in Shiraz apothecaries. *Journal of Islamic and Iranian Traditional Medicine*, 8:123-132.
8. Marinelli J. (Ed.). (2005). *Plant: The Ultimate Visual Reference to Plants and Flowers of the World*. DK Publishing Inc.
9. National Statistic and Information Authority (NSIA). (2021, April). Estimated Population of Afghanistan 2021–22 (PDF). Archived (PDF) from the original on June 24, 2021. Retrieved June 21, 2021.
10. Mojab, F., Mossadegh, M., & Moghimi, A. (2003). Investigating the reason for patients to refer to perfumers and herbal products delivered to them in perfumeries in Semnan province for one year. *Pajouhandeh*, 8(4), 9-15.
11. Pelt, J. M. (1967). Aperçu sur la végétation et la flore d'Afghanistan. *Bulletin de la Société de Philomatique de Strasbourg, Fr.* 13(2), 227-232.
12. Shafique, Y., Jacques, F., Dominique, N., Guy, M., Francois, M., & Jean, M. P. (1987). Repertory of Drugs and Medicinal Plants Used in Traditional Medicine of Afghanistan. *J. Ethnopharmacol* 20: 245-90.
13. Shah, S. M., & Hussain, F. (2021). Weed diversity in Maize fields of Mastuj valley, Hindukush range, Pakistan. *Pure and Applied Biology*, 5(4), 1044-1050.
14. Steenhuisen, J. (2007). *Mother Nature Still A Rich Source Of New Drugs*. Reuters Limited.

15. The State of Afghan Cities Report 2015". Archived from the original on 31 October 2015. Retrieved 21 October 2015.
16. The United Nations Human Settlements Programme. (2015). State of Afghan Cities report 2015 (Volume-II). https://unhabitat.org/soac2015_volume2
17. Traditional medicine. (2003). Available from: [http://www.who.int/mediacentre/factsheets/2003/fs13%204/en/](http://www.who.int/mediacentre/factsheets/fs13%204/en/)
18. Vickers, A., Zollman, C., & Lee R. (2001). Herbal medicine. *Western Journal of Medicine*, 175(2), 125-128.
19. Wang, J., et al. (2006). Platensimycin is a selective FabF inhibitor with potent antibiotic properties. *Nature*, 441, 358-361.



Study the Types of Medical Crimes and Medical Infractions in the Penal Code of Afghanistan

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ABSTRACT

Doctors are regarded as lifesavers in the world. Earlier, people who practiced medicine used to be precise in their treatment and more concerned with curing patients, it was considered as a service to humanity. However, currently, the profession has evolved into a money-making enterprise rather than a noble work. This has devolved into misuse of a noble deed. One of the ways to make money is medical crimes and medical infractions. So one of the most important issues discussed in today's law is the Medical Crimes and Medical Infractions. All countries have complete laws on prosecuting delinquent doctors. Afghanistan's legal system has incomplete laws regarding medical crimes and infractions. Unfortunately, no research has been done in this field in Afghan law. This is the first research that discusses medical crimes and medical infractions in the Afghan penal code. The penal code of Afghanistan is incompletely criminalized medical crimes and has not criminalized medical infractions. Therefore, it is necessary that the Afghan penal code should criminalize and recognize the medical infractions. This research wants to provide a legal solution to compensate the physical damage and mental damage of the patient, explaining the difference between medical infractions and medical crimes, study the challenges of the Afghan penal code regarding the criminalization of medical infractions cases and providing a suitable solution to prevent medical infractions and crimes in the Afghan society. Overall, there is a need for a fair and effective method to monitor and punish physician misconduct that will be conducive to the honest delivery of quality health care.

Keywords: Doctor, Crime, Infraction, Penal Code of Afghanistan, Owners of Pharmacies and Laboratories

INTRODUCTION

Crime means an activities that involve breaking the law (the Oxford English Dictionary) or crime is defined as an act punishable by laws forbidden by statute or injurious to public welfare. The Penal Code of Afghanistan has criminalized many behaviors. Since, The medical profession has been one of the most rooted professions in the history of human life (Sharivar,1998). This profession has always been important and challenging due to its connection with the life and death of people. Although doctors try to do their work with all their care and effort, sometimes they may commit a fault and negligence or carelessness in doing their work. For example; Some

people who visit the doctor for treatment, but they commit medical crimes, which are more vulnerable than fraudulent crimes (Rosoff, 2009). The reason for their vulnerability is that health care seekers have a medical problem that they have no ability to dictate its treatment procedures and need to get well as soon as possible. Health care providers take advantage of the desperate needs of a health care seeker to defraud them (King & Wheeler, 2006). Therefore, it is the right of the countries to impose criminal and non-criminal responses in order to prevent medical malpractice. Today, one of the biggest challenges in the Afghan society is the issue of medical crimes and medical infractions. Because many of the behaviors of the doctor and those involved in medical affairs have caused physical and mental harm to the patient and remain unanswered by the criminal justice system. Therefore, medical crimes and infractions are one of the important challenges of the Afghan criminal justice system.

Medical crime is a kind of occupational crime (Friedrichs, 2010). So, medical crime is a type of occupational crime which includes a wide range of illegal activities committed within the medical profession (Sara, 2016). Unfortunately The Penal Code of Afghanistan incompletely criminalized Medical Crimes. For example Afghan Penal Code is silent about the types of Abortion and the allowed disclosure of secrets. While, medical crimes include abortion and violation of professional secrecy, which can be subject to public prosecution in criminal courts (Fathi, 2016).

The basic goals of this research; fixing the shortcomings of the penal code and providing a suitable solution to reduce the illegal and ineffective behavior of doctors and those involved in medical affairs. The important questions of this research are (1) what are the legal solutions of the penal code regarding medical infractions? (2) Does the penal code distinguish between an infraction and a medical crime? (3) In the current situation, how does the judicial system of the country deal with the criminal and delinquent doctor? (4) How are the material and mental damages of the sick person compensated? Therefore, the hypotheses are that the penal code has defined an incomplete legal solution for medical crimes, and it has been silent on responding to medical infractions. It is disheartening to come across this news which medical crimes and medical infractions are not separated in the penal code of Afghanistan. Unfortunately, due to the lack of legal solutions, no cases regarding medical malpractice have been registered in the country's judicial system.

The most basic importance and necessity of this issue are that to Preventing illegal, negligent and careless behaviors of doctors and medical officials. Also, with studying this research by the legislators, it will be possible to prevent medical crimes and infractions in the country through the criminalization of some medical criminal behaviors and to have a healthy society free from illegal behaviors. Finally, this research discusses the key concepts of medical crimes and medical infractions, regardless of whether they are legislated in the penal code or not as follows. This research aims to provide appropriate and accurate answers to the questions by using analytical-descriptive method.

KEY CONCEPTS

The word of "Crime" means sin, error, transgression and offense (Dekhoda, 1994). In the expression, it means an illegal act [behavior] for which someone can be punished by the government or Crime means implies a serious offense punishable by the law of the state (Merriam Webster Dictionary).

Crime is a topic that is often discussed among law-makers as well as members of the general public (Sara,2016). Therefore, the crime is the behavior that is prohibited by the law approved by the Assembly. But, medical crime is fraudulent occupation of a medical job without legal requirements and over obedience from medical laws and regulations in performing legal duties and tasks (Abbasi, 1997). It would be better if the word fault was used in this definition. It has been discussed in the definition to committing an act by knowledge, intention and awareness. Therefore, a medical crime is a behavior that a doctor or people related to the medical profession knowingly committed and the law has considered a punishment for it.

The word of delinquency or infraction means going against, disobeying, and turning away (Moin, 1997). In the expression, illegal acts or actions that are prohibited by the laws and regulations of the country and the offender deserves to be blamed (Farjad, 1990). Therefore, the word fraction is general and includes infraction and crime. But, the meaning of infraction is the behaviors that are prohibited by the regulations that approved by the executive branch or the government. And Medical infraction is malpractice, negligence, non-observance of special systems related to the medical profession and other persons related to the medical profession (Sirati Nouri, 2017). Malpractice means a behavior that the perpetrator does not know that the behavior is forbidden.

TYPES OF MEDICA CRIMES WHICH ARE CRIMINALIZED IN PENAL CODE

The crimes under the title of medical crimes that the legal system of Afghanistan has criminalized and has been considered punishment for those are:

First; Importing illegal drugs: according to Article 886 of the Penal Code, a person who produces, imports, buys, sells, supplies, demands, transfers and stores non-standard drugs, In addition to forfeiting and destroying the drug, they will be sentenced to medium imprisonment up to three years. (1)When the crime specified in paragraph (1) of this article is committed by a legal person, in addition to the punishment of the perpetrator and forfeiting and destruction of drugs, the perpetrator, depending on the circumstances, will be sentenced to a fine of twenty thousand to two hundred thousand Afghani or his license will be taken. Therefore, if a person commits the material element of the crime knowingly that the drug is non-standard, in addition to destroying the drug, they will be sentenced to medium imprisonment up to three years. However, if a legal person or any company or pharmacy, commits the above-mentioned material element, in addition to the punishment of the person and destruction of the drug, the legal person will also be sentenced to a fine of twenty thousand to two hundred thousand Afghani or their license will be taken.

Second; Abuse of the drug whose date expires: Article 887 of the penal code provides: "A person who produces, maintain, supply, demand, buy, sell or transmit the drug, in addition to forfeiting and destruction of drugs, It is sentenced to twice the price of the forfeiting drug. Whenever the offense in paragraph (1) of this Article is committed by a legal person, in addition to punishing the perpetrator, forfeiting and destruction of the drug, the perpetrator shall be sentenced to four times the price of the drug or his license will be taken. Finally, if the offenses referred to in Article 886 and paragraphs (1 and 2) of this Article, the person's health is harmed, the perpetrator shall also be sentenced to pay the perpetrators in addition to punishing the offense. Therefore, the word person in this article is general and includes all medical staff or ordinary person. That is, if the natural person commits the material element of this crime, in addition to forfeiting and disappearance of the drug, he or she will be sentenced to the cash punishment of two several drug prices. But if the crime is committed by a legal

person such as a pharmaceutical or non-pharmaceutical manufacturing company, in addition to the perpetrator and forfeiting and disappearance of the drug, he or she will be denied a fine of four times as much as the drug or permitted of the license will be taken.

Third; Refusal of a doctor to treat a patient: When a health institution or a medical official refuses to treat a patient while performing his duty, if he/she is causing physical or mental harm, the perpetrator, in addition to compensation, will be punished for the crime specified in this law. However, if the health institution or the relevant medical officer refuses to cooperate with the forensic medical expert, he will be sentenced to a fine of five thousand to fifteen thousand Afghani (Criminal Code, Article 888). The content of this article, contrary to article 887 of this law, only includes natural persons and a legal person who responsible for medical affairs. That is, if these people, knowing that the patient needs treatment, voluntarily refuse to treat the patient or to cooperate with a specialist doctor, the following two situations are relevant; First case: If the issue is physical and mental harm, the perpetrator will be sentenced to the punishment of the crime committed in addition to compensating the damage. But in the second case, when the medical institution or the medical staff refuse to cooperate with the forensic expert, they will be sentenced to a fine of five thousand to fifteen thousand Afghani.

Fourth; Carelessness, imprudence or non-observance of medical regulations by a medical official: whenever a medical official commits such an error (act) in the diagnosis or treatment of a patient as a result of negligence, carelessness or non-observance of medical regulations which causes physical or mental harm to the patient, will be sentenced to the punishment of the crime of error specified in this law (Criminal Code, Article 889). In the interpretation of this article, it should be said that if, as a result of a criminal error, a doctor or a person employed in medical affairs, material or mental harm is caused to a sick person, According to the principle of proportionality of crime and punishment, the mentioned person is required to compensate the damages.

Fifth; Entry, processing and distribution of poisonous or harmful drugs: A person who imports or exports animals or animal products, biological materials or other controllable substances contaminated with infectious organisms or poisonous or harmful drugs, processes, manufactures, buys, sells, maintains, supplies, transfer or provide its bed. He will be sentenced to a fine of 20,000 to 30,000 Afghani (Criminal Code, Article 890). In the interpretation of this article, it should be said that whenever, due to a criminal error, a doctor or a person working in medical affairs causes material or mental harm to a sick person, the said person is required to compensate for the damages in accordance with the principle of proportionality of crime and punishment.

Sixth; Transmission of diseases to others: Article 891 of the Penal Code states: "A person who commits or refuses to commit one of the following acts that leads to the transmission of diseases to others, will be sentenced to short imprisonment".¹ If committing the crimes listed in paragraph (1) of this article causes the death of a person, the perpetrator will be sentenced to long imprisonment. The emphasis of these two clauses is on the real person (Explanation of Penal Code, 2018). That is, if the transmission of the disease is the result of his conscious abstinence, he will be sentenced to short imprisonment. However, if one of the actions leads to the

¹. Failure to establish a tracking system, failure to comply with food hygiene, failure to comply with regulations, procedures and standards, failure to comply with food safety measures, failure to comply with warnings, production, procurement, supply, storage, export, distribution and sale of non-food items Healthy, buying unhealthy food for the purpose of reselling it, publishing deceptive and misleading advertisements and illegal activities.

death of a person, he will be sentenced to long imprisonment. Finally, if a person who produces, supplies, exports and distributes, sells or buys for the purpose of selling HARAM food, he will be sentenced to a maximum of medium imprisonment.

Seventh; Negligence and refusal of duty principles: Whenever a veterinary medicine worker neglects to perform his duty and as a result of this neglect, harm is caused to the animals under treatment, he will be sentenced to a fine of five thousand to ten thousand Afghanis (Criminal Code, Article 892). The meaning of the crime of neglecting the principles of duty is the negligence of the veterinary worker in the performance of his duty, which results in harm to the animals under his treatment (Explanation of Penal Code, 2018). That is, if the animals under treatment are harmed due to the carelessness of the veterinary worker, the person will be sentenced to a fine of five thousand to ten thousand Afghani.

TYPES OF MEDICAL CRIMES WHICH ARE UNCRIMINALIZED IN PENAL CODE

Afghan penal code is silent to recognize some types of medical crimes. The most basic kinds of medical crimes, which unfortunately have not been criminalized by the Afghan Penal Code, are:

First; a false medical certificate: The meaning of medical certificate is a document that is prepared by a doctor about health and illness or something related to medicine (Qazaiee, 1368). One of the major problems in Afghanistan medicine is giving false medical certificate. the most common false certificates is the certificate of a government employee or student to justify their absence. unfortunately, the issuers of such false certificates are not prosecuted. Whiles, if the doctor does not comply with his professional duties in presenting the certificate, such behavior is a crime and can be prosecuted and punished. In other words, if the doctor does not fulfill his legal obligations in presenting the certificate, his action is considered a crime and can be prosecuted. Because the incorrectness of the issued certificate not only destroys the dignity and credibility of the doctor, but also provides the basis for corruption and injustice in the society.

Second; Disclosure of secrets: Secrecy is considered as one of the good and very desirable qualities, which is highly recommended in all religions and moral books. A secret is something that, revealing it, causes harm to a person's reputation and dignity (Jaafari Langroudi, 1368). But medical secrecy refers to all the information that a doctor obtains from his patient. But the Penal Code Afghanistan does not criminalize this issue and has remained silent on this matter. Therefore, the doctor's profession includes the obligations they has made and what he/she sees or hears during the treatment, even if the patient is not aware of those things. So, it is emphasized that the doctor should try to keep all these secrets and should not disclose them. It should be said that not only doctors but also laboratory owners, surgeons, midwives and pharmacists should keep secrets. If they reveal the client's secrets, they commit a crime and can be prosecuted. As a result, the disclosure of secrets by the doctor and those involved in medical affairs is prohibited unless the disclosure is for the benefit of the patient, or the disclosure by the doctor is in the position of self-defense, or the disclosure is in the capacity of an expert, or the disclosure by the doctor is as a witness. Either the disclosure of secrets is due to the announcement of infectious diseases or the disclosure of medical secrets in front of insurance companies (Guderzi, 2010).

Third; Abortion: abortion means is the deliberate ending of a pregnancy at an early stage (the Oxford English Dictionary) or carrying before the normal due date of birth in such a way that it is not alive or able to live (Goldozian, 2014). The types of abortion include normal, medical and criminal. Normal and medical abortion is

considered permissible according to the legal conditions when it is necessary for the health of the mother. That is, the doctor is obliged to take action after consulting at least two other doctors and submit the report to the hospital board within 24 hours (Azizi, 2014). While, criminal abortion is an abortion that does not conform to statutory provisions governing the performance of abortions or dismissal of the pregnancy before the normal time by the mother. For example the mother manipulating the uterus or using drugs or hitting the uterus (Ebadi, 2013). If the doctor intentionally performs an abortion in which the soul has grown, he/she or they will be sentenced to retribution, otherwise he/she will be sentenced to financial punishment and imprisonment.

TYPES OF MEDICAL INFRACTION WHICH ARE UNRECOGNIZED IN PENAL CODE

First; Employment in the medical profession without a legal license: The principle is that the establishment and provision of medical services must be approved by the Ministry of health. For example, if the Ministry of Health determines that there is no need to establish and operate a new maternity hospital in a certain area, it will not issue an establishment permit. Therefore, anyone who tries to establish a medical institution without having an official license, or transfers his license to someone else, or uses another license, or exports and imports medicine without a license, commits a medical infraction and should be prosecuted. Unfortunately, what has become more common in Afghanistan at the moment is the issue of using another license and importing and buying and selling drugs without obtaining a license. I hope this research will open the way for the authorities to prevent these medical infractions in the future.

Second; Unnecessary prescription of the medicine: Without a doubt, buying, selling and using drugs is legally prohibited in the country. The criminal law has imposed severe punishments for the perpetrators.² But sometimes, due to medical necessity, some drugs can be used. In this case, doctors are not allowed to prescribe narcotic drugs (opium) for their patients more than the amount required for three days' use in one prescription, and repeating these prescriptions more than once is not allowed. Therefore, if the doctor prescribes narcotic drugs in more than one prescription and required amount, or if the prescription of narcotic drugs leads to the patient's addiction the doctor commits an infractions and they should be punishment.

Third; Infraction of owners of medical diagnostic laboratories: In general, a medical diagnostic laboratory is a place where microbiology, parasitology, toxicology, biological chemistry and other causes related to that's be checked. Therefore, the first step to establish such laboratories is to have expertise, and then a license must be obtained. Therefore, the first step to establish such laboratories is to have expertise, and then a license must be obtained. infractions that happen in these laboratories are things like selling blood (Ebadi, 1368), issuing a false diagnosis to a patient in exchange for money, using outdated kits, changing some samples related to other patients, etc. Unfortunately, there has been no supervision and inspection of medical necessities by the authorities and those involved in medical affairs, and today we have witnessed many problems in this field.

Fourth; infractions of pharmacy owners: some major medical infractions are committed by pharmaceutical companies and pharmacies. Infractions of pharmacy owners: some major medical infractions are committed by pharmaceutical companies and pharmacies. What can be mentioned as infractions of pharmacy owners in our country today are the issues of employing unqualified people in pharmacies, running pharmacies without the presence of technical officials, selling medicine without a doctor's prescription (news report, 1402), not

². See: penal code of Afghanistan, The fifth chapter, drug section.

including the price of medicine in Doctor's prescription, overselling drugs, prescribing more than the patient needs, selling unconventional and unethical products in pharmacies, selling low-quality and counterfeit drugs, etc.

DISCUSSION

Nowadays, due to medical advances, medical crimes and infractions have also become a serious challenge for lawyers. In countries like Afghanistan, the lack of strict supervision of medical professionals and People's lack of awareness of the law, It has paved the way for more medical crimes, medical infractions and violated the rights of patients. On the one hand, the legislators of the country have not criminalized important medical crimes. For example, the types of abortions have not been discussed in the Penal Code. While, abortion can be classified into normal, medical and criminal. Also, for the medical community, it has not listed the cases of permission to disclose secrets. While, the doctor can reveal the secrets in the following cases. If the interest of the patient demands it, if the doctor defends his position, if the doctor takes the position of an expert, if the doctor acts as a witness, and if the doctor wants to announce infectious diseases. On the other hand, the Penal Code is silent on medical infractions, such as the infractions of pharmacy owners and owners of medical diagnostic laboratories, which are more common in the country today are that: employing unqualified people in pharmacies, running pharmacies without the presence of technical officials, selling medicine without a doctor's prescription, not including the price of medicine in Doctor's prescription, overselling drugs, prescribing more than the patient needs, selling unconventional and unethical products in pharmacies, selling low-quality and counterfeit drugs, selling blood, issuing a false diagnosis to a patient in exchange for money, using outdated kits, changing some samples related to other patients. Therefore, it must be said that the supervision and inspection of medical supplies has not been paid much attention by medical professionals, and today we have witnessed many problems in this field.

CONCLUSION & SUGESSTION

Crime has been defined in different social and humanistic sciences as behavior against order, behavior against public feelings and emotions and behavior incongruent with social conscience and common sense. But medical crime is a type of occupational crime which includes a wide range of illegal activities committed within the medical profession and medical infraction is malpractice, negligence, non-observance of special systems related to the medical profession and other persons related to the medical profession.

The Penal Code of Afghanistan incompletely criminalized Medical Crimes in articles 886 to 892. And Afghan Penal Code is silent about the types of Abortion and the allowed disclosure of secrets. Most importantly, The Penal Code of Afghanistan has not criminalized Medical Infractions. For example the Afghan Penal Code is silent on the discussion about the employment of medical techniques without legal permission, unnecessary prescription of drugs to the patient, deceiving the patient, infractions of the owners of medical diagnostic laboratories and the managers of pharmacies, which are among the types of medical infractions. While today, the people of Afghanistan suffer more from medical infractions matters and the Penal Code should criminalize and recognize that.

Finally, in order to prevent medical infractions and medical crimes, the author suggests the following for those involved in the legal, medical and judicial affairs of the country:

First; The concepts of medical infractions and medical crimes should be defined and separated by the legislators.

Second; Those involved in legal affairs should include the guarantee of appropriate executions for behaviors caused by medical crimes and medical infractions, according to the principles of Islamic Sharia.

Third; guarantee of executions such as retribution, Property damage, compensation for material and moral damage, warning, closure of the institution, exclusion from practicing medicine, etc.

Fourth; The complete law in health and medical affairs should be approved by the government based on Islamic Sharia and principle of legality.

Fifth; Normal abortion, medical abortion and criminal abortion should be approved by the relevant departments according to the characteristics and conditions of each other's in separate regulations and made available to the medical officials.

Sixth; The medical community can disclose secrets only the disclosure is for the benefit of the patient, or the disclosure by the doctor is in the position of self-defense, or the disclosure is in the capacity of an expert, or the disclosure by the doctor is as a witness. In other cases, in order to preserve the patient's dignity, disclosure of secrets is prohibited and considered a crime.

Seventh; The Penal Code is silent on all kinds of medical infractions, the author suggests that in order to prevent injustice and maintain order in the society, engaging in medical techniques without legal permission, prescribing unnecessary drugs for the patient, Failure to provide medical services (defrauding the patient, failure to comply with medical regulations, and infractions by the owners of pharmacies and medical diagnosis laboratories) as medical infractions and considering appropriate criminal or financial responses for each of them.

CONFLICT OF INTEREST:

The authors declared no conflicts of interest.

REFERENCES

1. Abbasi, Mahmoud. (1997). A collection of medical law articles. First Edition. Tehran: *Hayan Publishers*, page 45.
2. Azizi, Fereydoun. (1995). jurisprudence and medicine Third edition. Tehran: *Islamic Culture Publishers*, page 132.
3. Collective Authors. (2010). Description of Penal Code, Volume IV, First Edition. Kabul: *Asia Foundation Publishers*, pages 557- 559.
4. Dekhoda, Ali Akbar. (1991). Dekhoda dictionary. The fourth volume. First Edition. *Tehran: University of Tehran Press*, under the word infraction.
5. Dekhoda, Ali Akbar. (1994). Dekhoda dictionary. The fifth volume, first edition, Tehran: *Tehran University Press*, page 6722.
6. Ebadi, Shirin. (1994). Disciplinary infractions of doctors, *a collection of articles on medical ethics, The fourth volume*. page 265.

7. King, T. ,E. and wheeler, M., B. (2006). Medical Management of Vulnerable and Underserved Patients: principle, Practice and Populations. McGraw-Hill Professional. Available at: <https://accessmedicine.mhmedical.com/book.aspx?bookID=1768>
8. Shahrivar, Farhad. (1998). Medical crimes and violations in Iranian law. A thesis submitted to the Graduate studies office in partial fulfillment of the requirements for the degree of Master of science in criminal law and criminology. *Qom University*. Page 52.
9. Farjad, Mohammad Hossein. (1990). Criminal psychology and sociology. First Edition. Tehran: *Hamrah Publishers*, page 32.
10. Fathi, Mohammad Javad. (2016). Examination of crime and similar concepts in the medical law, *Journal of Medical Ethics and History of Medicine*, published online 1 May 2016. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4958928/>
11. Friedrichs, D. O. (2010). Trusted Criminals: White Collar Crime in Contemporary Society. *Belmont, CA: Wadsworth*. Page 97.
12. Goldouzian, Iraj. (1995). Special criminal law. first volume. Fifth Edition. *Tehran: University Jihad Publishers*, page 174.
13. Gooderzi, Farmarez. (1991). Forensic medicine. First Edition. Tehran: *Einstein Publishers*, page 48.
14. Jafari Langroudi, Mohammad Jaafar. (1989). fourth edition. Tehran: *Ganj Danesh Publishers*, page 356.
15. Merriam Webster Dictionary, online, under the words of crime. Available at: <https://www.merriam-webster.com/dictionary/crime>
16. Moin, Mohammad. (1997). Persian culture. Volume 1, 11th edition. Tehran: *Amir Kabir Publishers*, under the word Takhlaf.
17. News report, Tamadan TV. (2023). News at 8:00 p.m., *Tadaman TV channel*, dated 2/2/1402.
18. Penal code. (2016). official journal. Kabul: *Publishers of the Ministry of Justice*, No. 1260.
19. Qazaiee, Samad. (1989). Forensic medicine. second edition. Tehran: *Tehran University Press*, page 32.
20. Rosoff, S. (2009). Profit without honor. New York: Prentice Hall. Quoted from; medical crimes in the Health Industry Essay: Available at: <https://ivypanada.com/essays/medical-crimes-in-the-health-industry/>
21. Hogan, Sara. (2016). Medical crime: occupational crime at its worst, *sociological imagination. westerns undergraduate sociology student journal*, vol. 5. Abstract. Available at: <https://ir.lib.uwo.ca/si/vol5/iss1/5>
22. Serati Nouri, Nasim. (2017). The criminalization principles of medical crimes. A thesis submitted to the Graduate studies office in partial fulfillment of the requirements for the degree of Master of science in criminal law and criminology. *University of Tehran*, page.45.



The Ethical and Criminal Responsibilities of Medical Doctors: A Perspective from Afghanistan Laws and the International Islamic Charter of Health and Medical Ethics

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ABSTRACT

Medical doctors have the responsibility to observe all medical ethical principles during the treatment of patients to ensure their rights and strengthen trust in the medical sector. Some non-observance of these principles remains in ethical responsibility and some of them bring under criminal responsibility due to their high value. Whereas, if these principles are not observed, the medical sector will face serious challenges. This study discusses the ethical and criminal responsibilities of medical doctors, which are listed in the laws of Afghanistan and the International Islamic Charter of Medical and Health Ethics, and the causes which the thorough observance of these responsibilities has faced challenges. The study aims to inform medical doctors about their ethical and criminal responsibilities and to understand responsible organizations about the causes of the scarce care to it. The study is qualitative in nature and employs an analytical-descriptive research design. Secondary sources such as books, laws, regulations, online journals, scientific articles, and the International Islamic Charter of Medical and Health Ethics were utilized, and any terms requiring definition and clarification were provided after each main paragraph. The finding of this study shows that if medical doctors do not care about those responsibilities which include the laws of Afghanistan, the violator of ethical principles will be disciplined according to the regulation of medical ethics, and those who act against the penal code, will be punished accordingly. Moreover, the consideration of limited action as a crime in the penal code caused scarce care for the complete observance of those responsibilities. In the law of Afghanistan, medical doctors have a series of obligations in addition to their rights, such as respect for the patient, compliance with clinical standards, providing appropriate facilities, accurate diagnosis and treatments of the patient and so on that should be followed.

Keywords: Medical ethics, Criminal responsibility, Forensic prosecution, Penal code, Patient

INTRODUCTION

Medicine is a pure and sacred profession that ensures the health survival and growth of human society. To achieve these goals, it is necessary to maintain the sanctity of this profession. The purest it remains, the better the goals can be achieved. However, the more the sanctity is tarnished, the more difficult it becomes to achieve these goals. The question then arises: How can this profession remain sacred? In answer to this question, it can be said that the observance of medical ethics and the proper discharge of professional responsibilities by doctors can guarantee the sanctity of medicine.

Medical ethics is a systematic set of evaluations of actions in terms of merits, and social responsibility in the field of medicine (Nazari Tavakoli, & Nejdarsari, 2013). Such as respect for human dignity, upholding principles of justice, acting with benevolence (Hamdil Saddiqi, 2010), protecting the secrets of the patient, prescribing appropriate medicines, offering sympathy and reassurance to patients, and actively listening to them, etc. Advocates for the ethical purpose of punishments argue that individuals who violate ethical principles are primarily ethically culpable and responsible for their actions. As a result, they are considered to be both criminally guilty and responsible for their actions (Danish, 2020).

Due to the continuous instability in the country and the weakness of the governments, various institutions of the Afghan society have suffered, and unfortunately one of these institutions is the medical one. To restore this trust and strengthen the country's medical sector, it is most important to make special attention to the observance of medical ethics and responsibilities by medical doctors and institutions. This study was conducted for the better observance of medical ethics and responsibilities that are directed to medical doctors, to be analyzed in detail according to the laws of Afghanistan and the International Islamic Charter of Medical and Health Ethics, and it should also be clear that due to what factors the medical sector in Afghanistan is weak the observance of these responsibilities.

To discuss the main topic it is important to illustrate the rights and obligations of medical doctors for further information and well-known first. It is worth mentioning to know that the term doctor means medical doctors in this study.

1. Rights and obligations of a medical doctor

To clarify the rights and obligations of a medical doctor, first, we explain the rights and obligations, separately below:

1.1. The rights of medical doctors

According to the regulation of medical ethics, the doctor has the right to be respected by the patient and the Caregiver of the patient, Governmental, non-governmental, and private health centers are obliged to take necessary measures for good and balanced nutrition for medical doctors and health workers. Additionally, Governmental, non-governmental, and private health centers are obliged to establish capacity building programs in cooperation with the medical council to ensure the professional competence of medical doctors and health workers (regulation of medical ethics, 2020).

1.2. The obligations of a medical doctor

The medical doctor and health worker are obliged to observe the following points, taking into account the medical professionalism and medical ethics:

Medical doctors must apply the national medical ethics guidelines and should respect the patient and the personality of the patient. According to the principles of medical professionalism, knowledge, and professional skills should be updated and improved, and he must be ingenious (Competence includes having the knowledge, skill, and independence to perform relevant professional services). In his profession, he should observe the professional standards and ethics of medicine, and the Clinical standards must be followed, including infection prevention standards. Creating suitable and acceptable facilities for patients is his other responsibility. Communicating with the patient and their Caregiver should be in accordance with the pillars/elements of medical ethics. Health services should be provided according to the patient's needs. The doctor should diagnose and treat the patient with full accuracy, while providing health services, and Use available resources to provide equal health services to all patients (Medical Ethics Regulation, 2020). The pillars of medical ethics which have mentioned above are the principles of medical ethics that include patient autonomy, beneficence, non-maleficence, and justice.

2. Medical Ethics

Medical ethics is concerned with the obligations of the doctors and the hospital to the patient along with other health professionals and society (Markose et al.2016). Medical ethics deals with those ethical principles which govern the professional conduct of medicine. Medical ethics implies the obligation of the doctor toward the patient as well as some obligations toward another doctor (Bankowski, 1989). Medical ethics is a systematic set of evaluations of actions in terms of merits, and social responsibility in the field of medicine (Nazari Tavakoli, & Nejadsarori, 2013).

2.1. Medical Ethics in the International Islamic Charter of Health and Medical Ethics

According to the Charter, the doctor should observe the following ethical principles:

2.1.1. Listening to the patient's complaints, sympathizing, and not insulting him

It is necessary for the doctor to listen carefully to the complaint of the patient and to show sympathy in reducing his pain and suffering. To treat the patient very well and kindly, and in case of encountering the patient, Use gentleness and compassion (The International Islamic Charter of Health and Medical Ethics, 2005).

It is not permissible to consider oneself better than the patient and look down on him or her, even if the patient's academic level or social status is at any level or is attributed to any relationship. The doctor should pay attention to the opinion of the patient, especially to his personal affairs (The International Islamic Charter of Health and Medical Ethics, 2005).

2.1.2. Ensuring justice

The doctor must pay attention to justice in his behavior and attitude toward the patients and be bound to it. Avoid injustice in medical care due to personal, religious, sexual, and national tendencies and feelings (The International Islamic Charter of Health and Medical Ethics, 2005).

2.1.3. The presence of a *Mahram* or third person During a patient`s check-up

The doctor should fear Allah for the sake of the patients. At the time of check-up, diagnosis, and treatment, consider the belief, religion, and customs of the patient, and insist that do not commit an illegal act (such as being alone with a *non-mahram* (With Whom Marriage is admissible) person, or inspecting the private parts of the patient more than necessary) and this will also in the presence of a third person after the permission of the patient in case of Sharia necessity (The International Islamic Charter of Health and Medical Ethics, 2005).

2.1.4. Prohibition of additional tests or medications

The doctor is obliged to carry out the tests that confirm the patient's medical condition and refrain from additional tests. The whole process of diagnosis and treatment of the patient should be carried out transparently, avoiding any unreliable, unusual, or informal treatment or diagnosis method. Also, he is obliged to look at the condition of the patient when prescribing medicine and performing surgical operations and do it accordingly, that is, prescribing medicine and performing surgical operations without the need is against medical ethics (The International Islamic Charter of Health and Medical Ethics, 2005).

The doctor is obliged to give an honest explanation to the patient or the person acting on behalf of the patient about the type of the disease, its causes, its complications, and the benefits of the diagnostic tests and treatment. And considering the physical and mental condition of the patients, to give them a full understanding of the suitable alternatives for diagnosis and treatment in a suitable, transparent, and clear way. Doctors should prescribe medicines, materials, and medical equipment according to medical professional principles and according to the needs of the patient. He should not come under the pressure of any person during treatment, and also he should not accept gifts from any authority (The International Islamic Charter of Health and Medical Ethics, 2005).

2.1.5. Referring the patient to another specialist doctor

The doctor is obliged not to delay or reduce anything in sending the patient to another doctor who is a specialist in this disease or well-equipped, active, and has good and advanced equipment and facilities (The International Islamic Charter of Health and Medical Ethics, 2005).

2.1.6. Not refusing to treat the patient

It is not permissible for a doctor to refuse to treat a patient unless the condition of the patient is beyond the scope of the competence of the doctor. In emergency and emergency situations, the doctor is obliged to continue treating the patient until the patient's needs are met, or until he/she comes under the care of another doctor (The International Islamic Charter of Health and Medical Ethics, 2005).

The doctor is obliged to reduce the pain and suffering of the patient with the possible therapeutic, material, and spiritual means that he has in his power, and to make the patient understand that the doctor is interested in his health, and he is also obliged to use his skills to comfort the patient and use your skills to reduce the disease (The International Islamic Charter of Health and Medical Ethics, 2005).

The doctor is obliged to inform the patient about the disease in particular and about health in general, and also to give information about hygiene and preventive measures desirably and efficiently (The International Islamic Charter of Health and Medical Ethics, 2005).

2.1.7. Obtaining the patient's consent for treatment

Treatment without the patient's consent is not admissible, unless the condition of the patient requires medical intervention and it is not possible to obtain the patient's consent, or if the patient's illness threatens the health of other people, or to pollute others. Of course, the intervention should take place according to Islamic laws. In the case of full legal capacity of the patient, consent is obtained based on the express or implied consent of the patient or the consent of the person who legally represents the patient, if the patient is incapacitated, deaf, or has any disability. Or if any condition of legal capacity is less, the agreement should be written, and in clear form and be introduced to operations and surgical interventions (The International Islamic Charter of Health and Medical Ethics, 2005).

During a check-up, it is the duty of the doctor to accurately document and record the patient's health status, disease history, disease type, and relevant family history. The medical examination should be conducted with precision and correctness, allowing for an appropriate amount of time to address the patient's needs. Prescriptions must be written clearly, specifying the dosage and form of medication. In critical situations, doctors should provide important and anticipated information regarding the potential side effects of surgical procedures and medical treatments to the patient or their relatives. These obligations align with the principles outlined in medical ethics, emphasizing the importance of thorough documentation, precise examinations, clear prescriptions, and effective communication of information to ensure patient well-being (The International Islamic Charter of Health and Medical Ethics, 2005).

In the first paragraph, the word full legal capacity means to reach the age of adulthood and mentally being fit, according to Article 39 of the Civil Law of Afghanistan 1977/1355, the age of maturity is eighteen solar years. He is considered to be fully qualified to exercise his rights

2.1.8. Informing about the consequences of not taking treatment

If the patient does not want to be treated, the doctor is obliged to inform him of the consequences of this action without exaggeration. At the same time, the doctor is obliged to record the patient's words and get his profession, that he does not want to be treated. A doctor and a nurse should be part of the delegation (The International Islamic Charter of Health and Medical Ethics, 2005).

2.1.9. Not ending the patient's life

It is not permissible for the doctor to end the patient's life, even if it is for the sake of compassion and mercy, but to advise him to be patient. Especially in Intentional murder, where a person wants to end his life by his will, Doctor-assisted suicide, and deliberate killing of a newborn child who is born with a disability, whether this disability is life-threatening or not (The International Islamic Charter of Health and Medical Ethics, 2005):.

2.1.10. Keeping Secret the illness patient

Whenever the doctor discovers the secret of the patient, he is not allowed to reveal it, but he can in the following cases:

- A: Based on the written request of the patient or if it is in the interest of the patient to disclose this secret.
- B: When the Sharia requires disclosure or the court orders it.
- C: If the secret has been disclosed to avoid harm from the wife or husband, on the condition that both of them will be present.
- D: When the secret is revealed to prevent the spread of diseases.

Doctors and other health workers must try as much as possible to protect the confidentiality of the medical information recorded in the file, including the information recorded on the computer. Regarding the protection and confidentiality of the patient's secrets, the bill of professional workers of mental health clinics has also been emphasized in article 8, paragraph 2, and the mental health manual too.

2.1.11. No false advertising and propaganda

It is not permissible for a doctor to present misleading and distorted information in health advertisements or the media, hide the side effects of drugs or treatment, or present issues that are against public ethics to the public (Islamic Charter of Medical and health ethics, 2005). For example, a doctor who has gone to a foreign country for a seminar, should not write on the board or card that higher education in the foreign country.

The doctor should also refrain from the following actions (Islamic Charter of Medical and health ethics, 2005):

A: Using illegal means to attract patients.

B: Using his/her name to advertise the sale of medicines and other medical materials for commercial purposes.

C: Demanding or accepting a fee for sending a patient to another clinic or institution (including a pharmacy, laboratory, or a center for selling medical equipment and accessories) or for prescribing any medicine, and more.

2.1.12. Conditions for sending the patient to another health institution

If the doctor sends the patient to any other health institution where the doctor is a partner, is obliged to consider the following points (The International Islamic Charter of Health and Medical Ethics, 2005):

A: This organization should provide excellent services, which are not less than others in terms of quality and type.

B: The patient should be sent when there is a need for him and he has better chances of treatment compared to the first doctor.

2.2. Medical Ethics from the Perspective of the Laws of Afghanistan

Medical doctors are not allowed to provide health services contrary to established principles and standards, perform medical procedures in abnormal environments, or exhibit prejudice or discrimination based on linguistic, sexual, ethnic, religious, social, political, or cultural factors. Additionally, they must not abuse their functional and professional authority or engage in excessive physical, verbal, or non-verbal closeness with patients beyond what is professionally necessary. The use of outdated diagnostic and treatment methods is discouraged, as is prescribing tests and medicine that patients do not require. Negligence and carelessness in medical practice are strictly prohibited, as is the cessation of health services for patients in need. Doctors must avoid causing harm to patients in physical, psychological, economic, or social realms, and they should always ensure proper diagnosis is made through available means. Inappropriate and abusive behavior towards patients and caregivers is deemed unacceptable, as is using diagnostic tools beyond the level of knowledge of medical professionals and health workers. By adhering to these regulations, medical doctors aim to prioritize patient well-being and maintain the highest standards of ethical care (Medical Ethics Regulations, 2020).

If we look here, a series of actions are mentioned that are against ethical principles, and the medical doctor should keep himself from it. However some of the important ethical principles which our society has been in trouble with it, and the International Islamic Charter of Medical and Health Ethics has also mentioned them, they have not been mentioned in the regulation of medical ethics. Such as, Except for the emergency need, there should be a *Mahram* present with the patient during the checkup, also the doctor should show his sympathy for the patient and not insult him in any way.

In addition, if a doctor does not have enough knowledge about the illness of the patient, he should refer him to another related specialist, moreover, the doctor has not been obliged to write the condition of the patient, history of the disease, recommended tests and other necessary information in the prescription. Apart from this, it does not exist in terms of ethical principles that the doctor should not advertise falsely to deceive the public. Many doctors who have gone abroad for a seminar have written on their billboards, that (higher education in London, India and so on).

In public hospitals, the patient should not be invited to the private clinic by doctors. In Afghanistan, a problem that the author has faced many times is that when a patient undergoes an operation or other treatment in a government hospital, the doctor requests that the patient should come to his private clinic for the rest treatment.

If the patient does not take it serious and comes back to the hospital, He does not pay attention to him, from the sincerity of his heart.

3. Disciplinary proceedings

According to the regulation of medical ethics, in case of violation of the provisions of this regulation, the doctor is disciplined according to the circumstances by recommendation, written notice, a cash fine of five thousand (5,000) to fifty thousand (50,000) Afghani depending on the circumstances, and cancellation of the activity license (regulation of medical ethics, 2020).

4. Criminal responsibilities

A medical doctor is subject to forensic prosecution after an investigation by the Medical Council in the following situations (Medical Ethics Regulation, 2020). Forensic prosecution is the pursuit of a criminal incident and its perpetrator, which includes the discovery process and the investigation of the crime, trial, and execution of the order (Criminal Procedures Law, 2013). The whole process until the implementation is called forensic prosecution.

Firstly, In the case of medical and duty negligence, indifference, imprudence, non-observance of medical regulations, or refusal of treatment due to which physical or mental harm is inflicted on the patient.

To clarify this issue, we must define and clarify the above terms:

Medical Negligence: Medical negligence is the non-execution of assigned duties or inaccurate manner of execution or non-assigned (non-assigned) action by a medical doctor or health worker.

For example, if a doctor is on duty in a hospital at night, a patient comes during his shift and he does not treat him, as a result of which the patient's suffering increases or he dies, the doctor is criminally responsible.

Imprudence: imprudence is when a person acts without paying attention to the consequences of that action, which is predictable.

For example, the doctor does not know for himself whether the patient is allergic to the said medicine or not, prescribes medicine to the patient, due to which the patient's condition deteriorates or dies, in this case, the doctor is criminally responsible (penal Code, 2017).

Negligence and indifference: It is said that an action that should be done is not done due to inattention or lack of care (penal Code, 2017), such as the doctor should sterilize the operating room, but he does not sterilize it. He performs the operation and traumatizes the patient. In this case, based on the Penal Code of 2017, the medical officer, if due to carelessness, imprudence, or non-observance of medical regulations, makes such a mistake in the diagnosis or treatment of the patient's disease that if the patient suffers physical or psychological harm because of that, he will be sentenced to the punishment of the crime of unintentional (penal Code, 2017).

Secondly, if he/she has violated the standards of medical ethics and caused the death or disability of the patient.

Thirdly, If a person does not follow the prescribed procedures and standards and..., in the case of spreading diseases to other people, he will be sentenced to a short imprisonment (from 3 months to 1 year) and if it ends in his death, he will be sentenced to a long imprisonment (more than 5 years to 16 years) (penal Code, 2017).

Finally, also, if a doctor aborts a woman's fetus (a young human before it is born, especially a human more than eight weeks after fertilization (Oxford advanced learner's Dictionary, 2010) In the dictionary, the same embryo can be hidden or covered, it is called the sperm hidden in the mother's womb is called (Ibn Manzoor, 1955) and it has the same meaning in the term (Durani, 2017). Abortion of the fetus before the term of birth in such a way

that the fetus is not alive, or it is not possible to continue its life (Wasil, 2018.), even if the woman consents to it, he will be sentenced to medium imprisonment for more than two years (penal Code, 2017). But if the doctor aborts the fetus with medicine for rescuing the mother's life, in this case, the doctor is not punished (penal Code, 2017). Before the doctor comes under legal prosecution, the Medical Council should investigate this area (Medical Ethics Regulation, 2020).

CONCLUSION

In the law of Afghanistan, medical doctors have a series of obligations in addition to their rights, such as respect for the patient and his/her Caregiver, compliance with clinical standards, providing appropriate facilities for patients, accurate diagnosis and treatments of the patient and so on that should be followed. The medical ethics that are listed in the medical ethics regulations, such as providing standard health services in a standard environment, avoiding any kind of prejudice and discrimination, not getting too close to the patient beyond professional need, not prescribing additional drugs and tests to the patient, not treating the patient inappropriately, and not performing medical negligence.

However, some important medical ethics are not listed in the medical ethics regulation, especially those mentioned in the International Islamic Charter of Medical and Health Ethics. It should also be included, such as checking the patient in the presence of a mahram, introducing the patient to another specialist doctor related to the condition of the patient, not avoiding the treatment of the patient, informing the patient of the consequences of not treating the patient, not to advertise falsely and so on.

The main reason for the lack of attention to medical ethics is that only a few and limited medical violations have been brought punishable in the criminal code. Many important ethical principles are not observed seriously, because it does not cause the criminal responsibility of a doctor. Such as not prescribing additional drugs and tests, not treating the patient against the profession, etc. Introducing the expert, not making false advertisements and some other cases do not exist in the penal code of the country.

RECOMMENDATIONS

1. To know the status of observance and non-observance of ethical responsibilities of medical doctors in society, it is necessary to work on field research to determine the level of attention to ethical responsibilities in the medical sector.
2. The government should launch public awareness programs to make doctors aware of medical ethical and criminal responsibilities.
3. The government should include the necessary ethical principles in the penal law and consider them punishable, which the non-observance of them harms the trust and growth of the medical sector.

CONFLICT OF INTEREST

All authors express no conflict of interest in any part of the research, manuscript, and submission to the journal.

REFERENCES

1. Bankowski, Z. (1989). Ethics and health. *World Health* 1989; April: 2-6.
2. *Da aami roghtia qanoon* [Public Health Act]. (2009). Retrieved from <http://laws.moj.gov.af>.
3. *Da jazaee ijraato qanoon* [Criminal Procedure Law]. (2014). Retrieved from <http://laws.moj.gov.af>.

4. Danish, H. (2020). Penology (3rd ed.). Jalalabad, Afghanistan: Muslim Publisher.
5. Hamdil Saddiqi, A. (2010). Medical Ethics (2nd ed.). [E-book]. Retrieved from <http://balkh-un.edu.af>.
6. Ibn Manzūr, M. (1955). Lisān al-‘Arab [Arabes tongue]. Beirut House
7. *Jaza code* [Penal Code]. (2017). Retrieved from <http://laws.moj.gov.af>.
8. *Madani qanoon* [Civil Law]. (1977/1355). Retrieved from <http://laws.moj.gov.af>.
9. Markose, A., Krishnan, R., & Ramesh, M. (2016). Medical ethics. Journal of pharmacy & bioallied sciences, 8(Suppl 1), S1–S4. <https://doi.org/10.4103/0975-7406.191934>
10. Medical Ethics Regulation. (2020). Retrieved from <http://laws.moj.gov.af>
11. Nazari Tavakoli, & NejadSarori. (2013). *Cheesti akhlaq pazeshki; bayedha wa na bayedha* [What is medical ethics; Dos and do nots]. Ethics and medical history of Iran, 6(5), 1-11.
12. *Rahnamood-e-cehat-e-rawani* [Guide of Mental Health]. (____). Ministry of Public Health.
13. *Rahnamood-e-mille wa miyari barie marakiz khedamat cehi awalya* [National Guide of Standard Therapy for Centers of early health services]. (1392). Ministry of Public Health
14. Asia Foundation. (2019). *Sharha-e-code jaza* [Explanation of Pinal Code]. Kabul, Afghanistan: Saeed press.
15. Turnbull, J., Lea, D., Parkinson, D., Phillips, P., Francis, B., Webb, S., ... & Ashby, M. (2010). Oxford advanced learner’s Dictionary. International Student’s Edition.
16. Wasil, A. (2019). Allocated Criminal Law. Jalalabad, Afghanistan: New Moustaqble Publisher.
17. Wasiq Durani, A. (2017). Allocated Criminal Law (3rded.). Jalalabad, Afghanistan: Yaar Publisher.
18. World Health Organization. (2005). Islamic Charter of Medical and health ethics (No. EM/RC52/R. 10).

Family Status and its Impacts on Livestock Management in Three Districts of Balkh Province

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ABSTRACT

Background: Balkh is one of the strategic and high populated provinces in the north region of Afghanistan that has 14 districts which occupied 2.5% of the total land area of Afghanistan. Livestock is a dominant sector in the province; however, there is lack of knowledge and information in this regards.

Materials and Methods: Primary data were collected from 44 households. The survey contained the questionnaires on family structure including the number of male, female and children per household and education status as well as the type and number of animals, animal diseases, quantity of milk production, source of income and water availability.

Findings: Average number of the family member was higher in the Aziz Abad (11.2 persons), while Langar Khana had the lower family number (7 persons). Most of the children were educated in Poshti Bagh (85.1%) but only 27.3% of the children were educated in Dihdadi. There was none of the father and mother educated in Dihdadi. The most populated district was Poshti Bagh with 1400 family residence followed by Haji Kot (400) and Aziz Abad (320), respectively. The highest average number of dairy cattle per family and sheep per family belongs to Aziz Abad. Average number of layer chicken per family was 13.2, 7.5 and 2 in Dihdadi, Balkh and Nahri Shahi districts, respectively. Poshti Bagh had the highest milk production rate (9.8 kg/day) as compared to other villages. Foot and mouth diseases in cows, peste des petits ruminants (ovine rinderpest), parasite in sheep, and Newcastle in chicken are the most common diseases of livestock in the province. Source of drinking water in Poshti Bagh was deep well (20%) and river (80%), while people in Aziz Abad and Haji Kot used hand pumps.

Conclusion: In Balkh province, livestock provides great opportunities to the upsurge of income and employment. The most of the dairy production comes from small-scale farms. To support the sustainability of small farms and livelihood in the villages, the supporters (NGOs or government) should focus more on families especially women skills related to livestock sector.

Key words: Livestock; Family situation; Dairy production; Balkh province

INTRODUCTION

Balkh province is one of the high populated and strategic provinces in the north region of Afghanistan that has 14 districts including Balkh, Char Bolak, Char Kint, Chimtal, Dawlat Abad, Dihdadi, Kaldar, Khulm, Kishindih,

Marmul, Nahri Shahi, Sholgara, Shortepa, and Zari as shown in (Figure 1) (Grace, 2004). This province covered 16,186.3 km² areas which is 2.5% of the total land area of Afghanistan (CSOA, 2015). Balkh province has border with three countries including Turkmenistan, Uzbekistan, and Tajikistan (Fouache et al., 2012). The capital of Balkh is Mazar-e-sharif city which is located at 36^o 42' N and 67^o 12' E (CIA, 2015). Agriculture and livestock are the main employment for the people of the province. Balkh province has temperature greater than 30 °C and seven continuous months with no or negligible precipitation. During these months, livestock faced with heat stress along with other stressed factors.

As the other part of the country, agricultural activities are important sector in this province. Livestock production system is integrated to agricultural activities. Balkh province is one of the important centers for Karakul production (a special breed of sheep) in Afghanistan (West, 2003). Karakul is the symbol of Balkh province (Fortmann, 2009). In addition, dairy productions are important for the local diet either in winter and summer. The most common homemade dairy products are Ghee (clarified butter oil), buttermilk, curd, Qurut (dried curd), cheese and yogurt that generally prepared and supplied to the markets by women (Fouache et al., 2012). Women play a key role in animal husbandry in Afghanistan especially in Balkh province (Zafar, 2005).

After 2001, most of NGOs started their activities to reestablish the agriculture sector and to improve the dairy industry including some projects implemented by United State Agency for International Development (USAID). In addition, The Dairy Industry Revitalization Project for Afghanistan (DIRPA) was a project that focused on milk production, dairy processing and marketing of dairy products during 2004-2007. This project collaborated with Balkh Livestock Development Association (BLDA) in Mazar-e-Sharif to improve milk hygiene, production and balance rations, animal husbandry and veterinary services (MAIL, 2005; Rlung, 2008; Siddiky, 2017). In June 2007, with the efforts of Food and Agriculture Organization (FAO), Ministry of Agriculture, Irrigation and Livestock (MAIL) and dairy farmers of Balkh province, the Balkh Livestock Development Union (BLDU) was established. Later on, USAID builds and equipped the Balkh Dairy Plant in Shir Abad village of Dihdadi district by collaborating of BLDU and FAO. This dairy plant had the capacity to process 5,000 liters of milk per day and covered 450 farmers.

Traditionally, women care about one or two cows, other dairy animals and a number of chickens on a family farm (IDEA-NEW, 2012), but their participation in the farm practices is differed by some factors such as age, wealth and marital status (Grace, 2004). Generally, there is lack of information regarding family status involved in the livestock sector in the province as well as the production capacity and management of livestock in Balkh province. Thus, this study aimed to survey collect primary and secondary data regarding the family status and livestock production capacity in three districts of Balkh province.

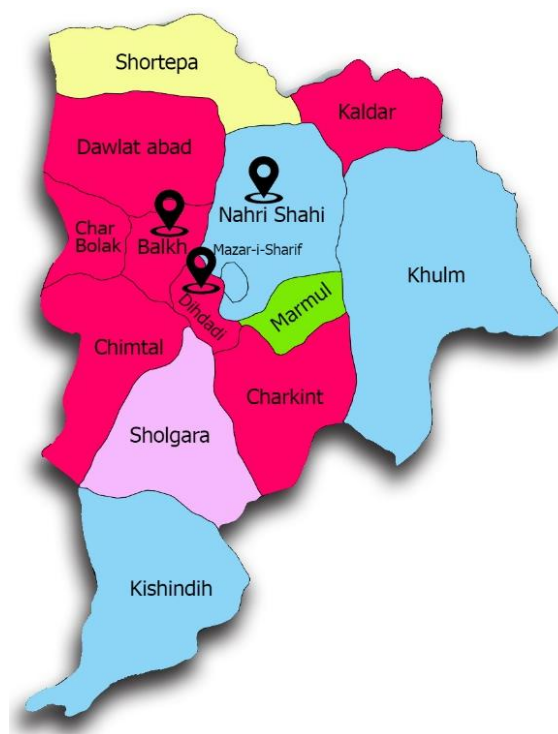


Figure 1. Map of Balkh province, Northern Afghanistan (the figure has been created by using Microsoft Office PowerPoint 2013 v.15.0.4.1). Sources: (<https://paintmaps.com/blank-maps/85c/samples>)

MATERIALS AND METHODS

In 2015, a questionnaire based survey was conducted in five villages from three districts of Balkh province, including Balkh, Dihdadi, and Nahri Shahi. The survey contains 44 households from Aziz Abad, Haji Kot villages in Balkh district, Poshti Bagh (Oliya and Sofla) and Dihdadi villages in Dihdadi district and Langar Khana village in Nahri Shahi district. The questionnaire of the survey contains the questions regarding the family structure, the number of male, female and children per household, education status, types and number of animals, quantity of milk production, the source of income, and the availability of drinking water. All the interviewed responded in this survey were women. Most of the female were not aware of the economy status of the family. Some secondary data were collected from the Ministry of Agriculture, Irrigation and Livestock (MAIL), Division of Animal Husbandry of Balkh province (DAHb) and Food and Agriculture Organization (FAO) based on their reports from (2005 - 2012).

RESULTS

Family Structure, Residency and Source of Income

Family status and educational background of the residence of the three districts in Balkh province are presented in the Table 1. Balkh district had the highest number of family member followed by Dihdadi and Nahri Shahi. Average number of the family member was higher in the Aziz Abad (11.2) and Haji Kot (8.7) villages of Balkh district than other villages, while Langar Khana of Nahri Shahi was the lowest (7). Generally, the females were

higher in all districts than males. Average number of female was high in Aziz Abad (5) while it was low in Langar Khana (3.7). Most of the children were educated in Poshti Bagh (85.1%) village of Dihdadi district followed by Balkh (Aziz Abad, 71.7% and Haji Kot, 78.8%) and Nahri Shahi (42.9%) but only 27.3% of the children were educated in Dihdadi village of Dihdadi district. Higher educated father and mother were recorded in Haji Kot followed by Nahri Shahi. However, none of the fathers were educated in Aziz abad and Dihdadi village, and none of the mothers were educated in Dihdadi district.

Population and Sources of Income and Drinking Water

Population rate, source of income and drinking water are illustrated in the Table 2. Based on the available data, the oldest village is Haji Kot followed by Poshti Bagh and Aziz Abad. The most populated district is Poshti Bagh with 1400 family residence with 8400 population followed by Haji Kot and Aziz Abad with 400 and 320 family residence and 2700 and 1920 population, respectively, as shown in the Table 2. Most of the people are labor and they as well have their small business shops which are the income source of them. Majority of the villagers are raising animals doing farm practices and agricultural activities. There are three sources of drinking water in all the surveyed districts which are hand pump, deep wells and rivers. Source of drinking water in Dihdadi was deep well (20%) and river (80%), while people in Balkh district used hand pump for drinking water. However, people in Nahri Shahi use hand pump and river for drinking water (Table 2).

Livestock Status and their Products

Table 3 shows the status of livestock management and dairy production in the surveyed districts. The highest number of dairy cattle per family belongs to Aziz Abad (2.7) while the lowest is recorded in Haji Kot which is 0.9. In addition, Aziz Abad had the highest number of sheep per family (13.7) followed by Dihdadi (10.1), Nahri Shahi (8.6), Poshti Bagh (5.1) and Haji Kot (3). Goat management is another asset of livestock with higher number in Aziz Abad (6.4) and the lower number in Haji Kot (1.7). The highest number of layer chicken per family was in Dihdadi followed by Balkh and Nahri Shahi districts, respectively. The highest milk production per day is observed in Poshti Bagh (9.8 kg/day), while the lower milk production is in Langar Khana of Nahri Shahi (4.5 kg/day) as shown in Table 3. One of the important by-products of cow is the dung production which uses for soil fertilization, fuel and so on. The highest dung production is recorded in Dihdadi (11 kg/day) followed by Balkh and Nahri Shahi with the lowest rate in Haji Kot (8.3 kg/day).

Table 1. Family structure in five villages of Balkh province including average number of male, female, children, son and daughter per family.

Districts	Villages	FM	Male	Female	Children	Son	Daughter	EC at age of school (%)	NEC at age of school (%)	EF (%)	EM (%)
Balkh	Aziz Abad	11.2	3.7	5	6.3	2.3	3	71.7	28.3	0	8.3
	Haji Kot	8.7	4	4.7	5.9	3.1	2.8	78.8	21.1	20	30
Dihdadi	Poshti Bagh (Oliya & Sofla)	8.3	4	4.3	6.3	3.2	3.1	85.1	14.9	11.1	0
	Dihdadi	7.7	4	3.7	3.7	2	1.7	27.3	72.7	0	0
Nahri Shahi	Langar Khana	7	3.4	3.6	4.8	2.5	2.3	42.9	57.1	9	9

FM: family members, EC, educated children, NEC: not educated children, EF: educated father, EM: educated mother

Table 2. Establishment year, population, family residence, source of income and drinking water in different districts of Balkh province

Location		Established year and population		Family residence		Source of income				Source of drinking water
Districts	Villages	Established (year)	Population (person)	Beginning of establish	2015	Labor	Small family business (shop)	Raising animal	Farming	
Balkh	Aziz Abad	1941	1920	30	320	Yes	Yes	Yes	Yes	Hand pump
	Haji Kot	1851	2700	15	400	Yes	Yes	Yes	Yes	Hand pump
Dihdadi	Poshti Bagh (Oliya & Sofla)	1881	8400	30	1400	Yes	Yes	Yes	Yes	Deep well (20%), River (80%)
	Dihdadi	NR	NR	NR	NR	NR	NR	Yes	Yes	Hand pump and river
Nahri Shahi	Langar Khana	NR	NR	NR	NR	NR	NR	Yes	Yes	Hand pump and river

NR: no record

Table 3. Average number of dairy cattle, sheep, goat and layer chicken as well as milk and dung production per family in different districts of Balkh province.

Districts	Villages	Dairy cattle	Sheep	Goat	Layer chicken	Milk production (kg/day)	Dung production (kg/day)
Balkh	Aziz Abad	2.7	13.7	6.4	1.5	7.1	10
	Haji Kot	0.9	3	1.7	6	9	8.3
Dihdadi	Poshti Bagh (Oliya & Sofla)	1.9	5.1	3.2	6.2	9.8	9.6
	Dihdadi	2	10.1	2	7	5.5	11
Nahri Shahi	Langar Khana	2.3	8.6	2.7	2	4.5	8.8

Common Animal Diseases

There are several species of animals including cattle, sheep, goat and poultry which are managed in small farms in the Balkh province. The diseases such as foot and mouth disease (FMD) in cows, Peste des petits ruminants (PPR) and parasite in sheep, and Newcastle in chicken are the most common diseases as shown in the Table 4. In addition, huge number of sheep and goat are affected and died due to the harsh weather. Based on the report of MAIL in 2012, a huge number (2,500) cattle were affected by FMD, of them 500 cattle were died in Balkh province. Furthermore, 2,000 sheep were infected by PPR, of them 300 sheep were died at the same year. In total approximately 14,500 animals were suffered from the harsh weather in 2012. The harsh weather killed about 1,000 sheep and 500 goats only in Balkh province. Another common disease was Newcastle in chicken that infected a huge number of poultry farms, of them about 3,000 chickens were died due to Newcastle in 2012.

Table 4. Animal species, diseases as well as number of infected and died animals in Balkh province.

Species	Type of disease	Number infected/affected	Number died
Cattle	FMD	2,500	500
Sheep	PPR	2,000	300
Sheep	Harsh weather	10,000	1,000
Goat	Harsh weather	4,500	500
Poultry	Newcastle	NR	3,000

FMD = Foot and Mouth Disease, PPR = Peste des petits ruminants, NR: not record.

DISCUSSION

The majority of household in Afghanistan, particularly in Balkh province relay upon agriculture and livestock practices (Gang, 2010). Their source of income is also from agricultural and livestock farming productions. The present study demonstrated that majority of family elder ages are uneducated, however, the new and younger generation are educated. Also, the number of female is more than the male in all districts. Thus, females take care of small scale farms in the province. In some societies, women are increasingly involved in managing the small enclosures reserved for milking cows and young calves (Wangui 2014). It shows that traditional livestock practices will be replace with modern farming management in the coming decades.

The research on the status of livestock in three districts of Balkh province was demonstrated that most of the farmers struggle with insecure life due to unstable livestock and agriculture products and the problem doubled by rapid increase of annual population about 2% and urbanization (Zafar, 2005). As livestock and agriculture activity is the only source of income for most of the families, thus the traditional status of this sector have a strong impact in different aspects of farmer's life.

The study reported that several kinds of livestock are managed in Balkh province, of them the common are cattle, sheep, goat and poultry. In addition, milk and dung production are the main output of these livestock

in three districts. These productions are mostly related with the quality of feeding, management practices, environmental stress and diseases (Rojas-Downing et al., 2017). The quality of feeding is one of the greater issues in the case of animal rising in Balkh province which comes from the shortage of feed supply. Erskine et al. (2011) reported protein deficiency of animals in Afghanistan and indicated that in north, people using feeding management and supply the cotton seeds as a dietary supplement to limit the extend of protein deficiency in animals but the availability of that supplement is not sure in all villages. This survey presents that due to harsh weather, inappropriate feeding and breeding management, there is low milk productivity in dairy cattle. The most of the dairy cows are local breed that adapted to the environment but they have low milk production. During last decade, artificial insemination become available for the farmers to improve their cows breed but it was not widely spread in all villages within province because of their distance from the city.

Environment and climate conditions are one of the effective factors in raising animals in Balkh province. The maximum temperature ($>30^{\circ}\text{C}$) is recorded for seven months of a year in Balkh province with five months without rainfall (Palka, 2001; Pineda et al., 2016) or a negligible precipitation which provide a special condition for animal to face with severe or moderate heat stress. Heat stress has detrimental change on feed intake, production, metabolic and reproduction of dairy cows (West, 2003). A study indicated that the temperature reaches 21 to 36°C , the milk yield starts to decline (Bernabucci et al., 2014) and a negative correlation between raising the temperature and dry mater intake (DMI) was confirmed in dairy cows (West, 2003). Consequently, the milk yield efficiency, protein and fat reduce by the decrease of DMI (Fouache et al., 2012). Therefore it is a significant financial burden and low quality dairy production.

Animal diseases are one of the influencing factors studied in livestock situation in Balkh province, which mainly causes economic defects to the farmers. In recent years, there were several diseases that become the causes of mortality in livestock due to the uncontrolled import of animals from neighboring countries due to the mismanagement of quarantine system and disease control. On the other hand, veterinary clinics are not available in each village as well as the medicines are expensive for farmers to purchase due to their low income. Cattle, sheep and goat mortality were 3.8%, 7.4%, and 5.4%, respectively, in the covered districts. According to (MAIL, 2012) report, most common diseases for cows, sheep and poultry were FMD, PPR, and Newcastle, respectively, in Balkh province. The report showed that animals in the north of Afghanistan face with several stress including environmental stress, mismanagement on housing low quality of feeds, various diseases, water availability and unmodified genetic breed. As the result of these stresses, the farmers face with high mortality of animals and low efficiency on animal's production that is similar to (Silanikove, 2000) findings. The current management for preventing diseases which is implemented by the veterinary program handles by MAIL Mazar and Dutch (non-government organization) to manage disease control is not effective. Using appropriate management system on housing, feed and water supply system, disease control and genetic modification will help to deal with numerous stresses on dairy cattle (Karimi et al.,

2015). Also, an appropriate stress management could be reducing the level of oxidative stress which correlated with improving the antioxidant level by feeding the biological substance to the animals.

CONCLUSION

In Balkh province, livestock provides great opportunities to the upsurge of income and employment. The most of the dairy production comes from small-scale farms. To support the small farm sustainability in the villages, the supporters (NGOs or government) should have more focus on women skill training on the subjects of animal husbandry, feeding management and hygiene. By considering to a sensitive cultural factor, it is important to train few female of each village extensively and target them to train other villagers. On the other hand, improved livestock practices, genetic improvement and advanced quarantine system can help farmers to well manage the current animals and achieved resistance breeds to heat and diseases, consequently increase dairy production and national economy.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

1. Bernabucci, U., Biffani, S., Buggiotti, L., Vitali, A., Lacetera, N., & Nardone, A. (2014). The effects of heat stress in Italian Holstein dairy cattle. *Journal of dairy science*, 97(1), 471-486.
2. Central Intelligence Agency (CIA), United State of America, (2015). PP: 45-56.
3. Central Statistic Organization of Afghanistan. (2015). Demographics report. PP. 154-162
4. Erskine, W., Sarker, A., & Kumar, S. (2011). Crops that feed the world 3. Investing in lentil improvement toward a food secure world. *Food Security*, 3, 127-139.
5. Food and Agriculture Organization (FAO). (2007). First draft country report on the status and perspectives of the animal genetic resources development and conservation in Islamic Republic of Afghanistan, Kabul, Afghanistan. PP: 98-112.
6. Food and Agriculture Organization (FAO). (2008). National livestock census 2002-2003, Rome, Italy. ISBN 978-92-5-105950-0. PP: 37-49.
7. Fortmann, L. (2009). Gender in Afghanistan Sourcebook. The World Bank, Food and Agricultural Organization, and International Fund for Agricultural Development. Washington DC: The World Bank ISBN 978-0-8213-7587-7. *Experimental Agriculture*, 45(4), 515-515.
8. Fouache, E., Besenval, R., Cosandey, C., Coussot, C., Ghilardi, M., Huot, S., & Lamothe, M. (2012). Palaeochannels of the Balkh river (northern Afghanistan) and human occupation since the Bronze Age period. *Journal of Archaeological Science*, 39(11), 3415-3427.
9. Gang, R. (2010). Community-based dispute resolution processes in Balkh Province. Universitäts-und Landesbibliothek Sachsen-Anhalt. Kabul; Afghanistan Research and Evaluation Unit
10. Grace, J. (2004). Gender roles in agriculture: Case studies of five villages in northern Afghanistan (No. 298-2016-4726).
11. Karimi, M. T., Ghorbani, G. R., Kargar, S., & Drackley, J. K. (2015). Late-gestation heat stress abatement on performance and behavior of Holstein dairy cows. *Journal of dairy science*, 98(10), 6865-6875.

12. Ministry of Agriculture, Irrigation and Livestock of Afghanistan (MAIL), Agriculture prospect report (2005). PP: 8-15.
13. Ministry of Agriculture, Irrigation and Livestock of Afghanistan (MAIL), Agriculture prospect report. (2012). PP: 10-12.
14. Palka, E. J. (2001). Afghanistan: A Regional Geography, Military Academy, west point, NY. Department of Geography and Environmental Engineering.
15. Pineda, A., Drackley, J. K., Garrett, J., & Cardoso, F. C. (2016). Effects of rumen-protected niacin on milk production and body temperature of middle and late lactation Holstein cows. *Livestock Science*, 187, 16-23.
16. Rojas-Downing, M. M., Nejadhashemi, A. P., Harrigan, T., & Woznicki, S. A. (2017). Climate change and livestock: Impacts, adaptation, and mitigation. *Climate risk management*, 16, 145-163.
17. Rlung, L. (2008). Dairy Projects in Afghanistan. Poverty Reduction that Works: Experience of Scaling Up Development Success, 109.
18. Siddiky, M. N. A. (2017). Dairying in South Asian region: opportunities, challenges and way forward. *SAARC Journal of Agriculture*, 15(1), 173-187.
19. Silanikove, N. (2000). Effects of heat stress on the welfare of extensively managed domestic ruminants. *Livestock production science*, 67(1-2), 1-18.
20. Wangui, E. E. (2014). Gender, livelihoods and the construction of climate change among Masai pastoralists. In *Global Perspectives on Gender and Space* (pp. 163-180). Routledge.
21. West, J. W. (2003). Effects of heat-stress on production in dairy cattle. *Journal of dairy science*, 86(6), 2131-2144.
22. Zafar, M. (2005). First draft country report on the status and perspectives of the animal genetic resources development and conservation in Islamic Republic of Afghanistan. Kabul, Afghanistan:FAO

Effect of Fennel (*Foeniculum vulgare*) Seed Powder and Furazolidone Supplementation on Growth Performance of Broilers

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ABSTRACT

Background: Residues of AGPs (antibiotic growth promoters) caused bacterial resistance in humans. This study has been conducted to assess how broiler chicks responded to various amounts of fennel seed powder as a natural growth promoter and as an alternative to antibiotics. Furazolidone was fed as an antibiotic growth promoter to evaluate the effects of this substance on the feed consumption, feed conversion ratio, and body weight gain of chickens.

Materials and Methods: 120-days-old broiler chicks were used in this study. They were placed into five groups randomly with three replicates in each group and eight chicks in each replication, over the course of 35 days. From these five groups, the T1 (control) group was given a normal diet, the T2, T3, and T4 groups were given one, two, and three grams of fennel seed powder per kilogram of diet, respectively. However, the T5 group was given 0,2 g furazolidone per kilogram of diet. The feed which used in this study was obtained from Habib Hassam trading company and it was of two types (4 number feed and 9 number feed).

Findings: It was found that the experimental groups who received 2 and 3 grams of fennel seed powder per kilogram of diet not only ate less food overall, but also had the best feed conversion ratio, and the greatest weight gains ($P < 0.01$). There was no significant difference between the group given 1gr of fennel seed powder per kilogram of a diet with the control group ($P > 0.05$). On the other hand, the group that was fed 0,2 g of furazolidone was not significant as compared to the first (control) group.

Conclusion: As a natural growth promoter, feeding broiler chicks 2-3 grams of ground fennel seed per kilogram of diet will have the best effects on growth performance as well as avoiding furazolidone.

Keywords: Fennel, Furazolidone, Broiler, Growth performance.

INTRODUCTION

The use of poultry products as a food source is increasing regularly, the reason for this is the high production of chickens at low cost in a short period of time, high nutritional value, and low capital and it leads to the economic development of farmers. The primary goal of the poultry industry nowadays is to transform low-value nutrients into high-value ones (Parks et al., 2000; Ahmed, 2015). As a result of the poultry industry in the past few years, chicken meat represents 80 percent of all meat production, which is the rapid grower segment in the meat industry. The development of the poultry industry has a vital role in increasing the production of eggs and meat.

People can find employment in poultry farming, which is often regarded as a reliable source of income. Chicken meat is the best food in terms of health because it has low fat and high protein. On the other hand, it has good taste, low cost, and short production period (Lemrabt et al., 2018).

Growth-promoting feed additives are of the ingredients that are very rarely added to chicken feed, accelerate the process of growth and weight gain, and also have a significant influence on egg production, nutrition utilization efficiency and lowering mortality rate in order to bring out desirable features or to minimize weak characteristics (AL-Zuhairi et al., 2018; Leeson & Summers, 2001).

The poultry industry in hot regions is extraordinarily vulnerable to high ambient temperatures, the optimum temperature for broilers is 32-34, 28-32, 26-28, 24-26, 18-24, and 18-24 degree centigrade in the first, second, third, fourth, fifth and sixth weeks, respectively. Therefore, it is necessary to manage the temperature in a proper way to save the chickens from the threat of high temperatures. When the temperature rises above 30 degrees Celsius, symptoms of heatstroke appear in chickens, high temperatures can result in severe physiological disturbances because chickens do not have sweat glands and evaporate their excess sweat by panting. It takes 0.5 kcal of energy to evaporate one gram of water. In the second method, excess body heat is released through the urinary system as a result of drinking too much water, with this there is a decrease in chicken production and less income is obtained economically. In addition, diet and proper management are essential to alleviate high temperature (heat) stress (Al-Sagan et al., 2020; Nasir & Grashom, 2006; Ruberto et al., 2000).

Feeding additive antibiotics as health and growth promoters have played a major role in animal production, but the use of antibiotics as growth promoters has been banned by the European Association since January 2006. A significant result has been done in the last two decades to find out alternatives for antibiotic growth promoters (AGPs), different categories of non-antibiotic growth promoters has recommended as natural growth promoters (NGPs) which are used as alternatives to antibiotic growth promoters. The advantage of natural growth promoters is the prevention of bacterial resistance in consumers, no harmful chemical remains in chickens' eggs and meat, Vis versa in antibiotic growth promoters as well. The addition of natural growth promoters in poultry feed has some beneficial effects with itself as the rapid growth of microflora, digestive stability, quick development of the immune system, reduction in the incidence of diarrhea and etc. Organic acids, probiotics, prebiotics, symbiotic, dietary enzymes are including in natural growth promoters (NGPs), (Ahmed., 2015; Dibner & Richards, 2005; Griggs & Jacob, 2005).

Aromatic plants are important in stimulating the digestive system to secrete more digestive enzymes and have antimicrobial activities. It is proved that fennel (*Foeniculum vulgare L.*) is one of the aromatic plants which has a high percentage of fatty acids, linolenic and citric in composition (Hassan & Mukhtar, 2015; Mohammed et al., 2009; Ostad et al., 2001). Fennel is a plant that has been used as an aromatic plant in the Mediterranean zone, and it also has some medical traits such as antispasmodic, carminative, diuretic, analgesic, antioxidant, antimicrobial, antifungal, anti-parasitic, and growth-stimulating effects in poultry, also using against heat stress as well (AL-Zuhairi et al., 2018; Ragab, 2007; Rezq, 2012). There are a lot of carbohydrates, proteins, fats, minerals (calcium, phosphorus, iron, potassium), and vitamins (thiamin, riboflavin, niacin) in the composition of fennel seeds (Ahmed, 2015).

Furazolidone is a nitrofurantoin antimicrobial agent used in human and veterinary medicine against bacteria and protozoa, Nitrofurans specifically furazolidone, furaltadone, nitrofurantoin, and nitrofurazone, belong to a class of broad-spectrum synthetic antibiotics that all contain a 5-nitrofurantoin ring, with use of antibiotics as growth promoters, the main problem is their residues in meat and eggs and thus the increase of bacterial resistance in humans (Bywater, 2005; Sahin et al., 2002; Vass et al., 2008). Therefore, based on this reason, we have conducted this research, the purpose of this experiment is to determine and clarify the effects of fennel seed powder and furazolidone on the growth performance of the broiler.

MATERIALS AND METHODS

Experimental Location

This experiment was conducted in 2022, in the poultry research farm of Nangarhar University, agriculture faculty, with the ambient temperature of 27-40 °C.

Housing

The poultry research farm in Nangarhar University is built of concrete. First, the farm was washed with water and disinfected with lime and sanitizer, and all hygienic rules had applied before and during of experiment. Fan, air cooler, heater, and dry bulb were used to control temperature, moisture and lighting of the farm, shown in (Table 1). There were fifteen cages with disinfected feeders and drinkers in the farm for different groups of broilers, and the farm temperature, moisture, lighting, and ventilation were adjusted before chicks' arrival.

Experimental Design and Birds

Totally 120-days-old chicks were purchased from SB hatchery of Peshawar, after being brought to the farm divided into five groups (T1, T2, T3, T4 & T5) in a complete randomized design. There were three replicates in each treatment and 8 chicks in each replication. Feed for chicks obtained from Habib Hasaam trading company which has the best quality feed. The chicks were fed with 4 number (commercial Name) feed as a starter till the 14th day, and from 14th to 16th day has fed with a mix of 4 and 9 number feed, after 16th days till the end of the experiment has fed with 9 number feed. Fennel seed was purchased from the market and ground by machine, Furazolidone antibiotic (Furazole 24.4%) was purchased from the market which has been produced by (Hilton Pharma Pvt Ltd) and used in the experiment.

The chicks were fed with normal feed first day to control initial stress and adapt well, from the second day till the end of the experiment the control group (Treatment 1) has fed with normal feed, treatment two (T2) has fed with normal diet plus 1gr fennel seed powder per kg diet, treatment three (T3) has fed with normal diet plus 2gr fennel seed powder per kg feed, treatment four (T4) has fed with normal diet plus 3gr fennel powder per kg feed and treatment five (T5) has fed with normally fed plus 0,2 g furazolidone per kg feed.

Age of Bird/Week	Temperature	Lighting	Humidity
1	34-31	23/24	40%
2	31-30	22/24	50%
3	30-28	20/24	55%
4	28-27	22/24	60%
5	28-26	23/24	60%

Statistical Analysis

Data were statistically analyzed using SPSS one-way analysis of variance (ANOVA). Data in the tables presented as mean \pm standard deviation. Different letters in a column indicated significant difference at 0.05 probability level based on Duncan grouping test.

RESULTS

Feed Intake

The results of total five weeks' feed intake of T1, T2, T3, T4, and T5 are 3303 gr, 3263 gr, 3242 gr, 3234 gr and 3294 gr, respectively. The fennel powder-fed groups (T3) and (T4) have significant difference ($p < 0.01$) with control group (T1) and furazolidone fed group (T5). There is no significant difference among fennel powder fed groups (T2, T3, and T4). In addition, the group which is fed with 1gr fennel powder per kg diet (T2) was not significant different with other groups. There was no significant difference between control (T1) and furazolidone fed groups (T5). These results are presented in the Table 2.

Weight Gain

The results of total five weeks' weight gains of T1, T2, T3, T4 and T5 are 2078 ± 4.24 gr, 2088.2 ± 6.93 gr, 2118.2 ± 7.21 gr, 2119.3 ± 4.45 gr and 2079.8 ± 6.22 gr, respectively. T3 and T4 had significant difference ($p < 0.01$) with control, T2 and T3 groups. On the other hand, there were no significant differences between the control group, T2 and T5. The results are illustrated in the Table 3.

Feed Conversion Ratio

The final results of feed conversion ratio have been achieved in such a way by the formula shown below and are summarized in the Table 4:

$$\text{FCR} = \text{Feed given} / \text{Chick weight gain}$$

The results showed that the T3 and T4 groups had significant better ($p < 0.05$) FCR than the control, T2, and T5 groups.

Table 2. Shows weekly and total feed intake results.						
Treatments	1 st week	2 nd week	3 rd week	4 th week	5 th week	Total food intake
Control (T1)	187.5 \pm 2.6	430.9 \pm 4.7 ^a	755.3 \pm 5.87 ^a	1033 \pm 3.90 ^a	896.5 \pm 5.46 ^a	3303 \pm 4.38 ^a
T2	188.3 \pm 2.3	426.3 \pm 2.2 ^{ab}	744.3 \pm 3.48 ^{ab}	1025 \pm 4.12 ^{ab}	879.8 \pm 1.37 ^{ab}	3263 \pm 2.29 ^{ab}
T3	186.8 \pm 4.1	418.0 \pm 3.2 ^b	738.2 \pm 6.58 ^b	1023 \pm 2.93 ^b	876.6 \pm 1.24 ^b	3242 \pm 3.25 ^b
T4	187.5 \pm 5.8	417.6 \pm 3.5 ^b	733.2 \pm 7.86 ^b	1021 \pm 2.11 ^b	875.4 \pm 6.94 ^b	3234 \pm 6.15 ^b
T5	189.4 \pm 2.9	425.8 \pm 7.7 ^{ab}	749.1 \pm 6.94 ^{ab}	1032 \pm 3.57 ^a	894.0 \pm 10.64 ^a	3294 \pm 5.44 ^a
Significant differences	Ns	*	*	**	**	**
	Ns=non-significant, *=significant difference at $P < 0.05$, **=significant difference at $p < 0.01$.					

Table 3. Shows weekly and total weight gain results.

Treatments	Initial weight (gr)	1 st week	2 nd week	3 rd week	4 th week	5 th week	Total weight gain (gr)
Control (T1)	49.5	147.1±1.24 ^{ab}	327.4±1.03 ^{ab}	510.0±1.48 ^b	612.4±1.48 ^b	481.1±2.30 ^b	2078± 4.24 ^b
T2	51.5	146.0±1.70 ^{ab}	330.5±1.89 ^{ab}	515.8±1.63 ^{ab}	615.4±1.14 ^b	480.5±1.63 ^b	2088.2± 6.93 ^b
T3	50.0	153.8±3.28 ^a	332.8±2.93 ^{ab}	520.2±3.97 ^a	622.6±1.53 ^a	488.8±1.20 ^a	2118.2± 7.21 ^a
T4	50.9	148.6±1.65 ^{ab}	335.0±2.64 ^a	523.7±2.07 ^a	623.0±2.87 ^a	489.0±1.41 ^a	2119.3± 4.45 ^a
T5	51.3	145.9±2.44 ^b	326.6±2.71 ^b	510.6±3.96 ^b	614.3±4.64 ^b	482.4±1.68 ^b	2079.8± 6.22 ^b
Significant differences		*	*	**	**	**	**
	Ns=Non-significant, *=significant difference at P<0.05, **=significant difference at p<0.01.						

Table 4. Shows weekly and average FCR results.

Treatments	1 st week	2 nd week	3 rd week	4 th week	5 th week	Average FCR
Control (T1)	1.301 ± 0.030 ^a	1.302± 0.010 ^a	1.454± 0.012 ^a	1.680± 0.061 ^a	1.846± 0.019 ^a	1.516± 0.007 ^a
T2	1.300 ± 0.029 ^a	1.293± 0.017 ^{ab}	1.456± 0.020 ^a	1.683± 0.013 ^a	1.832± 0.013 ^{ab}	1.512± 0.005 ^a
T3	1.204± 0.045 ^b	1.249± 0.014 ^c	1.410± 0.010 ^b	1.592± 0.018 ^b	1.790± 0.021 ^{bc}	1.449± 0.008 ^b
T4	1.270± 0.036 ^{ab}	1.253± 0.016 ^{bc}	1.409± 0.010 ^b	1.587± 0.013 ^b	1.779± 0.010 ^c	1.459± 0.014 ^b
T5	1.295 ± 0.034 ^{ab}	1.282± 0.017 ^{abc}	1.440± 0.015 ^{ab}	1.676± 0.010 ^a	1.841± 0.026 ^{ab}	1.506± 0.014 ^a
Significant difference	*	**	**	**	**	*
	Ns=Non-significant, *=significant difference at P<0.05), **=significant difference at p<0.01)					

DISCUSSION

Based on this study, there was a significant difference in the consumption of chicken feed among all groups, the T1 (control) group had the most food consumption, followed by the T5 and T2 groups, while on the contrary, the T3 and T4 groups had consumed the least amount of food, which is different from the T1 and T5 groups, according to (P<0.01). Similar findings were obtained from the research of AL-Zuhairi et al., (2018) that 5% fennel seed powder has a significant difference (P<0.05) in the feed intake and body weight gain (BWG) with the control group.

There was significant difference in the BWG among all groups, the T3 and T4 groups had highest BWG than T1, T2 and T5 groups. The T3 and T4 had significant difference with control, T2 and T5 groups according to (P<0.01). Similar result was presented by Lember et al. (2019) that 750 gr fennel seed powder + 50 kg diet had a significant effect on broiler BWG, as well similar report was published by Ahmed., (2015) that the 3% fennel seed powder has a significant effect (P<0.05) on broiler feed intake, FCR, and BWG.

In this study T3 and T4 had better FCR than control, T2 and T5, so the difference was significant according to (P<0.05). Similar result was reported from Mohammed et al. (2009) that 1, 2, and 3 gr fennel seed powder per

kg diet has a significant effect ($P < 0.05$) on FCR and BWG, also in the experimental study by AL-Sagan et al., (2019) and presented that 3.2% fennel seed powder had a significant effect ($P < 0.05$) on FCR and BWG.

There was significant difference among the all groups due to use of fennel, feed intake was significantly decrease in T3 and T4, BWG was significantly increased in T3 and T4 and the FCR was also better in T3 and T4 (which were fed 2 and 3 gr fennel per kg diet, respectively). Similar findings were reported by (Fatima et al., 2022) that overall feed intake and weight gain were considerably greater.

In this study there was no significant difference among the all groups by used of furazolidone. Similar finding was obtained from the research of Wekhe and Nyeche, (2002) which used furazolidone as feed additives and reported that the groups which have fed with furazolidone did not significantly effect on the feed intake, FCR, and BWG with the control group. As well Ahmed et al. (2012) reported that use of different level (0, 100, 200, 300 mg) of furazolidone and reported that they did not have significant effect among all groups.

CONCLUSION

Uses of fennel have positive effect on growth performance of broiler and also it has heat stress removal traits, it must be used in hot regions. Furazolidone did not have significant effect, it is better to use fennel instead of furazolidone.

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Conflict of interest

All authors express no conflict of interest in any part of research, manuscript and submission to the journal.

REFERENCES

1. Ahmed, H. D., Elamin, K. M., & Ati, K. A. A. (2012). Effects of Dietary Furazolidone on the Performance of Broiler Chicks under Sudan Conditions. *Research Journal of Veterinary Sciences* 6 (1): 20-26
2. Ahmed, S. A. H. (2015). Evaluation of Shamar (*Foeniculum vulgare*) Seeds as Natural Growth Promotion (NGP) in Broiler Chicks (Doctoral dissertation, Sudan University of Science and Technology).
3. Al-Sagan, A. A., Khalil, S., Hussein, E. O., & Attia, Y. A. (2020). Effects of fennel seed powder supplementation on growth performance, carcass characteristics, meat quality, and economic efficiency of broilers under thermoneutral and chronic heat stress conditions. *Animals*, 10(2), 206.
4. AL-Zuhairi, Z. A. J., Abdullah, W. S., & Majal, R. K. (2018). Effect the dietary supplementation of Cariander (*Coriandrum sativum* L.) and Fennel (*Foeniculum vulgare*) seed powder and their mixture in productional and physiological performance of broiler. *Al-Qadisiyah Journal of Veterinary Medicine Sciences*, 17(2), 143-148.
5. Bywater, R. J. (2005). Identification and surveillance of antimicrobial resistance dissemination in animal production. *Poultry science*, 84(4), 644-648.
6. Dibner, J. J., & Richards, J. D. (2005). Antibiotic growth promoters in agriculture: history and mode of action. *Poultry science*, 84(4), 634-643.
7. Fatima, F., Chand, N., Naz, S., Saeed, M., Khan, N.U., Khan, R.U. (2022). Coping heat stress by crushed fennel (*foeniculum vulgare*) seeds in broilers: Growth, redox, balance and humoral immune response, University of Agriculture, Peshawar, Pakistan. *Journal of Livestock Science*, 265, 156-163
8. Griggs, J. P., & Jacob, J. P. (2005). Alternatives to antibiotics for organic poultry production. *Journal of applied poultry research*, 14(4), 750-756.

9. Hassan, S. A., & Mukhtar, M. A. (2015). Evaluation of shamar (*Foeniculum vulgare*) seeds as natural growth promotion (NGP) in broiler chicks. *World Journal of Pharmacy and Pharmaceutical Sciences*, 5(1), 225-236.
10. Hernandez, F., Madrid, J., Garcia, V., Orengo, J., & Megias, M. D. (2004). Influence of two plant extracts on broilers performance, digestibility, and digestive organ size. *Poultry science*, 83(2), 169-174.
11. Hruska, K. (2006). Veterinary Research Institute, Brno, Czech Republic: Analysis of papers published from 1990 to 2005. *Veterinarni Medicina*, 51(5), 161-167.
12. Kahn, C. M., Line, S., & Aiello, S. E. (2005). The merck veterinary manual. Merck & Co. Inc., Whitehouse Station, NJ.
13. Leeson, S., & Summers, J. D. (2001). Nutrition of the chicken 4th Ed. *Guelph, Ontario, Canada: University Books*.
14. Lemrabt, S., Ram Pal Singh, & Nagar, S. (2018). Efficacy of *Foeniculum vulgare* seeds powder on growth performance in broilers, *International Journal of Food Science and Nutrition*, pp167-169.
15. Mohammed, A. A., & Abbas, R. J. (2009). The effect of using fennel seeds (*Foeniculum vulgare* L.) on productive performance of broiler chickens. *International Journal of Poultry Science*, 8(7), 642-644.
16. Nasir, Z., & Grashorn, M. A. (2006). Use of Black cumin (*Nigella sativa* Linn.) as alternative to antibiotics in poultry diets. 9. Tagung Schweine-und Geflügelernährung, Martin-Luther-Universität Halle-Wittenberg, Halle, Germany, 28-30 November 2006, 210-213.
17. Niewold, T. A. (2007). The nonantibiotic anti-inflammatory effect of antimicrobial growth promoters, the real mode of action? A hypothesis. *Poultry science*, 86(4), 605-609.
18. Ostad, S. N., Soodi, M., Shariffzadeh, M., Khorshidi, N., & Marzban, H. (2001). The effect of fennel essential oil on uterine contraction as a model for dysmenorrhea, pharmacology and toxicology study. *Journal of ethnopharmacology*, 76(3), 299-304.
19. Parks, C. W., Grimes, J. L., Ferket, P. R., & Fairchild, A. S. (2000). The case for mannanoligosaccharides in poultry diets. An alternative to growth promotant antibiotics. In *Proceedings of Alltech's Sixteenth Annual Symposium*. Nottingham University Press (pp. 45-60).
20. Ragab, M. S. (2007). Effects of using fennel seeds in growing Japanese quail diets varying in their protein content with or without enzyme supplementation. *Fayoum Journal of Agricultural Research and Development*, 21(2), 113-136.
21. Revington, B. (2002). In 2002 Multi-State Poultry Feeding and Nutrition Conference, Feeinfo, Indianapolis, Indiana, USA, Pp 1-14.
22. Rezq, A. A. (2012). Beneficial health effects of fennel seeds (Shamar) on male rats feeding high fat-diet. *Med. J. Cairo Univ*, 80(2), 101-113.
23. Romila, R. M. A. (2001). *Hacked By SOSO H. H Iraqi-Cracker. M. Sci* (Doctoral dissertation, University of Cairo Egypt).
24. Ruberto, G., Baratta, M. T., Deans, S. G., & Dorman, H. D. (2000). Antioxidant and antimicrobial activity of *Foeniculum vulgare* and *Crithmum maritimum* essential oils. *Planta medica*, 66(08), 687-693.
25. Sahin, O., Morishita, T. Y., & Zhang, Q. (2002). *Campylobacter* colonization in poultry: sources of infection and modes of transmission. *Animal Health Research Reviews*, 3(2), 95-105.
26. Vass, M., Hruska, K., & Franek, M. (2008). Nitrofurant antibiotics: a review on the application, prohibition and residual analysis. *Veterinarni medicina*, 53(9), 469-500.
27. Wekhe, S. N., & Nyeche, V. N. (2002). Performance of broilers on furazolidone additive. *Nigerian Journal of Animal Production*, 29(1), 16-20.

Genetic relationship, antibiotic resistance pattern and virulence factors of *Klebsiella pneumoniae* strains isolated from meningitis patients

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ABSTRACT

Background: Meningitis due to *Klebsiella pneumoniae* is increasingly reported from several Asian countries. Microbiological characteristic of *K. pneumoniae* strains causing meningitis is not yet explored in Iran. The aim of this study was to demonstrate antibiotic resistance pattern and virulence factors, as well as the genetic relationship of *K. pneumoniae* strains isolated from meningitis patients.

Materials and Methods: Eight *K. pneumoniae* isolates were collected from hospitalized patients at Imam Khomeini hospital, Tehran-Iran from 14 May 2018 to 15 Mar 2020. The antibiotic resistance pattern was determined by disc diffusion method. Antibiotic resistance genes and virulence-associated genes were traced by Polymerase Chain Reaction. Repetitive Extragenic Palindromic Polymerase Chain Reaction was used for evaluation of the genetic relationships among isolates.

Findings: Six out of eight isolates were resistant to almost all the 15 antibiotics tested. These six isolates harbored triple antibiotic resistance genes: *bla*_{OXA-48}, *bla*_{SHV}, and *aac* (6')-Ib. Five of these isolates co-harbored *aac* (3)-IIa gene. Besides, *bla*_{TEM} and *bla*_{CTXM-1} were detected in five and three isolates respectively. *bla*_{NDM-1} was identified in one isolate. All isolates harbored *acrAB*, *ompK36*, and *tolC* resistance genes. None of the isolates were related to K1 or K2 capsular serotypes. The most commonly detected virulence genes were *entB* (100%), *mrkD* (100%), *ybtS* (62.5%) and *kfu* (25%). rep-PCR fingerprinting discriminated seven isolates into three clusters.

Conclusion: Simulations presence of the genes coding for Extended Spectrum Beta Lactamases, Carbapenemases, and Aminoglycoside Modifying Enzymes narrows therapeutic alternatives and imposes a heavy load on the public health system. Antibiotic susceptibility test is recommended however, Carbapenems can still be considered as first-line medications for preliminary empirical treatment of *K. pneumoniae* meningitis before obtaining susceptibility test results. Low genetic diversity suggests the circulation of certain *K. pneumoniae* clones in hospital which highlights the establishing of effective infection observation and prevention program.

Keywords: *Klebsiella pneumoniae*, antibiotic resistance, virulence genes, cerebrospinal fluid

INTRODUCTION

Despite the introduction of new antibiotics, the mortality rate of acute bacterial meningitis (ABM) is still high globally and the comparative proportion of organisms causing the disease has changed recently (Lu et al., 2002). Even though meningitis caused by *K. pneumoniae* was rare in the past, after implementation of vaccination against Gram positive organisms that more frequently caused bacterial meningitis an increase has been observed in the incidence of the meningitis caused by Gram-negative pathogens such as *Escherichia coli* and *K. pneumoniae* (Ellis et al., 2019; Scheld et al., 2002). Currently two evolutionary divisions exist for *K. pneumoniae*: classical *K. pneumoniae* (cKP) which most commonly infects people with immune defect, especially diabetics and patients struggling with malignancies, and hyper virulent *K. pneumoniae* (hvKP) that can infect immune deficient as well as immunocompetent hosts (Paczosa et al., 2016).

The capability of *K. pneumoniae* for establishing a successful infection stems from possession of numerous virulence factors most of which fall in the following classes: Capsule, lipopolysaccharide (LPS), siderophores, pili, outer membrane proteins (OMPs), iron transport systems, and genes that contribute in allantoin metabolism (Li et al., 2014; Paczosa et al., 2016). *K. pneumoniae* capsular polysaccharide (K antigen) is a crucial virulence factor that can be used for typing of *K. pneumoniae* isolates to different serotypes (Choi et al., 2020). Amongst 78 capsular serotypes that are currently identified in *K. pneumoniae*, a few serotypes (chiefly K1 and K2) have hyper-mucoid phenotype and the measure of mucoidy seems to be associated with the invasiveness of infections they produce (Li et al., 2014).

Infections caused by multidrug-resistant (MDR) *K. pneumoniae* frequently result in failure of treatment and longstanding hospitalization (Paterson et al., 2005). *K. pneumoniae* utilizes two efflux pump systems: The *AcrAB-TolC* and *mdtK*, to expel antimicrobial drugs from its cell that can lead to emergence of MDR organisms (Wasfi et al., 2016). Two outer-membrane proteins: *ompK35* and *ompK36* are in charge of creating pores in the outer membrane of Gram-negative bacteria thorough which cephalosporin and carbapenem antibiotics enter the cell. Loss of or mutation in these porins can bring about organisms that might have reduced susceptibility to the mentioned antibiotics (Shi et al., 2013).

Treatment of infections caused *K. pneumoniae* has become more complex since it acquired plasmid-encoded extended-spectrum beta-lactamase (ESBL) genes (El Fertas-Aissani et al., 2013). ESBLs are enzymes produced by certain bacteria that hydrolyze penicillins, the first three generations of cephalosporins, and aztreonam (Paterson et al., 2005). Hence, carbapenems are extensively used as the last line agents for the treatment of infections caused by ESBL producing *K. pneumoniae* strains as their β -lactam ring is more resistant to hydrolysis by these enzymes (Shi et al., 2013). However, resistance to carbapenems can be created by several mechanisms mainly combination of porin loss with the expression of ESBLs, and the production of carbapenemases (Paterson et al., 2005). Carbapenemases are recently emerged β -lactamase enzymes that are able to hydrolysis carbapenems (Queenan et al., 2007).

Therapeutic options for treatment of carbapenemase-producing *K. pneumoniae* strains are limited and mainly relies on use of tigecycline, colistin, fosfomycin, and aminoglycosides (Falagas et al., 2007). Aminoglycosides are most commonly used for this purpose because of their wide availability and cost effectiveness (Forge et al., 2000). Nevertheless, aminoglycoside resistance is a growing challenge that occurs principally due to two

phenomena: enzymatic modification of drugs and target site modification in bacteria that are mediated by aminoglycoside modifying enzymes (AMEs) and 16S rRNA methylase genes, respectively (Krause et al., 2016). Molecular genetic techniques are valuable tools for the assessment of genetic relationship between bacterial isolates, especially if there is a suspected outbreak. Repetitive Extragenic Palindromic Polymerase Chain Reaction (rep-PCR) offers speedy typing results that are cheap and reproducible with a fairly high differentiating power (Nielsen et al., 2011). Studies have already shown the applicability of rep-PCR method for the study of *K. pneumoniae* epidemiology (Hou et al., 2015; Nielsen et al., 2011).

In recent decades increasing reports of *K. pneumoniae* meningitis has been published from several Southeast Asian countries (Ku et al., 2017; Xu et al., 2019). This high prevalence has been linked with the emergence of hvKP strains in that region and the mortality rate is reported from 48.5% to 66% (Ku et al., 2017). Yet there is no data regarding antibiotic resistance pattern and virulence profile of *K. pneumoniae* strains causing meningitis in Iran. In the current study, we investigated antibiotic resistance pattern, most common virulence genes, and genetic relationships among *K. pneumoniae* strains isolated from CSF of meningitis patients admitted to a large hospital in Tehran.

MATERIALS AND METHODS

Bacterial isolates and identification

In this descriptive cross-sectional study conducted during 14 May 2018 to 15 March 2020, a total of eight *K. pneumoniae* CSF isolates were obtained from laboratory staff of Imam Khomeini hospital (a 1400 bed teaching hospital in Tehran). *K. pneumoniae* isolates were identified by conventional biochemical tests and were further confirmed by PCR amplification of *khe* gene which is specific for *K. pneumoniae* (Wong et al., 2014). Table 1 shows characteristic of primers used for amplification of *Khe* gene.

Table 1. characteristic of primers used for genotypic detection of *K. pneumoniae* isolates

Gene	Primer sequence (5' to 3')	Product size (bp)	Tm (C)
<i>khe</i>	F: GGCGAGGTTTACGTCTCAAC	271	57
	R: GTACTTCTTGTTGGCCTCGC		57

Antimicrobial susceptibility testing

Antibiotic susceptibility of *K. pneumoniae* isolates was determined by the disc diffusion method (Kirby Bauer) according to Clinical and Laboratory Standard Institute (CLSI) guidelines (CLSI, 2019). The following antibiotic discs were used for this purpose: ciprofloxacin (5µg), augmentin (10 µg), cefoxitin (10 µg), cefotaxime (10µg), ceftriaxone (10µg), azterionam (30 µg), trimetoprim/sulfamethoxazole (1.25/23.75 µg), ampicillin (10 µg), piperacillin tazobactam (100/10µg), tetracycline (30µg), cefipime (30 µg), gentamicin (10 µg), ceftazidime (30 µg), imipenem (10µg) and meropenem (10µg). *Escherichia coli* ATCC 25922 was used as the positive control.

PCR amplification of antibiotic resistance genes

Extraction of DNA was performed by boiling method. PCR was conducted for amplification of ESBL genes (*blaSHV*, *blaTEM*, *blaCTXM-1*), carbapenemase encoding genes (*blaKPC*, *blaNDM-1*, *blaIMP*, *blaOXA-48*), and genes encoding aminoglycoside modifying enzymes (AMEs) and 16 SrRNA methylases (*aac* (6')-Ib, *aac* (3)-IIa, *aac* (3)-Ia, *aac* (3)-IVa, *ant* (2'')-Ia, *ant* (4')-IIa, *aph* (3')-Ia, *armA*, *rmtB* and *rmtC*) using previously

described primers. Table 2 demonstrates sequences and characteristics of primers used for amplification of most of these genes.

Table 2. Characteristics of primers used for amplification of antibiotic resistance genes.

Gene	Primer sequence (5' to 3')	Product size (bp)	Tm (C)	Reference
<i>aac(6')-Ib</i>	F: TTGCGATGCTCTATGAGTGGCTA	482	59	(Fernández et al., 2015)
	R: CTCGAATGCCTGGCGTGTTT			
<i>aac(3')-IIa</i>	F: GGCAATAACGGAGGCGCTTCAAAA	536	63	(Fernández et al., 2015)
	R: TTCCAGGCATCGGCATCTCATACG			
<i>aac(3)-Ia</i>	F: GCAGTCGCCCCCTAAACTAA	464	62	(Fernández et al., 2015)
	R: CACTTCTTCCCGTATGCCCAACTT			
<i>aac(3)-IVa</i>	F: TCGGTCAGCTTCTCAACCTT	314	56	(Fernández et al., 2015)
	R: GATGATCTGCTCTGCCTGTG			
<i>ant(2'')-Ia</i>	F: ACGCCGTGGGTTCGATGTTTGATGT	572	65	(Fernández et al., 2015)
	R: CTTTTCCGCCCCGAGTGAGGTG			
<i>ant(4')-IIa</i>	F: CCGGGGCGAGGCGAGTGC	423	66	(Fernández et al., 2015)
	R: TACGTGGGCGGATTGATGGGAACC			
<i>aph(3')-Ia</i>	F: CGAGCATCAAATGAAACTGC	624	55	(Fernández et al., 2015)
	R: GCGTTGCCAATGATGTTACAG			
<i>armA</i>	F: CCGAAATGACAGTTCCTATC	846	55	(El-Badawy et al., 2017)
	R: GAAAATGAGTGCCTTGGAGG			
<i>bla_{IMP}</i>	F: GGAATAGAGTGGCTTAAYTCTC	232	58	
	R: GGTTTAAAYAAAACAACCACC			
<i>bla_{NDM}</i>	F: GGTTTGGCGATCTGGTTTTTC	621	58	
	R: CGGAATGGCTCATCACGATC			
<i>rmtB</i>	F: ATGAACATCAACGATGCCCTC	769	60	(El-Badawy et al., 2017)
	R: CCTTCTGATTGGCTTATCCA			
<i>Bal_{OXA-48}</i>	F: GCGTGGTTAAGGATGAACAC	438	58	(Nordmann et al., 2011)
	R: CATCAAGTTCAACCCAACCG			
<i>rmtC</i>	F: CAGGGGTTCCAACAAGT	246	55	(Güven et al. 2016)
	R: GAAGAGTATATAGCTTGAACATAAGTA			
<i>bla_{KPC}</i>	F: CGTCTAGTTCTGCTGTCTT	796	58	
	R: CTTGTCATCCTTGTTAGGC			

Detection of capsular serotypes and virulence-associated genes

Genes specific for two invasive capsular serotypes (K1 and K2) were investigated by PCR as described previously (Compain et al., 2014). Virulence-associated genes (*entB*, *iutA*, *ybtS*, *magA*, *rmpA*, *mrkD*, *allS*, *kfu*, and *traT*), and genes encoding efflux pump systems and outer-membrane porins (*acrAB*, *mdtK*, *tolC*, *ompK35*, and *ompK35*) were traced by PCR using primers previously described. Table-3 presents characteristics of primers used for amplification of K1 and K2 specific genes and some important virulence associated genes.

Table 3. Primers used for amplification of K1 and K2 specific genes and some important virulence genes

Gene	Primer sequence (5' to 3')	Product size (bp)	Tm (C)	Reference
<i>ybtS</i>	F: GACGGAAACAGCACGGTAAA	242	53	(Compain et al., 2014)
	R: GAGCATAATAAGGCGAAAGA			
<i>entB</i>	F: GTCAACTGGGCTTTGAGCCGTC	400	63	(Compain et al., 2014)
	R: TATGGGCGTAAACGCCGGTGAT			
<i>K2</i>	F: CAACCATGGTGGTCGATTAG	532	55	(Compain et al., 2014)
	R: GGGTCTGGCGCAGAGTATGC			
<i>Kfu</i>	F: GGCCTTTGTCCAGAGCTACG	637	61	(Compain et al., 2014)
	R: GGGTCTGGCGCAGAGTATGC			
<i>iutA</i>	F: GGGAAAGGCTTCTCTGCCAT	920	56	(Compain et al., 2014)
	R: TTATTCGCCACCACGCTCTT			
<i>magA/K1</i>	F: GGTGCTCTTACATCATTC	1283	54	(Compain et al., 2014)
	R: GCAATGGCCATTTGCGTTAG			

Analysis of genetic relationships between isolates

Genetic similarities between isolates were explored by rep-PCR method. Two inosine-containing primers: REP1R-I “IIICGICGICATCIGGC” and REP2-I “ICGICTTATCIGGCCTAC” were used for amplification of regions between noncoding repetitive DNA sequences in bacterial genome. PCR products were run on 1.5% agarose gel with a voltage of 80 volts for 120 minutes, using 1kb DNA ladder. The DNA fingerprints obtained were analyzed in http://insilico.ehu.es/dice_upgma/ using Dice similarity coefficient. Isolates with DNA similarity of $\geq 90\%$ were assigned to the same cluster.

RESULTS

Antibiotic Susceptibility Pattern

All isolates were resistant to ampicillin. Six out of eight isolates showed resistance to at least one agent of more than three antimicrobial classes and hence were MDR. All of these isolates were resistant to quinolones, cephalosporins, amoxicillin-clavulanate, and aztreonam. Two isolates were susceptible to all of these antibiotics, except for resistance to Augmentin in one isolate. The lowest resistance rate was detected to tetracycline (25%) followed by imipenem (50%). The antibiotic susceptibility patterns of all isolates are presented in Table 4.

Table 4. antibiotic susceptibility of *K. pneumoniae* strains isolated from meningitis patients

Antibiotic	No. (%)		
	Sensitive	Intermediate	Resistant
Imipenem	3(37.5)	1(12.5)	4 (50)
Meropenem	2 (25)	2 (25)	4 (50)
Gentamicin	2 (25)	0 (0)	6 (75)
Ciprofloxacin	1 (12.5)	1 (12.5)	6 (75)
Cefoxitin	2 (25)	0	6 (75)
Cefotaxime	0 (0)	2 (25)	6 (75)
Ceftriaxone	2 (25)	0 (0)	6 (75)
Augmentin	1 (12.5)	0 (0)	7 (87.5)
Aztrionam	2 (25)	0 (0)	6 (75)
Ampicillin	0 (0)	0 (0)	8 (100)
Trimethoprim- sulfamethoxazole	3 (37.5)	0 (0)	5 (62.5)
Piperacilin- tazobactam	2 (25)	1 (12.5)	5 (62.5)
Tetracycline	6 (75)	0 (0)	2 (25)
Cefipime	2 (25)	0 (0)	6 (75)
Ceftazidime	2 (25)	0 (0)	6 (75)

Detection of Antibiotic Resistance Genes

The six MDR isolates harbored at least one gene of three different classes of antibiotic resistance genes (ARGs): ESBLs (*bla_{SHV}*), carbapenemases (*bla_{OXA-48}*), and AMEs (*aac(6')-Ib*). The most frequent ESBL gene was *bla_{SHV}* which was detected in 7/8 (87.5%) of isolates followed by *bla_{TEM}* (62.5%) and *bla_{CTXM-1}* (37.5%). Simultaneous presence of *bla_{SHV}* and *bla_{TEM}* was detected in 62.5% of isolates and co-occurrence of three genes was found in 25% of isolates. The most frequent carbapenemase gene was *bla_{OXA-48}* with a prevalence rate of 75% among isolates, while *bla_{NDM-1}* was detected only in one isolate (12.5%). No PCR product was found for *bla_{IMP}* and *bla_{KPC}*. Amongst the AMEs and 16 SrRNA methylases genes that were traced, *aac(6')-Ib* and *aac(3)-IIa* were found in 75% and 62.5% of isolates respectively, while *aac(3)-Ia*, *aac(3)-IVa*, *ant(2'')-Ia*, *ant(4')-IIa*, *aph(3')-Ia*, *armA*, *rmtB*, and *rmtC* were not detected. The two isolates that were susceptible to most of the tested antibiotics were negative for all three classes of ARGs except for the presence of *bla_{SHV}* in one of these isolates. Table 5 presents the frequency of ARGs detected in eight isolates. Figure 1 presents pictures of gel electrophoresis of the most commonly detected ARGs.

Table 5. Frequency of antibiotic resistance genes (ARG) detected in *K. pneumoniae* strains isolates from meningitis

isolates	<i>bla_{SHV}</i>	<i>bla_{TEM}</i>	<i>bla_{CTXM}</i>	<i>bla_{OXA-48}</i>	<i>bla_{NDM-1}</i>	<i>aac(6')-Ib</i>	<i>aac(3)-IIa</i>
1	+	+	-	+	-	+	+
2	+	+	-	+	-	+	-
3	+	+	-	+	-	+	+
4	+	+	+	+	+	+	+
5	-	-	-	-	-	-	-
6	+	-	+	+	-	+	+
7	+	+	+	+	-	+	+
8	+	-	-	-	-	-	-
No. (%) positive	7 (87.5)	5 (62.5)	3 (37.5)	6 (75)	1 (12.5)	6 (75)	5 (62.5)

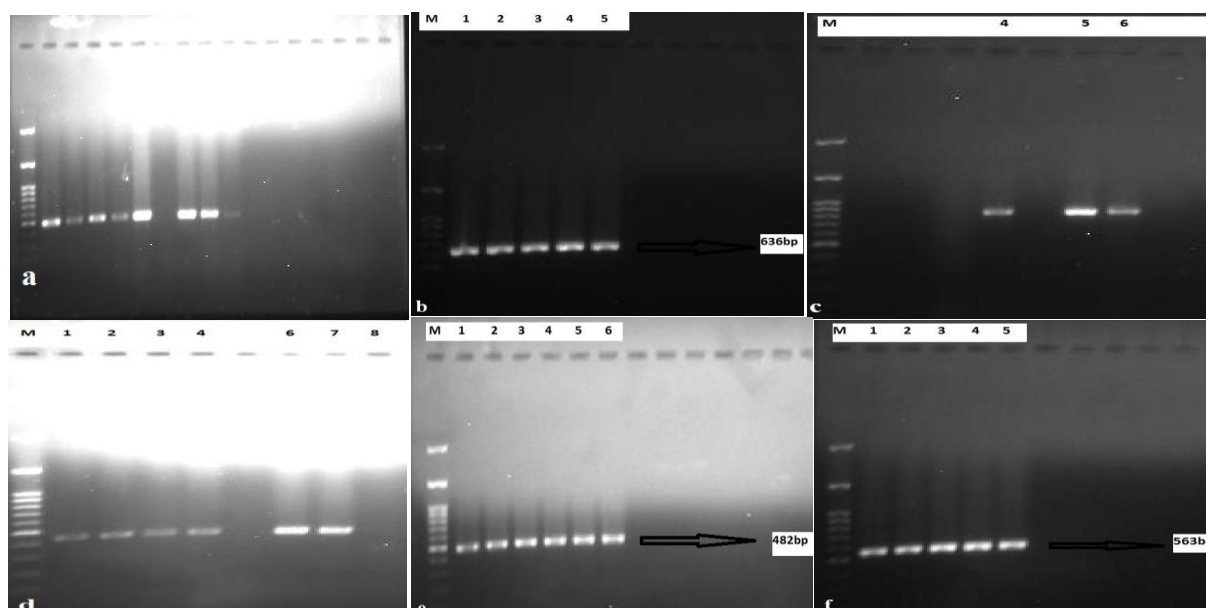


Figure 1. Pictures of gel electrophoresis of antibiotic resistance genes; a: *bla_{SHV}*, b: *bla_{TEM}*, c: *bla_{CTXM}*, d: *bla_{OXA-48}*, e: *aac(6')-Ib*, f: *aac(3)-IIa*

Detection of Genes Coding for Outer Membrane Porins, MDR Efflux Pumps, and Virulence Factors

All eight isolates harbored *acrAB-tolC* efflux pump system but were negative for *mdtK*. Of the two genes coding for outer membrane porins, *ompK36* was observed in all isolates while *ompK35* was not detected. PCR assay of *wzi* genes specific for K1 and K2 capsular serotype demonstrated that none of the isolates were related to K1 or K2 capsular serotypes. Genes encoding for type 3 fimbrial adhesin (*mrkD*) and enterobactin (*entB*) were present in 100% (8/8) isolates. Additionally, genes coding for yersiniabactin (*ybtS*), ABC iron transport system (*kfu*), and serum resistance (*traT*) were detected in 62.5%, 25%, and 12.5% of isolates respectively. Genes in charge of overproduction of capsule: mucoviscosity associated gene A (*magA*) and regulator of mucoid phenotype (*rmpA*), as well as gene coding for aerobactin siderophore (*iutA*) and a gene involved in allantoin metabolism (*allS*), were not detected in this study. The frequency of virulence-associated genes detected in all isolates is shown in Table 6. In addition, Figure 2 shows pictures of gel electrophoresis of virulence associated genes.

Table 6. frequency of virulence-associated genes detected in *K. pneumoniae* strains isolates from meningitis

isolates	<i>entB</i>	<i>ybtS</i>	<i>kfu</i>	<i>mrkD</i>	<i>traT</i>	<i>acrAB</i>	<i>TolC</i>	<i>ompK36</i>
1	+	+	+	+	-	+	+	+
2	+	-	-	+	-	+	+	+
3	+	+	+	+	-	+	+	+
4	+	-	-	+	+	+	+	+
5	+	-	-	+	-	+	+	+
6	+	+	-	+	-	+	+	+
7	+	+	-	+	-	+	+	+
8	+	+	-	+	-	+	+	+
No. (%) positive	8 (100)	5 (62.5)	2 (25)	8 (100)	1 (12.5)	8 (100)	8 (100)	8 (100)

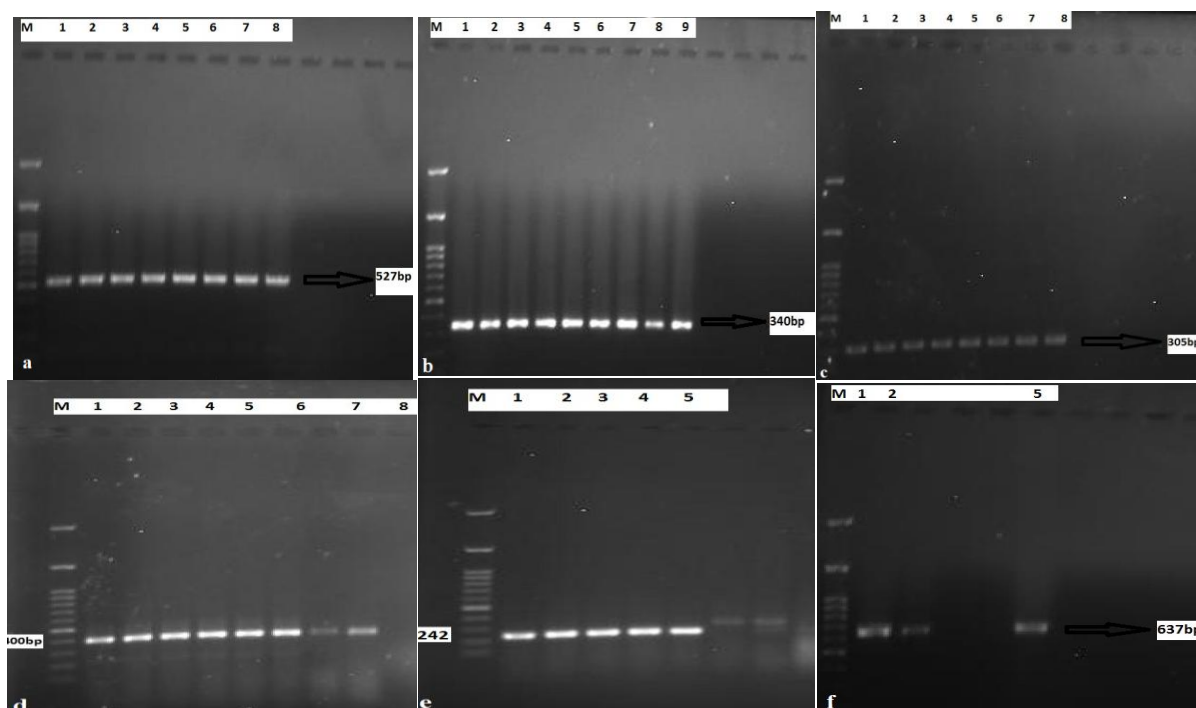


Figure 2. Pictures of gel electrophoresis of most commonly detected virulence associated genes; a: *tolC*, b: *mrkD*, c: *ompK36*, d: *entB*, e: *ybtS*, f: *kfu*

Determination of genetic relationships between isolates

rep-PCR analysis using a similarity cut-off of $\geq 90\%$, discriminated eight isolates into three clusters (three strains in cluster A and two strains in each of clusters B and C) and one sporadic strain. Isolates 1, 2, 3 that were assigned to cluster A, harbored *bla*_{OXA-48} and either one or two of AME genes. Isolates 5 and 8 were assigned to cluster B, both were negative for *bla*_{OXA-48} and AME genes and were phenotypically susceptible to imipenem and gentamicin. Isolates 6 and 7 which were assigned to cluster C also harbored *bla*_{OXA-48} and AME genes. Isolate 4 which represented the sporadic strain was the only *bla*_{NDM1} positive isolate. Figure 3 shows dendrogram and virtual gel images obtained by rep-PCR.

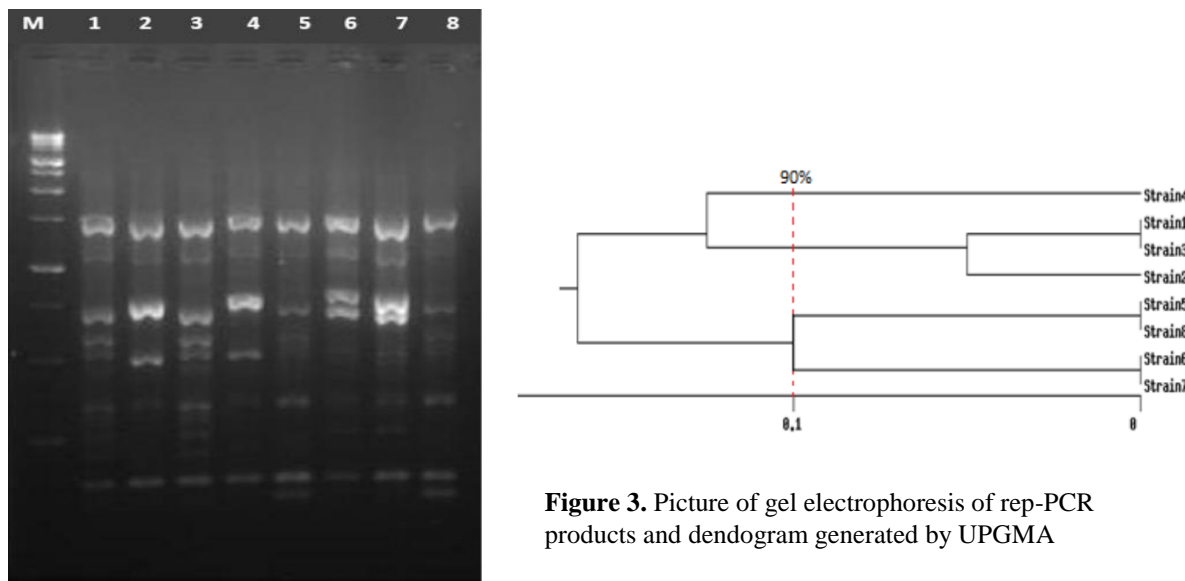


Figure 3. Picture of gel electrophoresis of rep-PCR products and dendrogram generated by UPGMA

DISCUSSION

In this study, we collected 8 *K. pneumoniae* isolates during almost two years' period. Meningitis caused by *K. pneumoniae* had been relatively uncommon in the past but its frequency seems to be growing over time (Tang et al., 1994). A retrospective study in Qatar found only ten cases of adult *K. pneumoniae* meningitis from 2007 to 2012 (Khan et al., 2014). Although *K. pneumoniae* meningitis is much more common in Taiwan, Hong Kong, and China, these high reports are primarily due to the emergence of hvKP strains in that geographical region (Xu et al., 2019).

Carbapenems are the most widely used antibiotics for treatment of MDR *K. pneumoniae* globally (Grundmann et al., 2017). In our study 75% of isolates were MDR and 50% of isolates showed resistance to carbapenems. This rate is higher than previous reports from Iran which found that resistance to imipenem was 25.7% (Kiaei et al., 2019) and 37.9% (Rastegar et al., 2019) but lower than reports from turkey 68% (Aksöz et al., 2015) and congruent with a report from Saudi Arabia (Al Bshabshe et al., 2020). These data suggest an annual increase in the frequency of carbapenem-resistant *K. pneumoniae* (CRKP) in Iran. Our results showed that tetracycline with a resistance rate of 25% was the most effective antibiotic. Efficacy of tetracycline against CRKP is previously reported from Iran (Jafari et al., 2018).

Limited numbers of antibiotics can be used as alternatives to carbapenems for treatment of CRKP (Grundmann et al., 2017), amongst which aminoglycosides are the most commonly accessible and cheap drugs (Forge et al., 2000). In our study resistance to gentamicin was detected in 75% of isolates. Resistance to gentamicin is

reported between 34.6% to 52.4% from different parts of Iran (Nasiri et al., 2018; Mokhtari et al., 2018; Rastegar et al., 2019), 60% from Egypt (El-Badawy et al., 2017), and 57.1% from Saudi Arabia (Al Bshabshe et al., 2020). Jafari et al. (2018) reported that 93.3% of CRKP isolates were resistant to gentamicin. Similarly, in our study, 100% (4/4) of CRKP isolates were resistant to gentamicin. These data show that resistance to aminoglycosides is higher among CRKP strains compared to carbapenem-susceptible strains.

We explored two general ESBL genes: *bla*_{SHV} and *bla*_{TEM}, and one common variant of *bla*_{CTXM-1} gene: *bla*_{CTXM-1}. The redundancy rates were 87.5% for *bla*_{SHV}, 62.5% for *bla*_{TEM}, and 37.5% for *bla*_{CTXM-1}. The prevalence rate of these genes was reported 67.4%, 54%, and 46.51% respectively in a study published from Iran in 2010 (Parvin et al., 2010). Previous studies have shown a higher frequency of ESBL genes among CRKP isolates (Moghadampour et al., 2018). As per our study, 100% (4/4) of CRKP isolates harbored *bla*_{SHV} and *bla*_{TEM} genes and 50% (2/4) carried *bla*_{CTXM-1} gene.

Amongst the genes coding for aminoglycoside-resistance *aac* (6') *Ib* and *aac* (3) *Ila* were detected in 75% (6/8) and 62.5% (5/8) of isolates respectively and 62.5% were positive for both genes. The prevalence of *aac* (6') *Ib* and *aac* (3) *Ila* was reported 44.6% and 43% respectively in a study from Spain (Fernández et al., 2018) and 84% and 25% respectively in a study from Greece (Galani et al., 2019). In accordance with our study, several studies from Iran found that *aac* (6') *Ib* and *aac* (3) *Ila* were the most common AME genes in aminoglycoside resistant isolates (Nasiri et al., 2018; Harir et al., 2018).

We explored four carbapenemase encoding genes in the current study, the most prevalent of which was *bla*_{OXA-48} (75%) followed by *bla*_{NDM-1} (12.5%). The presence rates of these genes were reported 58% and 2% respectively in a study from Turkey (Aksöz et al., 2015), and 29% and 75% respectively in a study from Egypt (Ragheb et al., 2020). In line with our study, several studies from Iran found that *bla*_{OXA-48} was the most frequent carbapenemase gene followed by *bla*_{NDM-1} (Moghadampour et al., 2018; Jafari et al., 2018). However, studies from south of Iran mostly reported *bla*_{NDM-1} as the main carbapenemase gene (Kiaei et al., 2019; Shoja et al., 2018). This discrepancy may be explained by traffic with neighboring countries. As previously suggested high prevalence of *bla*_{OXA-48} gene among *K. pneumoniae* strains in Tehran might be due dissemination of clones harboring this gene from Turkey (Jafari et al., 2018). Travel of passengers from south of Iran to India, Pakistan, and Bangladesh and vice versa might have caused dissemination of NDM1 positive strains in that part of the country.

In this study, rep-PCR fingerprints distinguished seven isolates into three clusters and one strain was assigned to be sporadic. This pattern of genetic similarity suggests that three clones of *K. pneumoniae* might have been transmitted from patient to patient in hospital, while one patient might have acquired the infection from a distinct clone. Comprehension of mode of transmission is vital for planning effective control schemes for prevention of *K. pneumoniae* dissemination (Granov et al., 2020). Unfortunately, our study suffers from the lack of patients' demographical data. For this reason, we were not able to correlate the result of genotypic epidemiology of strains with the situation in which patients acquired the infection.

CONCLUSION

Our work revealed high resistance to aminoglycoside and medium resistance to carbapenems among *K. pneumoniae* strains isolated from meningitis patients, with the existence of *aac* (6')-*Ib* and *aac* (3)-*Ila*, and *bla*_{OXA-48} genes being primarily responsible for this resistance. This suggests that carbapenems can be considered as first-line drugs for initial empirical treatment of *K. pneumoniae* meningitis before obtaining

susceptibility test results. Low diversity in genetic relationships among isolates could be due to circulation of some clones in hospital which emphasizes on the establishment of effective infection surveillance and prevention program.

Acknowledgment

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Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. Aksöz, E. D. C. A. N. (2015). *Klebsiella pneumoniae*: characteristics of carbapenem resistance and virulence factors. *ABP, Vol. 62 No 4*, 867–874
2. Al Bshabshe, A., Al-Hakami, A., Alshehri, B., Al-Shahrani, K. A., Alshehri, A. A., Al Shahrani, M. B., Hamid, M. E. (2020). Rising *Klebsiella pneumoniae* Infections and Its Expanding Drug Resistance in the Intensive Care Unit of a Tertiary Healthcare Hospital, Saudi Arabia. *Cureus, 12*(8), e10060. doi:10.7759/cureus.10060
3. Choi, M., Hegerle, N., Nkeze, J., Sen, S., Jamindar, S., Nasrin, S., Tennant, S. M. (2020). The Diversity of Lipopolysaccharide (O) and Capsular Polysaccharide (K) Antigens of Invasive *Klebsiella pneumoniae* in a Multi-Country Collection. *Front Microbiol, 11*, 1249. doi:10.3389/fmicb.2020.01249
4. *Clinical and Laboratory Standard Institute*. (2019). Wayne: CLSI document M-100.
5. Compain, F., Babosan, A., Brisse, S., Genel, N., Audo, J., Ailloud, F., Decré, D. (2014). Multiplex PCR for detection of seven virulence factors and K1/K2 capsular serotypes of *Klebsiella pneumoniae*. *J Clin Microbiol, 52*(12), 4377-4380. doi:10.1128/JCM.02316-14
6. El Fertas-Aissani, R., Messai, Y., Alouache, S., & Bakour, R. (2013). Virulence profiles and antibiotic susceptibility patterns of *Klebsiella pneumoniae* strains isolated from different clinical specimens. *Pathol Biol (Paris), 61*(5), 209-216. doi:10.1016/j.patbio.2012.10.004
7. El-Badawy, M. F., Tawakol, W. M., El-Far, S. W., Maghrabi, I. A., Al-Ghamdi, S. A., Mansy, M. S., Shohayeb, M. M. (2017). Molecular Identification of Aminoglycoside-Modifying Enzymes and Plasmid-Mediated Quinolone Resistance Genes among *Klebsiella pneumoniae* Clinical Isolates Recovered from Egyptian Patients. *Int J Microbiol, 2017*, 8050432. doi:10.1155/2017/8050432
8. Ellis, J., Luintel, A., Chandna, A., & Heyderman, R. S. (2019). Community-acquired acute bacterial meningitis in adults: a clinical update. *Br Med Bull, 131*(1), 57-70. doi:10.1093/bmb/ldz023
9. Falagas, M. E., & Kopterides, P. (2007). Old antibiotics for infections in critically ill patients. *Current Opinion in Critical Care, 13*(5), 592-597. doi:10.1097/MCC.0b013e32827851d7
10. Fernández-Martínez, M., Ruiz Del Castillo, B., Lecea-Cuello, M. J., Rodríguez-Baño, J., Pascual, Á., & Martínez-Martínez, L. (2018). Prevalence of Aminoglycoside-Modifying Enzymes in *Escherichia coli* and *Klebsiella pneumoniae* Producing Extended Spectrum β -Lactamases Collected in Two Multicenter Studies in Spain. *Microb Drug Resist, 24*(4), 367-376. doi:10.1089/mdr.2017.0102
11. Forge, A., & Schacht, J. (2000). Aminoglycoside antibiotics. *Audiol Neurootol, 5*(1), 3-22.

12. Galani, I., Nafplioti, K., Adamou, P., Karaiskos, I., Giamarellou, H., & Souli, M. (2019). Nationwide epidemiology of carbapenem resistant *Klebsiella pneumoniae* isolates from Greek hospitals, with regards to plazomicin and aminoglycoside resistance. *BMC Infect Dis*, *19*(1), 167. doi:10.1186/s12879-019-3801-1
13. GelarehNasiri, A., TaghiNaserpourFarivar, Peyman Hosseini. (2018). Molecular epidemiology of aminoglycoside resistance in clinical isolates of *Klebsiella pneumoniae* collected from Qazvin and Tehran provinces, Iran. *Infection, Genetics and Evolution*.
14. Granov, D., Dedić-Ljubović, A., & Salimović-Bešić, I. (2020). Characterization of Carbapenemase-Producing *Klebsiella pneumoniae* in Clinical Center University of Sarajevo, Bosnia and Herzegovina. *Microb Drug Resist*, *26*(9), 1038-1045. doi:10.1089/mdr.2019.0188
15. Grundmann, H., Glasner, C., Albiger, B., Aanensen, D. M., Tomlinson, C. T., Andrasević, A. T., Monnet, D. L. (2017). Occurrence of carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* in the European survey of carbapenemase-producing Enterobacteriaceae (EuSCAPE): a prospective, multinational study. *Lancet Infect Dis*, *17*(2), 153-163. doi:10.1016/s1473-3099(16)30257-2
16. Hou, X. H., Song, X. Y., Ma, X. B., Zhang, S. Y., & Zhang, J. Q. (2015). Molecular characterization of multidrug-resistant *Klebsiella pneumoniae* isolates. *Braz J Microbiol*, *46*(3), 759-768.
17. Khan, F. Y., Abukhattab, M., AbuKamar, M., & Anand, D. (2014). Adult *Klebsiella pneumoniae* meningitis in Qatar: clinical pattern of ten cases. *Asian Pac J Trop Biomed*, *4*(8), 669-672.
18. Kiaei, S., Moradi, M., Hosseini-Nave, H., Ziasistani, M., & Kalantar-Neyestanaki, D. (2019). Endemic dissemination of different sequence types of carbapenem-resistant *Klebsiella pneumoniae* strains harboring bla (NDM) and 16S rRNA methylase genes in Kerman hospitals, Iran, from 2015 to 2017. *Infect Drug Resist*, *12*, 45-54. doi:10.2147/idr.s186994
19. Krause, K. M., Serio, A. W., Kane, T. R., & Connolly, L. E. (2016). Aminoglycosides: An Overview. *Cold Spring Harb Perspect Med*, *6*(6). doi:10.1101/cshperspect.a027029
20. Ku, Y. H., Chuang, Y. C., Chen, C. C., Lee, M. F., Yang, Y. C., Tang, H. J., & Yu, W. L. (2017). *Klebsiella pneumoniae* Isolates from Meningitis: Epidemiology, Virulence and Antibiotic Resistance. *Sci Rep*, *7*(1), 6634. doi:10.1038/s41598-017-06878-6
21. Li, B., Zhao, Y., Liu, C., Chen, Z., & Zhou, D. (2014). Molecular pathogenesis of *Klebsiella pneumoniae*. *Future Microbiol*, *9*(9), 1071-1081. doi:10.2217/fmb.14.48
22. Lu, C. H., Huang, C. R., Chang, W. N., Chang, C. J., Cheng, B. C., Lee, P. Y., Chang, H. W. (2002). Community-acquired bacterial meningitis in adults: the epidemiology, timing of appropriate antimicrobial therapy, and prognostic factors. *Clin Neurol Neurosurg*, *104*(4), 352-358.
23. Mahsa Harir Foroush M.Sc., L. S. P. D. M. M. D. (2018). Prevalence of Genes Encoding Aminoglycoside Modifying Enzymes in Clinical Isolates of *Klebsiella Pneumoniae* in the Hospitals of Borujerd *I J M L*, *5*(1), 35-41.
24. Moghadampour, M., Rezaei, A., & Faghri, J. (2018). The emergence of blaOXA-48 and blaNDM among ESBL-producing *Klebsiella pneumoniae* in clinical isolates of a tertiary hospital in Iran. *Acta Microbiol Immunol Hung*, *65*(3), 335-344. doi:10.1556/030.65.2018.034
25. Mohammad Mehdi Feizabadi, S. D., Nafiseh Raji, Araz Majnooni, Marzieh Aligholi, Fereshteh Shahcheraghi, Mahmood Parvin, and Davud Yadegarinia. (2010). Distribution of blaTEM, blaSHV, blaCTX-M Genes Among Clinical Isolates of *Klebsiella pneumoniae* at Labbafinejad Hospital, Tehran, Iran. *MICROBIAL DRUG RESISTANCE, Volume 16, Number 1*.
26. Mokhtari, H., Eslami, G., Zandi, H., Dehghan-Banadkouki, A., & Vakili, M. (2018). Evaluating the Frequency of aac(6)-IIa, ant(2'')-I, int11, and int12 Genes in Aminoglycosides Resistant *Klebsiella*

- pneumoniae* Isolates Obtained from Hospitalized Patients in Yazd, Iran. *Avicenna J Med Biotechnol*, 10(2), 115-119.
27. Nielsen, J. B., Skov, M. N., Jørgensen, R. L., Heltberg, O., Hansen, D. S., & Schønning, K. (2011). Identification of CTX-M15-, SHV-28-producing *Klebsiella pneumoniae* ST15 as an epidemic clone in the Copenhagen area using a semi-automated Rep-PCR typing assay. *Eur J Clin Microbiol Infect Dis*, 30(6), 773-778. doi:10.1007/s10096-011-1153-x
 28. Paczosa, M. K., & Meccas, J. (2016). *Klebsiella pneumoniae*: Going on the Offense with a Strong Defense. *Microbiol Mol Biol Rev*, 80(3), 629-661. doi:10.1128/membr.00078-15
 29. Paterson, D. L., & Bonomo, R. A. (2005). Extended-spectrum beta-lactamases: a clinical update. *Clin Microbiol Rev*, 18(4), 657-686. doi:10.1128/cmr.18.4.657-686.2005
 30. Queenan, A. M., & Bush, K. (2007). Carbapenemases: the versatile beta-lactamases. *Clin Microbiol Rev*, 20(3), 440-458, table of contents. doi:10.1128/cmr.00001-07
 31. Ragheb, S. M., Tawfick, M. M., El-Kholy, A. A., & Abdulall, A. K. (2020). Phenotypic and Genotypic Features of *Klebsiella pneumoniae* Harboring Carbapenemases in Egypt: OXA-48-Like Carbapenemases as an Investigated Model. *Antibiotics (Basel)*, 9(12). doi:10.3390/antibiotics9120852
 32. Rastegar, S., Moradi, M., Kalantar-Neyestanaki, D., Ali Golabi, D., & Hosseini-Nave, H. (2019). Virulence Factors, Capsular Serotypes and Antimicrobial Resistance of Hypervirulent *Klebsiella pneumoniae* and Classical *Klebsiella pneumoniae* in Southeast Iran. *Infect Chemother*.
 33. Scheld, W. M., Koedel, U., Nathan, B., & Pfister, H. W. (2002). Pathophysiology of bacterial meningitis: mechanism(s) of neuronal injury. *J Infect Dis*, 186 Suppl 2, S225-233. doi:10.1086/344939
 34. Shi, W., Li, K., Ji, Y., Jiang, Q., Wang, Y., Shi, M., & Mi, Z. (2013). Carbapenem and cefoxitin resistance of *Klebsiella pneumoniae* strains associated with porin OmpK36 loss and DHA-1 β -lactamase production. *Braz J Microbiol*, 44(2), 435-442. doi:10.1590/s1517-83822013000200015
 35. Shoja, S., Ansari, M., Faridi, F., Azad, M., Davoodian, P., Javadpour, S., Karmostaji, A. (2018). Identification of Carbapenem-Resistant *Klebsiella pneumoniae* with Emphasis on New Delhi Metallo-Beta-Lactamase-1 (blaNDM-1) in Bandar Abbas, South of Iran. *Microb Drug Resist*, 24(4), 447-454.
 36. Tang, L. M., & Chen, S. T. (1994). *Klebsiella pneumoniae* meningitis: prognostic factors. *Scand J Infect Dis*, 26(1), 95-102. doi:10.3109/00365549409008596
 37. Tang, L. M., Chen, S. T., Hsu, W. C., & Chen, C. M. (1997). *Klebsiella meningitis* in Taiwan: an overview. *Epidemiol Infect*, 119(2), 135-142. doi:10.1017/s0950268897007930
 38. Wasfi, R., Elkhatib, W. F., & Ashour, H. M. (2016). Molecular typing and virulence analysis of multidrug resistant *Klebsiella pneumoniae* clinical isolates recovered from Egyptian hospitals. *Scientific Reports*, 6, 38929-38929. doi:10.1038/srep38929
 39. Wong, Y. P., Chua, K. H., & Thong, K. L. (2014). One-step species-specific high resolution melting analysis for nosocomial bacteria detection. *J Microbiol Methods*, 107, 133-137.
 40. Xu, M., Fu, Y., Fang, Y., Xu, H., Kong, H., Liu, Y., Li, L. (2019). High prevalence of KPC-2-producing hypervirulent *Klebsiella pneumoniae* causing meningitis in Eastern China. *Infect Drug Resist*, 12, 641-653.
 41. Zeinab Jafari, A. A. H., Mehri Haeili, Jalil Kardan-Yamchi, Sirous Jafari, Fereshteh Jabalameli, Alipasha Meysamie, Alireza Abdollahi, and Mohammad Mehdi Feizabadi. (2018). Molecular Epidemiology and Drug Resistance Pattern of Carbapenem-Resistant *Klebsiella pneumoniae* Isolates from Iran. *MICROBIAL DRUG RESISTANCE, Volume 00, Number 00*.

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