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First Report of an Edible Mushroom, *Termitomyces umkowaan* from Punjab, Pakistan

Ukasha Iqbal[1]*, Nousheen Yousaf[1], Alishba Chudhrey[1], Shumaila Rasheed[1], Muhammad Hanif[1], Samina Sarwar[2]

1. Department of Botany, Government College University Lahore, Katchery Road, 54000, Lahore, Pakistan
2. Department of Botany, Lahore College for Women University, Lahore, Pakistan

Corresponding Author's Email: iqbalukasha09@gmail.com

ABSTRACT: *During the fungal forays for the collection of macro-fungi from different districts of Punjab province of Pakistan three specimens of Termitomyces were collected in the rainy seasons of the year 2021 and 2022s. In current study, micro and macroscopic characterization of collected samples are done and taxonomic description, macrographs of basidiomata, illustrations and light micrographs of microscopic features are given. Morpho-anatomical comparison with similar species is also provided. After detailed morpho-anatomical studies, these specimens were recognized as T. umkowaan, which an edible taxon, and first time reported from two districts (Khanewal and Lahore) of Pakistan.*

Keywords: Biodiversity, Termitomyces, Mushroom, Pakistan, Taxonomy

INTRODUCTION

Termitomyces, a group of gilled mushrooms that coexist with termites in a mutualistic or symbiotic manner, was first described in 1942 (Heim, 1942). Termitomyces is an agaric genus that termites have created, and basidiomata are found developing near termite nests (Izhar et al., 2020). By employing plant material that passes through the stomachs of eusocial insects (termites) of the subfamily Macrotermitinae (Isoptera), they are propagated (Karun & Sridhar, 2013). Termite farming of Termitomyces began in rain forests of Africa as the primary centre before spreading to Asia and Madagascar. However, molecular analyses have shown that the Termitomyces in Africa and Asia does not share any identical sequences (Frøslev et al., 2003; Karun & Sridhar, 2013), suggesting their geographical dissimilarity as well as a potential distinct evolution. There is an ongoing discussion about the complex co-evolution of termites and Termitomyces, including their life cycles, sexual and asexual phases (Nobre and Aanen, 2012). Termites create a structure called the comb (fungus garden), which shows similarity to an external rumen, by gathering asexual spores of fungus together with bacterial origin through faeces, foraged plant material and lignocellulolytic enzymes of fungal (Nobre and Aanen, 2012; Karun and Sridhar, 2013).

These mushrooms are palatable and valued for their chemical and nutritional properties in addition to their texture and flavour (Batra and Batra, 1979; Chakraborty et al., 2006). Protein content in Termitomyces mushroom ranges from 15.1 to 19.1 g/100 g, lipids range from 2.5 to 5.4 g/100 g, crude fiber ranges from 17.5 to 24.7 g/100 g, and minerals range from 2.4 g/100 g (Kansci et al., 2003; Hussain et al., 2015).

Termitomyces are a naturally occurring resource that is economically useful and can be used in place of foods derived from plants and animals. In addition to nutritional value, members of Termitomyces also have industrial uses and therapeutic qualities (Tibuhwa, 2012). For instance, Termitomyces clypeatus R. Heim 1951, has significant levels of ascorbic acid (10–14%), antioxidants, proteins (31%), and carbohydrates (32%) (Ogundana and Fagade, 1982; Karun and Sridhar, 2013). Members of this genus are also a source of enzymes mainly in culture media that are valuable as food additives, leavening agents for bread, silage processors, and in other industrial processes (such as the clarification

of fruit juices other than citrus) (Khowala et al., 1992; Karun and Sridhar, 2013).

From southern and southeastern Asia to all sub-Saharan regions including America, Cameroon, India, Indonesia, Pakistan this genus is distributed widely. Out of 38 validated species of genus Termitomyces by Index Fungorum only seven species of this genus have been reported from Pakistan (Izhar et al., 2020).

MATERIALS AND METHODS

Sample collection

To explore fungal diversity, extensive field surveys were conducted, and mushroom specimens were collected from two different sampling sites. During the years, 2021 and 2022, 3 collections of this species were made from two districts (Khanewal and Lahore) of Punjab, Pakistan. The semi-arid conditions of Lahore and the subtropical desert-type climate of Khanewal are two places with quite variable weather patterns. After collection of mushrooms, voucher number was given in the field and photographs from different angles were captured. A lot of care was taken while collecting basidiocarps to avoid any harm to them.

Macroscopic Identification of Mushroom

Different morphological characteristics such presence or absence of annulus, pileus (size, shape, ornamentation), lamellae (spacing, attachment to the stipe), stipe (size, shape), odor etc., were recorded in the field. However, detailed notes and macro-morphological descriptions were prepared after macroscopic examination of specimens in the lab by observation of their macrographs.

Microscopic Identification of Mushroom

Anatomical investigation was done by preparing the slides of each part of mushrooms using 5 % aqueous KOH or 1 % Congo red as mounting media. Microscopy was done at 40× and 100x magnification under binocular Labomed microscope. Different anatomical characters such as Basidia, cystidia, pileal hyphae/ elements, stipe hyphae and basidiospores were observed (Wei et al., 2009). About 20 readings for basidiospores and ten readings for other microscopic characters were noted down using ocular micrometry technique. Quotient and average values for basidiospores were also calculated and included in the microscopic description.

RESULTS

Termitomyces umkowaan **(Cooke and Massee) D.A. Reid [as 'umkowaani'], Contr. Bolus Herb. 7: 118 (1975)**

= *Schulzeria umkowaan* (Cooke and Massee) Sacc., Syll. fung. (Abellini) 9: 11 (1891)

= *Agaricus umkowaan* Cooke and Massee [as 'umkowaani'], in Cooke, Grevillea 17(no. 83): 70 (1889)

Macroscopic characterization

Basidiocarps 2.8–6.5 cm long, epigeous, white to bone white, soft, fleshy. **Pileus** 2.7–5.5 cm wide, shiny, silky, smooth, thick, fleshy, initially campanulate or convex, later upturned with conical umbo center, white base with brown to light greyish center, moist, margins split and forming star like shape at maturity, fibrillose, shiny. **Lamellae** bone white, adnexed to free, regular, broad, wavy edges, closed to crowded. **Stipe** 3.9–9.7 × 0.8–1.7 cm, whitish, fibrillose, cylindrical, central, solid, stuffed, smooth, soft, slightly bulbous base. **Annular ring & rhizomorphs** not observed. **Odor** pleasant. **Volva** absent. **Taste** not recorded (Fig. 1 and Fig. 2).

Microscopic characterization

Basidiospores 2.8–8.7 × 2.8–5.6 µm, Q= 1–2, Q_{Avg}= 1.5, ellipsoid to ovoid, apical pore present, thick-walled, oil droplets, hyaline in 5 % KOH. **Basidia** 25.6–34.4 × 6.4–10 µm, outer surface smooth, clavate to slightly clavate, hyaline in 5 % KOH, thick-walled, abundant, 2 to 4 sterigmate. **Cheilocystidia** 26.9–34.4 × 17.4–19.9 µm, clavate to slightly clavate, thin-walled. **Pleurocystidia** 22.7–25.6 × 5.6–11.6 µm, broadly clavate, hyaline in 5 % KOH. **Pileipellis** 6.3–14.4 µm, thick-walled, frequently septate, branched, hyaline in 5 % KOH. **Stipitipellis** 6.4–7.8 µm, thick-walled, broad to narrow, smooth, branched, septate, hyaline in 5 % KOH. **Clamp connections** present (Fig. 3 and Fig. 4).

Material Examined: PAKISTAN: Punjab province**, 2 Collections from District Lahore: 1: AC–4** found growing scattered on the soil in Shalimar Garden Lahore, 3rd September 2021 (Collector: Alishba Chudhery). **2: AC–5** found growing scattered near pond of Bara-Dari Lahore, 2nd September 2021 (Collector: Alishba Chudhery); **1 Collection from District Khanewal**: **MC–67** found scattered on grassy ground in Mian Channu, 29th September 2022 (Collector: Ukasha Iqbal).

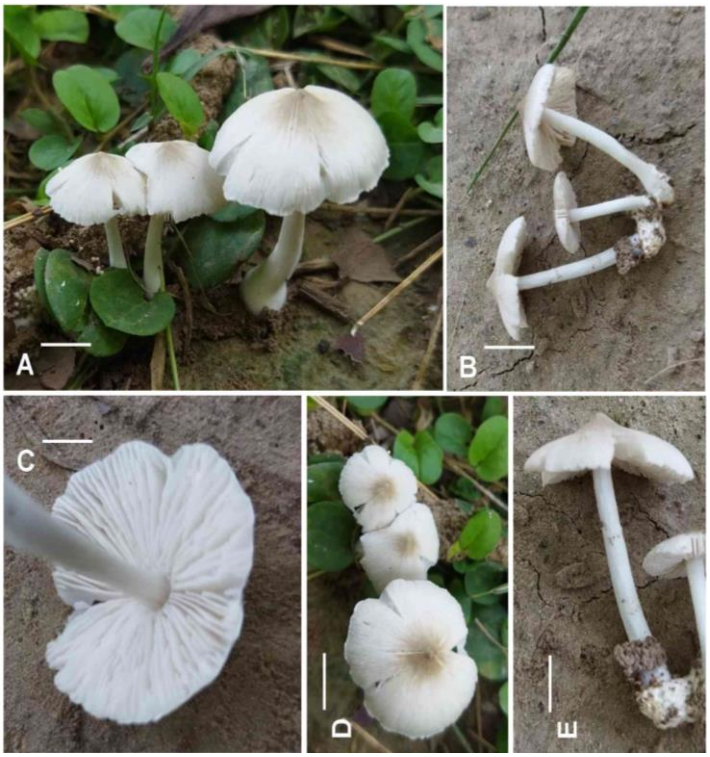

Fig. 1. A–E. Morphology of *Termitomyces umkowaan* (MC–67). A & B. Basidiomata. C. Gills. D. Pileus. E. Stipe. Scale bar: A = 1.5 cm, B = 6.5 cm, C = 1 cm, D & E = 2 cm

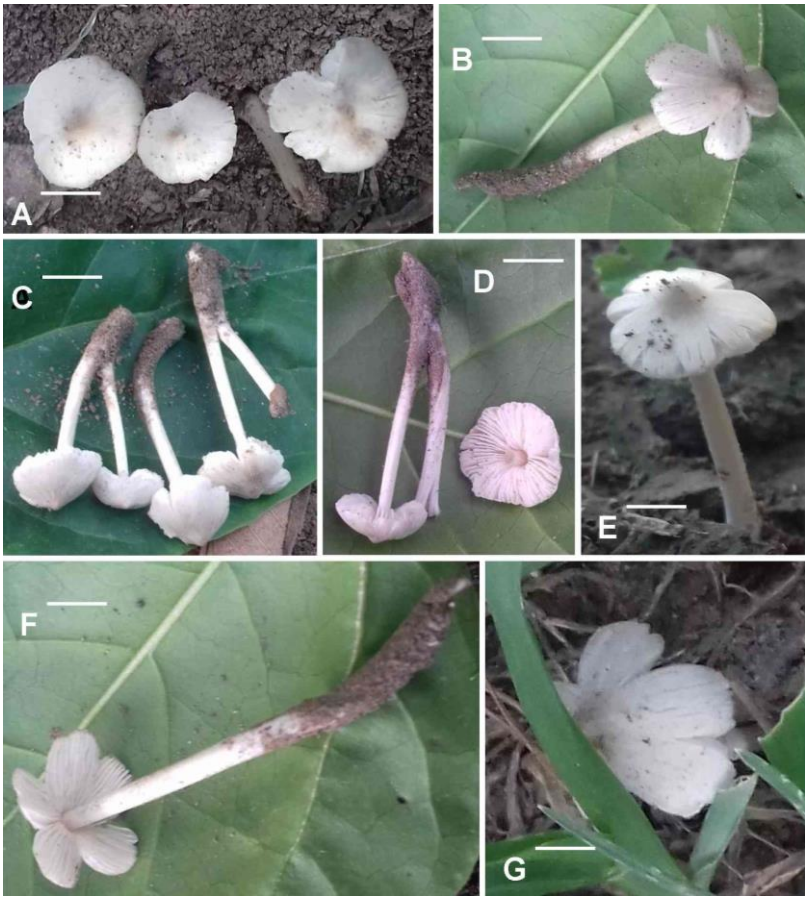

Fig. 2. (A–G) Morphology of *Termitomyces umkowaan*. A, C & D. AC–4. B, E–G. AC–5. Scale bar: A & B = 2 cm, C & D = 4 cm, E = 1.5 cm, F = 2.5 cm, G = 1 cm

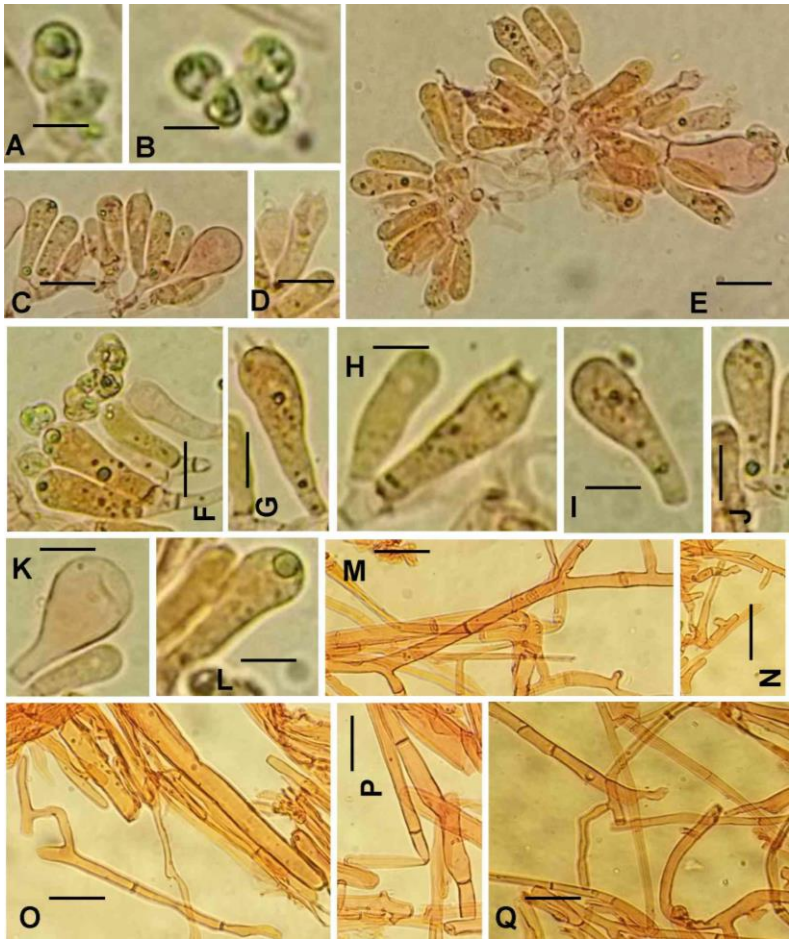

Fig. 3. (A–Q) Light micrographs of microscopic features of *Termitomyces umkowaan* A & B. Basidiospores. C & E. Portion of hymenium showing Basidia and cistidia. D, F–H, J. Basidia. I & L. Pleurocystidia K. Cheilocystidia. O & P. Pileipellis. M, N & Q. Stipitipellis. Scale bars: A = 8.5 µm, B = 8 µm, C = 3 µm, D = 16 µm, E = 21 µm, F = 13.5 µm, G = 12 µm, H = 10 µm, I & J = 11 µm, K = 17 µm, L = 10.5 µm, M & Q = 36 µm, N = 72 µm, O & P = 34 µm

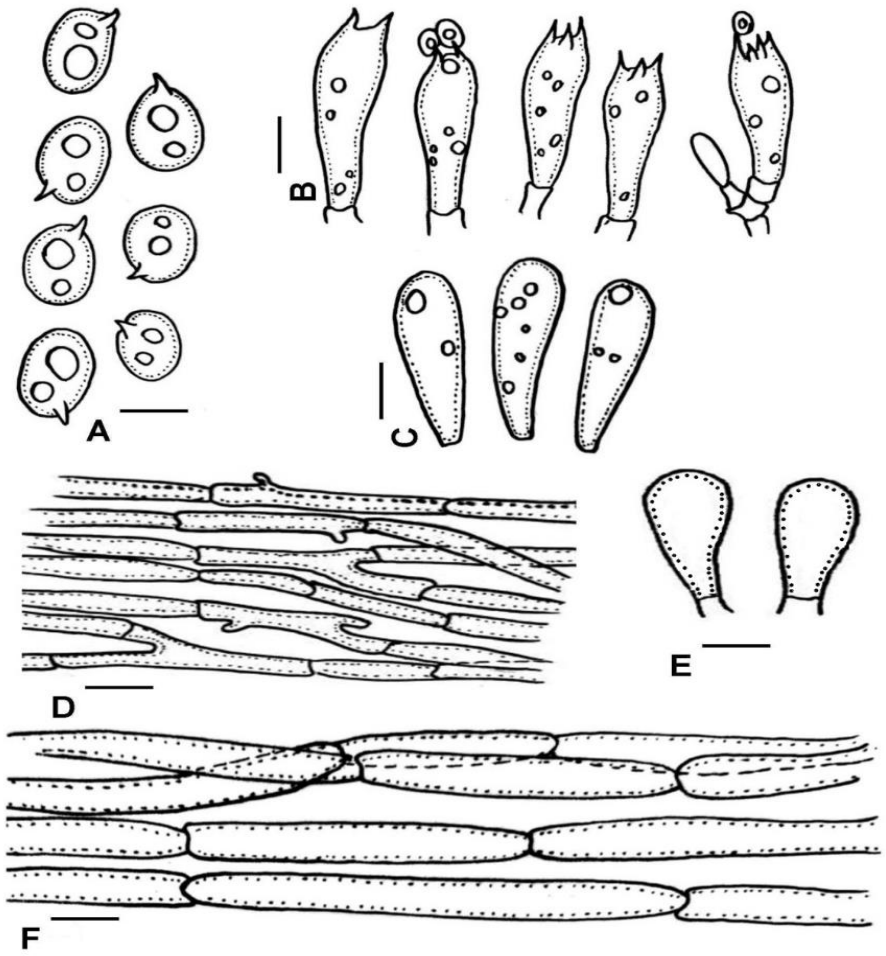

Fig. 4. (A–F) Illustrations of microscopic features of *Termitomyces umkowaan* A. Basidiospores. B. Basidia. C. Pleurocystidia. D. Stipitipellis. E. Cheilocystidia. F. Pileipellis. Scale bars: A = 7 µm, B = 11.7 µm, C = 10 µm, D = 36 µm, E = 23 µm, F = 20 µm

A detailed morpho-anatomical comparison of *T. umkowaan* with closely related species of the genus is given in Table 1.

Table 1: Comparison of *Termitomyces umkowaan* with closely related species of same section

Characters	Geographical location	Morphological characters			Anatomical characters			References
		Pileus	Stipe	Gills	Basidiospores	Basidia	Cystidia	
1. Termitomyces umkowaan	Punjab, Pakistan	2.8–5.6 cm wide, white with light greyish, brown center, initially campanulate then convex	4.5–9.6 × 0.9–1.7 cm, whitish, central, cylindrical	approximate to adnexed and free, white, broad, cloased to crowded, regular, wavy edges	2.9–8.6 × 2.9–5.7 µm, ellipsoid to ovoid	25.7–34.2 × 6.3–10 µm, clavate	Cheilocystidia 27.1–34.2 × 17.1–20 µm, Pleurocystidia 22.8–25.7 × 5.7–11.4 µm	Current study
2. Termitomyces microcarpus	China	1.4–3.4 cm, conical to applanate at maturity, white surface, grey, brown at centre, glabrous, margin splits when mature, smooth	1.7–6.4× 0.3–0.6 cm, cylindrical, central, white surface, smooth, solid, fibrous	white at first become pink at maturity, crowded	5.6–8.3 × 3.6–5.7 µm, ellipsoid to ovoid	16.3–25 × 6.3–8.0 µm, clavate	Cheilocystidia 14.8–46 × 9.4–19 µm, Pleurocystidia 22–43 × 9.4–26.3 µm	Wei et al. (2009)
3. Termitomyces heimii	China	1.5–8.9 cm, subglobose, grey to dark brown at centre, white towards margin, splitting radially	2.3–7.9 × 1.3–2.4 cm, central, conical above annulus and cylindrical below, white, annulate surface, soft to solid, fibrous	free, white, wide, crowded	6.2–8.9 × 4.3–6.4 µm, ellipsoid to ovoid	18.0–29 × 6.0–8.5 µm, clavate	Cheilocystidia 21–35 × 14.0–20 µm, Pleurocystidia 20–34 × 11.0–18.0 µm	Wei et al. (2009)

4. Termitomyces acriumbonatus	Punjab, Pakistan	15–30 mm, convex, fleshy, creamy white with greyish lines radiating towards margin	free, edges serrate, thick, light grey	centrally attached, smooth, cylindrical, smooth, whitish grey to light–gray	6.3–8.5 × 4.6–6.6 µm, globose to ellipsoid	15–22 × 7–8 µm, narrowly clavate to clavate	Cheilocystidia 15–29 × 5.4–7.3 µm, Pleurocystidia 15.2–24.3 × 4.6–8.4 µm	Usman et al. (2020)
5. Termitomyces sheikhupurensis	Punjab, Pakistan	1.8–3 cm, conical to plano–convex at maturity, light brown fading towards margin, dull orange near margins	3.4–4.3 × 0.3–0.6 cm, solid, subcylindrical from upper part, strigose, surface pale yellow	dull orange, free, distant, crisped near margins, regular	5.7–7.9 × 4.7– 7.9 µm, ellipsoid to ovoid	18–26 × 9.3–12.4 µm, clavate	Cheilocystidia 17.3–34.2 × 6–13µm, Pleurocystidia 15.9–33.3 × 7.8–12 µm	Izhar et al. (2020)

DISCUSSION

The mushroom genus *Termitomyces* Heim (1942) is distinguished by a prominent umbo called a perforatorium and the presence of a pseudorrhiza. *Termitomyces* species can be recognized by their fleshy agaricoid fruiting bodies, pluteoid carpophores, which frequently have a distinct, obvious umbo, free to annexed lamellae despite having a decurrent tooth stipe, and underground pseudorhiza linked to termite nests (only a few species are deficient in this characteristic). *Termitomyces* species often have spheroid, smooth, inamyloid basidiospores, monomitic tramal system, inamyloid hyphae, and clamp connections as part of their anatomical makeup. (Frøslev et al., 2003; Aryal and Budathoki, 2015; Tang et al., 2020). A mutualistic or symbiotic relationship exists between *Termitomyces* and termites. The termites employ the *Termitomyces* colonies as "fungus gardens" in their nests, and the fungi provide the termites with food by decomposing the lignin and cellulose of plant material. There have been eighty-one (81) members published under the name *Termitomyces*, forty (40) of which are listed in the Dictionary of Fungi (Kirk et al., 2008; Razaq et al., 2023). It is also clear that some of the six (6) taxa of *Termitomyces* are found in Pakistan, although the information on these species' endemism is not properly reported (Sultana et al., 2011; Razaq et al., 2023).

Termitomyces umkowaan, collected and described in current study has already been reported from district Malakand of KP province, Pakistan (Hussain et al., 2015). However, it is reported for first time from two districts (Khanewal and Lahore) of Punjab province, Pakistan.

T. umkowaan (Cooke and Massee) D.A. Reid, can be confused with *Termitomyces microcarpus* (Berk and Broome) R. Heim, in appearance (both have white stipe) and both these species grow in clusters and groups. Basidiospores and basidia shape is same in both species but size vary. Both have ellipsoid to ovoid spore shape but latter basidiospores (5.6–8.3 × 3.6–5.7 µm) are more in size as compared to former (2.9–8.6 × 2.9–5.7 µm). *T. umkowaan* basidia are more elongated (25.7–34.2 × 6.3–10 µm) than *T. microcarpus* (16.3–25 × 6.3–8.0 µm). These mushrooms are edible (Wei et al., 2009). *T. umkowaan* is closely related to *Termitomyces pakistanensis* Razaq due to similar appearance (white color) and umbonate pileus with brittle margins. Both can be different from basidiocarps size at maturity, latter is smaller in size (rarely 2 cm). *T. umkowaan* has pseudorhiza of 10 cm length and large pileus size up to 13 cm which make it different from *T. pakistanensis* (Hussain et al., 2015; Razaq et al., 2023).

CONCLUSION

It is concluded that identified specimens of *Termitomyces* were collected from 2 districts of Punjab, with varying climatic conditions ranging from semi-arid (Lahore) to hot desert (Khanewal). Current study showed taxon from 2 districts of Punjab province, Pakistan.

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ETHICAL APPROVAL

The study have been approved by the Department of Botany, Government College University Lahore, Katchery road, 54000, Lahore, Pakistan.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

REFERENCES

1. Aryal HP, Budathoki U (2015). Systematics of Nepalese *Termitomyces*. Our Nature. 13: 31-44. http://dx.doi.org/10.3126/on.v13i1.14207
2. Batra LR, Batra SWT (1979). Termite–fungus mutualism. Batra LR, Batra SWT editors. Insect-fungus symbiosis. New York, USA. https://lccn.loc.gov/78020640
3. Chakraborty I, Mondal S, Rout D, Islam SS (2006). A water-insoluble (1→ 3)-β-d-glucan from the alkaline extract of an edible mushroom *Termitomyces eurhizus*. Carbohydr. Res. 341: 2990-2993. https://doi.org/10.1016/j.carres.2006.09.009
4. FrØSlev TG, Aanen DK, Laessøe T, Rosendahl S (2003). Phylogenetic relationships of *Termitomyces* and related taxa. Mycol. Res. 107: 1277-1286. https://doi.org/10.1017/S0953756203008670
5. Heim R (1942). New descriptive studies on *termitophilous agarics* of tropical Africa. J. Nat. Hist. 6: 107-166.

https://sciencepress.mnhn.fr/en/periodiques/archives-serie6/18/1/2

6. Hussain S, Afshan NS, Ahmad H, Khalid AN (2015). New report of edible mushroom, *Termitomyces umkowaan* from Pakistan. Sylwan. 159: 185-197. https://www.researchgate.net/publication/292158545_Title_New_Report_of_edible_mushroom_Termitomyces_umkowaan_from_Pakistan
7. Izhar A, Khalid AN, Bashir H (2020). *Termitomyces sheikhupurensis* sp. nov.(Lyophyllaceae, Agaricales) from Pakistan, evidence from morphology and DNA sequences data. Turk. J. Bot. 44: 694-704. https://journals.tubitak.gov.tr/cgi/viewcontent.cgi?article=1069&context=botany
8. Kansci G, Mossebo DC, Selatsa AB, Fotso M (2003). Nutrient content of some mushroom species of the genus *Termitomyces* consumed in Cameroon. Food/Nahrung. 47: 213-216. https://pubmed.ncbi.nlm.nih.gov/12866626/
9. Karun NC, Sridhar KR (2013). Occurrence and distribution of *Termitomyces* (Basidiomycota, Agaricales) in the Western Ghats and on the west coast of India. Ceska. Mykol. 65: 233-254. http://www.czechmycology.org/_cmo/CM65207.pdf
10. Khowala S, Ghosh AK, Sengupta S (1992). Saccharification of xylan by an amyloglucosidase of *Termitomyces clypeatus* and synergism in the presence of xylanase. Appl. Microbiol. Biotechnol. 37: 287-292. https://link.springer.com/article/10.1007/BF00210979
11. Kirk PM, Cannon PF, Minter DW, Stalpers JA (2008). Dictionary of Fungi, 10th edn. CABI press, UK. https://www.researchgate.net/publication/311424562_Dictionary_of_the_Fungi10th_ed
12. Nobre T, Aanen DK (2012). Fungiculture or Termite Husbandry? The Ruminant Hypothesi. Insects. 3: 307-323.
13. Ogundana SK, Fagade OE (1982). Nutritive value of some Nigerian edible mushrooms. Food chem. 8: 263-268. https://doi.org/10.1016/0308-8146(82)90028-0
14. Razaq A, Ishaq A, Ilyas S, Niaz S, Sadia S (2023). *Termitomyces pakistanensis*, a new mushroom species from Pakistan based on scanning electron microscopy and ITS-rDNA barcoding. Microsc. Res. Tech. 86: 115-121. https://doi.org/10.1002/jemt.24265
15. Sultana K, Rauf CA, Riaz A, Naz F, Irshad G, Haque MI (2011). Checklist of agarics of Kaghan valley. Pak. J. Bot. 43: 1777-1787.
16. Tang SM, He MQ, Raspe O, Luo X, Zhang XL, Li YJ, Su KM, Li SH, Thongklang N, Hyde KD (2020). Two new species of *Termitomyces* (Agaricales, Lyophyllaceae) from China and Thailand. Phytotaxa. 439: 231-242. 10.11646/PHYTOTAXA.439.3.5
17. Tibuhwa DD (2012). *Termitomyces* species from Tanzania, their cultural properties and unequalled basidiospores. J. Bio. Life Sci. 3:

2157-6076. http://dx.doi.org/10.5296/jbls.v3i1.1723

18. Usman M, Khalid AN (2020). *Termitomyces acriumbonatus* sp. nov. (Lyophyllaceae, Agaricales) from Pakistan. Phytotaxa. 477: 217-228.
19. Wei TZ, Tang BH, Yao YJ (2009). Revision of *Termitomyces* in China. Mycotaxon. 108: 257-285.

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Assessment of Reproductive and Renal Profiles in Photocopier Operators: Implications of Occupational Exposure to Emitted Pollutants

Ayesha Zahoor, Yaqut Umm-E-Hani, Kaleem Maqsood*, Farwa Liaqat, Husna Ahmad, Nabila Roohi

1. Institute of Zoology University of the Punjab Lahore -54590

Corresponding Author's Email: kaleemmaqsood4@gmail.com

ABSTRACT: *Photocopy machines emit several harmful pollutants that include ozone, VOCs, Ultraviolet, particulate matter and fumes of heavy metals. The operators are exposed to all these toxic toner components. The objective of the current investigation was to assess the effects of these emitted pollutants on reproductive and renal profile of photocopiers. For this study, photocopy operators (n=40) being occupationally exposed to photocopier emissions and non-exposed healthy controls (n=25) were recruited fulfilling the inclusion criteria. Serum level of testosterone and renal biomarkers of all the participants were evaluated. Independent sample "t"-test was applied at significance level P<0.05 by using GraphPad Prism. Significant decrease in testosterone levels in the photocopier group (5.19 ± 0.19) compared to the control group (8.04 ± 0.22, P < 0.001) was observed. The renal profile revealed notable increases in serum uric acid and creatinine levels in the photocopier group, with uric acid at 4.76 ± 0.18 versus 3.91 ± 0.10 in the control group (P < 0.001) and creatinine at 0.93 ± 0.04 compared to 0.78 ± 0.03 in the control group (P < 0.02). Additionally, serum albumin levels were significantly lower in the exposed group (3.63 ± 0.10) compared to the control group (4.47 ± 0.11, P < 0.001). The potential acute or chronic kidney disorders and reproductive damage associated with exposure in the photocopier workers.*

Keywords: Testosterone, Renal Profile, Photocopiers, Hypogonadism, Occupational exposure

INTRODUCTION

Photocopy machines effortlessly and affordably generate paper duplicates of documents and various visual images (Pakpahan et al., 2019). In advanced nations, the rapid progress in home and office automation has elevated the utilization of photocopiers (Gminski et al., 2011). Numerous individuals worldwide are employing photocopiers, irrespective of the economic advantages (Elango et al., 2013a).

In nations undergoing development, like Pakistan, a significant number of photocopy shops operate without constraints or safety control steps (Nandan et al., 2020). In photocopiers, dry toner is used, which include polycyclic aromatic hydrocarbons and styrene (Saritha et al., 2016) which are potential human carcinogens (Lee et al., 2006). The operators are exposed to all these toxic toner components (Saritha et al., 2016). This situation can potentially pose serious risks to the health of the workers (Lyu et al., 2021). The substances within photocopiers that pose health risks encompass VOCs, ozone, formaldehyde, particulates, heavy metals, nitrogen oxides, carbon monoxide, UV light (Hunashal, 2011) carbon black (CB), PAHs and heavy metals including zinc, Fe, Cr, and Ni (Gminski et al., 2011; Henschel et al., 2001).

Subjection to these chemicals can cause many damages, including irritation of lung tissues, eye irritation, headaches, and itching of the skin (Bai et al., 2010). VOCs especially can cause discomfort (Sarkhosh et al., 2012) even at very low concentration in photocopy workers. While prolonged Exposure may result in symptoms such as headaches, shortness of breath, allergies, fatigue, nausea, mental confusion, and the potential development of cancer (El-Hashemy and Ali, 2018).

The reproductive health of adults is of growing concern of this age (Gubhaju, 2002). In males, reproductive hormone, testosterone production is essential for the maintenance of sexual characteristics and the development of sex organs (Aydogdu and Swerdloff, 2016). In man, low levels of sex hormones associated with infertility are referred to as hypogonadism (Basaria, 2014). Extended exposure to photocopier toner powder may pose a risk of anemia due to potential renal damage (Osadolor and Ezegbogu, 2015). Furthermore, the nephrons exhibit sensitivity to exposure to lead, cadmium and mercury leading to persistent renal failure (Kum et al., 2014). In abnormal condition of

kidney, amount of creatinine released via urine reduced and accumulated in blood. Elevated urea concentration in comparison to creatinine levels may indicate the presence of certain kidney issues. When there is an elevation of urea levels in serum as compared to creatinine, some kidney malfunctioning may arise (Levey et al., 1999) and these renal impairments resulted in uremia (Hawkins and Dugaiczyk, 1982).

MATERIALS AND METHODS

This study was approved by the ethical review committee of the Institute of Zoology, University of the Punjab, Lahore. Informed consent was obtained from all participants. A questionnaire was designed and distributed before blood sampling to measure their BMI, age, medical record and other details related to occupational exposure of subjects.

The research involved 65 participants, consisting of 25 healthy male subjects of same age groups as controls and 40 male workers recruited from different photocopy shops present in Punjab University, Lahore. The subjects in both groups having any medical history were excluded from the study. All healthy and hygienic measures have opted as the investigation involved human sampling. Participants of the investigation were in good health condition. A registered technician was engaged for blood sampling. Blood samples (5cc) were collected after 12 hours of fasting in the morning. The blood was allowed to coagulate by placing it in a clot activator vial for almost 30 minutes at a temperature of 25°C. Afterwards, the blood underwent serum separation through centrifugation at 3000rpm. Using a micropipette, the serum was carefully transferred to a labelled plastic Eppendorf tube, taking approximately 10 minutes. Subsequently, the collected serum was stored at -80°C for further analysis.

The ELISA technique was employed for hormonal analysis. Specifically, the testosterone estimation assay was conducted on serum samples from the subjects using a PerkinElmer ELISA kit. The sample was allowed to thaw for 10 minutes at 25°C prior to utilization. Renal profile (Urea, Uric acid, Creatinine and Albumin) was measured by commercially available kits of Monlab, Spain using photometer (5010) *v5plius* ROBERT RIELE GmbH & Co KG, Germany.

Statistical Analysis

Data obtained from control and exposed group subjects have been analyzed through a two-tailed

independent sample t-test at a significance level of $P<0.05$ by using software GraphPad Prism-5 and presented as mean±SEM.

RESULTS

No significant difference was observed in the Body Mass Index (BMI) between the exposed and control groups (Fig. 1). While a highly prominent decrease ($P<0.001$) of 43% in serum testosterone level in photocopiers exposed group was observed when compared to the control group (Fig. 2). Among the renal profile, in the photocopiers exposed group, serum urea concentration was non-significantly high when compared to control group (Fig. 3). Serum uric acid concentration in the photocopier's exposed group was increased significantly ($P<0.001$) by 19% on comparing with control group (Fig. 4). While serum creatinine level in photocopiers exposed group was decreased significantly ($P<0.05$) by 11% while comparing with control group (Fig. 5) as well as serum albumin level was also significantly high ($P<0.001$) with an increase of 14% in exposed group (Fig. 6) (Table: 1).

Table 1: Overall Comparison of Body Mass Index (BMI), Testosterone and Renal parameters in Control and Exposed Group

Parameters	**Mean±SEM**		***P*-value**	**Percentage difference**
	Control (25)	**Exposed (n=40)**		
BMI (Kg/m^2)	23.21 ± 0.58	22.77 ± 0.35	0.49	2↓
Testosterone (ng/mL)	8.04 ± 0.22	5.19 ± 0.19	<0.001***	43↓
Urea (mg/dL)	22.80 ± 0.85	25.92 ± 1.43	0.10	8 ↑
Uric acid (mg/dL)	3.91 ± 0.10	4.76 ± 0.18	<0.001***	19↑
Creatinine (mg/dL)	0.78 ± 0.03	0.93 ± 0.04	0.02*	11 ↑
Albumin (mg/dL)	4.47 ± 0.11	3.63 ± 0.10	<0.001***	14 ↓

* and *** indicates significance at $P < 0.05$ and 0.001, respectively: ↑ Increase, ↓ Decrease.

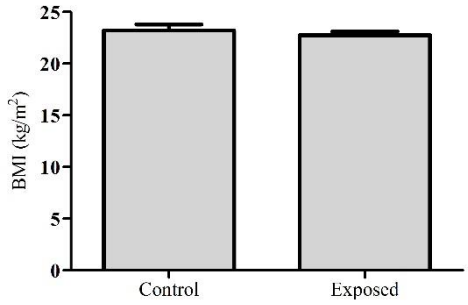

Fig. 1: Comparison of BMI (Kg/m^2) between Control and exposed groups. Values are Mean±SEM

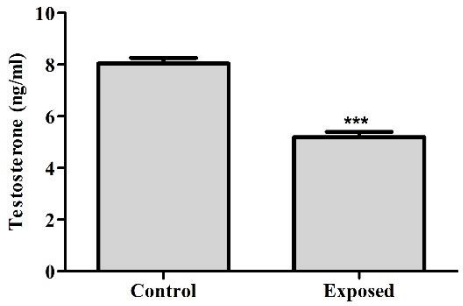

Fig. 2: Comparison of serum testosterone (ng/mL) in control and exposed group. Values are Mean±SEM. *** indicates significance at $P< 0.001$

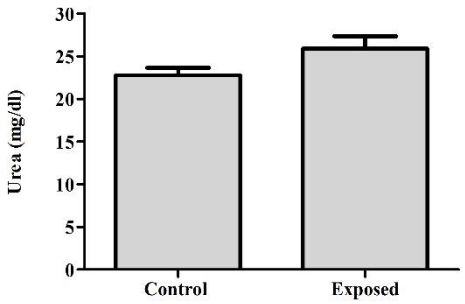

Fig. 3: Comparison of serum urea (mg/dL) in control and exposed group. Values are Mean±SEM

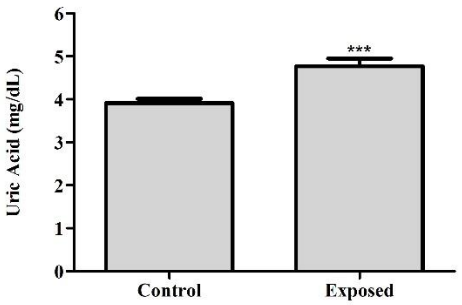

Fig. 4: Comparison of serum uric acid (mg/dL) in control and exposed group. Values are Mean±SEM*** indicates significance at $P<0.001$

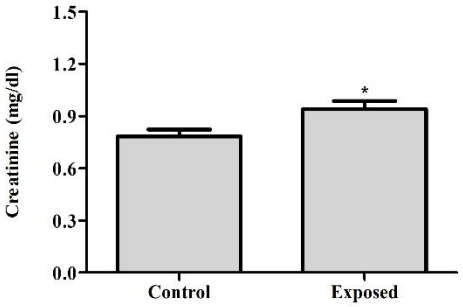

Fig. 5: Comparison of serum creatinine (mg/dL) in control and exposed group. Values are Mean±SEM. * indicates significance at $P<0.05$.

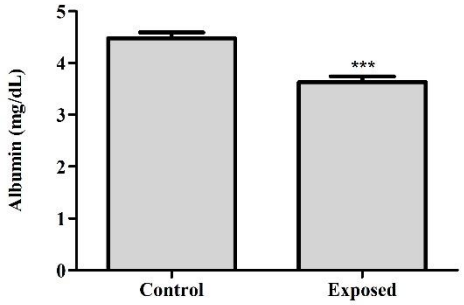

Fig. 6: Comparison of serum albumin (mg/dL) in control and exposed Group. Values are Mean±SEM*** indicates significance at $P<0.001$

DISCUSSION

Within workplaces and other business organizations, photocopiers are deemed crucial utilities (Elango et al., 2013b). Our current investigation of adult males, working in photocopier shops, indicated significant ($P<0.001$) decrease of 43% in serum testosterone concentration compared to the control group which is aligned by the findings of a previous study demonstrated a significant decrease in serum testosterone levels associated with exposure to heavy metals pollutants (Eisenegger et al., 2011). Our findings are consistent with previous research showing that exposure to heavy metals can disrupt endocrine functions (Aronson et al., 2000). In an in vitro setting, when cadmium was administered on Leydig cells of rats exhibited a diminished action of testosterone synthesis. In another experiment, rats were treated with manganese, resulting in lowering of blood testosterone concentration (Laskey and Phelps, 1991).

The results of our research are corroborated by another study which suggests that PM 2.5 can disrupt hormone concentrations and potentially pose a significant risk to the fertility of males by impacting the sperm production process (Qiu et al., 2018; Yang et al., 2019).

The result of our research that hypogonadism in males take place due to exposure to heavy metals is aligned by a previous study in which exposure to heavy metal such as nickel (Ni) in occupational settings may lead to a reduction in inhibin B by Sertoli cells or testosterone release by Leydig cells. Nickel was reported to interact with reproductive hypothalamic hormones LH and Follicle stimulating hormone along with testosterone (Sancini et al., 2014).

A deficiency in testosterone level, known as hypogonadism in males, gives rise to various health implications, including erectile dysfunction, diminished libido, reduced bone mass, dizziness, fatigue, mood swings, loss of body hair, diminished vitality, metabolic syndrome, insulin resistance, visceral obesity, and infertility resulting from hypogonadism (Basaria, 2014; Dohle et al., 2012).

According to the results, exposed group exhibited a notable rise

in creatinine levels compared to the control group, supported by a previous study that elevations in creatinine levels in either blood serum samples or urine samples may have adverse effects on the kidney profile. The elevation of creatinine corresponded with a decrease in the levels of ascorbic acid and vitamin E in the body. These vitamins play a crucial role in protecting cells against such contaminants (Ilahi et al., 2012). In our study, there was a notable 19% increase in uric acid levels in the exposed group when compared to the control group. Our findings are corroborated by a previous study in Taiwan, which reported that elevated uric acid levels are predominantly observed in individuals exposed to heavy metals. This may be lead to chronic kidney disease, where the kidneys fail to eliminate uric acid efficiently because of the accumulation of heavy metals, leading to hyperuricemia (Lu et al., 2023).

There was a noteworthy 14% reduction in albumin levels in the exposed group. As in previous research has supported that the decreased quantity of albumin is a result of abnormal filtration by the kidney (Jude et al., 2002). These deviations in parameters were linked to disorders affecting both kidney and reproductive hormones. The potential accumulation of toner particles in the kidney was proposed to impact its filtration functions for blood and urine. As a result, heightened levels of urea, uric acid and creatinine were observed in the serum, but albumin and testosterone levels experienced a decline. These abnormal concentrations suggested kidney dysfunction, potentially leading to complete kidney failure. Additionally, the typical testosterone levels could be attributed to a diminished number of interstitial cells and impairment in the testosterone synthesis pathway, contributing to suboptimal sperm production and male infertility or hypogonadism.

This study with relatively small sample size limits generalizability, Further research with larger cohort is needed. However, this is the first-time study on photocopier workers from Pakistan. Further validations are required to reach a conclusive understanding.

CONCLUSION

It can be concluded from this study that photocopy operators are at risk of hypogonadism and renal destruction due to exposure to various harmful chemicals that jeopardize their reproductive sterility. Therefore, it is recommended that health officials should initiate collaboration between photocopier workers, administration, and health professionals to endorse technical precautionary measures. Undertake large-scale, detailed epidemiological studies to further

investigate the prevalence and severity of hypogonadism and renal damage among photocopy workers. Data should be collected on the types and levels of chemical exposure in photocopy shops to establish a clear link between specific chemicals and health outcomes. Invest in research and development of environmentally friendly and health-safe photocopying alternatives.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

1. Aronson KJ, Miller AB, Woolcott CG, Sterns EE, McCready DR, Lickley LA, Fish EB, Hiraki GY, Holloway C, Ross T (2000). Breast adipose tissue concentrations of polychlorinated biphenyls and other organochlorines and breast cancer risk. Cancer Epidemiology and Prevention Biomarkers. 9(1):55-63.
2. Aydogdu A, Swerdloff RS (2016). Emerging medication for the treatment of male hypogonadism. Expert opinion on emerging drugs. 21(3):255-266.
3. Bai R, Zhang L, Liu Y, Meng L, Wang L, Wu Y, Li W, Ge C, Le Guyader L, Chen C (2010). Pulmonary responses to printer toner particles in mice after intratracheal instillation. Toxicol Lett. 199(3):288-300.
4. Basaria S (2014). Male hypogonadism. The Lancet. 383(9924):1250-1263.
5. Dohle G, Arver S, Bettocchi C, Kliesch S, Punab M, De Ronde W (2012). Guidelines on male hypogonadism. European Association of Urology. 4:1-28.
6. Eisenegger C, Haushofer J, Fehr E (2011). The role of testosterone in social interaction. Trends Cogn Sci. 15(6):263-271.
7. El-Hashemy MA, Ali HM (2018). Characterization of btex group of vocs and inhalation risks in indoor microenvironments at small enterprises. Sci Total Environ. 645:974-983.
8. Elango N, Kasi V, Vembhu B, Poornima JG (2013a). Chronic exposure to emissions from photocopiers in copy shops causes oxidative stress and systematic inflammation among photocopier operators in india. Environ Health. 12(1):78. doi:10.1186/1476-069x-12-78.
9. Elango N, Kasi V, Vembhu B, Poornima JG (2013b). Chronic exposure to emissions from photocopiers in copy shops causes oxidative stress and systematic inflammation among photocopier operators in india. Environmental Health. 12(1):1-12.
10. Gminski R, Decker K, Heinz C, Seidel A, Könczöl M, Goldenberg E, Grobéty B, Ebner W, Gieré R, Mersch-Sundermann V (2011). Genotoxic effects of three selected

black toner powders and their dimethyl sulfoxide extracts in cultured human epithelial a549 lung cells in vitro. Environ Mol Mutagen. 52(4):296-309.

11. Gubhaju BB (2002). Adolescent reproductive health in asia. Asia Pacific population journal. 17(4):97-119.
12. Hawkins JW, Dugaiczyk A (1982). The human serum albumin gene: Structure of a unique locus. Gene. 19(1):55-58.
13. Henschel DB, Fortmann RC, Roache NF, Liu X (2001). Variations in the emissions of volatile organic compounds from the toner for a specific photocopier. J Air Waste Manage Assoc. 51(5):708-717.
14. Hunashal RB (2011). Study of health effects on photostat workers in kolhapur, maharashtra. Nat, Environ Pollut Technol. 10(4):633-636.
15. Ilahi I, Khan A, Ali M, Ullah U, Ali J, Khan M (2012). Effects of stone dust exposure on some liver and kidney related serum parameters of stone crush plant workers. J Biol Life Sci. 3(1):211-217.
16. Jude EB, Douglas JT, Anderson SG, Young MJ, Boulton AJ (2002). Circulating cellular adhesion molecules icam-1, vcam-1, p-and e-selectin in the prediction of cardiovascular disease in diabetes mellitus. Eur J Intern Med. 13(3):185-189.
17. Kum K, Kim EC, Yoo YJ, Zhu Q, Safavi K, Bae K, Chang S (2014). Trace metal contents of three tricalcium silicate materials: Mta a ngelus, m icro m ega mta and b ioaggregate. Int Endod J. 47(7):704-710.
18. Laskey J, Phelps P (1991). Effect of cadmium and other metal cations on in vitro leydig cell testosterone production. Toxicol Appl Pharmacol. 108(2):296-306.
19. C-W, Dai Y-T, Chien C-H, Hsu D-J (2006). Characteristics and health impacts of volatile organic compounds in photocopy centers. Environ Res. 100(2):139-149.
20. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D, Group* MoDiRDS (1999). A more accurate method to estimate glomerular filtration rate from serum creatinine: A new prediction equation. Ann Intern Med. 130(6):461-470.
21. Lu L-H, Tsai C-C, Lin C-Y, Wang C-W, Wu P-Y, Huang J-C, Chen S-C, Chang J-M (2023). Association and interaction between heavy metals and hyperuricemia in a taiwanese population. Diagnostics. 13(10):1741.
22. Lyu L, Li Y, Ou X, Guo W, Zhang Y, Duan S, Gao Y, Xu Y, Yang T, Wang Y (2021). Health effects of occupational exposure to printer emissions on workers in china: Cardiopulmonary function change. NanoImpact. 21:100289.
23. Nandan A, Siddiqui NA, Kumar P (2020). Estimation of indoor air pollutant during photocopy/printing operation: A computational fluid dynamics (cfd)-based study. Environ Geochem Health.1-31.

24. Osadolor B, Ezegbogu M (2015). Toxicity of vanadium, cadmium, chromium and iron on the kidney status of occupational photocopier operators at the university of benin, benin-city, nigeria-a pilot study. Nigerian Journal of Pharmaceutical and Applied Science Research. 4(1):20-24.
25. Implementation of certainty factor method for diagnoses of photocopy machine damage. Journal of Physics: Conference Series; 2019: IOP Publishing.
26. Qiu L, Chen M, Wang X, Qin X, Chen S, Qian Y, Liu Z, Cao Q, Ying Z (2018). Exposure to concentrated ambient pm2. 5 compromises spermatogenesis in a mouse model: Role of suppression of hypothalamus-pituitary-gonads axis. Toxicol Sci. 162(1):318-326.
27. Sancini A, De Sio S, Gioffrè P, Casale T, Giubilati R, Pimpinella B, Scala B, Suppi A, Bonomi S, Samperi I (2014). Correlation between urinary nickel and testosterone plasma values in workers occupationally exposed to urban stressors. Ann Ig. 26(3):237-254.
28. Saritha V, Dwarapureddi BK, Bhavannarayana C (2016). Occupational health effects of self employed personnel with reference to auto drivers and photocopy workers. Nature Environment and Pollution Technology. 15(1):35.
29. Sarkhosh M, Mahvi AH, Zare MR, Fakhri Y, Shamsolahi HR (2012). Indoor contaminants from hardcopy devices: Characteristics of vocs in photocopy centers. Atmos Environ. 63:307-312.
30. Yang Y, Yang T, Liu S, Cao Z, Zhao Y, Su X, Liao Z, Teng X, Hua J (2019). Concentrated ambient pm2. 5 exposure affects mice sperm quality and testosterone biosynthesis. PeerJ. 7:e8109.

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Antimicrobial Polyester Textiles Based on Organic Compounds

Shama Sadaf[1*], Shagufta Naz[2], Wajeeha Salamat[2], Komal Hassan[1], Ayesha Saeed[1], Saima Sharif[2]

1. Department of Home Economics, Lahore College for Women University, Lahore, Pakistan
2. Department of Zoology, Lahore College for Women University, Lahore, Pakistan

Corresponding Author's Email: sadaf.shama@gmail.com

ABSTRACT: Microorganisms though present everywhere, but they can be prevented by a simple antimicrobial finish. In this cross-sectional study, eco-friendly antimicrobial finishes were extracted from leaves of Azadirachata indica, Butea monosperma and Litchi chinensis plants and applied on 100% polyester. The antimicrobial finish was applied by the pad dry cure method and was fixed by using of polyurethane binder. Plants 'extractions were manipulated by making two concentration levels, in one level pure plant extraction was applied and on the other level, 50% concentration dilute solution was applied. The results were analyzed through analysis of variance (ANOVA), Spectrum Analysis, Scanning Electron Microscope (SEM), photographic images, and Crosstab. The eco-friendly antimicrobial finish made 89% reduction in microbial growth. The antimicrobial finish lasted up to 25 washes. Antimicrobial fabric is suitable to provide protection against microorganisms and can be used for the medical industry, paramedical staff, sportswear, home furnishing as well as common people.

Keywords: Antimicrobial finish, Polyester, Azadirachata Indica, Butea Monosperma, Litchi Chinensis

INTRODUCTION

Microorganisms are present everywhere in surroundings such as in homes and hospitals, so human beings are frequently exposed to them. Textiles provide enormous surface zone and moisture which are required for bacterial development (Sauperl, 2016). Polyester fibres which are obtained from natural sources such as cotton and silk provide dampness, oxygen, nutrients and temperature which are the basic requirements for bacteria development and duplication. It causes bad smell, skin infection, colour and product deterioration, allergic and other allied sicknesses (Maghsoudi et al., 2021).

The new focus developed for antimicrobial treated fabric is to guard the wearer from germs as well as to guard the fabric from fibre deterioration. Now-a-days, antimicrobial textiles have received importance in industry as well as educational research because its importance is to give good quality life and protection benefits to human beings. Every antimicrobial treatment applied to textiles must be non-toxic to both human health and the ecosystem (Dehari et al., 2023).

Recently customers are demanding in textiles commonly functional treatment but specifically antibacterial treatment to guard people from bacteria (Shibly et al., 2021). There is a wide range of materials on which antimicrobial finish can be applied such as apparel for doctors, nurses, patient, premature babies, sportswear, socks, babies, older people, undergarments, soldiers, miners and in home furnishing it can be apply on bed sheets, curtains, carpets as well as common people (Rajput et al., 2017).

MATERIALS AND METHODS

In this study antimicrobial finish was extracted from three plants leaves i.e. *A. indica*, *B. monosperma* and *L. chinensis* (carried out in laboratory of Botany Department, Government College University) were applied on 100% polyester. The weights of dry powder of leaves *A. indica* (Neem), *B. monosperma* and *L. chinensis* were 2 kg each. Three airtight containers were prepared and labelled as A (*A. indica*), B (*B. monosperma*), and L (*L. chinensis*). These containers, along with distilled water, were then autoclaved at a temperature of 110 degrees to ensure sterilization. In Laminar Air Flow Hood, poured powder of leaves of *A. indica* in autoclave container A then add autoclaved distilled water. The ratio of grinded leaves and distilled

water was 100 g/250 ml. This process was repeated for *B. monosperma* and *L. chinensis*. Leave this soaked material for 7 days and stirred it twice a day. After that it was filtered by using muslin cloth then filtered again by using What man filter paper. The filtered extracts of *A. Indica* (Neem), *B. monosperma* and *L. chinensis* were concentrated by a rotary film evaporator.

Antimicrobial finish was applied in National Textile University (NTU) Faisalabad. Plants 'extractions were manipulated by making two concentration levels, in one level pure plant extraction was applied and in other level 50% concentration solution was used. The fabric samples were cut, treated with antimicrobial finish and then tested to govern their effectiveness as antimicrobial fabrics. Antimicrobial agents were extracted from leaves of *A. indica, B. monosperma* and *L. chinensis*. Binder was used to improve the durability of finish. The binder was obtained from CHT Pakistan. Antimicrobial testing was carried out in Centre of Excellence in Molecular Biology (CEMB). To check the presence of antimicrobial finish on fabrics pre-test post-test, FTIR test was conducted at the Institute of Chemistry, University of the Punjab, SEM test was conducted in The Centre for Solid State Physics, University of the Punjab, Lahore.

The study included a control group consisting of untreated fabrics without antimicrobial finish. To mitigate the impact of extraneous variables such as temperature and humidity, the research was conducted in controlled testing laboratories with standardized atmospheric conditions, ensuring a consistent environment for all experiments. There were one fabric and three plants. The antimicrobial finish was developed from plants in two concentrations that were 50% and 100% then applied on fabrics. Microorganisms' presence was checked by pre-test, post-test control group design. Antimicrobial finish was applied by making two concentration levels, in one level 100% (pure) plant extraction were applied and in other level 50% concentration solution was made. Durability of antimicrobial finish to washes was checked at 50% concentration of antimicrobial finish by repeated number of wash cycle i.e. 5 washes intervals up to 25 washes.

Microorganisms Observation

The microorganisms examined in this study were those that were isolated during the experiment. The isolates consisted of various morphological forms, including Gram-positive microorganisms

such as small thick rods, clusters, cocci, and coccus clusters, as well as Gram-negative microorganisms like thin short rods, diplococci with short tails, rounded cocci, coccus-bacilli, coccus diploids, and fungi (yeast). These microorganisms were studied in Centre of Excellence in Molecular Biology (CEMB) under standard conditions.

Application of Concentrated Antimicrobial Finish

To check antimicrobial activity 100% (pure) antimicrobial leaves extract was used. The researcher took 200 ml plant leaves extract of *A. indica* which was obtained from rotary film evaporator in a beaker. Cut one foot width and three feet length fabric sample randomly from 100% polyester fabric. Four more samples of same measurements were taken, one for untreated control group and three were for experimental group. On experimental group *A. indica*, *B. monosperma* and *L. chinensis* leaves extract finish was applied by using pad dry cure method. After applying this finish microorganism's detection was checked against control group in CEMB.

The dilute concentration of finish was prepared in ratio of 200 ml leaves extract of *A. indica* (Neem), 50 ml poly urethane binder and 150 ml distilled water. Same ratio was used for *B. monosperma* and *L. chinensis*. The three meter fabric sample was taken as length and twelve inch as width from cotton fabric; label untreated (un), *A. indica* (A), *B. monosperma* (B) and *L. chinensis* (L). So, there were four samples from polyester fabric. On untreated cotton samples no finish was applied. On sample A, *A. indica* antimicrobial finish was applied, on sample B, *B. monosperma* leaves extract antimicrobial finish was applied and on sample *L. chinensis* leaves extract antimicrobial finish was applied respectively. The untreated polyester sample was the control group and the polyester samples treated with *A. indica*, *B. monosperma* and *L. chinensis* leaves extract antimicrobial finish were experimental group.

The antimicrobial finish was applied by using the pad dry cure machine in NTU. On pad dry cure machine (process) drying was done at 120°C temperatures for 2 minutes and curing was done 150°C temperatures for 3 minutes. After applying this concentration antimicrobial finish, microorganisms' presence was checked in CEMB. Sustainability of antimicrobial finish to home laundry was checked by five washes interval up to 25 washes

and samples were cut according to each test requirement.

A 0.5 kg sample of Poly Urethane Binder was obtained from CHT. The binder was applied to enhance the wash durability of the antimicrobial finish. To assess the antimicrobial properties of the binder, a 1x1 feet cotton fabric sample was treated with a solution of 10ml poly urethane binder and 90ml distilled water, applied using a pad dry machine. The ASTM E2149 Shake Flask Method was employed to test the antimicrobial properties, which revealed that the binder did not exhibit any antimicrobial activity. The polyurethane binder, used as a polymeric finishing agent, had a slightly yellow colour and a pH range of 4.0-5.0.

To check the effectiveness of plant leaves, extract as antimicrobial finish on polyester fabric, cut fabric samples in Laminar Air Flow hood. The sample size for antimicrobial testing was one inch width and one inch length. Random sample was taken from untreated control group and from experimental group same size sample were cut i.e. one sample from each *A. indica, B. monosperma* and *L. chinensis* treated polyester fabric respectively.

To check presence of antimicrobial finish on fabrics ASTEM E2149 shake flask method was used. It was a quantitative screening test. The temperature of autoclave was 110oC.All the fabrics samples were cut 3cm in length and in width. Each sample was dipped in both concentrated and dilute solution of 100% *A. indica*, *B. monosperma* and *L. chinensis* for two hours. These samples were kept in room temperature to dry. In sterilized petri dishes took 50 ml PBS (phosphate buffer solution) in which fabric samples were soaked individually for one hour with continuous shaking. Label all petri dishes which have both treated and untreated fabric samples. Next, inside a Laminar Air Flow Hood, agar plates were prepared by precisely dispensing 50 microliters of solution using a pipette adjusted to a volume of 50 microliters.

In Laminar Air Flow Hood took a spreader, first dipped in spirit then put on spirit lamp until it became red, cooled down it. Fifty micro litre solution of plant leaves extract (with sterilized nozzle, which change every time) and poured on agar plate. Petri dish was put on rotator and with the help of spreader, spread the drop in clockwise direction. Cover it and put it in incubator which set at 37-40℃ temperature. First reading was taken after 22 hours and then counted the number of colonies in

range of 20-200, 30-300, colonies more than 2000 which called uncountable or lawn. After six days reading was taken again.

In Laminar Air Flow Hood, put petri dishes, slides, spirit lamp, pipette man, wire stick and distilled water. Adjust pipette man at 10 microliters. Took a slide, put a drop of distilled water. Then took iron wire stick, dipped in spirit, heated it on spirit lamp until its colour was red, cooled down it. Took only those petri dishes on which microorganism's presence was shown by use of colony counter and spread gently on glass slide until it was fully dissolved in distilled water. Dried it on spirit lamp with help of tweezer and staining was carried out.

Put slide on frame. Flood smear with methylene blue (injected methylene blue in the smear) and left it for one minute. Drained it with iodine solution and left it for one minute. Washed it with distilled/tap water. Drained it with decolourizer. At the end floods with methylene red dye and left for one minute. Then washed it. After drying these slides microorganisms' presences were checked on microscope and observation were noted.

Sustainability in home laundry

The washing of fabrics samples was checked by using AATCC test method 135-2003. Following apparatus was used, Automatic washing machine **,** Automatic tumble dryer, Conditioning/drying racks with pull-out screens or perforated shelves**,** Facilities for drip drying and line drying and 1993 AATCC Standard Reference Detergent.

Cut the sample from fabric in standard testing atmosphere. Samples were placed on the flat surface. Automatic washing machine did laundry by the following steps as washing, rinsing, and drying. In washing, automatic washing machine weights the fabric samples. According to sample size water level was selected. The temperature for washing and rinsing was less than 29°C. Add 1993 AATCC standard reference detergent by the ratio of 1g/l. Add fabric samples to washing machine, set the washer cycle and time. After that rinsed and dried the samples then line dries the samples. In line dry, hung each sample in vertical direction by clipping it in two corners. Subsequently, the fabric samples were air-dried at room temperature, which was maintained at a maximum of 26°C (79°F) to prevent any heat-induced damage or degradation.

RESULTS

The results of treating untreated polyester fabrics with *A. indica*, *B. monosperma,* and *L. chinensis* demonstrate high applicability, justifying its use in textile finishing due to its potential to provide durable antimicrobial properties and enhance fabric performance.

Table 1. Quantitative analysis test results of treated and untreated polyester sample

	Untreated	***A. indica***	***B. monosperma***	***L. chinensis***	**Reduction %**
Reading after 22 hours					
1st reading	0	0	0	0	100%
2nd reading	0	0	0	0	100%
3rd reading	0	0	0	0	100%
Reading after 6 days					
1 st reading	4	0	0	0	100%
2nd reading	5	0	0	0	100%
3rd reading	3	0	0	0	100%

There was no microorganism's growth found after 22 hours and even after 6-day interval. Result revealed that polyester fabric showed 89% reduction using *A. indica, B. monosperma* and *L. chinensis* leaves extract antimicrobial finishes as compared to control group. Significant difference (.013) between plant extract and microorganisms' presence on polyester fabric and the effect size was large (η^2=.409).

Table 2. Effect of antimicrobial finish on Microorganisms presences of Polyester Fabric (Univariate Analysis)

Antimicrobial			**Plant Extract**			
	Df*	**SS***	**MS***	**F**	**P**	**η^2**
Between-group	3	18.00	6.00	4.615	.013	.409
Within-group	20	26.00	1.30			
Total	23	44.000				

*DF= degree of freedom

*SS= sum of squared differences from the mean

*MS= Mean Square

Table 2 showing significant difference (.013) between plant extract and microorganisms' presence on polyester fabric and the effect size was large (η^2=.409)

A. indica and *B. monosperma* and *L. chinensis* plant extracts had effect on microorganism's presence of polyester fabric as compared to control group.

Table 3. Effect of Antimicrobial finish on Microorganisms presences of polyester fabric

	Plant Name				Mean Difference (I-J)		Std. Error	Sig.[b]
Microorganisms' presences	Control vs Experimental (*A. indica*)				2.000*		.658	.006
	Control vs Experimental (*B. monosperma*)				2.000*		.658	.006
	Control vs Experime2ntal (*L. chinensis*)				2.000*		.658	.006
	Control Group		*A. indica*		*B. monosperma*		*L. chinensis*	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
	2.00	2.28	.00	.00	.00	.00	.00	.00

One way ANOVA showed that the difference in antimicrobial finish between control group (M=2.00, SD=2.28), the first experimental group *A. indica* (M=.00, SD=.00), second experimental group *B. monosperma* (M=.00, SD=.00) and third experimental group *L. chinensis* (M=.00, SD=.00) were statistically significant (F=4.615, p=0.013, η^2=.409).

It revealed that control group scored significantly higher than the experimental groups. However, the three experimental groups (*A. indica*, *B. monosperma* and *L. chinensis)* did not differ significantly. The significant difference between control group and the experimental group is also evident from the big difference in the mean values and remarkable difference in standard deviation (control=2.28, *A. indica*=.00, *B. monosperma* =.00, *L. chinensis*=.00). The hypothesis that antimicrobial finish has no significance effect on polyester fabric was not accepted for *A. indica*, *B. monosperma* and *L.*

chinensis. The antimicrobial finish made a significance difference on polyester fabric as microorganism's colony.

Table 4. Colony Characteristics

	Pigments	Microscopy Structure	Surface	Colony Form	Elevation	Margins
Untreated fabric samples						
Polyester	Orange	Gram -ve short thin rods	Smooth	Circular	Raised	Entire
Polyester	Yellow	Gram –ve coccus	Rough	Irregular	Flat	Curled
Polyester	Yellow	Gram –ve Coccus bacilli	Rough	irregular	Flat	Serrate
Polyester	Yellow	Gram –ve coccus	Rough	Irregular	Flat	Serrate
Polyester	Off white	Gram +ve Cocci cluster	Smooth	Circular	Flat	Entire
Polyester	Yellow	Gram –ve coccus	Rough	Irregular	Flat	Serrate
Polyester	Orange	Gram –ve coccus	Rough	Irregular	Flat	Serrate

The readings were taken after 22 hours as mentioned in ASTM 2149 Shake Flask Method and after six days interval to check the effectiveness of antimicrobial finish. There are 8 colonies on untreated polyester fabric. The results showed that the untreated fabric (control group) harbored microorganisms, whereas no colonies were observed on the treated fabrics. The microorganisms present on the untreated fabric were identified as Gram-positive (small thick oval rods, cocci clusters, thick short rods with rounded ends) and Gram-negative (diplococci with short tails, short thin rods, cocci, coccus-bacilli) bacteria, as well as fungi. Based on these findings, a percentage reduction in microorganisms was calculated to assess the efficacy of the treatment

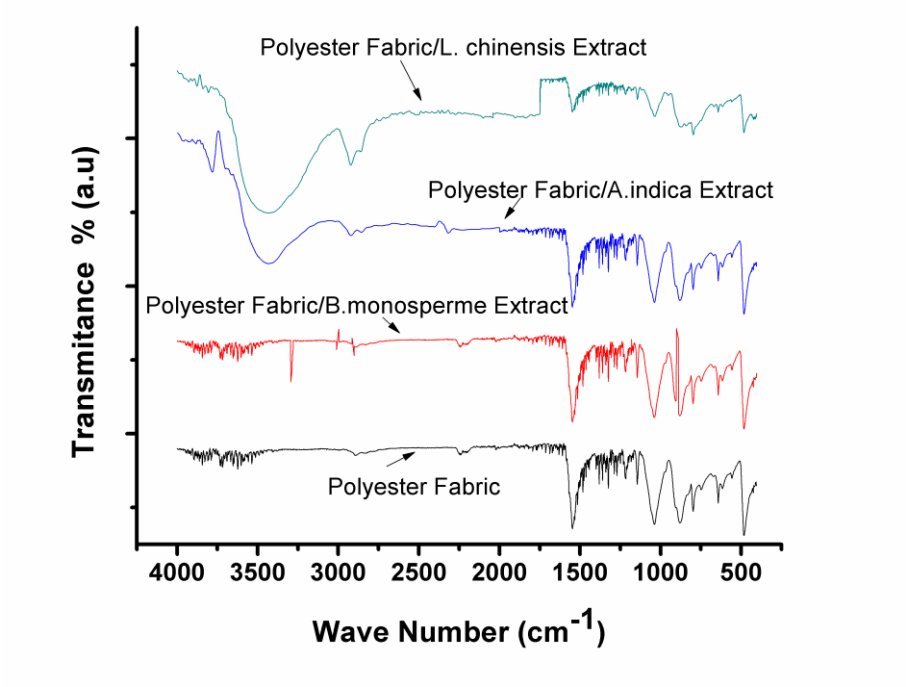

Fig. 1. FTIR Spectra of untreated vs treated polyester fabrics

The FTIR spectrum of polyester fabric is shown in Figure 2. The high peak from 1700 cm^{-1} to 600 cm^{-1} indicates the original signals, such as characteristics spectra of stretching vibration band of C=O at 1730 cm^{-1} and O-C-O stretching vibration band at 1097 cm^{-1} and 1240 cm^{-1}. All these peaks confirm the existence of ester linkage. A broad band region 3435 cm^{-1} which shows the presence of hydroxyl group.

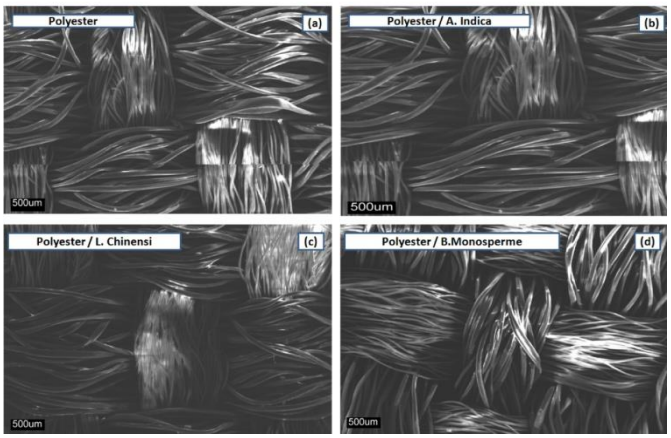

Fig. 2. SEM micrographs of untreated and treated polyester fabric

Fig. 3 portrayed the outcome of treatment of extract on polyester fabric. Figure 3a is the SEM illustration of pure polyester, Figure 3b is *A. indica*, Figure 3c is *L. chinensis*, and Figure 3d is B. monosperma processed polyester fabric. It is revealed that with the dealing of extract on polyester fabric is unaffected for the structure of woven polyester fabric expect the *L. chinensis* in which little bit of breakage has appeared on the surface of the fabric. The treated polyester fabric treated shows presence of finish as compared to untreated fabric. The result indicates that hypothesis is not accepted.

DISCUSSION

In this study, an eco-friendly antimicrobial finish was developed from the leaves of *A. indica, B. monosperma,* and *L. chinensis.* The antimicrobial finish was applied using the pad dry cure method on 100% polyester fabric. The results were discussed in comparison to the untreated fabric (Control Group). The presence of microorganisms was checked on both untreated (Control Group) and treated fabrics. The readings were taken after 22 hours, as referenced in the ASTM 2149 Shake Flask Method, and after six days to test the efficacy of the antimicrobial finish (Emam, 2019). The microorganisms studied were Gram-positive (small thick oval rod, cocci cluster, thick short rods with rounded ends), Gram-negative (diplococci short tail rounded, short thin rod, coccus, coccus bacilli), and fungi, which were observed on the untreated fabric (Darwesh, 2018).

Recent studies have also explored the use of neem oil as an antimicrobial finish. For instance, Guedes (2016) extracted and characterized a surfactant from neem oil (SNO) that showed a yield of approximately 100%. The surfactant demonstrated reasonable soap qualities with a high potential for use as a cleansing agent for textile applications, such as high pH value (10.1), moderate foaming of 1.5 cm, and a critical micelle concentration of almost 0.12 g mL−1. However, the surfactant from neem oil (SNO) showed moderate bactericidal activity against *Escherichia coli* and bacteriostatic activity against *Staphylococcus aureus*, both common nosocomial microorganisms (De Smet, 2019). This suggests that the surfactant from neem oil (SNO) has a good potential for use in clinical textile applications due to its soap and bactericidal properties, as it is also biodegradable.

Other studies have explored the use of polyester/aluminum (PET/Al) filters for the high-

efficiency simultaneous capture and inactivation of airborne microorganisms that survive on fabric for several days (*E. coli* can survive for 21 days on polyester fiber) (Gressier, 2019). In this study, nonorganic developed on the fibers, and the antimicrobial activity against airborne *E. coli* and *S. epidermidis* improved to around 94.8% and 96.9%, respectively, due to the sustained hydrophobicity and surface roughness of the filter.

Triclosan is a strong candidate for obtaining antibacterial capability against microorganisms for textiles, including clinical applications such as face masks, sterile garments, and wound dressings. Purwar (2009) researched the characterization, antibacterial properties, and durability of triclosan on polyester, polyester/cotton, and cotton surfaces. The pure triclosan and presence of triclosan in solutions were identified by gas chromatography and mass spectrometry chromatograms. In this study, surfaces were homogeneously coated by triclosan, as observed by scanning electron microscope micrographs, and new bands appeared on Fourier transform infrared spectra after treatments. Triclosan showed strong biocidal activity against microorganisms for 3 hours. Although they lost their antibacterial properties after washing, they showed good antibacterial (bactericidal) properties and long-term stability to washes (Gressier, 2019). This suggests that triclosan is a highly effective and durable compound on polyester and cotton surfaces for clinical textile applications.

Additionally, El-Khatib (2012) found that natural antibacterial agents had long-term biocidal effects without being harmful to the environment. Due to their specific targets of action, small molecular antibiotics can cause the growth of microbial resistant species. However, in the case of the currently evaluated antimicrobial agents, destroying microbial cell membranes is reported to be the primary mechanism for preventing microbial growth. Natural antimicrobial finishing compounds (natural and synthetic in origin) have been widely described as one of the main classes of antimicrobial textile finishing agents in this review. Textile antimicrobial compounds derived from natural sources, such as chitosan, cyclodextrins, and natural dyes, were considered environmentally friendly.

CONCLUSION

The study demonstrates the effectiveness of antimicrobial

finishes using *A. indica, B. monosperma,* and *L. chinensis* leaves extract on polyester fabric. The results show a significant reduction (89%) in microorganism growth on treated fabrics compared to the control group, with no growth observed even after a 6-day interval. The statistical analysis confirms the significance of the antimicrobial finish, with a large effect size (η^2=.409). The study rejects the hypothesis that antimicrobial finish has no significant effect on polyester fabric, highlighting the potential of these plant extracts as natural antimicrobial agents for textile applications. The findings have important implications for the development of sustainable and eco-friendly antimicrobial finishes for polyester fabrics, reducing the reliance on synthetic chemicals and promoting a safer and healthier environment

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CONFLICT OF INTEREST

Authors declare there is no conflict of interest.

REFERENCES

1. Choi DY, Heo KJ, Kang J, An EJ, Jung SH, Lee BU, Jung JH (2018). Washable antimicrobial polyester/aluminum air filter with a high capture efficiency and low pressure drop. J. Hazard. Mater. 351: 29-37.
2. De Smet N (2019). Surfactant from Neem Oil (SNO): A Novel, Eco-Friendly and Multifunctional Agent for Textile Applications. Text. Res. J. 22: 537-547.
3. Dehari D, Chaudhuri A, Kumar DN, Nath G, Agrawal AK (2023). Fiber and textile in drug delivery to combat multidrug resistance microbial infection. In *Fiber and textile engineering in drug delivery systems* (pp. 359-387). Woodhead Publishing.
4. Emam HE (2019). Antimicrobial cellulosic textiles based on organic compounds. J. Biotech. 9: 1-14.
5. Emam HE, Darwesh O M, Abdelhameed RM (2018). In-growth metal organic framework/synthetic hybrids as antimicrobial fabrics and its toxicity. Colloids Surf B. 165: 219-228.
6. Gressier P, De Smet D, Behary N,

Campagne C, Vanneste M (2019). Antibacterial polyester fabrics via diffusion process using active bio-based agents from essential oils. Ind Crops Prod. 136: 111-120.

7. Gressier B (2019). Triclosan: A Review of Its Antimicrobial Properties, Applications, and Toxicology. J. Appl. Microbiol.126: 1221-1235.
8. Guedes RM (2016). Extraction and Characterization of a Surfactant from Neem Oil (SNO) and Its Application in Textile Finishing. J. Text. Appar. Technol. Manag, 6: 1-9.
9. Joshi M, Ali SW, Purwar R, Rajendran S (2009). Ecofriendly antimicrobial finishing of textiles using bioactive agents based on natural products. Ind. J. Fibers Tex. Res. 34: 295-304
10. Maghsoudi S, Nasiri PP, Ebrahimnejad H, Jalali E, Zangiabadi M (2021). Decoration of cotton fiber with biosynthesized Ag/ZnO nanocomposite for durable antibacterial textile. J. Nat. Fibers. 52: 1-11.
11. Martirosyan I, Pakholiuk O, Semak B, Lubenets V, Peredriy O (2019). Investigation of wear resistance of cotton-polyester fabric with antimicrobial treatment. In: Grabchenko's International Conference on Advanced Manufacturing Processes. Springer, Cham. 433-441.
12. Morais DS, Guedes RM, Lopes MA (2016). Antimicrobial approaches for textiles: from research to market. Mater. Res. 9: 498.
13. Orhan M (2020). Triclosan applications for biocidal functionalization of polyester and cotton surfaces. Eng. Fibers Fabr. 15: 1-11.
14. Prabhu K H, Teli MD (2011). Eco-dyeing using *Tamarindus indica* L. seed coat tannin as a natural mordant for textiles with antibacterial activity.J. Saudi Chem. Soc. 12: 753-759.
15. Rajput A, Ramachandran M, Gotmare VD, Raichurkar PP (2017). Recent bioactive materials for development of eco-friendly dippers: an overview. J. Pharm. Res. 9: 1844-1848.
16. Raja ASM, Thilagavathi C (2011). Influence of enzyme and mordant treatments on the antimicrobial efficacy of natural dyes on wool materials. Asian J. Text. 1: 138-144.
17. Sauperl O (2016). Textiles for protection against microorganisms. AIP Conf. 1727: 20021.
18. Shibly MMH, Hossain MF, Rahman M, Nur MG (2019). Development of cost-effective menstrual absorbent pad with eco-friendly antimicrobial finish. Eur. Sci. J. 15: 438-445.
19. Siqueira de Azevedo C, Ladchumananandasivam R, Rossi CG, Silva RKda, Camboim Wda, S, Zille A, Padrão J, Silva KKde OS (2021). Characterization of a natural surfactant from an essential oil from neem (*Azadirachta indica A. Juss*) for textile industry applications. Text. Res. J. 91: 1241-1253.

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Evaluation of phytochemicals and antioxidant potential of *Cymbopogon citratus*

Muhammad Atif Sohail[1], Syeda Mona Hassan*[2], Muhammad Sabir Tehseen[3], Hafiza Hadiya Shahzad[4], Gulshan Perveen[5], Muhammad Asif Ibrahim[6], Syed Khuram Hassan[7]

1. Department of Applied Chemistry and Biochemistry, GC University, Faisalabad
2. Department of Chemistry, University of Agriculture, Faisalabad
3. Department of Chemistry, The University of Lahore, Sargodha Campus
4. Department of Chemistry, University of Education, Lahore
5. Department of Chemistry, Bahauddin Zakariya University, Multan
6. Department of Mathematics and Statistics, The University of Lahore, Lahore
7. Institute of Quality and Technology Management, PU, Lahore

Corresponding Author's Email: s.monahassan@lgu.edu.pk

ABSTRACT: *Cymbopogon citratus is an important medicinal plant belongs to family Gramineae. It originates from Ethiopia, India Africa America and is broadly extended all over humid, subtropical and warm temperate regions of the world. This present research work has been designed to evaluate the antioxidant potential of Cymbopogon leaves. The antifungal and antioxidant components of Cymbopogon leaves were extracted by means of four solvent systems (80% methanol, 100% methanol 80% ethanol, and 100% ethanol) and maximum extract yield (48.1 g/100g DW) was obtained in 80% methanolic solvent system. Cymbopogon leaves were analyzed in terms of total phenolic contents, showed that 80% methanolic leaves extract offered highest total phenolic contents (35.2 mg GAE/g DW) Antioxidant activity was investigated by DPPH radical scavenging activity and by measuring reducing power. Results showed that 80% methanolic leaf extract showed maximum radical scavenging activity and reducing potential. Overall results of the present study showed that 80% methanolic Cymbopogon leaf extract can be used effectively to make antioxidant agents which can be utilized in different industries like pharmaceutical, food and cosmetics.*

Keywords: Antioxidant, *Cymbopogon,* Phenolics, Reducing Potential, Lemon Grass

INTRODUCTION

The aromatic plant Lemon grass belongs to the family Gramineae (Akhila., 2010). The leaf-blade of this plant is linear, elongated at both ends and it can raise to a 1.5 cm in width and 50 cm in length. The tubular shape of the leaf sheath acts as a pseudo stem. This plant at mature stage of growth produces flowers (Tajidin et al, 2012). Lemon grass or Cymbopogon citratus (C. citratus widely cultured in warm, tropical and subtropical regions. On dry basis it has 1% to 2% essential oil and its chemical composition may be different as a genetic diversity function, habitat and cultural agronomic treatment (Hadjilouka, 2012).

The important phytoconstituents of lemon grass are essential oils which includes Citral α, Citronellal, phenolic and flavonoids compounds (Vanisha et al.,2012).

Lemongrasses possess antioxidant, bactericidal, antidepressant, astringent, sedative, fungicidal, nervine and antiseptic properties (Naik et al., 2010). According to biological effects C. citratus extracts describe to its main bioactive elements, resulting from its stem, roots and leaves, in addition to secondary metabolites of these compounds (Christopher et al., 2014). The biologically active citral component of lemon grass consists of its essential oil (Huynh et al., 2008).

The current research work was performed to evaluate the biological potential of lemon grass. The phytochemicals and the antioxidant activity were evaluated for C. citratus.

Materials and methods

Collection of plant materials

C. citratus leaves were obtained from the vicinity of Lahore Garrison University, Lahore, Pakistan.

Pretreatment of plant materials

The leaves of C. citratus were washed with tap water and then dried out at 41oC in an oven (Memmert, Jarmany) until stable weight. By using a commercial blender, dried leaves were grounded into fine powder. Then the ground material was conceded through 79-mesh strainer. The passed material was also used for extraction purposes. Polythene bags were used to store the ground samples at 4oC till further analysis.

Extraction of bioactive compounds

For extraction, four solvent systems (100% ethanol and 80% ethanol,100% methanol, 80% methanol,) are being used. In this regard powdered leaves (20g) were also extracted with 200mL in an orbital shaker for 6 hours at room

temperature (Gallenkamp, UK). To separate the extract from residue Whatman No. 1 filter paper was used. Two times resulting residues were extracted with the same solvent system. Drying of extracts was done at temperature of 45˚C and their yield was calculated by weighing extracts. The extracts were reserved in a refrigerator at 4˚C for further analysis (Hassan et al., 2016).

Phytochemical studies of medicinal plant extract

Total phenolic contents

The method which was used to establish the total phenolic contents of C. citratus was based on the procedure of Zafar et al., (2016). The results were presented in gallic acid equivalent (GAE) per gram of extract.

Antioxidant potential

DPPH radical scavenging assay

DDPH radical scavenging analyzes was applied to determine the free radical scavenging activity of C. citratus. 2, 2-diphenyl-1-picrylhydrazyl radical was used to determine the scavenging action as described by Suleman et al., (2018) with little amendment.

Determination of reducing power

The reducing power of the leaf extracts was resolute according to the procedure explained by (Hassan et al., 2016) with slight modification.

Statistical analysis

By performing all experiments in triplicate (n=3), mean ± SD was applied. Data analysed at 5% significant level through statistical software Minitab 2000 Version 13.2 (Minitab Inc. Pennsylvania, U.S.A).

RESULTS AND DISCUSSION

The current research work was conducted to display the phytochemical constituents and antioxidant potential of C. citratus leaves.

Percentage yield (g/100g DW) of extracts

The percentage yield of plant extracts is based on different factors like amount of solvent used, nature of plant material and method of extraction (Hsu and Coupar, 2006). Methanol is known to be a superior and broadly used solvent to pull out antimicrobial components and natural antioxidative components from plants (Anwar et al., 2010).

The extraction yields from leaves of C. citratus against different solvent systems are presented in Table 1. Comparatively, 80% methanol showed significantly ($p<0.05$) higher extraction yields from leaves (48.1%). The extraction capability of different solvent systems from leaves followed the order: 80%

methanol> 80% ethanol> absolute methanol> absolute ethanol.

Table 1: Percentage yield (g/100g DW) of extracts of Cymbopogon leaves

Sr. no	Solvent System	Percentage yield (g/100g DW)
1	80 % Methanol	48.1±0.42[a]
2	80 % Ethanol	45.7±0.23 [b]
3	Absolute Methanol	41.3±0.40 [c]
4	Absolute Ethanol	39.2±0.25 [d]

Values mean ± SD of three samples analysed individually in triplicate at p <0.05. Superscripts alphabets within the column depicted significant differences among different solvent systems.

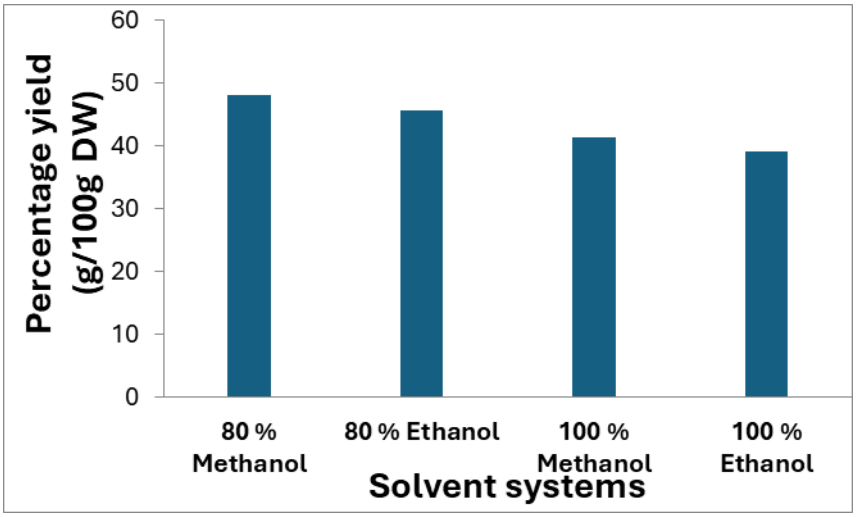

Fig. 1: Percentage yield (g/100g DW) of extracts of *C. citratus* leaves

Total phenolic content

In plants, phenolic compounds are the most important antioxidants (Sakihama et al., 2002). They contribute an essential role in scavenging free radical action (Agbo et al., 2015). Scavenging activity of the potent free radical of phenolic compounds is due to the presence of their hydroxyl groups and it directly contributes to antioxidant potential (Wojdylo et al. 2007). Results of phenolic contents medicinal plant extract are given in Table 2 showed total phenolic content in leaves of cymbopogon was found to be

varied significantly from 29.3-35.2 (mg GAE/g DW), respectively. It was investigated that maximum TPC were obtained from leaves in 80% methanolic extract followed by: absolute methanol>80% ethanol>absolute ethanol. Overall, results showed that the maximum phenolic contents were obtained from methanolic leaf extracts.

Our results are supported by the previous study of Butsat and Siriamornpun (2016) who reported that higher phenolic contents were obtained from 80% methanol, than 80% ethanol for plant extracts. Ghasemzadeh et al. (2011) reported that methanol solvent was found to be more effective in extracting phenolic components as compared to other solvents that also support our results.

Liao et al. (2012) also determined the phenolic contents in different parts of Cymbopogon leaves of the methanol extracts. The maximum phenolic content was obtained from Cymbopogon leaves. Namvar et al. (2017) examined that 80% methanolic extract was found to possess higher total phenolic contents than other extracts. Thus, for extraction of phenolic compounds, an aqueous methanolic solvent system is being used that is a better and more efficient solvent.

Table 2: Total phenolic contents (mg GAE/g DW) of Cymbopogon leaves

Sr. no	Solvent System	Total phenolic contents (mg GAE/g DW)
1	80 %Methanol	35.2±0.56 [a]
2	80 % Ethanol	30.9±0.58 [b]
3	absolute Methanol	32.1±0.43 [ab]
4	absolute Ethanol	29.3±0.35 [c]

Values mean ± SD of three samples investigated individually in triplicate at $p < 0.05$. Superscripts alphabets within the column showed significant difference among different solvent systems

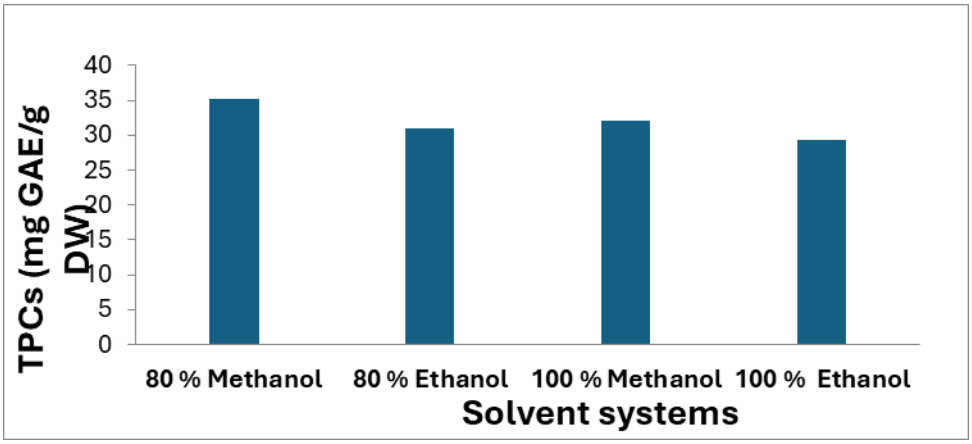

Fig. 2: Total phenolic contents (mg GAE/g DW) of Cymbopogon leaves

Antioxidant activity

There are various numbers of medicinal plants that are being used due to their antioxidant properties. These chemical constituents are very helpful to prevent the destructive actions caused by their oxidative stress (Selvam et al., 2012). In the present study, two assays were used to find the antioxidant activity of Cymbopogon leaves.

DPPH radical scavenging activity

Natural constituents such as polyphenols, flavonoids, phenolics, terpenes and tannins possess antioxidant property to scavenge free radicals (Hassan et al., 2009). Antioxidant activity of these products can be evaluated by using DPPH radical scavenging assay. This assay has been used widely to test the scavenging capability of compounds which act as free radical or hydrogen donors to DPPH (Patel et al., 2015). A nitrogen centred free radical compound is present in stable form of DPPH. Upon reduction, its colour changes from violet to yellow by hydrogen or electron donation. Substances which can execute such types of reactions are known as good antioxidants and better radical scavengers. It has been also found that with increasing the extract concentration, DDPH free radical scavenging ability also increases (Ebrahimzadeh et al., 2010).

So, DPPH free radical scavenging method is based on phenomenon of transfer of electron. It is an antioxidant assay that produces a violet coloration in methanol solution (Garcia et al.,

2012) It is an important mechanism that explains the oxidation process of proton radical scavenger. By decreasing the absorbance of DPPH solution to 517 nm, its reduction capability was evaluated suggesting that antioxidant activity of plant extract is owing to its proton donating capability (Chougule et al., 2012). The antioxidant molecule has the hydrogen donating atom which contributes to its free radical scavenging nature which is an important quality of antioxidants (Sathisha, 2011). DPPH radical assay has been used because it is a quick, reliable, easy and rapid method to investigate the general antioxidant activity of plants extracts as well as pure compounds. This method is also used for showing a lot of samples for radical scavenging potential and is self-governing on the polarity of sample (Aliyu et al., 2009).

The antioxidant potential of medicinal plant C. citratus was evaluated by using the DPPH free radical scavenging assay. This assay also explored its new potential sources for natural antioxidants. DPPH concentrations of medicinal plant leaves were tested and found to be reduced due to scavenging potential. Table 3 showed that there is a significant difference of DPPH radical scavenging activities of C. citratus extracts among different solvent system. The aquoues alcoholic extracts of C. citratus leaves exhibited satisfactory DPPH radical scavenging ability. The C. citratus leaves exhibited highest DPPH radical scavenging potential significantly ($p < 0.05$) in 80% methanolic extract followed by 80% ethanol, absolute methanol and absolute ethanol.

Present results are supported by the preceding report of Liao et al., (2012) who investigated that Cymbopogon leaves exhibit higher free radical scavenging activity. Wu et al., also (2009) reported that methanolic extracts of cymbopogon also showed highest DDPH radical scavenging ability (71.1%).

Table 3: DPPH radical scavenging activity of the *C. citratus* leaves

Sr. no	Solvent System	DPPH (%) radical scavenging activity
1	80 % Methanol	60.1±1.20 [a]
2	80 % Ethanol	58.9±0.45 [b]
3	100 % Methanol	57.3±2.48 [bc]
4	100 % Ethanol	56.4±0.65 [c]

Values mean ± SD of three samples investigated individually in triplicate at p <0.05. Superscripts alphabets within the column showed significant differences among different solvent systems. Reductive abilities of the plant extracts can be an indication of their potential towards antioxidant activities (Zhang et al., 2011). The antioxidant ability of phenolic compounds is generally due to their redox properties. These properties allow them to react as a reducing agent such as an oxygen quencher electron donor. Studies on medicinal plants and vegetables revealed that plants are the great source of antioxidant properties. In biological systems, these plants can apply the protection effects against certain oxidative stress (Sylvie et al., 2014). Electrons are donated to reactive radical species due to the presence of antioxidant substances by the process in which these are neutralized into stable and nonreactive species (Nishaa et al., 2012).

In this assay, lessening of the Fe3+ to the ferrous form occurs due to presence of reducers which is also known as antioxidant. So reducing power is measured by donation of electron and reduction of Fe3+(CN−)6 to Fe2+(CN−)6. Perl Prussian blue colour product formation indicates the presence of Fe2+ concentration that can be monitored at the wavelength of 700 nm (Ahmed et al., 2015). Higher absorbance values were indication of high antioxidant properties (Nishaa et al., 2012). Hence, activity of reducing power increases with increasing the concentration of extracts (Senguttuvan et al., 2014).

The reducing power of C. citratus leaves extracts is offered in table 2. The reducing potential values of the examined extracts were observed at different concentrations ranging from 2.5 to 10.0 mg/mL. It was experimental

that leaves extracts showed considerably (P< 0.05) high reducing potential, irrespective to which type of solvent used. However, 80% methanolic leaves extract showed the highest reducing power. The results revealed that antioxidant power was a function of concentration. By increasing the extract concentration, antioxidant activity was also increased. Results of present research work are supported by the previous analysis of Geng et al., (2015) who investigated that the reducing power of C. citratus leaves linearly increased with increasing the extract concentration. Furthermore, Chang et al. (2007) described the reducing power of C. citratus extracts which also showed greatest reduction potential at 2.5mg/mL concentration.

Table 3: Reducing Power of Cymbopogon leaves extract

Plant Parts	**Solvent system**	**Concentration (mg/ml)**			
		2.5[a]	**5.0[b]**	**7.5[c]**	**10[d]**
Leaves[a]	**80% methanol**	**0.131±0.03**	**0.137±0.02**	**0.145±0.01**	**0.158±0.06**
	80% ethanol	**0.128±0.01**	**o.131±0.04**	**0.145±0.03**	**0.150±0.05**
	Absolute Methanol	**0.121±0.06**	**0.127±0.02**	**0.134±0.01**	**0.151±0.06**
	Absolute Ethanol	**0.127±0.04**	**0.132±0.01**	**0.138±0.06**	**0.145±0.02**

Values mean ± SD of three samples investigated individually in triplicate at p <0.05. The superscripts alphabets within the column showed significant differences among different plant parts. Superscripts alphabets within the rows depicted significant differences among different concentrations.

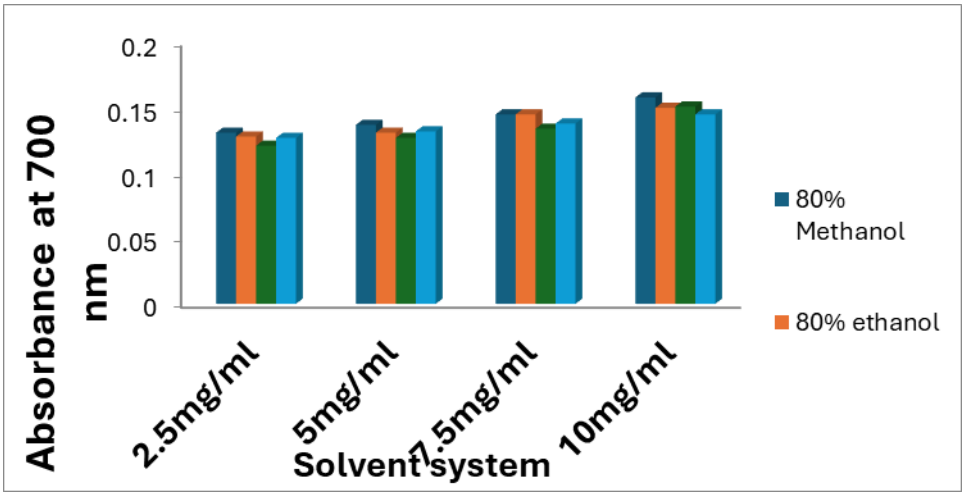

Fig. 3: Reducing Power of *C. citratus* leaf extracts

CONCLUSION

The present research work was conducted to investigate the biological activities of leaf extracts of C. citratus. A considerable quantity of total phenolics was found in examined leaf extracts of C. citratus. It was revealed that leaf extracts of C. citratus exhibited excellent antioxidant activity. Phytoconstituents of plants can be used in food and cosmetic industries to stop the process of oxidation.

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CONFLICT OF INTEREST

Authors declare there is no conflict of interest.

REFERENCES

1. Agbo MO, Uzor PF, Akazie-Nneji UN, Eze-Odurukwe CU, Ogbatue UB, Mbaoji EC (2015). Antioxidant, Total Phenolic and Flavonoid Content of Selected Nigerian Medicinal Plants. Journal of Pharmaceutical Sciences. 14(1): 1-7.
2. Ahmed D, Khan MM, Saeed R (2015). Comparative Analysis of Phenolics, Flavonoids, and Antioxidant and Antibacterial Potential of Methanolic, Hexanic and Aqueous Extracts from Adiantumcaudatum Leaves. Antioxidants. 4: 394-409.
3. Akhila A, (2010). Essential Oil-bearing Grasses: The genus Cymbopogon. Medical and aromatic plants-industrial profile. Taylor and Francis Group, 4: 43-51.

4. Aliyu AB, Musa AM, Ibrahim MA, Ibrahim H, Oyewale AO (2009). Preliminary phytochemical screening and antioxidant activity of leave extract o Albizia Chevalieri harms (Leguminosease-Mimosoideae). Bayero Journal of Pure and Applied Sciences. 2(1): 149 -153.
5. Butsat S, Siriamornpun S (2016). Effect of solvent types and extraction times on phenolic and flavonoid contents and antioxidant activity in leaf extracts of Amomumchinense C. International Food and Research Journal. 23(1): 180-187.
6. Chang H, Ho Yl, Sheu Mj, Lin Y, Tseng M, Wu S, Huang G, Chang Y (2007). Antioxidant and free radical scavenging activities of Phellinus merrillii extracts. Botanical Studies. 48: 407-417.
7. Chougule P, Pawar R, Limaye D, Joshi YM, Kadam V (2012). In-Vitro Antioxidant Activity of Ethanolic Extract of Centaureabehen. Journal of Applied and Pharmaceutical Sciences. 2 (4): 106-110.
8. Christopher E, Ekpenyong EE, Akpan NE, Daniel (2014). Phytochemical Constituents, Therapeutic Applications and Toxicological Profile of Cymbopogoncitratus Stapf (DC) Leaf Extract. Journal of Pharmacognosy and Phytochemistry. 3 (1): 133-141.
9. Ebrahimzadeh MA, Nabavi SF, Nabavi SM, Eslamib B (2010). Antihypoxic and antioxidant activity of Hibiscus esculentus seeds. Grasas Y Aceites. 61 (1): 30-36.
10. Felhi S, Daoud A, Hajlaoui F, Mnafgui K, Gharsallah N, Kadri A (2017). Solvent extraction effects on phytochemical constituents' profiles, antioxidant and antimicrobial activities and functional group analysis of Ecballium elaterium seeds and peels fruits. Journal of Food Science and Technology. 37(3): 483-492.
11. Garcia EJ, Oldoni TLC, Alencar SMD, Reis A, Loguercio AD, Grande, RHM (2012). Antioxidant Activity by DPPH Assay of Potential Solutions to be Applied on Bleached Teeth. Brazilian Dental Journal. 23(1): 22-27.
12. Geng S, Liu Y, Ma H, Chungang C (2015). Extraction and Antioxidant Activity of Phenolic Compounds from Okra Flowers. Tropical Journal of Pharmaceutical Research, 14(5): 807-814.
13. Ghasemzadeh A, Jaafar HZE, Rahmat A (2011). Effects of solvent type on phenolics and flavonoids content and antioxidant activities in two varieties of young ginger (Zingiber officinale Roscoe) extracts. Journal of Medicinal Plants and Research. 5(7): 1147-1154.

14. Hadjilouka A, Polychronopoulou M, Paramithiotis S, Tzamalis P, Eleftherios H, Drosinos EH (2012). Effect of Lemongrass Essential Oil Vapors on Microbial Dynamics and Listeria monocytogenes Survival on Rocket and Melon Stored under Different Packaging Conditions and Temperatures. Microorganisms. 3: 535-550.
15. Hasan SMR, Hossain MM, Akter R, Jamila M, Mazumder MEH, Rahman S (2009). DPPH free radical scavenging activity of some Bangladeshi medicinal plants. Journal of Medicinal Plant and Research. 3(11): 875-879.
16. Hassan SM, Sultana B, Jahan N, Iqbal T (2016). Evaluation of phenolic profile and antioxidant potential of medicinal plants. Oxidation communication. 39(1): 2222-2236.
17. Hsu B, Coupar M (2006). Antioxidant activity of hot water extract from the fruit of the Doum palm, Hyphaene thebaica. Food Chemistry. 98: 317-328.
18. Huynh KP, Maridable J, Gaspillo P, Hasika M, Malaluan R, Kawasaki J (2008). Essential oil from lemongrass extracted by supercritical carbon dioxide and steam distillation. The Phillippine Agricultural Science. 91: 36-41.
19. Liao H, Liu H, Yuan K (2012). A new flavonol glycoside from the Abelmoschusesculentus Linn. Pharmagnosy Magazine. 8(29): 12-15.
20. Naik MI, Fomda BA, Jaykumar E, Bhat JA (2010). Antibacterial activity of lemongrass (Cymbopogon citratus) oil against some selected pathogenic bacterias. Asian Pacific Journal of Tropical Medicine. 31: 535-538.
21. Namvar K, Mohammadi A, Salehi EA, Feyzi F (2017). Evaluation of Solvent Effect (Methanol: Water Mixture) on the Phenolic Content and Antioxidant Activities of Stachys turcomanica Trautv. Journal of Pharmaceutical Sciences. 23: 244-248.
22. Nishaa S, Vishnupriya M, Sasikumar JM, Christable HP, Akrishnan VNKG (2012). Antioxidant activity of ethanolic extract of Maranta arundinacea. L Tuberous Rhizomes. Asian Journal of Pharmaceutical and Clinical Research. 5(4): 85-88.
23. Patel R, Patel Y, Kunjadia P, Kunjadia A (2015). DPPH free radical scavenging activity of phenolics and flavonoids in some medicinal plants of India. International Journal of Current Microbiology and Applied Sciences. 4(1): 773-780.
24. Sakihama Y, Kohen MF, Grace SC, Yamasaki H (2002). Plant phenolic antioxidant and prooxidant activities:

phenolics-induced oxidative damage mediated by metals in plants. Toxicology. 177(1): 67-80.

25. Sathisha AD, Lingaraju HB, Prasad KS (2011). Evaluation of Antioxidant Activity of Medicinal Plant Extracts Produced for Commercial Purpose. European Journal of Chemistry. 8(2): 882-886.
26. Selvam K, Arunprakash S, Selvankumar T, Govarthanan M, Sengottaiyan A (2012). Antioxidant prospective and secondary metabolites in Abutilon indicum at different environment. International Journal of Pharmaceutical Sciences and Research. 3(7): 2011-2017.
27. Senguttuvan J, Paulsamy S, Karthika K (2014). Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, Hypochaeris radicata L. for in vitro antioxidant activities. Asian Pacific Journal of Tropical Biomedicine. 4(1): 359-367.
28. Shabbir G, Anwar F, Sultana B, Khalid ZM, Afzal M, Khan MQ, Ashrafuzzaman M (2011). Antioxidant and antimicrobial attributes and phenolics of different solvent extracts from leaves, flowers and bark of Gold Mohar. Molecules. 16: 7302-7319.
29. Suleman M, Nouren S, Hassan SM, Faiz AH, Sahr GA, Soomro GA, Tahir MA, Iqbal M, Nazir A (2018). Vitality and Implication of Natural Products from Viburnum Grandiflorum: an Eco-Friendly Approach. Polish Journal of Environmental Studies. 27(3): 1-5.
30. Sultana B, Anwar F, Mushtaq M, Aslam M, Ijaz S (2014). In vitro antimutagenic, antioxidant activities and total phenolics of clove (Syzygium aromaticum L.) seed extracts. Pakistan Journal of Pharmaceutical Sciences. 27(4): 893-899.
31. Sylvie DD, Anatole PC, Cabral BP, Veronique PB (2014). Comparison of in vitro antioxidant properties of extracts from three plants used for medical purpose in Cameroon: Acalypha racemosa, Garcinia lucida and Hymenocardia lyrata. Asian Pacific Journal of Tropical Biomedicine. 4(2): 625-632.
32. Tajidin NE, Ahmad SHl, Rosenani AB, Azimah H, Munirah M1 (2012). Chemical composition and citral content in lemongrass (Cymbopogon citratus) essential oil at three maturity stages. African Journal of Biotechnology. 11(11): 2685-2693.
33. Vanisha S, Nambiar, Matela H (2012). Review article Potential Functions of Lemon Grass (Cymbopogon citratus) in Health and Disease. International Journal of Pharmaceutical and Biological Archives. 3(5):1035-1043.

34. Wojdylo A, Oszmianski J, Czemerysr R (2007). Antioxidant activity and phenolic compounds in 32 selected herbs. 105(3): 940-949.
35. Wu N, Fu K, Fu YJ, Zu YG, Chang FR, Chen YH, Liu XL, Kong Y, Liu W. Gu, CB (2009). Antioxidant Activities of Extracts and Main Components of Pigeonpea [Cajanus cajan (L.) Millsp.] Leaves. Molecules. 14: 1032-1043.
36. Zafar F, Jahan N, Rahman KU, Aslam S (2016). Synergistic free radical scavenging potential of polyphenolic phytotherapeutics in various plants combinations. Oxidation communication. 39, 3(1): 2213-2221.
37. Zhang A, Fang Y, Wang H, Li H, Zhang Z (2011). Free-Radical Scavenging Properties and Reducing Power of Grape Cane Extracts from 11 Selected Grape Cultivars Widely Grown in China. Molecules. 16: 10104-10122.

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Evaluating the Varied Effects of Micronutrients on Wheat Variety TJ-83 Cultivation in Tando Jam

Asif Ali Kaleri*[1], Bakht Nisa Mangan[1], Muzamil Hussain Awan[2], Ghulam Mustafa Banbhan[3], Nabila Maryam[4], Farman Ali Kaleri[2], Danish Manzoor[1], Akhtar Jamali[1], Mena Hameed[5], Muhammad Sadiq[6], Salman Adil[7]

1. Department of Agronomy, SAU, Tando Jam, Pakistan
2. Department of PBG, SAU, Tando Jam, Pakistan.
3. Agriculture Research, Government of Sindh
4. Senior Scientist, Cotton Research Institute, Tandojam
5. Department of Agronomy, College of Agriculture, University of Sargodha, Pakistan.
6. Department of Agronomy, Balochistan Agriculture College Quetta, Pakistan.
7. Department of Plant Pathology, SAU, Tando Jam, Pakistan

Corresponding Author's Email: asifalikaleri2013@gmail.com

ABSTRACT: *In Pakistan, wheat is a staple food crop and this field experiments were department of agronomy at SAU Tando Jam. The present study revealed significant variations in the growth and yield of the wheat variety TJ-83. The extreme values were observed under treatment T9 (1750g ha-1), including a plant height of 95.50 cm, tillers at 325.00 m-2, spike length of 11.75 cm, grains per spike totaling 47.00, the weight of grain per spike at 2.50 g, seed index (1000 grain weight) of 48.00 g, a biological yield of 11960 kg ha-1, a grain yield of 5780 kg ha-1, and a harvest index of 48.90%. Following closely, treatment T7 (1500 g ha-1) exhibited a plant height of 94.80 cm, tillers at 320.00 m-2, spike length of 11.60 cm, grains per spike amounting to 47.80, weight of grain per spike at 2.45 g, seed index (1000-grain weight) of 47.80 g, biological yield of 11900 Kg ha-1, grain yield of 5680 Kg ha-1, and a harvest index of 47.90%. Conversely, the lowest values were recorded in the control group (T1 = No fertilizer), with a plant height of 70.10 cm, tillers at 240.10 m-2, spike length of 8.06 cm, grains per spike totalling 30.80, the weight of grain per spike at 1.40 g, seed index (1000-grain weight) of 32.10 g, biological yield of 7125 kg ha1, grain yield of 2920 kg ha1, and a harvest index of 40.80%.. The results suggest that enriching the soil with micronutrients significantly enhances wheat production.*

Keywords: Wheat, Micro NutrientsVarity TJ-83

INTRODUCTION

Wheat is positioned as the world's second-largest grain produced (Samar et al., 2019). It is the main crop grown by many people in Pakistan and makes up most of the country's cultivated area, which is about 9.1 million hectares (Abdullah, 2023). Wheat is paramount as Pakistan's mainstay crop, playing a vital role in safeguarding the nation's food security. The cultivation of wheat makes a substantial contribution, constituting 9.2 percent of agricultural value added and 1.8 percent of Pakistan's total GDP. Irshad et al., 2022. Wheat is a primary global food source, supplying carbohydrates and essential micronutrients such as iron, zinc, and vitamin B (Peter and Sandra, 2015). Micronutrient deficiency affects half of the world's population. The enrichment of food crops, particularly grains, which are widely consumed around the world, could assist in solving the problem. Plant nutrition management is one approach for enriching cereals with micronutrients (Cho et al., 2008; Bouis and Welch, 2010). Micronutrients have been shown to be important in agriculture all around the world. Micronutrient insufficiency is an expected trend among cereal crops, limiting grain output and nutritional value. Boron, iron, manganese, and zinc are micronutrients that perform significant physiological roles in humans and animals (Aref et al., 2012). Zinc is one of the most significant fundamentals in a carbohydrate's metabolism; most of the enzymes that play an important role in carbohydrate metabolism are stimulated by zinc. Zinc is the foremost building block of many enzymes, and it is essentially necessary for the development of some significant plant enzymes. In accumulation, it initiates many enzymatic responses. It is essential in many enzymes for their appropriate functioning and plays a vital role in the transcription of DNA (Kumar et al., 2016). Zinc is an essential micronutrient for plants, engaging actively in key cellular functions that are vital for important metabolic and physiological activities. It is critical for enzyme activation and the regulation of ion balance within the plant's system (Alsafran et al., 2022). Boron is essential for preserving the structural integrity of wheat plant cell walls, which is crucial for their overall stability. A boron deficiency can negatively impact root growth, ultimately leading to a reduced grain yield (Reid et al., 2014). Micronutrient deficiencies are prevalent globally; more than half of the soil utilized for cereal crop cultivation suffers from micronutrient

deficiencies. In Pakistan, a considerable number of soils are deficient in crucial micronutrients such as boron (B), attributed to their alkaline and calcareous nature, limited organic matter, regular cropping patterns, and poor nutrient management strategies. Research conducted by Cakmak in 2006, Rashid in 2006, and Shah et al. in 2017 has underscored this problem. Plants require boron for essential functions, and recent research on the biological role of this element in various metabolic, nutritive, hormonal, and physiological contexts has supplied evidence suggesting that boron is similarly indispensable for humans and animals (Kabu et al., 2013). In the wheat crop, insufficient boron can cause irregular growth patterns, impede the development of shoots and roots, and ultimately lead to a reduction in crop yield (Hu et al., 2013). This study's primary goal was to identify the optimal growth stage for wheat at which zinc (Z) and boron (B) application would result in the highest yield. Furthermore, it sought to determine the appropriate amounts of Z and B to apply and to evaluate the impact of these nutrients on different yield characteristics of wheat.

MATERIAL AND METHODS

The investigation was carried out at the Student Experimental Farm, situated in the Department of Agronomy at SAU Tando Jam in Sindh, Pakistan, during the Rabi season of 2022–23. The experiment's specifics followed the guidelines of the randomized complete block design.

Replication = 03

Net plot size: 3 m x 4 m, 12 m2.

Variety =TJ-83

Treatments=08

T1 = untreated (control)

T2 = 250g ha^{-1}

T3 = 500g ha^{-1}

T4 = 750g ha^{-1}

T5 = 1000g ha^{-1}

T6 = 1250g ha^{-1}

T7 = 1500g ha^{-1}

T8 = 1750g ha^{-1}

Culture practices

The soil underwent careful preparation involving two comprehensive plowings, followed by leveling of the land to establish an optimal seedbed. The recommended quantity of DAP fertilizer was evenly distributed across all plots during the sowing process. Throughout the research, zinc, boron, and iron were provided at different stages of wheat growth. To evaluate the plant characteristics, five plants were selected from each plot every five days during the initial ten days following the planting of crops.

The observation was recorded. Key parameters for evaluating crop performance include plant height, tillers per square meter, spike length, grains per spike, grain weight per spike, seed index (weight of 1000 grains), biological yield per hectare, grain yield per hectare, and harvest index.

Statistical analysis: The data underwent statistical analysis using Statistix 8.1 software. The LSD test was employed to compare the means of different treatments at a significant level of 5%.

Table 1: Evaluation of the soil at the field trial location involved both physical and chemical analyses.

Characteristics	**Units**	**Value (2017–18)**
Soil depth	(cm)	0–15
		15–30
Texture	(Class)	Sandy clay loam
pH		7.5
		7.7
EC	(dS m−1)	2.31
		2.36
Organic matter	(%)	0.72
		0.67
Total nitrogen	(%)	0.041
		0.038
Available B	(mg kg−1)	0.7
		1.4
DTPA Zn	(mg kg−1)	0.51
		0.47

Table 2: Zinc, Boron and Iron fertilization assessing their differential impact on wheat crops

Micronutrients Levels	Plant height (cm)	Tillers m^{-2}	Spike Length (cm)	Grain per $spike^{-1}$	Grain weight per $spike^{-1}$	Seed index (1000) grain weight, g)	Biological yield (kg ha^{-1})	Grain Yield (kg ha^{-1})	Harvest Index (kg ha^{-1})
T1=Untreated (Control)	70.10 f	240.10 g	8.06 g	30.80 h	1.40 d	32.10 e	7125 h	2920 g	40.80 d
T2=250g ha^{-1}	75.55 e	260.10 f	9.55 e	34.40 f	1.60 d	37.65 d	7895 f	3740 e	46.90 bc
T3=500g ha^{-1}	80.65 d	272.00 e	9.90 d	36.11 e	1.69 cd	27.65 cd	8380 e	3870 e	45.90 c
T4=750g ha^{-1}	83.50 c	280.00 d	10.00 c	38.77 d	1.70 cd	41.19 c	9120 d	4360 d	47.60 ab
T5=1000g ha^{-1}	84.30 c	290.30 c	10.25 c	40.55 c	1.95 bc	43.20 c	9870 c	4670 c	46.80 bc
T6=1250g ha^{-1}	91.10 b	310.00 b	11.50 b	45.80 b	2.00 b	46.50 b	11718 b	5490 b	47.90 bc
T7=1500g ha^{-1}	94.80 a	320.00 a	11.60 ab	47.80 ab	2.45 a	47.80 a	11900 ab	5680 a	47.90 ab
T8=1750g ha^{-1}	95.50 a	325.00 a	11.75 a	47.00 a	2.50 a	48.00 a	11960a	5780 a	48.90 a
S.E± =	1.11	1.89	0.059	0.52	0.15	5. 19	40.26	93.48	0.57
$LSD_{0.05}$ =	2.51	4.14	0.14	1.09	0.33	11. 07	87.59	190.28	1.42

RESULTS

Plant height (cm)

The utilization of micronutrients zinc and boron on wheat crops substantially and positively influenced numerous physiological yields and yield constituent characteristics. (Table 1). The plant height (cm) of wheat is affected by different levels of micronutrients. The treatments T8 = 1750 g ha^{-1} produced a maximum plant height of 95.50 cm, while the crops receiving T_7 =1500g ha^{-1} and T_6 = 1250g ha^{-1} resulted in mean plant heights of 94.80 cm and 91.10 cm, respectively. Similarly, the following mean plant heights (84.30 cm, 83.50 cm, 80.65 cm, and 75.55 cm) were observed when crop treatments with T5, T_4, T_3, and T_2 were applied. Further,

the lowest mean plant height (70.10 cm) was noted with T_1 = Control, no fertilizer, 00 kg ha^{-1}.

Number of tillers: m^2

The number of tillers per m2 of wheat is affected by different levels of micronutrients. The treatments T8 = 1750 g ha-1 had a maximum number of tillers m^{-2} of 325.00, while the crops receiving T_7 =1500g ha^{-1} and T_6 = 1250g ha^{-1} had a mean number of tillers per hectare of 320.00 and 310.00, respectively. Similarly, the following mean number of Tillers m^{-2} (290.30, 280.00, 272.00, and 260.10) were observed when crop treatments with T_5, T_4, T_3, and T_2 = were applied. Further, the lowest mean number of tillers (m^{-2}) (240.10) was noted with T_1 = control, no fertilizer, 00 kg ha^{-1}.

Spike Length (cm)

The spike length (cm) of wheat is affected by different levels of micronutrients. The treatments T8 = 1750 g ha-1 had a maximum spike length of 11.75 cm, while the crops receiving T_7 =1500g ha^{-1} and T_6 = 1250g ha^{-1} had spike lengths of 11.60 cm and 11.50 cm, respectively. Similarly, the following mean spike length cm (10.25, 10.00, 9.90, and 9.55) were observed when crop treatments with T_5, T_4, T_3, and T_2 were applied. Further, the lowest mean spike length (cm (8.06)) was noted with T_1 = control, no fertilizer, 00 kg ha^{-1}.

Grain per spike

The grain per spike of wheat is affected by different levels of micronutrients. The treatments T8 = 1750 g ha-1 had a maximum grain per spike of 47.00, while the crops receiving T_7 =1500g ha^{-1} and T_6 = 1250g ha^{-1} resulted in grain per spike of 47.80 and 45.80, respectively. Similarly, the following mean grain per spike (40.55, 38.77, 36.11, and 34.40) were observed when crop treatments with T_5, T_4, T_3, and T_2 were applied. Further, the lowest mean grain per spike (30.80) was noted with T_1 = control, no fertilizer, 00 kg ha^{-1}.

Grain weight per spike

The grain weight per spike of wheat is affected by different levels of micronutrients. The treatments T8 = 1750 g ha-1 had a maximum grain weight per spike of 2.50, while the crops receiving T_7 =1500g ha^{-1} and T_6 = 1250g ha^{-1} had a grain weight per spike of 2.45 and 2.00, respectively. Similarly, the following mean grain weight per spike (1.95, 1.70, 1.69, and 1.60) were observed when crop treatments with T_5, T_4, T_3, and T_2 were applied. Further, the lowest mean grain weight per spike (1.40) was noted with T_1 = control, no fertilizer, 00 kg ha^{-1}.

Seed index: 1000; grain weight: g

The seed index (1000) and grain weight (g) of wheat are affected by different levels of micronutrients. The treatments T8

= 1750 g ha^{-1} had a maximum seed index (1000) grain weight (g) of 48.00, while the crops receiving T_7 =1500g ha^{-1} and T_6 = 1250g ha^{-1} resulted in seed index (1000) grain weight (g) of 47.80 and 46.50, respectively. Similarly, the following mean grain weight per spike (43.20, 41.19, 27.65, and 37.65) were observed when crop treatments with T_5, T_4, T_3, and T_2 were applied. Further, the minimum seed index (1000) grain weight (g) (32.10) was noted with T_1 = control, no fertilizer, 00 kg ha^{-1}.

Biological yield (kg ha^{-1})

The biological yield (kg ha^{-1}) of wheat is affected by different levels of micronutrients. The treatments T8 = 1750 g ha-1 had a maximum biological yield (kg ha^{-1}) of 11960, while the crops receiving T_7 =1500g ha^{-1} and T_6 = 1250g ha^{-1} resulted in a maximum biological yield (kg ha^{-1}) of 11900 and 11718, respectively. Similarly, the following mean biological yields (kg ha^{-1}) (9870, 9120, 8380, and 7895) were observed when crop treatments with T_5, T_4, T_3, and T_2 were applied. Further, the lowest mean biological yield (kg ha^{-1}) (7125) was noted with T_1 = control, no fertilizer, 00 kg ha^{-1}.

Grain yield kg ha^{1}

The grain yield (kg ha^{-1}) of wheat is affected by different levels of micronutrients. The treatments T8 = 1750 g ha-1 had a maximum grain yield (kg ha^{-1}) of 5780, while the crops receiving T_7 =1500g ha^{-1} and T_6 = 1250g ha^{-1} had a grain yield (kg ha^{-1}) of 5680 and 5490, respectively. Similarly, the following mean grain yields (kg ha^{-1}) (4670, 4360, 3870, and 3740) were observed when crop treatments with T_5, T_4, T_3, and T_2 were applied. Further, the lowest mean grain yield (kg ha^{-1}) (2920) was noted with T_1 = control, no fertilizer, 00 kg ha^{-1}.

Harvest Index (kg ha^{-1})

The harvest index (kg ha^{-1}) of wheat is affected by different levels of micronutrients. The treatments T8 = 1750 g ha-1 had a maximum harvest index (kg ha^{-1}) of 48.90, while the crops receiving T_7 =1500g ha^{-1} and T_6 = 1250g ha^{-1} had a harvest index (kg ha^{-1}) of 47.90 and 47.90, respectively. Similarly, the following mean Harvest Index (kg ha^{-1}) (46.80, 47.60, 45.90, and 46.90) were observed when crop treatments with T_5, T_4, T_3, and T_2 were applied. Further, the lowest mean harvest index (kg ha1) of 40.80 was noted with T_1 = control, no fertilizer, and 00 kg ha1.

DISCUSSION

The agricultural challenge extends beyond merely feeding the masses; it also involves delivering nutrient-rich food to impoverished individuals. To address this, there is a need to design agriculture systems that prioritize the overall

health and well-being of the population. (Maberly et al., 2010). Typically, integrating micronutrient fertilizers such as zinc and boron into the soil can significantly improve the growth, yield, and overall health of wheat crops. Farmers and agricultural specialists advocate for the inclusion of these fertilizers in their crop management plans to boost productivity and enhance profitability. The findings of the research underscore the vital significance of trace elements in fostering the development and productivity of the wheat variety TJ-83. The maximum plant height is 95.50 cm, the number of tillers is 325.00, the spike length is 11.75 cm, the grain per spike is 47.00, the grain weight per spike is 2.50, the seed index (1000) of grain weight is 48.00, the biological yield (kg ha^{-1}) is 11960, the grain yield (kg ha^{-1}) is 5780, and the harvest index (kg ha^{-1}) is 48.90. The test results showed that trace elements are significant in the growth and yield characteristics of the wheat variety TG-83 when applied at levels of treatment T8 = 1750 g ha^{1} Respectively. The total number of tillers remained largely unaffected by the application of zinc (Z), boron (B), and iron (Fe) at various growth stages. This lack of significant change might be since B was not applied during the tillering phase. This observation aligns with findings previously reported by Khan et al. (2010) and Hussain et al. (2005). The results of the experiment indicated that trace elements are vital in affecting the growth and yield of the wheat variety TJ-83. Micro-nutrient fertilizers were administered at a rate of 1750 g/ha. Subsequently, various plant parameters were assessed, including plant height, tillers per square meter, spike length, grains per spike, grain weight per spike, seed index, biological yield, grain yield, and harvest index. In comparison, the untreated control treatment exhibited minimal growth and yield characteristics. The findings of this study are consistent with the research conducted by Rahman et al. (2014). Likewise, the results from this investigation closely represent those reported by Zeidan et al. (2010). Adding zinc to the soil was found to enhance several aspects of wheat production, including grain weight, grain count per spike, total grain yield, biological yield, zinc concentration in flag leaves and

grains, and the protein content of the wheat grains. As reported by Debnath et al. (2014), soil application of zinc contributed to improvements in the weight of a thousand grains, the number of grains per spike, overall grain yield, total biological yield, zinc levels in flag leaves and grains, and the protein content in the grains. The research findings indicated that applying zinc and boron significantly enhanced numerous plant growth metrics, including plant height, number of tillers m2, spike length cm, grain per spike, grain weight per spike, seed index 1000 grain weight g, biological yield kg ha^{-1}, grain yield kg ha-1, and harvest index kg ha^{-1} (Anjum et al., 2017). In a 2019 study by Hassan et al., it was discovered that applying zinc to the soil during the tillering and ear stages of wheat growth significantly enhanced both the yield and quality of the grain compared to untreated plants. Moreover, research conducted by Pahlavan and Pessarakli in 2009 underscored the synergistic impact of zinc and iron on wheat grain weight, demonstrating a substantial increase in yield when both nutrients were supplied. The highest weight of 1000 grains were achieved through the application of 20 kg of zinc and iron. Muhammad et al. (2006) investigated the influence of soil zinc levels on the physiology, phenology, yield indices, and zinc and iron content of wheat grains. Results indicated that leaf area, tiller m2, productive tiller, and yield components. Mekkei and Eman (2010) achieved comparable findings, noting that the soil application of zinc significantly elevated plant height (in centimeters), spike length (in centimeters), grains per spike, 1000-grain weight (in grams), and wheat grain yield (in tons per hectare). Metwally and co-authors (2012) discovered that employing zinc fertilizer in soil has been effective in enhancing grain quality. Similarly, Bameri et al. (2012) corroborated this finding, asserting that zinc soil fertilizer effectively improves grain quality. Furthermore, Esfandiari et al. (2016) documented that applying zinc to the soil had a notably positive influence on wheat grain production and its assorted components. Many studies have indicated that the augmentation of agronomic traits can be attributed to the foliar application of zinc. According to Jiang and Huang

(2002), the increase in wheat yield and its components is associated with zinc influencing the volume of chlorophyll and the concentration of abscisic acid. The elevation of chlorophyll levels contributes to enhanced yield by promoting photosynthesis. The use of boron positively influenced the grain yield of wheat crops, an effect attributable to boron's distinctive distribution within the plant's dry matter, as noted by Hussain and Yasin (2004). Conversely, a deficiency in boron can cause grain sterility, leading to a marked reduction in grain yield, as documented by Subedi et al. (2000). Generally, adding micronutrient fertilizers, including zinc and boron, can significantly boost the growth, yield, and quality of wheat. It is strongly advised for farmers and agronomists to include these fertilizers in their crop management plans to improve productivity and increase profits.

CONCLUSIONS

The study concludes that the yield-affecting characteristics of the wheat variety TJ-83 are notably influenced by various soil-applied micronutrients. The results suggest that the application of micronutrients to the soil significantly enhances wheat yield and that various factors contribute to yield. After conducting research, it was established that the most efficient approach to boosting wheat growth and augmenting grain yield involves applying micronutrients at a rate of 1750 grams per hectare alongside the recommended dose of fertilizer.

REFERENCES

1. Abdullah N (2023). agriculture and climate change. Profit Agriculture magazine.
2. Alsafran M, Usman K, Ahmed B, Rizwan M, Saleem MH, Al Jabri H (2022). Understanding the phytoremediation mechanisms of potentially toxic elements: A proteomic overview of recent advances. Front. Plant Sci. 13(1): 881242.
3. Aref F, Rad HE (2012). Physiological characterization of rice under salinity stress during vegetative and reproductive stages. Ind. J. Sci. Technol. 5(4): 2578-2586.
4. Blamey FP, Mould C, Chapman J (2017). Concentrations in plant tissues

of two wheat cultivars. J. Agron. 71: 243-247.

5. Bouis HE, Welch RM (2010). Biofortification is a sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. Crop Sci. 50: S-20.
6. Cakmak I (2006). Enriching grain with micronutrients: Benefits for crop plants and human health. IFA Ag. Conf, International Fertilizer Industry Association (IFA). Optimizing resource use efficiency for sustainable intensification of agriculture, February 27-March 2, Kunming, China.
7. Cho K, Wang XU, Nie S, Chen Z, Shin DM. (2008). Therapeutic nanoparticles for drug delivery in cancer. Clin. Can. Res. 14(5): 1310-1316.
8. Debnath C, H. A. N. D. A. N, Kader MA, Islam N (2014). Effect of nitrogen and boron on the performance of wheat. J. Environ. Sci. Nat. Res. 7(1): 105-110.
9. Esfandiari E, Abdoli M, Mousavi SB, Sadeghzadeh B (2016). Impact of foliar zinc application on agronomic traits and grain quality parameters of wheat grown in zinc deficient soil. Ind. J. Plant Physiol. 21(1): 263-270.
10. Irshad M, Hussain M, Baig MA (2022). Macroeconomic variables the indicators for the economic growth of Pakistan. Pak. Soci. Sci. Rev. 6(2): 58-72.
11. Hu X, Zhang K, Sun Z (2013). Numerical simulation and optimization of rotary drilling parameters for extended reach wells in offshore oil fields. J. Petrol. Sci. Eng. 107(1): 82-89.
12. Hussain N, Khan MA, Javed MA (2005). Effect of foliar application of plant micronutrient mixture on growth and yield of wheat (Triticum aestivum L.). Pak. J. Biol. Sci. 8: 1096-1099.
13. Hussain F, Yasin M (2004). Soil fertility monitoring and management in rice-wheat system. Ann. Rep. LRRP, NARC, Islamabad, Pakistan: 1-33.
14. Kabu M, Akosman MS (2013). Biological effects of boron. Rev. Environ. Contami. Toxicol. 57-75.
15. Kaleri AA, Khushk GM, Jogi Q, Lund MM, Magsi M, Baloch HN, Laghari R (2023).

Response of Wheat Crop to Various Foliar Applications of Nitrogen, Zinc and Boron Fertilizers. Plant Health. 2(2): 51-55.

16. Khan MB, Muhammad F, MF, Mubshar H, M H, Shahnawaz S, Ghulam S, GS. (2010). Foliar application of micronutrients improves the wheat yield and net economic return. Int. J. Agric. Biol. 12: 953-956.
17. Kumar M, Sarangi A, Singh DK, Rao AR, Sudhishri S (2016). Response of wheat cultivars to foliar potassium fertilization under irrigated saline environment. J. Appli. Nat. Sci. 8(1): 429-436.
18. Khurshid N, Ali K, Fiaz (2020). A. Pakistan Economic Review, Pakistan Economic Survey Team.
19. Peter R, Shewry, Sandra JH (2015). The contribution of wheat to human diet and health. Food Energy Secur. 4(3): 178–202.
20. Rashid A (2006). Incidence, diagnosis and management of micronutrient deficiencies in crops: Success stories and limitations in Pakistan. In IFA Int. Workshop Micronut. (27):1-23.
21. Rehman H U (2019). Impact of foliar applied boron on wheat yield and nutrient uptake under varying fertility levels. J. Plant Nut. 42(15): 1812-1821.
22. Reid GD, Spencer R, Elkamel A (2014). A comparison of exergy-based sustainability measures for energy systems. Energy. 69(1): 82-93.
23. Samar MC (2019). *On* the Precipice of Transition: Water, Crops and Adaptation in Pinal County, Arizona (Doctoral dissertation, Northern Arizona University. 1-24.
24. Shah JA, Sial MA, Abbas M (2017). Disparity in growth, yield and fibre quality of cotton genotypes grown under deficient and adequate levels of boron. Pak. J. Agric. Agric. Eng. Vet. Sci. 33(2): 163-176.
25. Singh U, Praharaj C S, Singh S S, Singh N P (2016). Biofortific. Food Crops. New Delhi: Springer. 480. pp. 3-18.
26. Subedi K D, Gregory P J, Summer field, R. J, Gooding, M J (2000). Pattern of grain set in boron-deficient and cold-stressed wheat (Triticum aestivum L.). J. Agric. Sci. 134(1): 25-31.
27. Tripathi DK, Singh S, Gaur S, Singh S, Yadav V, Liu S, Sahi S (2018). Acquisition and homeostasis of iron in higher plants and their probable role in abiotic stress tolerance. Front. Environ. Sci. 5(1): 86.

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CRISPR: An Elixir for Autoimmune Diseases? A Systematic Review

Amina Javid[1], Muhammad Numan[2], Sara Janiad[3], Mehboob Ahmed[1]*

1. Institute of Microbiology and Molecular Genetics, University of the Punjab, Quaid-e-Azam Campus, Lahore-54590, Pakistan
2. Department of Microbiology, Gulab Devi Educational Complex, Lahore, Pakistan
3. Department of Microbiology and Molecular Genetics, The Women University Multan, Multan, Pakistan

Corresponding Author's Email: mehboob.mmg@pu.edu.pk

ABSTRACT: *Genetic studies have linked the gene polymorphisms and autoimmune disorders. In response, the Clustered Regularly Interspaced Short Palindromic Repeats and its associated protein 9 (CRISPR-Cas9) has become a promising tool for treating these diseases. The broad acceptance of CRISPR, due to its simplicity, precision, and adaptability, has significantly rushed scientific research, and fostered radical discoveries in both model species and human cells. CRISPR-Cas9 offers versatile applications for rare diseases like urea cycle disorders or hepatorenal tyrosinemia and in reducing cholesterol by targeting Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9). It can also immunomodulate the autoimmune diseases by specifically targeting genes associated with these conditions. This targeted approach holds the potential to modify the immune response, leading to the potential alleviation of disease progression. Our review underscores the ongoing exploration of CRISPR-Cas9 therapy for autoimmune disorders, emphasizing its transformative possibilities in this field. We specifically highlight the potential target genes for CRISPR-Cas9 immunomodulation in prevalent autoimmune disorders such as systemic lupus erythematosus, multiple sclerosis, insulin-dependent diabetes mellitus, psoriasis, type 1 coeliac disease, and rheumatoid arthritis. The future holds immense promise as the remarkable advances in CRISPR-Cas9 therapies pave the way for a revolutionary transformation in the treatment of various autoimmune disorders.*

Keywords: *Autoimmune disorders, CRISPR-Cas9 therapy, Immunomodulation, Gene therapy, RNA silencing*

INTRODUCTION

The understanding of the human genome has been greatly enhanced due to the continued advancement of genome editing techniques in recent decades, which has virtually enabled us to grasp a deeper comprehension of the role of genes and gene products in disease processes (Pavel-Dinu et al., 2023). The marvellous achievements of genetic engineering (the modification of nucleic acids) caused a breakthrough in the field of genome editing, back in the decade of 1970s. Over the past ten years, scientists are successfully able to perform various astonishing roles in the domains of biomedical research and applied biotechnology by using nucleases enzymes either synthetic or extracted from bacteria. All these achievements are achieved at a more rapid rate than ever imagined.

The essence of genome editing is the ability to permanently alter DNA at the molecular level. Scientists have successfully availed the two most powerful biological strategies including Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9 and Transcription Activator-like Effector Nuclease (TALEN) systems. Both technologies are being used widely nowadays (Doudna, 2020; Ustiugova et al., 2023).

The history of genome editing techniques takes us back to the early years of molecular biology, where the foundational principles were laid by scientists such as Crick. It was Crick who first articulated the central dogma of the field, which states that information flows from DNA/RNA (nucleic acid) to proteins in a sequential manner. This fundamental concept highlights the importance of permanent DNA modifications, as they have the potential to reshape protein sequences and thereby profoundly influence their functionality.

Gene Editing vis-à-vis Immunological Disorders: Research in immunology experienced a paradigm shift in the late 1990s with the completion of the human genome reference sequence. Although the 3.2 billion DNA bases in our bodies were discovered as part of the Human Genome Project, yet functions of most of the genes remained unknown (Feng et al., 2023). The focus of genomic research has been primarily on immune cells, which tackle invading foreign pathogens and are thus essential for maintaining human health. For mapping the DNA along with its associated genetic regulators responsible for the types, states, and functions of immune cells, various techniques, such as

transcriptional and chromatin state profiling, have been employed to study these cells (Vockley et al., 2023).

Conversely, genetic manipulation is the only way to provide answers to important concerns regarding immunity and DNA. What is the biological significance of the functional sequences in our DNA? What is the genetic framework—encoded in genes, noncoding sequences, and trans-regulators—that is responsible for wiring certain cellular pathways as well as performing specific roles in immune cells? What changes in cellular function and risks of immune-mediated illness can be attributed to variation in important coding and non-coding sequences? Can we reprogram natural immune cell genetic circuits using what we know about them to create the next wave of synthetic cellular therapies? The ability of emerging technology to edit immune cells' genomes will determine the solutions to all such queries. Immunologists are already starting to modify immune cell genomes to unveil the genetic basis of immunity thanks to one of these tools, CRISPR (Guo et al., 2022; Akram F et al., 2023).

Currently, several researchers are working to modify the underpinnings of genomes of immune cells so that immunological diseases can be treated using CRISPR. In this article, we review some autoimmune diseases that can be treated using CRISPR. However, before delving into those diseases and exploring how CRISPR technology can be employed to treat them, we first discuss the basics of the CRISPR technique.

CRISPR: The revolutionary technology known as CRISPR, in conjunction with its associated protein Cas9, originates from the bacterial cell's adaptive immune response system. To put it simply, the CRISPR sequence within the bacterium incorporates small segments of the viral genome, acting as memory sequences. These sequences enable the bacterium to recognize and mount a defence against future infections by the same virus. Furthermore, the Cas9 protein, serving as an endonuclease, plays a crucial role in this process by targeting and inducing double-strand breaks in the viral genome. This mechanism potentially renders the virus inactive, providing an effective defence mechanism for the bacterium (Katti et al., 2022).

The CRISPR sequence consists of multiple short repeating sequences interspersed with longer sequences known as spacers. When a host bacterium is exposed to a viral infection, small segments of the viral genome are integrated into the CRISPR region of the bacterium's genome, resulting in the production of

spacer sequences. These spacer sequences are transcribed into a long RNA molecule called pre-CRISPR RNA (pre-crRNA) (Khanzadi and Khan, 2020). The processing of pre-crRNA and the efficient functioning of the CRISPR/Cas9 system rely on the involvement of a small RNA molecule called trans-activating CRISPR RNA (tracrRNA). The tracrRNA is produced from the upstream region of the CRISPR sequence. Within the CRISPR sequence, the short repetitive sequences exhibit complementarity to specific regions of the tracrRNA. This complementary binding allows the pre-crRNA to form a duplex RNA structure with the tracrRNA, facilitating further processing and activation of the CRISPR/Cas9 system (Guo et al., 2022). The formation of the mature crRNA: tracrRNA complex involves sequential processing steps, including catalysis by RNASIII and an unidentified nuclease. Through these processing phases, the spacer region of the crRNA, derived from viral DNA fragments, serves as a crucial memory component for the bacterial cell. This memory function allows the bacterial cell to recognize and mount a specific immune response against future encounters with the corresponding virus (Hillary and Ceasar, 2023). Fig. 1 visually represents this process.

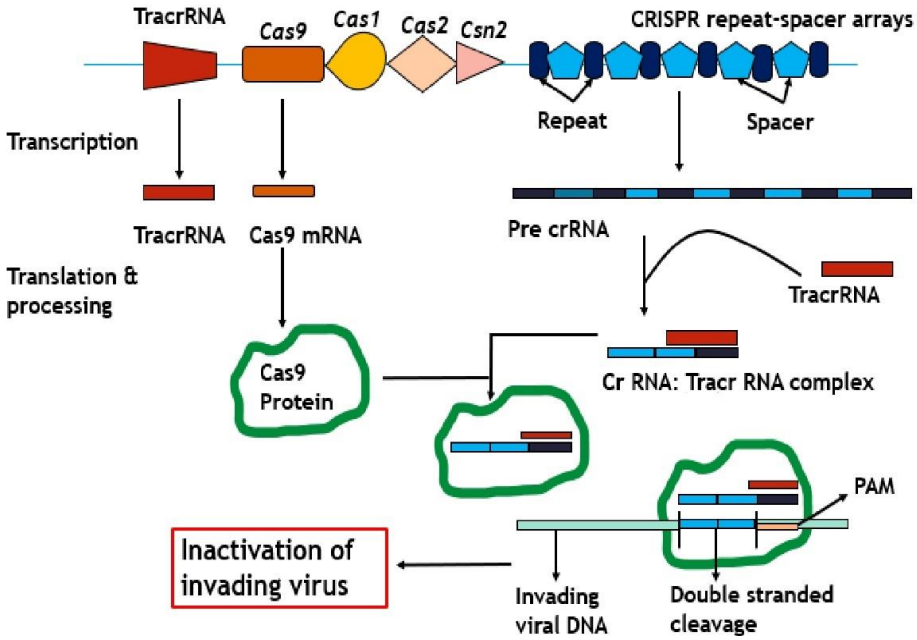

Fig. 1. CRISPR as a bacterial or archaeal adaptive immune system

The discovery of the CRISPR/Cas system was rooted in the intriguing observation of a distinct set of repetitive sequences downstream of the termination codon of the *Iap* gene's translation in *Escherichia coli* (*E. coli*) (Bharathkumar et al., 2022). These repetitive sequences, consisting of 14 base pairs and known as palindromic repeats, form the CRISPR locus. Throughout the genome of bacteria and archaea, these repeats are interspersed with 32-nucleotide sequences, creating a repetitive pattern. This CRISPR locus serves as a natural defence mechanism in these organisms, directed by RNA, to combat both RNA and DNA viruses (Pinilla-Redondo et al., 2022). Furthermore, the CRISPR locus not only consists of the palindromic repeats and spacer sequences but also contains foreign DNA segments known as CRISPR Array Regions. These CRISPR Array Regions are inserted between the palindromic repeats and play a crucial role in the system's functionality. By storing and translating information about previous infections, the CRISPR/Cas system serves as a paradigm for adaptive immune responses. This remarkable system enables the organism to retain a molecular memory of past encounters with specific pathogens, allowing for a more targeted and efficient immune response in future encounters (Sharma et al., 2021).

The technology has made significant progress since the initial report on CRISPR in 1987 (Fig. 2). The CRISPR-Cas9 technique has enabled genetic experiments to be conducted on a wide range of living species. This includes various organisms such as plants, *Drosophila*, zebrafish, mice, and even more complex organisms like humans. The versatility of CRISPR-Cas9 has expanded its applications across different species, facilitating precise genetic modifications and furthering our understanding of gene function and regulation.

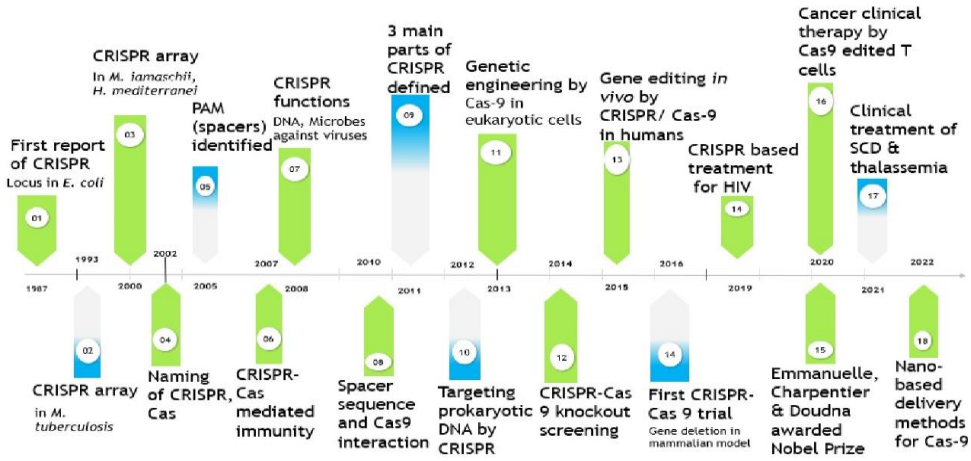

Fig. 2. Timeline of CRISPR-Cas9 System Development and Advancement

Phases of CRISPR mediated immunity: CRISPR-associated genes (Cas), which play a vital role in the CRISPR system, are typically found near the CRISPR region. These genes are known to be involved in various stages of adaptive immunity (Liu et al., 2019). The process of CRISPR-mediated adaptive immunity in bacteria or archaea can be broadly divided into three phases:

- Adaptation Phase: During this phase, the Cas genes, including Cas1 and Cas2, recognize and process the invading DNA. These Cas proteins facilitate the integration of short DNA fragments from the invading DNA into the spacer region of the CRISPR array, allowing the organism to acquire a genetic memory of past encounters with specific pathogens (Shmakova et al., 2022).
- Expression Phase: In this phase, the CRISPR array is transcribed into a precursor RNA called pre-crRNA. The pre-crRNA is then processed to generate mature CRISPR RNAs (crRNAs) with a specific 20-nucleotide sequence that targets the foreign DNA. The repeats in the crRNA interact with a trans-activating RNA molecule called tracrRNA. The Cas9 protein, along with the crRNA and tracrRNA complex, forms an active interference complex (Kanafi and Tavallaei, 2022).
- Interference Phase: In this final phase, the interference complex, consisting of the Cas9 endonuclease and the crRNA-tracrRNA complex, recognizes and binds to the

complementary sequence in the foreign DNA. The Cas9 endonuclease then cuts the double-stranded DNA, leading to the degradation or inactivation of the foreign genetic material (Dao et al., 2023).

Classification of CRISPR: The structure of CRISPR/Cas loci in bacteria and archaea can exhibit various variations, leading to the categorization of CRISPR systems based on their effector proteins. Presently, CRISPR is classified into two classes and six types, each characterized by a specific Cas protein (Makarova et al., 2020; Chaudhuri et al., 2022). The composition of the Cas protein involved in the interference phase serves as the primary distinguishing factor between Classes 1 and 2 (Burmistrz et al., 2020; Tian et al., 2022).

The Class 1 interference phase of the CRISPR system involves multiple Cas proteins, specifically Types I, III, and IV (Chakrabarti et al., 2019; Khan et al., 2022). On the other hand, Class 2 CRISPR systems rely on a single protein to perform multiple functions during the interference phase, encompassing Types II, V, and VI (Makarova et al., 2022). Among the Class 2 CRISPR systems, Cas9 is the most extensively studied and utilized protein due to its versatile capabilities. Cas9 belongs to Type II CRISPR and is responsible for various crucial tasks such as binding with the guide RNA (crRNA + tracrRNA), scanning the genome, and inducing double-strand breaks (Zhang et al., 2021).

The distinct roles and characteristics of Cas proteins in different CRISPR classes and types provide researchers with a diverse toolkit for manipulating and studying genetic material. Understanding the specific functions and mechanisms of these proteins is essential for harnessing the full potential of CRISPR technology.

Genome Editing Unlocked: The Contributions of Cas Proteins: The single guide RNA (sgRNA) serves as the instructional component for Cas endonucleases, such as Cas9, Cas12, Casx, etc., directing them to the desired location in the DNA where a double-strand break is to be introduced (Fuziwara et al., 2022). Cells possess a DNA repair system that facilitates the successful ligation of DNA, as this type of break can be highly detrimental and lead to genome loss. Gene editing techniques, including the insertion or deletion of nucleotides from the DNA sequence, are employed in this process to repair and ligate damaged DNA strands (Zhuo et al., 2021).

The two primary processes for repairing DNA double-strand breaks are non-homologous end joining (NHEJ) repair and homologous directed repair

(HDR) (Zhuo et al., 2021; Fuziwara et al., 2022).

- Non-homologous End Joining Repair (NHEJ):
  NHEJ is the primary mechanism for double-strand break repair and is effective at rejoining the broken ends. While NHEJ lacks specificity and may introduce errors, it is significant for gene disruption as it can lead to loss of gene expression or protein function through microdeletions, insertions, frameshift mutations, or premature stop codon insertions in target genes (Song et al., 2021).
- Homologous Directed Repair (HDR):
  HDR utilizes homologous DNA fragments, either exogenous or endogenous, to accurately repair double-strand breaks. HDR is less prone to errors but occurs at a lower frequency, primarily during the S-G2 phase of the cell cycle (Smirnikhina et al., 2022). Exogenous DNA templates can be introduced to provide the correct gene sequence required for gene repair through HDR, but it requires the addition of Cas9 and sgRNA.
  The frequency of HDR can be modified by various approaches, including cell synchronization, medication, or molecular manipulation, to enhance gene repair efficiency (Yoshimi et al., 2021). These strategies aim to increase the utilization of HDR for precise gene editing and repair.

Autoimmune Disorders:

Autoimmune diseases occur when the immune system mistakenly targets and attacks the body's own tissues, leading to organ damage or dysfunction (Marrack et al., 2001; Xiao et al., 2021). Maintaining immunological tolerance is a vital feature of the human immune system, as it helps prevent self-inflicted harm (Olivieri et al., 2021). However, in cases of autoimmunity, the immune system mistakenly launches an immune response against its own tissues and organs. Autoimmune disorders affect approximately 3 to 5% of the general population, and they have been identified as a leading cause of mortality among women in their 20s and 30s (Krovi and Kuchroo, 2022; Mubariki and Vadasz, 2022).

The consequences of autoimmune diseases extend beyond physical symptoms. Individuals with these conditions often experience a lower quality of life, as the chronic nature of the diseases can disrupt daily activities, limit mobility, and cause persistent pain or discomfort. Moreover, the emotional toll of living with a chronic illness should not be overlooked. Studies have indicated that individuals with autoimmune disorders are more prone to mental

health challenges, such as anxiety and depression, further impacting their overall well-being (Nadali et al., 2023).

Genetic factors, abnormal immune responses, and environmental influences are known to contribute to the development of autoimmune diseases. The intricate interplay between these factors, either individually or in combination, can disrupt the normal immune tolerance mechanisms and lead to the onset of autoimmune disorders. During the maturation of B and T cells, self-antigen-responsive cells are typically eliminated or rendered inactive (anergized) to prevent the formation of self-reactive cells (Olivieri et al., 2021; Xiao et al., 2021). In certain instances, the regulatory system responsible for eliminating self-reactive cells fails, allowing them to evade the typical checks and balances. The regulation of these cells involves various mechanisms, including the crucial role played by regulatory T cells (Marks and Rao, 2022).

Autoimmune diseases involve the production of autoantibodies by the immune system, which target the body's own tissues or organs. These autoantibodies recognize specific self-antigens and play a role in the development and advancement of various autoimmune disorders. The identification and understanding of these autoantibodies, as outlined in Table 1, are essential for accurate diagnosis, prognosis, and the development of targeted treatments for autoimmune conditions.

Table 1: Few diseases associated with autoantibodies

Autoantibody	Subtypes	Target antigens	Diseases	References
Anti-thyroid Antibody	Anti-TPO	Thyroid peroxidase (microsomal)	Graves' disease, Hashimoto's thyroiditis,	(Malandrini et al., 2022; Siddiq et al., 2023)
Anti-thrombin Ab	-	Thrombin	Systemic lupus erythematosus	(Szabó et al., 2021; Yamamoto et al., 2023)
Anti-nuclear Ab	Anti-dsDNA	Double stranded DNA	Systemic lupus erythematosus, Myasthenia	(Choi et al., 2020; Li et al., 2023)

			Gravis	
	Anti-centromere	Centromere	CREST (Calcinosis, Raynaud's phenomenon) syndrome	(Zian et al., 2020)
	Anti-SSA/Ro	Ribo-nucleoproteins	Systemic lupus erythematosus, Sjögren's syndrome	(Lin et al., 2022; Alduraibi FK et al., 2023)
	Anti-histone	histone	Systemic lupus erythematosus	(Bao S et al., 2023; Choi et al., 2023)
	Anti-ribosomal	Ribosomes	Systemic lupus erythematosus	(Bao S et al., 2023; Duca et al., 2023)
	Anti-gp210	Nuclear membrane	Primary biliary cirrhosis	(Rigopoulou and Bogdanos, 2023)
	Anti-p62	Nucleoporin 62		(Bauer et al., 2022; Tan et al., 2023)
	Anti-sp100	Sp100		(Lepri et al., 2023)
Anti-CP	-	Citrullinated peptide	Rheumatoid arthritis	(Yoshii et al., 2023)
Anti-ganglioside Ab	-	GD3/GM1/GQ1b	Guillain–Barré syndrome	(Koike and Katsuno, 2021; Zhu et al., 2023)
Anti-actin	-	Actin	Coeliac disease	(Mašić et al., 2022; Machado, 2023)
Anti-	Anti-p-	Myeloperoxidase	Microscopic	(Suwanchote et

neutrophil Ab	ANCA		polyangiitis, Eosinophilic granulomatosis with polyangiitis, Rheumatoid arthritis, Primary sclerosing cholangitis, Ulcerative colitis	al., 2018; Bianco and Allegra, 2021)
	Anti-c-ANCA	Neutrophil cytoplasm	Granulomatosis with polyangiitis, Ulcerative colitis	
Anti-Vinculin	-	Vinculin	Systemic Sclerosis	(Herrán et al., 2023)
RF	-	Fc portion of IgG	Rheumatoid arthritis	(Abdelhafiz D et al., 2023)
Anti-AChR	-	Nicotinic acetylcholine receptor	Myasthenia gravis	(Iacomino et al., 2023)

CRISPR and Rheumatoid Arthritis (RA): Several in-vitro as well as in-vivo studies have been conducted that report the importance of CRISPR application to ablate key genes responsible for the onset of different types of arthritis (Evans et al., 2023; Kumar et al., 2023). Moreover, three studies employing human cell models for rheumatoid arthritis (RA) have investigated the potential of gene therapy using CRISPR-Cas9 and identified potential targets. Notably, the *MYC1* and *FOXO1* genes have been implicated in the pathogenesis of RA (Poniewierska-Baran et al., 2023). In addition to the association of *MYC* and *FOXO1* genes with RA, the study has revealed that $CD4^+$ T-cells in RA patients exhibit

heightened autophagy. It was previously hypothesized that *MYC* regulates this pathway. The study provides evidence supporting the contribution of both *MYC* and *FOXO1* genes to RA through comprehensive analysis involving techniques such as Assay for Transposase-Accessible Chromatin with high-throughput (ATAC) sequencing, high-throughput chromosome conformation capture technique (Hi-C), Capture Hi-C, and nuclear RNA-sequencing. These methods were employed in studying activated helper T cells over a 24-hour period, further elucidating the role of these genes in the pathogenesis of RA.

In a genome-wide association study conducted by Lee et al. (2022), it was discovered that the single nucleotide polymorphism (SNP) rs6927172, located on chromosome 6q23, is a risk factor for the development of RA. The study further investigated the genes surrounding this SNP region and identified *TNFAIP3* and *OLIG3* as relevant genes. Disruption of the SNP region using CRISPR-Cas9 resulted in decreased expression of both *TNFAIP3* and *OLIG3*, indicating a significant association between rs6927172, *TNFAIP3*, *OLIG3*, and the progression of RA. These findings highlight the potential role of these genes in the pathogenesis of RA.

In studies conducted by Markovics et al. (2020) and Balchin C et al. (2023), microRNA 155 (*miR-155*) was identified as a significant pro-inflammatory component in patients with rheumatoid arthritis (RA). The researchers observed that the deletion of *miR-155* in RAW 264.7 cells resulted in the up-regulation of SHP1 and hindered the production of pro-inflammatory cytokines. Based on these findings, they propose that modifying the *miR-155* region could potentially lead to effective treatment strategies for RA. This suggests that targeting *miR-155* could have therapeutic implications for mitigating the inflammatory response associated with RA.

CRISPR and Systemic Lupus Erythematosus (SLE): In studies investigating gene therapy for systemic lupus erythematosus (SLE) using human cell culture, potential targets for CRISPR-based interventions were identified, including A20 deubiquitinase, chromosome X open reading frame 21 (*CXorf21*), transferrin receptor genes, and Semaphorin3A. Harris et al. (2019) specifically focused on the role of *CXorf21* in the development of SLE by conducting in-vitro knockdown experiments. They found that the removal of *CXorf21* led to a reduction in the expression of TNF-α and IL-6, suggesting that

CXorf21 expression, particularly in sexually dimorphic forms, may contribute to the pathogenesis of SLE. These findings highlight the potential of targeting *CXorf21* as a therapeutic strategy for SLE using CRISPR technology.

In a study by Voss et al. (2023), the role of transferrin receptor CD71 was investigated using CRISPR. The researchers found that iron uptake mediated by CD71 plays a crucial role in T cell dysfunction, contributing to the development of systemic lupus erythematosus (SLE). Additionally, Eiza et al. (2023) suggested Semaphorin3A as a potential target for CRISPR therapy. Semaphorin3A acts as a regulatory ligand for the CD72 receptor, which is involved in co-regulating B cells and is implicated in the pathogenesis of SLE. Furthermore, the expression of the *IRF5* rs4728142 SNP has also been associated with SLE, indicating its potential relevance in the disease.

CRISPR and Type-1 Coeliac Disease: In their study, Yu et al. (2023) not only analyzed the gene sequences of immunogenic epitopes, specifically α- or γ-gliadins found in gluten proteins from wheat, but they also developed CRISPR constructs to specifically target these epitopes. The study proposed that α- or γ-gliadin genes could be effective targets for CRISPR-based gene therapy and demonstrated the potential to create safe grain variants by editing these genes using CRISPR technology.

CRISPR and Multiple Sclerosis (MS): Several studies investigating gene therapy for multiple sclerosis (MS) using human cell models have identified potential targets for CRISPR therapy. These targets include the RNA for DDX39B (helicase DEAD box polypeptide 39B) as well as the genes for *IR7R*, *TNFRSF1A*, and *IL2RA*. Maier et al. (2009) described the genetic heterogeneity of the *IL2RA* in both MS and insulin-dependent diabetes mellitus (IDDM). The *IL2RA* variants were found to be independently associated with levels of soluble IL2RA and increased the risk of developing MS, suggesting that *IL2R* variants are significant risk factors for both MS and T1DM.

According to Galarza-Muñoz et al. (2017), there is an epistatic interaction in humans that increases the likelihood of developing MS. This interaction involves the RNA helicase DDX39B, which can activate exon 6 of *IL7R* and repress *IL7R* in its soluble form. Strong correlations were found between the risk of MS and the genetic variants rs6897932 in *IL7R* and rs2523506 in *DDX39B*. Additionally, the study suggests that the risk of MS is influenced by locally mutated *IL7R* as well as genetic and functional interactions involving

the *IR7R* and the rs2104286 SNP in intron 1 of *IL2RA*. These interactions contribute to the increased risk of MS.

In a study conducted by Zhao et al. (2022), it was revealed that the pathogenesis of MS is influenced by the immunogenic pathway, specifically involving IL7R and its soluble form. The research highlights the significance of rs6897932, particularly its C allele, which promotes the skipping of exon 6 in the *IL7R* gene. This variant, acting as an exon splicing silencer, is associated with MS and has the potential to affect the balance between soluble and membrane bound IL7R proteins, thereby directly impacting the risk of developing MS. In a separate study conducted by Gomez-Pinedo et al. (2022), it was demonstrated that anti-tumor necrosis factor (TNF) therapies, commonly used for the treatment of autoimmune disorders, have shown efficacy beyond MS. The research focused on the mutation in rs1800693 of the *TNFRSF1A*, which encodes TNFR1 (tumor necrosis factor receptor 1), and revealed its association with the etiology of MS. This finding suggests that the mutation in *TNFRSF1A* may contribute to the development of MS and highlights the potential of anti-TNF therapies in managing autoimmune disorders.

CRISPR and Psoriasis: Arakawa A et al. (2021) conducted a comprehensive study investigating the role of *ERAP1* (endoplasmic reticulum aminopeptidase 1) variants in the development of psoriasis. Through their research, they found that these *ERAP1* variants interact with HLA-C*06:02, a known genetic risk factor for psoriasis. This interaction suggests a critical involvement of *ERAP1* in the pathogenesis of psoriasis, potentially influencing the antigen processing and presentation pathways. The study underscores the importance of understanding the immunogenetics and immunological mechanisms underlying psoriasis, providing valuable insights for future therapeutic interventions.

In parallel, Roth-Carter et al. (2020) focused on exploring the function of Desmoglein 1 (Dsg1) in the context of psoriasis. Their investigation revealed that Dsg1 plays a regulatory role in inflammatory responses, barrier development, and epidermal differentiation. By employing Desmoglein 1 knockout mice, they demonstrated that the inhibition of Dsg1 led to barrier dysfunction and increased susceptibility to psoriatic processes. These findings shed light on the significance of Dsg1 in maintaining skin integrity and its involvement in the inflammatory cascade associated with psoriasis.

Targeting *ERAP1* and Dsg1 through CRISPR therapy holds promise for potential therapeutic interventions. By precisely editing the genetic sequences associated with these genes, it may be possible to modulate their expression or function, leading to a potential reduction in psoriatic symptoms and disease progression.

CRISPR and Insulin Dependent Diabetes Mellitus (IDDM): Zhu et al. (2019) investigated the role of *LCK* SNPs (Lymphocyte-specific protein tyrosine kinase) in Insulin-dependent diabetes. They utilized CRISPR technology to assess the activity of *LCK* SNPs in blood samples from individuals with the disease. Among the tested SNPs, rs10914542 demonstrated a significant correlation, indicating that the G allele of *LCK* rs10914542 is associated with an increased risk of Type 1 diabetes.

In a related study, Ratiu et al. (2017) used *AID* knockout mice to identify potential therapeutic targets for Insulin-dependent diabetes patients, highlighting *AID/RAD51* as a potential target. Based on these findings, both the *AID/RAD51* and the *LCK* rs10914542 are suggested as suitable targets for CRISPR therapy in the treatment of Insulin-dependent diabetes.

Limitations and Future Prospects:

One significant challenge in CRISPR/Cas9-based therapies is the potential for unintended genetic alterations in non-targeted areas (Uddin et al., 2020; Yang et al., 2021). These off-target effects can have unforeseen consequences. To address this concern, researchers are actively working on enhancing the precision of CRISPR/Cas9. Strategies such as base editing and prime editing have emerged as promising approaches. Base editing allows for precise changes in single DNA letters, reducing the risk of off-target effects (Satomura et al., 2017). Prime editing, on the other hand, offers even greater accuracy by directly rewriting DNA sequences without requiring double-strand breaks (Liu et al., 2021). These advancements hold the potential to make CRISPR/Cas9 therapies safer and more reliable.

Efficiently delivering CRISPR/Cas9 components to the specific tissues or cells that require modification is another hurdle (Salman et al., 2022). Developing safe and effective delivery methods is crucial for the success of CRISPR-based treatments. Researchers are exploring various approaches, including nanoparticle-based delivery systems and viral vectors (Sivakumar and Cherqui, 2022). Nanoparticles can protect the CRISPR cargo and deliver it precisely to the target cells (Khurana et al., 2022; Chavez et al., 2023). Viral vectors, modified

viruses, can efficiently carry CRISPR components into cells (Karimian et al., 2019). Continued progress in delivery technology is essential to ensure that CRISPR/Cas9 therapies can reach their intended destinations within the body.

The rapid advancement of CRISPR/Cas9 technologies has raised important ethical considerations. One prominent concern is the potential for unintended consequences, both in individual patients and at the societal level (Fogleman et al., 2016; Brokowski and Adli, 2019). The prospect of germline editing, where changes made to an individual's DNA could be passed on to future generations, has sparked significant debate (Schultz-Bergin, 2018; Shinwari et al., 2018). Researchers, policymakers, and the scientific community are actively engaged in discussions and regulations to address these ethical concerns (Nidhi et al., 2021). Ensuring that CRISPR/Cas9 applications adhere to strict ethical guidelines is essential to promote responsible and safe use of this powerful technology (Gostimskaya, 2022).

In a nutshell, CRISPR/Cas9 holds substantial promise for personalized therapies and cellular immunotherapy in the treatment of autoimmune disorders (Zhang, 2021). However, addressing limitations related to precision, delivery methods, and ethical considerations is crucial for realizing the full potential of CRISPR/Cas9-based treatments and ensuring their safety and ethical use in the future (Rasul et al., 2022). Researchers are committed to overcoming these challenges to benefit patients with autoimmune disorders.

Conclusion:

In our pursuit to unravel the mysteries of autoimmune disorders, we embarked on a groundbreaking exploration, fueled by the revolutionary potential of CRISPR-Cas9. Through diligent exploration, we delved into the complex genetic landscape underlying autoimmune disorders. Equipped with this knowledge, we embarked on a mission to harness the potential of precision gene editing. By targeting aberrant T cell activity and curbing inflammatory cytokines, our aim is to reshape the course of autoimmune battles. The remarkable promise of CRISPR-Cas9 as a therapeutic tool shine bright, instilling hope for those seeking relief. CRISPR-Cas9 can exhibit promising desirable effects in modulating defective genes in autoimmune disorders. Overcoming the specific challenges of CRISPR itself through precise manufacturing, we can deliberately use it to knock out defective genes and replacing it with correctly sequenced gene or can simply improvise to correct the disrupted nucleotide sequence.

While the road to clinical application may present challenges, our unwavering commitment propels us forward, envisioning a future where personalized gene therapies offer solace to those affected by autoimmune conditions. Together, we advance towards a future where the transformative potential of CRISPR-Cas9 paves the way for innovative solutions and renewed hope in the realm of autoimmune disorder treatments.

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Not Applicable.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

REFERENCES

1. Abdelhafiz D, Baker T, Glascow DA, Abdelhafiz Ah (2023). Biomarkers for the diagnosis and treatment of rheumatoid arthritis–a systematic review. Postgrad. Med. 135: 214-223.
2. Akram F, Sahreen S, Aamir F, Haq Ikram Ul, Malik K, Imtiaz M, Naseem W, Nasir N, Waheed H, Mariam (2023). An insight into modern targeted genome-editing technologies with a special focus on CRISPR/Cas9 and its applications. Mol. Biotechnol. 65: 227-242.
3. Alduraibi FK, Sullivan KA, Chatham W, Hsu Hui-C, Mountz JD (2023). Interrelation of T cell cytokines and autoantibodies in systemic lupus erythematosus: A cross-sectional study. Clin. Immunol. 247: 109239.
4. Arakawa A, Reeves E, Vollmer S, Arakaw Y, He M, Galinski A, Stöhr J, Dornmair K, James E, Prinz JC (2021). *ERAP1* controls the autoimmune response against melanocytes in psoriasis by generating the melanocyte autoantigen and regulating its amount for HLA-C* 06: 02 presentation. J. Immunol. 207: 2235-2244.
5. Balchin C, Tan AL, Wilson OJ, McKenna J, Stavropoulos K, Antonios (2023). The role of microRNAs in regulating inflammation and exercise-induced adaptations in rheumatoid arthritis. Rheumatol. Adv. Pract. 7: rkac110.
6. Bao S, Huang H, Jin Y, Ding F, Yang Z, Xu X, Liu C, Lu J, Jin Y (2023). Autoantibody-based subgroups and longitudinal seroconversion in juvenile-onset systemic lupus erythematosus. Lupus Sci. Med. 10: e000834.
7. Bauer A, Habior A, Gawel D (2022). Diagnostic and clinical value of specific autoantibodies against Kelch-like 12 peptide and nuclear envelope proteins in patients with primary biliary cholangitis. Biomedicines. 10: 801.
8. Bharathkumar N, Sunil A, Meera P, Aksah S, Kannan M, Saravanan K, Anand T (2022). CRISPR/Cas-Based modifications for therapeutic applications: A review. Mol. Biotechnol. 64: 355-372.
9. Bianco M, Allegra E (2021). Diagnosis of EGPA syndrome in a patient With chronic polypoid rhinosinusitis presenting as Loeffler Syndrome. ENT J. 100: NP216-NP217.

10. Brokowski C, Adli M (2019). CRISPR ethics: Moral considerations for applications of a powerful tool. J. Mol. Biol. 431: 88-101.
11. Burmistrz M, Krakowski K, Krawczyk-Balska A (2020). RNA-targeting CRISPR–Cas systems and their applications. Int. J. Mol. Sci. 21: 1122.
12. Chakrabarti A, Henser-Brownhill T, Monserrat J, Poetsch A, Luscombe N, Scaffidi P (2019). Target-specific precision of CRISPR-mediated genome editing. Mol. cell. 73: 699-713. e696.
13. Chaudhuri A, Halder K, Datta A (2022). Classification of CRISPR/Cas system and its application in tomato breeding. Theor. Appl. Genet. 135: 367-387.
14. Chavez M, Chen X, Finn P, Qi L (2023). Advances in CRISPR therapeutics. Nat. Rev. Nephrol. 19: 9-22.
15. Choi M, FitzPatrick R, Buhler K, Mahler M, Fritzler M (2020). A review and meta-analysis of anti-ribosomal P autoantibodies in systemic lupus erythematosus. Autoimmun. Rev. 19: 102463.
16. Choi S-E, Park D-J, Kang J-H, Lee S-S (2023). Significance of co-positivity for anti-dsDNA,-nucleosome, and-histone antibodies in patients with lupus nephritis. Ann. Med. 55: 1009-1017.
17. Dao F-Y, Liu M-L, Su W, Lv H, Zhang Z-Y, Lin H, Liu L (2023). AcrPred: A hybrid optimization with enumerated machine learning algorithm to predict anti-CRISPR proteins. Int. J. Biol. Macromol. 228: 706-714.
18. Doudna J (2020). The promise and challenge of therapeutic genome editing. Nature. 578: 229-236.
19. Duca L, Roman N, Teodorescu A, Ifteni P (2023). Association between inflammation and thrombotic pathway link with pathogenesis of depression and anxiety in SLE patients. Biomolecules. 13: 567.
20. Eiza N, Sabag A, Kessler O, Neufeld G, Vadasz Z (2023). CD72-semaphorin3A axis: A new regulatory pathway in systemic lupus erythematosus. J. Autoimmun. 134: 102960.
21. Evans C, Ghivizzani S, Robbins P (2023). Osteoarthritis gene therapy in 2022. Curr. Opin. Rheumatol. 35: 37-43.
22. Feng F, Tang F, Gao Y, Zhu D, Li T, Yang S, Yao Y, Huang Y, Liu J (2023). GenomicKB: A knowledge graph for the human genome. Nucleic Acids Res. 51: D950-D956.
23. Fogleman S, Santana C, Bishop C, Miller A, Capco D (2016). CRISPR/Cas9 and mitochondrial gene replacement therapy: promising techniques and ethical considerations. Am. J. Stem Cells. 5: 39-52.
24. Fuziwara C, de Mello D, Kimura E (2022). Gene editing with CRISPR/Cas methodology and thyroid cancer: Where are we? Cancers. 14: 844.
25. Galarza-Muñoz G, Briggs F, Evsyukova I, Schott-Lerner G K, Edward M, Tinashe W, Liuyang B, Laura W, Steven G, Georgia D (2017). Human epistatic

interaction controls IL7R splicing and increases multiple sclerosis risk. Cell. 169: 72-84. e13.
26. Gomez-Pinedo U, Matías-Guiu J, Torre-Fuentes L, Montero-Escribano P, Hernández-Lorenzo L, Pytel V, Maietta P, Alvarez S, Sanclemente-Alamán I, Moreno-Jimenez L (2022). Variant rs4149584 (R92Q) of the *TNFRSF1A* gene in patients with familial multiple sclerosis. Neurología (English Edition). S2173-5808: 00087-00086.
27. Gostimskaya I (2022). CRISPR–Cas9: A history of its discovery and ethical considerations of its use in genome editing. Biochemistry (Moscow). 87: 777-788.
28. Guo N, Liu J-B, Li W, Ma Y-S, Fu D (2022). The power and the promise of CRISPR/Cas9 genome editing for clinical application with gene therapy. J. Adv. Res. 40: 135-152.
29. Harris V, Koelsch K, Kurien B, Harley I, Wren J, Harley J, Scofield R (2019). Characterization of *cxorf21* provides molecular insight into female-bias immune response in SLE pathogenesis. Front. Immunol. 10: 2160.
30. Herrán M, Adler B, Perin J, Morales W, Pimentel M, McMahan Z (2023). Anti-vinculin antibodies in systemic sclerosis: associations with slow gastric transit and extra-intestinal clinical phenotype. Arthritis. Care. Res. 75: 2166-2173.
31. Hillary V, Ceasar S (2023). A review on the mechanism and applications of CRISPR/Cas9/Cas12/Cas13/Cas14 proteins utilized for genome engineering. Mol. Biotechnol. 65: 311-325.
32. Iacomino N, Scandiffio L, Conforti F, Salvi E, Tarasco M, Bortone F, Marcuzzo S, Simoncini O, Andreetta F, Pistillo D (2023). Muscle and muscle-like autoantigen expression in myasthenia gravis thymus: Possible molecular hint for autosensitization. Biomedicines. 11: 732.
33. Kanafi M, Tavallaei M (2022). Overview of advances in CRISPR/deadCas9 technology and its applications in human diseases. Gene. 830: 146518.
34. Karimian A, Azizian K, Parsian H, Rafieian S, Shafiei-Irannejad V, Kheyrollah M, Yousefi M, Majidinia M, Yousefi B (2019). CRISPR/Cas9 technology as a potent molecular tool for gene therapy. J. Cell Physiol. 234: 12267-12277.
35. Katti A, Diaz B, Caragine C, Sanjana N, Dow L (2022). CRISPR in cancer biology and therapy. Nat. Rev. Cancer. 22: 259-279.
36. Khan Z, Ali Z, Khan A, Sattar T, Zeshan A, Saboor T, Binyamin B (2022). History and classification of CRISPR/Cas system. in: Ahmad, A., Khan, S.H., Khan, Z. [Ed.]. The CRISPR/Cas Tool Kit for Genome Editing. Springer, Singapore.
37. Khanzadi M, Khan A (2020). CRISPR/Cas9: Nature's gift to prokaryotes and an auspicious tool in genome editing. J. Basic Microbiol. 60: 91-102.

38. Khurana A, Sayed N, Singh V, Khurana I, Allawadhi P, Rawat P, Navik U, Pasumarthi S, Bharani K, Weiskirchen R (2022). A comprehensive overview of CRISPR/Cas 9 technology and application thereof in drug discovery. J. Cell Biochem. 123: 1674-1698.
39. Koike H, Katsuno M (2021). Macrophages and autoantibodies in demyelinating diseases. Cells. 10: 844.
40. Krovi S, Kuchroo V (2022). Activation pathways that drive CD4+ T cells to break tolerance in autoimmune diseases. Immunol. Rev. 307: 161-190.
41. Kumar D, Sahoo S, Chauss D, Kazemian M, Afzali B (2023). Non-coding RNAs in immunoregulation and autoimmunity: Technological advances and critical limitations. J. Autoimmun. 134: 102982.
42. Lee M, Shin J, Yang J, Lee K, Cha D, Hong J, Park Y, Choi E, Tizaoui K, Koyanagi A (2022). Genome editing using CRISPR-Cas9 and autoimmune diseases: A comprehensive review. Int. J. Mol. Sci. 23: 1337.
43. Lepri G, Airò P, Distler O, Andréasson K, Braun-Moscovici Y, Hachulla E, Balbir-Gurman A, De Langhe E, Rednic S, Ingegnoli F (2023). Systemic sclerosis and primary biliary cholangitis: Longitudinal data to determine the outcomes. J. Scleroderma. Relat. Disord. 8: 210-220.
44. Li S, Chen J, Yang X, Huang X, Wang H, Feng H (2023). Anti-dsDNA is associated with favorable prognosis in myasthenia gravis: A retrospective study. Acta Neurol. Scand. 2023: 1-11.
45. Lin L, Hang H, Zhang J, Lu J, Chen D, Shi J (2022). Clinical significance of anti-SSA/Ro antibody in neuromyelitis optica spectrum disorders. Mult. Scler. Relat. Disord. 58: 103494.
46. Liu J-J, Orlova N, Oakes B, Ma E, Spinner H, Baney K, Chuck J, Tan D, Knott G, Harrington L (2019). CasX enzymes comprise a distinct family of RNA-guided genome editors. Nature. 566: 218-223.
47. Liu R, Liang L, Freed E, Gill R (2021). Directed evolution of CRISPR/Cas systems for precise gene editing. Trends Biotechnol. 39: 262-273.
48. Machado M V (2023). New developments in celiac disease treatment. Int. J. Mol. Sci. 24: 945.
49. Maier L, Lowe C, Cooper J, Downes K, Anderson D, Severson C, Clark P, Healy B, Walker N, Aubin C (2009). *IL2RA* genetic heterogeneity in multiple sclerosis and type 1 diabetes susceptibility and soluble interleukin-2 receptor production. PLoS Genet. 5: e1000322.
50. Makarova K, Wolf Y, Koonin E (2022). Evolutionary Classification of CRISPR-Cas Systems. in: Rodolphe Barrangou, Erik J. Sontheimer and Marraffini, L. A. [Eds.]. CRISPR: Biology and Applications. 1st ed. ASM Press.
51. Makarova K, Wolf Y, Iranzo J, Shmakov S, Alkhnbashi O, Brouns S, Charpentier E, Cheng

D, Haft D, Horvath P (2020). Evolutionary classification of CRISPR–Cas systems: A burst of class 2 and derived variants. Nat. Rev. Microbiol. 18: 67-83.
52. Malandrini S, Trimboli P, Guzzaloni G, Virili C, Lucchini B (2022). What about TSH and Anti-Thyroid antibodies in patients with autoimmune thyroiditis and celiac disease using a gluten-free diet? A systematic review. Nutrients. 14: 1681.
53. Markovics A, Toth D, Glant T, Mikecz K (2020). Regulation of autoimmune arthritis by the SHP-1 tyrosine phosphatase. Arthritis. Res. Ther. 22: 160.
54. Marks K, Rao D (2022). T peripheral helper cells in autoimmune diseases. Immunol. Rev. 307: 191-202.
55. Marrack P, Kappler J, Kotzin B (2001). Autoimmune disease: Why and where it occurs. Nat. Med. 7: 899-905.
56. Mašić M, Močić Pavić A, Gagro A, Balažin Vučetić A, Ožanić Bulić S, Mišak Z (2022). From Chilblains (Pernio) to coeliac disease—Should we still consider it random? Children. 9: 1972.
57. Mubariki R, Vadasz Z (2022). The role of B cell metabolism in autoimmune diseases. Autoimmun. Rev. 21: 103116.
58. Nadali J, Ghavampour N, Beiranvand F, Maleki Takhtegahi M, Heidari M E, Salarvand S, Arabzadeh T, Narimani Charan O (2023). Prevalence of depression and anxiety among myasthenia gravis (MG) patients: A systematic review and meta-analysis. Brain Behav. 13: e2840.
59. Nidhi S, Anand U, Oleksak P, Tripathi P, Lal J, Thomas G, Kuca K, Tripathi V (2021). Novel CRISPR–Cas systems: An updated review of the current achievements, applications, and future research perspectives. Int. J. Mol. Sci. 22: 3327.
60. Olivieri B, Betterle C, Zanoni G (2021). Vaccinations and autoimmune diseases. Vaccines. 9: 815.
61. Pavel-Dinu M, Borna S, Bacchetta R (2023). Rare immune diseases paving the road for genome editing-based precision medicine. Front. Genome Ed. 5: 1114996.
62. Pinilla-Redondo R, Russel J, Mayo-Muñoz D, Shah S, Garrett R, Nesme J, Madsen J, Fineran P, Sørensen S (2022). CRISPR-Cas systems are widespread accessory elements across bacterial and archaeal plasmids. Nucleic Acids Res. 50: 4315-4328.
63. Poniewierska-Baran A, Bochniak O, Warias P, Pawlik A (2023). Role of sirtuins in the pathogenesis of rheumatoid arthritis. Int. J. Mol. Sci. 24: 1532.
64. Rasul M, Hussen B, Salihi A, Ismael B, Jalal P, Zanichelli A, Jamali E, Baniahmad A, Ghafouri-Fard S, Basiri A (2022). Strategies to overcome the main challenges of the use of CRISPR/Cas9 as a replacement for cancer therapy. Mol. Cancer. 21: 64.
65. Ratiu J, Racine J, Hasham M, Wang Q, Branca J, Chapman H, Zhu J, Donghia N, Philip V,

Schott W (2017). Genetic and small molecule disruption of the AID/RAD51 axis similarly protects nonobese diabetic mice from type 1 diabetes through expansion of regulatory B lymphocytes. J. Immunol. 198: 4255-4267.

66. Rigopoulou E, Bogdanos D (2023). Role of autoantibodies in the clinical management of primary biliary cholangitis. World J. Gastroenterol. 29: 1795-1810.
67. Roth-Carter Q, Godsel L, Koetsier J, Broussard J, Burks H, Fitz G, Huffine A, Amagai S, Lloyd S, Kweon J (2020). 225 desmoglein 1 deficiency in knockout mice impairs epidermal barrier formation and results in a psoriasis-like gene signature in E18. 5 embryos. J. Invest. Dermatol. 140: S26.
68. Salman A, Kantor A, McClements M, Marfany G, Trigueros S, MacLaren R (2022). Non-viral delivery of CRISPR/Cas cargo to the retina using nanoparticles: Current possibilities, challenges, and limitations. Pharmaceutics. 14: 1842.
69. Satomura A, Nishioka R, Mori H, Sato K, Kuroda K, Ueda M (2017). Precise genome-wide base editing by the CRISPR Nickase system in yeast. Sci. Rep. 7: 2095.
70. Schultz-Bergin M (2018). Is CRISPR an ethical game changer? J. Agric. Environ. 31: 219-238.
71. Sharma G, Sharma A, Bhattacharya M, Lee S-S, Chakraborty C (2021). CRISPR-Cas9: A preclinical and clinical perspective for the treatment of human diseases. Mol. Ther. 29: 571-586.
72. Shinwari Z, Tanveer F, Khalil A (2018). Ethical issues regarding CRISPR mediated genome editing. Curr. Issues Mol. Biol. 26: 103-110.
73. Shmakova A, Shmakova O, Karpukhina A, Vassetzky Y (2022). CRISPR/Cas: History and perspectives. Russ. J. Dev. Biol. 53: 272-282.
74. Siddiq A, Naveed A, Ghaffar N, Aamir M, Ahmed N (2023). Association of pro-inflammatory cytokines with vitamin D in Hashimoto's thyroid autoimmune disease. Medicina. 59: 853.
75. Sivakumar A, Cherqui S (2022). Advantages and limitations of gene therapy and gene editing for Friedreich's ataxia. Front. Genome Ed. 4: 903139.
76. Smirnikhina S, Zaynitdinova M, Sergeeva V, Lavrov A (2022). Improving homology-directed repair in genome editing experiments by influencing the cell cycle. Int. J. Mol. Sci. 23: 5992.
77. Song B, Yang S, Hwang G-H, Yu J, Bae S (2021). Analysis of NHEJ-based DNA repair after CRISPR-mediated DNA cleavage. Int. J. Mol. Sci. 22: 6397.
78. Suwanchote S, Rachayon M, Rodsaward P, Wongpiyabovorn J, Deekajorndech T, Wright H, Edwards S, Beresford M, Rerknimitr P, Chiewchengchol D (2018). Anti-neutrophil cytoplasmic antibodies and their

clinical significance. Clin. Rheumatol. 37: 875-884.
79. Szabó G, Debreceni I, Tarr T, Soltész P, Østerud B, Kappelmayer J (2021). Anti-β2-glycoprotein I autoantibodies influence thrombin generation parameters via various mechanisms. Thromb. Res. 197: 124-131.
80. Tan C, Soh N, Chang H C, Yu V (2023). p62/SQSTM1 in liver diseases: the usual suspect with multifarious identities. FEBS J. 290: 892-912.
81. Tian Y, Liu T, Liu C, Xu Q, Liu Q (2022). Pathogen detection strategy based on CRISPR. Microchem. J. 174: 107036.
82. Uddin F, Rudin C, Sen T (2020). CRISPR gene therapy: Applications, limitations, and implications for the future. Front. Oncol. 10: 1387.
83. Ustiugova A, Ekaterina D, Nataliya M, Alexey D, Dmitry K, Marina A (2023). CRISPR/Cas9 genome editing demonstrates functionality of the autoimmunity-associated SNP rs12946510. Biochim. Biophys. Acta Mol. Basis Dis. 1869: 166599.
84. Vockley J, Aartsma-Rus A, Cohen J, Cowsert L, Howell R, Yu T, Wasserstein M, Defay T (2023). Whole-genome sequencing holds the key to the success of gene-targeted therapies. Am. J. Med. Genet. C. Semin. Med. Genet. 193: 19-29.
85. Voss K, Sewell A, Krystofiak E, Gibson-Corley K, Young A, Basham J, Sugiura A, Arner E, Beavers W, Kunkle D (2023). Elevated transferrin receptor impairs T cell metabolism and function in systemic lupus erythematosus. Sci. Immunol. 8: eabq0178.
86. Xiao Z, Miller J, Zheng S (2021). An updated advance of autoantibodies in autoimmune diseases. Autoimmun. Rev. 20: 102743.
87. Yamamoto T, Matsushita S, Endo D, Shimada A, Dohi S, Kajimoto K, Yokoyama Y, Sato Y, Machida Y, Asai T (2023). Management of cardiovascular surgery in patients with systemic lupus erythematosus including thromboembolism and multiple organ failure prevention: A retrospective observational study. Medicine. 102: e32979.
88. Yang Y, Xu J, Ge S, Lai L (2021). CRISPR/Cas: Advances, limitations, and applications for precision cancer research. Front. Med. 8: 649896.
89. Yoshii I, Chijiwa T, Sawada N (2023). The influence of anti-citrullinated polypeptide antibodies on bone mineral density decrease and incident major osteoporotic fractures in patients with rheumatoid arthritis: A retrospective case-control study. Osteology. 3: 47-60.
90. Yoshimi K, Oka Y, Miyasaka Y, Kotani Y, Yasumura M, Uno Y, Hattori K, Tanigawa A, Sato M, Oya M (2021). Combi-CRISPR: combination of NHEJ and HDR provides efficient and precise plasmid-based knock-ins in mice and rats. Hum. Genet. 140: 277-287.
91. Yu Z, Yunusbaev U, Fritz A, Tilley M, Akhunova A, Trick H, Akhunov E (2023). CRISPR-

based editing of the ω-and γ-gliadin gene clusters reduces wheat immunoreactivity without affecting grain protein quality. Plant Biotechnol. J. 22: 892-903.
92. Zhang B (2021). CRISPR/Cas gene therapy. J. Cell. Physiol. 236: 2459-2481.
93. Zhang S, Shen J, Li D, Cheng Y (2021). Strategies in the delivery of Cas9 ribonucleoprotein for CRISPR/Cas9 genome editing. Theranostics. 11: 614-648.
94. Zhao Z, Xue J, Zhuo Z, Zhong W, Liu H (2022). The association of *IL7R* rs6897932 with risk of Multiple Sclerosis in Southern Chinese. Neuropsychiatr. Dis. Treat. 18: 1855-1859.
95. Zhu Q, Wang J, Zhang L, Bian W, Lin M, Xu X, Zhou X (2019). *LCK* rs10914542-G allele associates with type 1 diabetes in children via T cell hyporesponsiveness. Pediatr. Res. 86: 311-315.
96. Zhu W, Li K, Cui T, Yan Y (2023). Detection of anti-ganglioside antibodies in Guillain-Barré syndrome. Ann. Transl. Med. 11: 289.
97. Zhuo C, Zhang J, Lee J-H, Jiao J, Cheng D, Liu L, Kim H-W, Tao Y, Li M (2021). Spatiotemporal control of CRISPR/Cas9 gene editing. Signal Transduct. Target Ther. 6: 238.
98. Zian Z, Mechita M, Hamdouch K, Maamar M, Barakat A, Nourouti N, El Aouad R, Valdivia M, Arji N (2020). Proteomics characterization of CENP-B epitope in Moroccan scleroderma patients with anti-centromere autoantibodies. Immunol. Lett. 221: 1-5.

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Breeding Behaviour and Management Practices of *Struthio camelus* under Captive Condition at Private Farm Tehsil Jhando Mari

Rameez Raja Kaleri*[1,2], Hubdar Ali Kaleri[2], Raza Ali Mangi[3], Ghulam Mustafa Solangi[4], Depeesh Kumar Bhuptani[5], Zainab Lanjar[6], Imran Ahmed[7], Sajid Mehmood Sajid[8]

1. Livestock and Fisheries Department Government of Sindh
2. Department of Animal Breeding and Genetics, SAU, Tandojam
3. Department of Veterinary Anatomy and Histology, SBBUVAS, Sakarand
4. Department of Veterinary Pathology, SBBUVAS, Sakarand
5. Department of Meat Technology, SBBUVAS, Sakarand
6. Department of Veterinary Microbiology, SBBUVAS, Sakarand
7. Deparment of Livestock and Poultry Production, UPR, Azad Kashmir
8. Faculty of Veterinary and Animal Sciences, UPR, Azad Kashmir

Corresponding Author's Email: rameezkaleri@gmail.com

ABSTRACT: *The study was conducted on a private farm in tehsil Jhando Mari, district Tando Allahyar, to observe the behavior of captive ostriches. Twenty birds, aged 10-150 days, were divided into groups of five, with four birds in each group ringed for identification. Observations were made for four consecutive days, six hours daily, in the morning, noon, and afternoon. Behavioral data were collected every 30 minutes, totaling 16 observations per bird per day. The study analyzed behaviors such as attacking, stone ingesting, picking, feces ingesting, running, standing, and walking. Non-parametric tests revealed significant differences in eating, lithophagia, standing, and walking behaviors during the morning and noon periods, especially in the first hour of the day. Age significantly influenced sand bathing behavior during morning and afternoon. Comparisons between noon and afternoon showed significant differences in eating, walking, dancing, running, lithophagia, sand bathing, and coprophagia, with maximum activity observed from dusk to dawn. The study concluded that ostrich behaviors vary with age and time of day under captivity.*

Keywords: *Breeding behaviour, Management practices, Ostrich, Captive condition*

INTRODUCTION

The Ostrich is the largest living bird on the earth omnivores, eat grains, and grass also some types of insects' lizard and small reptiles. Ostrich is flightless bird that has long neck with two toys with large body size with strong legs can walk more than 70 kilo meters per hour (Dikmen, 2024; Amado et al., 2011). Ostrich has high efficiency to tolerate heat, withstanding temperature rages 56^{o}C without getting stressful conditions (Tomuta et al., 2021). Ostrich feathers have excellent insulator ability to receive solar radiation and decrease loss of heat in the winter season (Dragon et al., 2019; Souza, 2004). The African ostrich species widely known as social species therefore it mainly reared in groups (Cooper, 2001 and Cooper et al., 2009). Ensuring that animals could exhibit their natural behaviours is a fundamental aspect of promoting good welfare. Newberry and others said in 2007 that pecking is normal behaviour seen outdoors, which helps set who ranks higher within a group (Amado et al., 2011; Menon et al., 2014). Chicken groups usually have one male who looks after a group of females and takes care of the baby chicks. Using ways to manage like putting ostrich babies of the same size together can stop more food being eaten by bigger ones compared to smaller ones. This makes conditions better for these animals (Fraser and Broom, 2007). The goal of this study was to look at how ostriches behave when they are kept on farms and held captive for ten days to five months. This is an important time in their growth and ability to live through these ways used for raising them.

METHODOLOGY

This study was performed at a private farm close to Nasarpur area in taluka Jhando Mari district Tando Allahyar. During the time of study, daily temperatures

changed from 20℃ in early morning to 38℃ at midday. Also, humidity levels varied between highs of 78% in mornings and lows around 45% during middle afternoons. There was no rain recorded during this time. The buildings were made following African ostrich farming rules (Souza, 2004). They had a yard surrounded by fence and a main shelter in the centre. Each open space was 20 meters by 20 meters for groups of twenty birds up to five months old. When they got older, the space for each group of 30 birds grew to be 20 meters by 30 meters. In both protected and open areas, the base was made of packed earth with rocks.

Behaviour observation and parameters

Ethograms:

Courtship behaviour

Standing (S): recorded when staying in one place with head up.

Running (R): is moving on two legs off the ground next to a wall a cross a playground.

Walking (W):

Management behaviour

Drinking: drinking from bowl counted at one time.

Feeding: feeding of food.

Coprophagia: Involves eating poop.

The birds under observation were divided into 5 groups: (1) Age ten to forty days, (2) Age between 41 and 60 days, (3) Age ranging from sixty-one to ninety days, (4) Time period of ninety-one up to one hundred twenty years old, and (5) the final set marked in this range which is the longest with age amounts reaching as high as up to a full total. Each age group had 30 birds in it, but only four randomly selected chicks from each group were marked with different colour Velcro rings and checked every day.

Total four numbers of people watched the behaviour for four days in every age group. We did this three times a day at different hours each time. Period 1 was from 8: From 12 to 5 PM time by midnight till noon, the second

phase started then ran up to. Then came the last instalment which occurred between those two other parts from 3 in afternoon until nightfall at late evening time, and so on each different timeslot has its go during.

Data Collection

In March, we gathered data on sunny and dry days that were between 20°C to 30°C temperature-wise. The humidity was at about half of what it could be or around 52%. We used the one-zero method for recording behaviour suggested Martin and Bateson during 1986, that all birds were kept under observation for five minutes after 30 minutes, So, during a day they observed them all together for a total of 80 minutes (16 times with each one lasting only 5 minutes). Since some variables didn't have a normal distribution, we used non-parametric stats instead. For each age group, the average number of times it happened every hour was added up. We compared how people act differently in different age groups using a special test called the Kruskal-Wallis. We used a method called Spearman rank correlation to check for connections between different factors.

RESULTS AND DISCUSSION

During the early months of life, ostriches exhibit a behavioural repertoire that is primarily focused on exploring and familiarizing themselves with various aspects of their enclosure. They also talk with other birds, which can cause them to claim areas and form families in production systems (Jayne and See, 2019; Kokoszynski, 2017). Young ostriches around 10 to 40 days old spent less time on average compared with those older now at between age of 41 and 60 days. Ostriches' ages, z=2245 for the ones who are over a month younger than that in group has higher trend when aged from range like below they were born after being shared out

Details are given in Table-1. The findings of our study showed that Ostrich in the age of 121 to 150 spend less time for different behaviours such as bust bath, dancing, running, aggression and drinking, feeding as compared with the age of 41 to 60 and 61 to 90 days during the daytime of period. The results of (Souza, 2004; Carrel et al., 2005), within their confines, captive birds can be seen walking, running, and eating. During the first month of life, younger birds prefer to walk and run more, whereas older birds stand still more (Dragon et al., 2019; Alvarenga, 2006), reported that this conduct is linked to frustration or a restricted environment; stress and frustration can be reduced by restoring a more natural setting. According to the study conducted by Souzo (2004), in morning dances are frequently performed by both wild ostriches and animals kept in captivity, and these behaviours occur more frequently in shelters. At six in the morning, the birds were still in the refuge and could be seen dancing (Engelbrecht, 2013; Csermely et al., 2006). The study found low water consumption in all age groups of ostriches, possibly due to food consumption, environmental dryness, and high temperatures. Adult ostriches only used 1.1% of their time for water intake activities (Ipek et al., 2003; Mukhtar and Mirza, 2017). The study suggests that water availability is related to young ostriches learning to drink water. The low use might be because there's only a little plastic in the water tanks, they aren't protected, and no new water is put in often. The research showed that ostriches kept in places like zoos and hot temperatures mostly drink water during early morning and evening times (Mohapatra et al., 2014; Cooper et al., 2010). They eat more food first thing in the day then again around afternoon hours.

Table 1: The results for average mean frequency of different behaviours of Ostrich during different stages of age under captive condition

Behavior	Age of Ostrich birds in days				
	10- 40 days	41-60 days	61–90 days	91- 120 days	120-150 days
Dust bath	----	0.10±0.08	0.13±0.05	0.11±0.07	0.08±0.01
Coprophagia	2.88±0.1	1.03±0.14	1.04±0.07	0.88±0.12	0.75±0.08
Walking	5.33±0.21	3.47±0.17	3.39±0.31	3.27±0.43	0.97±0.07
Drinking	0.77±0.03	1.16±0.15	1.16±0.12	0.66±0.04	0.57±0.05
Standing	0.22±0.07	1.11±0.15	0.45±0.13	0.6±0.11	0.98±0.14
Dancing	0.37±0.07	----	----	-----	------
Running	0.76±0.13	0.33±0.09	0.13±0.05	0.11±0.05	0.10±0.01
Aggression	0.87±0.21	0.001±0.001	0.001±0.01	0.02±0.01	0.01±0.01
Lithophagia	0.01±0.01	2.41±0.57	2.31±0.15	2.59±0.06	2.11±0.21
Feeding	3.21±0.11	2.45±0.33	2.87±0.17	2.76±0.23	0.67±0.07
Pecking	0.00±0.01	0.21±0.09	0.56±0.02	1.17±0.27	0.55±0.11

However, higher temperatures from 11:3:00 pm to 6:01 am might have caused a reduction in eating food so as not to gain more calories. The study's results got mixed up because of temperature and food offer, which makes it hard to tell what caused them. Details given in Table 2. Sauer and Sauer (1966) found that ostriches in nature spend most of their time walking, eating grass or searching for food to get the nutrients they need every day. The study found that ostriches exhibit calm behaviour near noon, spending more time standing. This behaviour is consistent with previous research (Csermely et al., 2007; Brasso et al., 2020) findings. The study also found greater expression of this behaviour in captive male ostriches. The result regarding

dust bathing suggested that a common ostrich behaviour was observed more frequently in late afternoons in Great Britain, but it wasn't very visible in the morning and got higher during afternoon. It was most noticeable at sunset time Croney et al. (2007).

Table 2: The results for average mean values of Ostrich during different stages of daytime under captive condition during the daytime

Behaviour	The time calculated during day period		
	8 AM to 11 AM	11 AM to 2 PM	2 PM to 5 PM
Dust bath	0.01±0.01	0.01±0.01	0.28±0.3
Coprophagia	2.01±0.19	0.98±0.22	1.76±0.34
Walking	3.11±0.07	3.014±0.09	3.51±0.11
Drinking	0.98±0.13	0.57±0.03	1.13±0.07
Standing	0.91±0.11	0.47±0.03	0.85±0.09
Dancing	0.24±0.14	0.28±0.03	0.49±0.11
Running	0.26±0.07	0.19±0.01	0.46±0.02
Lithophagia	3.01±0.37	1.76±0.71	2.41±037
Feeding	2.98±0.21	2.00±0.11	3.23±0.07

CONCLUSION

The study revealed that ostrich behaviour changed with age and even throughout the day. However, defining normal behaviour is challenging and further research is essential to understand ostrich needs under various production conditions.

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Not Applicable.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

REFERENCES

1. Alvarenga ABB (2006). Ontogeniacomportamental, stilosdeenfrentamentoecrescimentoemavestruzes (Struthio camelus)

(Doctoral dissertation, Dissertação (Mestradoem ProduçãoAnimal)-Faculdade de Agronomia e MedicinaVeterinária/Universidade de Brasília, Brasília).

2. Amado MF, Xavier DB, Boere V, Torres-Pereira C, McManus C, Bernal FE. 2011. Behaviour of captive Ostrich chicks from 10 days to 5 months of age. Rev. Brasil. de Zootec. 40:1613-8.
3. Bateson M, Martin P. Measuring behavior: an introductory guide. 2006. Cambridge university press; May 6.
4. Brassó DL, Béri B, Komlósi I (2020). Studies on ostrich (*Struthio Camelus*)-review. Acta. Agr. Debreceniensis. 20(1):15-22.
5. Broom DM, Fraser AF (1997). Domestic Animal Behaviou... CABI.
6. Carrer CD, Carrer CR, Elmôr RA, Kornfeld ME (2005). Estrutiocultura: planejamento, manejo e mercado. Zootec 2005; produção animal e responsabilidade.
7. Cooper RG (2001). Handling, incubation, and hatchability of ostrich (*Struthio camelus var. domesticus*) eggs: a review. J. Appl. Poult. Res. 10(3): 262-273.
8. Cooper RG, Horbańczuk JO, Villegas-Vizcaíno R, Kennou Sebei S, Faki Mohammed AE, Mahrose KM (2010). Wild ostrich (*Struthio camelus*) ecology and physiology. Trop. Anim. Health Prod. 42(1): 363-373.
9. Cooper RG, Mahrose KM, Horbańczuk JO, Villegas-Vizcaíno R, Kennou Sebei S, Faki Mohammed AE (2009). The wild ostrich (Struthio camelus): a review. Tropic. Anim. Health Prod. 41(1): 1669-1678.
10. Croney CC, Prince-Kelly N, Meller CL (2007). A note on social dominance and learning ability in domestic chicken (Gallus gallus). Appl. Anim. Behav. Sci. 105(1-3): 254-258.
11. Csermely D, Gaibani G, Dardani E (2007). Year-round behavioral sequences in captive ostrich (Struthio camelus domesticus) pairs. Appl. Anim. Behav. Sci. 103(1-2):156-166.
12. Deeming DC. A note on effects of gender and time of day on the winter time-activity budget of adult ostriches (Struthio camelus) in a farming environment. Applied Animal Behavior Science. Sep 1;59(4):363-71.
13. Dikmen BY (2024). Effect of Season, Sex and Time of Day On Ostrich Breeder. (Struthio camelus) Behavior. JAPS: J. Anim. Plant Sci. 34(1).
14. Dragan D, Birau A, Rada OA, Fericean LM. 2019. Observation regarding the pellets and food behaviour in captivity of Bubo bubo. 33-40.
15. EGF S (1966). The behavior and ecology of the South African ostrich. Living Bird. 5:45-75.
16. Engelbrecht A (2013). Establishing genetic and environmental parameters for ostrich (*Struthio camelus domesticus*) growth and slaughter

characteristics (Doctoral dissertation, Stellenbosch: Stellenbosch University).
17. Ipek AY, Sahan U, Yılmaz B (2003). The effect of different incubation temperatures on the incubation performance of ostrich (Struthio camelus) eggs. 271-274.
18. Jayne K, See A (2019). Behavioral research on captive animals: scientific and ethical concerns. In Animal Experimentation: Working Towards a Paradigm Change (pp. 517-547). Brill.
19. Kokoszynski D, (2017). Guinea Fowl, Goose, Turkey, Ostrich, and Emu Eggs. Egg Innovations and Strategies for Improvements. 33-43.
20. Menon DG, Bennett DC, Cheng KM (2014). Understanding the behavior of domestic emus: a means to improve their management and welfare—major behaviors and activity time budgets of adult emus. J. Anim.
21. Mohapatra RK, Panda S, Acharya UR (2014). Study on activity pattern and incidence of stereotypic behavior in captive tigers. J. Vet. Behav. 9(4): 172-176.
22. Mukhtar N, Mirza MW (2017). Understanding of social and mating behaviour of ostrich (*Struthio camelus*). J. World's Poult. Res. 7(2): 72-78.
23. Newberry RC, Keeling LJ, Estevez I, Bilčík B (2007). Behaviour when young as a predictor of severe feather pecking in adult laying hens: the redirected foraging hypothesis revisited. Appl. Anim. Behav. Sci. 107(3-4): 262-274.
24. Souza JS (2004). Criação de avestruz. Viçosa, Aprenda Fácil.
25. Tomuta R, Rada O, Fericean LM (2021). Ostrich reproduction behavior under farming conditions.

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A Review on the Synergistic Approaches for Heavy Metals Bioremediation: Harnessing the Power of Plant-Microbe Interactions

Iqra Arshad[1], Hifza Iqbal[1], Syeda Saira Iqbal[2], Muhammad Afzaal[2], Yasir Rehman[1]*

1. Department of Life Sciences, School of Science, University of Management and Technology, Lahore, Pakistan
2. Sustainable Development Study Center, Government College University, Lahore, Pakistan

Corresponding Author's Email: Yasir.rehman@umt.edu.pk; yasirehman@gmail.com

ABSTRACT: *Heavy metals contamination is a serious threat to all life forms. Long term exposure of heavy metals can lead to different life-threatening medical conditions including cancers of different body parts. Phytoremediation and bioremediation offer a potential eco-friendly solution to such problems. Different microbes can interact with heavy metals in a variety of ways such as biotransformation, oxidation/reduction, and biosorption. Phytoremediation of the heavy metals using plants mostly involves rhizofilteration, phytoextraction, phytovolatization, and Phytostabilization. A synergistic approach using both plants and microbes has proven much more efficient as compared to the individual applications of microbes or plants. This article aims to highlight the synergistic methods used in bioremediation, emphasizing the potent collaboration between bacteria and plants for environmental cleaning, along with the discussion of the importance of site-specific variables and potential constraints. While identifying the necessity for all-encompassing solutions, this review places emphasis on the combination of methodologies as a multifarious rehabilitation approach. This discussion offers insightful suggestions for scholars, scientists and decision-makers about the sustainable recovery of heavy metal-contaminated environments using a comprehensive strategy.*

Keywords: *Heavy metals, Phytoremediation, Bioremediation, Plant-Microbe Interaction*

INTRODUCTION

Life as well as ecosystems are at stake because of the environmental contamination, mainly caused by industrial processes. Bioremediation is a potential approach in the search for long-term pollution mitigation options. The general goal of bioremediation technology is to use biological processes to remove contaminants such as pesticides, heavy metals, radionuclides, organic waste, and others from contaminated sites or industrial discharges. It may frequently be completed on site, is well accepted by the people, and requires very little in the way of technology. The kind of pollutants, the amount of time, and the technique all affect the outcome of the remediation processes (Saha et al., 2021).

High concentrations of various contaminants are present in industrial effluent that is often dumped into water bodies. The toxicity of these toxicants not only harm the human health, but other life forms as well. It also disrupts the ecosystems and thus affects the overall quality of the environment (Choudhary et al., 2017). Because heavy metals are persistent and cannot be biodegraded in wastewater treatment, their toxicity especially at high concentrations has become a major concern worldwide. Lead, uranium, selenium, zinc, arsenic, cadmium, chromium, mercury, copper, and nickel are some of the most important contaminants in this regard. Typically, mining activities, ore refinement, sludge disposal, paints, alloys, batteries, fly ash from incinerators, radioactive material processing, metal plating, or the production of electrical equipment, insecticides, or preservatives generate these hazardous compounds (Junaid et al., 2017). Toxic metals are not just released by industry; occasionally, natural processes also release heavy metals into the environment. Climate change, volcanic eruptions, leaching and erosion are some of the natural processes that can release heavy metals into the environment (Wuana and Okieimen, 2011). These processes increase the human exposure to such contaminants as they persist in the environment. While small concentrations of these elements are essential for optimal health,

larger concentrations are hazardous both in the short and long-term exposure. Depending on the heavy metal and the individual exposure, heavy metals can have teratogenic, nephrotoxic, fetotoxic, and neurotoxic consequences (Leong and Chang, 2020).

Bioremediation methods are often categorized as *in situ* i.e., at the contaminated location, or *ex situ* which refers to removing a pollutant from its original location and treating it somewhere else. *In situ* bioremediation is an economical and ecologically acceptable way to treat polluted soil and groundwater. It needs the right climate, vital nutrients, a healthy population of microbes, and enough time for natural breakdown. Because the rates at which microorganisms break down / transform the toxicants vary, process optimization requires constant observation and modification (Sharma, 2020). *Ex situ* bioremediation is the process of transferring contaminated materials somewhere else for treatment. This is frequently done when *in situ* treatments are not feasible. Methods like bioreactors and biopiles provide better environmental control and can handle vast amounts of polluted medium (Azubuike et al., 2016).

One effective approach is to utilize the beneficial interactions between microbes and plants, utilizing the distinct advantages of each application. The goal of plant-microbe synergy is to increase the effectiveness of environmental cleaning. As phytoremediators, plants are very effective because they can draw many toxic compounds, including heavy metals, out of soil or water. Simultaneously, microbes that live in the rhizosphere of plants or create symbiotic relationships with plant roots provide their biochemical and metabolic skills to aid in the breakdown, immobilization, transformation, or uptake of the pollutants. This integrated bioremediation strategy's interactions between bacteria and plants result not only in greater pollutant remediation, but also better plant health. In addition to cleaning up polluted areas, the goal of these cooperative initiatives is to advance environmentally sound behaviours that support ecosystem restoration and balance. The framework for investigating the many aspects of plant-microbe interactions in bioremediation—

which include phytoremediation, rhizoremediation, myco-remediation, phyto-stabilization, and bioaugmentation—is discussed in this review.

1. BIOREMEDIATION

Microorganisms or their enzymes are used in bioremediation to eliminate pollutants from a contaminated location. The primary microorganisms utilized in the bioremediation process to clean up polluted soil and water are bacteria and fungus (Strong and Burgess, 2008). Methane, phenol, and toluene are examples of substrates that may be used to stimulate bioremediation activity through microorganisms. Other methods include adding nutrients (nitrogen and phosphorus) and electron acceptors (e.g., oxygen) (Romantschuk et al., 2023).

This decade has seen a rise in interest in the use of bacterial, fungal, and algal biomass as an absorbent medium for the removal of heavy metals. Because it may act as a bio trap for heavy metals, renewable biomass from a variety of microorganisms may prove to be an environmentally beneficial alternative to physicochemical remediation techniques. Biotrap is any living thing or part of a living thing that can connect with a poisonous metal and change its shape, allowing the metal to be removed and recovered from contaminated soil or water or turning it harmless (Crusberg and Mark, 2000). Microbial adsorbents have emerged as an eco-friendly and efficient material choice (Valls and De Lorenzo, 2002). Reclamation of contaminated places greatly depends on how microorganisms react to harmful heavy metals (Congeevaram et al., 2007). For growth, microorganisms need the ideal conditions of temperature, nutrition, and oxygen. Heavy metals have a variety of effects on the physiology of microorganisms, but many of them manage to resist these pressures. To survive in environments where metals are scarce, bacteria have developed a few strategies. These processes allow them to mobilize, immobilize, or change metals, making them inert enough to withstand the toxicity of heavy metal ions (Nies, 1999). These mechanisms include chelation (metals form complex with the metal-binding proteins, e.g., metallothionein's), extrusion (metals are pushed out of the cell through chromosomal/plasmid-mediated events), and exclusion

(the metal ions are kept away from the target sites or other cell components), methylation-demethylation, and biotransformation (conversion to less hazardous ones) (Umrania, 2006). These mechanisms enable metabolic activity of bacteria in environments polluted by metals. These processes may be inducible or constitutive. Bacteria most likely pick up their resistance to heavy metals by natural changes on plasmids and transposons or gene transfer. For example, the *czc* system is responsible for resistance against cadmium, zinc, and cobalt in Gram-negative bacteria (such *Ralstonia eutropha*). These metals are exported via the cation-proton antiporter (CzcABC), which is encoded by the *czc* genes (Nies and Silver, 1995). Many fungi and bacteria have been employed for the removal of heavy metals, each with a unique mechanism, as explained below.

1.1. Microbial Mechanisms Involved in Bioremediation of Heavy Metals

Many microorganisms interact with heavy metals in the environment and find applications in bioremediation. The following are the most common microbial mechanisms involved in this process (Fig. 1).

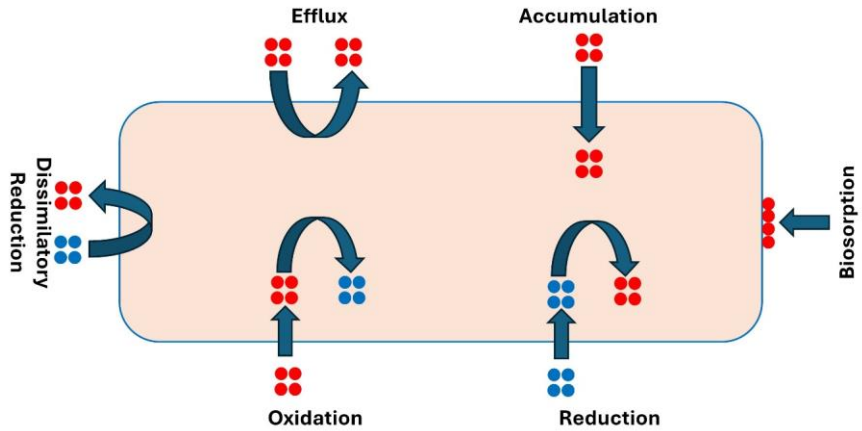

Fig. 1. Most common modes of microbial interactions with heavy metals

a) **Biosorption:** Many functional groups are present on the cell surface of microorganisms, such as carboxyl, amino, and phosphate groups. Many bacteria are also known to

produce extracellular polymeric substances (EPS). In the case of fungi, they also contain chitin as a main constituent of their cell walls. These and other functional groups found on cell walls of microbes can bond with heavy metal ions, causing a buildup of metals on the surface of the cells. This interaction leads to biosorption and not only helps in reducing the bioavailability of the heavy metals but can also help to remove them (Wu et al., 2021).

b) Bioaccumulation

Many bacteria have been reported to accumulate heavy metals in their cells. This is possible due to several channels that exist in the membranes of the microorganisms through which the heavy metals are transported inside, followed by their interaction and complexation with different proteins (Igiri et al., 2018). Microorganisms such as fungi can also accumulate heavy metals in their mycelia. It includes the sequestration of metals in intracellular compartments and the creation of metal complexes. Heavy metals can be absorbed by algae into their cells. The metals can be attached to certain cellular elements or kept in vacuoles of cells (Chugh, M et al., 2022).

c) Bio-mineralization: Bacteria use this process to immobilize heavy metals. Some bacteria can change reduce the solubility of metals, rendering them less hazardous, and may even lead to their precipitation (Kapahi and Sachdeva, 2019).

d) Oxidation / Reduction: Some bacteria have the capacity to oxidize or reduce heavy metals, thus rendering them less toxic and less soluble too. This is also a detoxification strategy in case of many heavy metals (Pande et al., 2022). Many microbial species are known to oxidize or reduce different heavy metals (Silver 2011; Silver and Phung, 2005).

Many bacterial species have been reported to interact with heavy metals including *Pseudomonas*, *Bacillus*, *Aeromonas*, *Shewanella*, and many other bacterial species. Sulfate reducing bacteria such as *Desulfovibrio* species can cause the precipitation of metal sulfides, and thus can aid in the

bioremediation of metals. Applications for bioremediation using bacteria include the removal of polluted sediments, wastewater, and soils. Applying them in in-situ or ex-situ treatment techniques is contingent upon the environmental circumstances. (Kapahi and Sachdeva, 2019). Fungi are used for cleaning up soils, sediments, and water bodies polluted with metals. They have capacity to form mutualistic connections with plants and prove advantageous in augmenting plant well-being and absorption of metals (Joshi et al., 2011). Examples include *Aspergillus*, *Trichoderma*, and many other fungal species. Microalgae and macroalgae alike microorganisms are more important when there is need to eliminate heavy metals from aquatic environments. Wastewater treatment frequently uses algae-based bioremediation, particularly to remove heavy metals. Before being released, tainted water can be treated in artificial wetlands with algal mats (Ankit et al., 2022). Examples include *Chlorella*, *Spirogyra*, *Ulva* (Sea Lettuce), and many other algal species.

1.2. Microbial Strategies Used in Bioremediation

The most important microbial strategies used in bioremediation of heavy metals include biosorption, bioaugmentation, bioaccumulation, biomineralization, microbial transformation, and rhizoremediation.

a) Bioaugmentation

It can be used for increasing microbial populations' ability to withstand and immobilize heavy metals in polluted settings. Microorganisms that can bind metals, either natural wild type or genetically modified, are introduced at the site of the contamination. This leads to higher number of these microorganisms at these sites which enhance the efficiency of bioremediation. The microorganisms then remediate the heavy metals by the processes as mentioned above.

b) Biosorption

Heavy metals can be extracted by biosorption from polluted water sources, mining runoff, and industrial effluents. On their cell surfaces, microorganisms including bacteria, fungus, and algae have functional groups that bind to heavy metal ions. These groups consist of phosphate, amino, and carboxyl groups.

c) Rhizoremediation

Rhizoremediation can be used for soils and streams polluted with heavy metals. Plants exude root exudates, which facilitate the development of fungi and bacteria that interact with the metals in the rhizosphere. In the root zone, these microbes help to convert or immobilize heavy metals. Many plants such as willows (*Salix* spp.) and sunflowers (*Helianthus annuus*) are used in phytoextraction of heavy metals. Plant roots are home to mycorrhizal fungus, which improve the intake of nutrients and metals.

d) Biotransformation

Heavy metals can have their chemical forms changed via microbiological transformation to reduce their toxicity or facilitate their immobilization. Heavy metal ions are enzymatically changed by microorganisms into less hazardous or transportable forms. This might entail procedures like complexation, oxidation, or reduction. Insoluble metal sulfides can precipitate from the reduction of sulfate to sulfide by sulfate-reducing bacteria, such as *Desulfovibrio* species. Additionally, certain fungi aid in the transformation of heavy metals.

By improving microbial communities' capacity to deal with metals, by using biological entities for metal adsorption, by taking advantage of plant-microbe interactions for metal uptake, or enzymatically converting heavy metals into less hazardous forms, these microbial techniques are essential to the process of heavy metal bioremediation. The characteristics of the pollutants as well as the physiochemical properties of the polluted site determines which treatment is best (Emenike et al., 2018).

2. PHYTOREMEDIATION

Phytoremediation utilizes plants to bio-transform or remove the toxic compounds from soil or water. Lots of different pollutants such as heavy metals, pesticides, insecticides, hydrocarbons and others can be removed through this approach (Hong-Bo et al., 2010). Many plant species have been reported to not only withstand but also uptake large concentrations of heavy metals. Both wild type as well as transgenic plants have been used in phytoremediation (Sabreena et al., 2022). The interactions between plants and metals,

environmental conditions, physiochemical properties of the site (water or soil) as well as the presence of other toxicants effect the performance of phytoremediation (Pandey and Bajpai, 2019). A lack of precise knowledge of these factors continues to impede the wide-scale use of this technology (Shen et al., 2022).

2.1. Strategies of Phytoremediation

The following are the most common processes employed by plants to reduce the toxicity of heavy metals (Fig. 2):

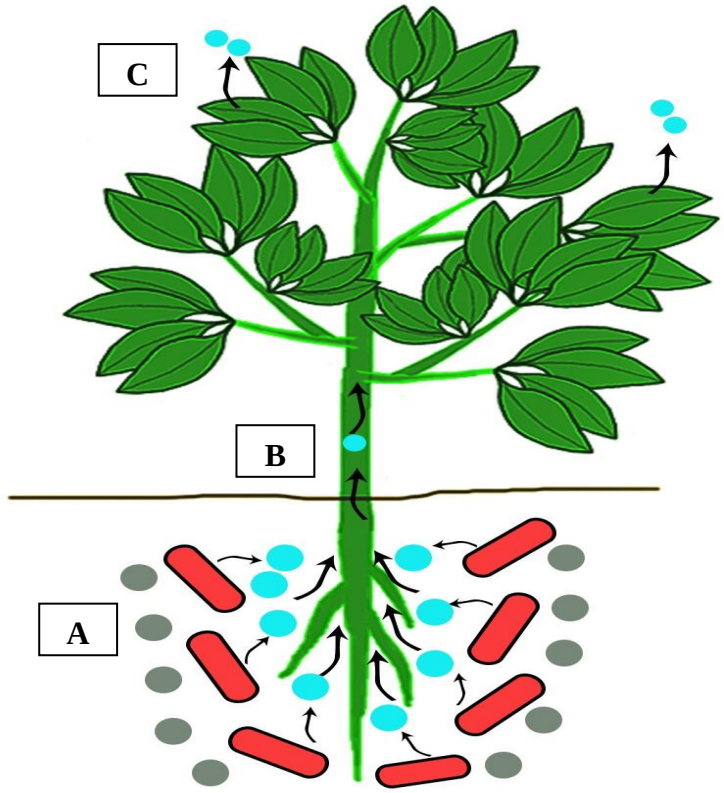

Fig. 2. Effects of microbial transformation of heavy metals on phytoremediation. **A:** microbes in the rhizosphere increase the mobility of the heavy metals through biotransformation, **B:** Plants efficiently uptake these heavy metals and store in their shoots and leaves i.e., phytoextraction, **C:** Plants are also able to release the heavy metals in the atmosphere via transpiration i.e., phytovolatilization

a) Rhizofiltration

It is described as a mechanism by which plants adsorb onto, or absorb into, their roots the pollutants from contaminated water sources. Acid mine drainage, agricultural runoff, and industrial discharge can all be partially mitigated via rhizofiltration. It is feasible to remove heavy metals as well as radionuclides using this technique (Jabeen et al., 2009). Plant species that have a strong affinity for specific heavy metals are selected for hydroponic growing or artificial wetland creation. Mathematical formulas for metal adsorption, root development, and moisture uptake have been generated through various studies. Designing phytoremediation programs requires knowledge of heavy metal transport in soil, water, and root system (Verma et al., 2006). One of the benefits of rhizo-filtration is that species other than hyperaccumulators can also be employed, and it can be applied both *in situ* and *ex situ*. Studies have been done on the capacity of many plants, including sunflower, Indian mustard, tobacco, rye, spinach, and corn, to extract metals from wastewater (Ghosh and Singh, 2005).

b) Phytostabilisation

The process of developing a plant cover on the surface of a contaminated site in order to reduce off-site pollution by limiting the transport of pollutants inside the vadose zone by root accumulation or immobilization inside the rhizosphere. It also increases the organic content of the soil that also binds the heavy metals. Certain plant species like grasses or ferns are chosen because of their capacity to withstand heavy metals and store them in their root systems. Heavy metals are taken up and transported from the soil to the roots of plants by metal transporters. Its primary application is in the remediation of sludges, sediments, and soil (Mueller et al., 1999). This process relies heavily on roots' ability to decrease contaminant movement and soil bioavailability. Phytostabilization can occur by sorption, precipitation, complex action, or metal valence reduction. Plant roots play a primary role in this regards, as they reduce the water movement through soil matrix, thus preventing not only soil erosion but also the movement of the toxic heavy metals to other areas (Fig. 3). It also prevents

leaching of the heavy metals (Berti and Cunningham, 2000).

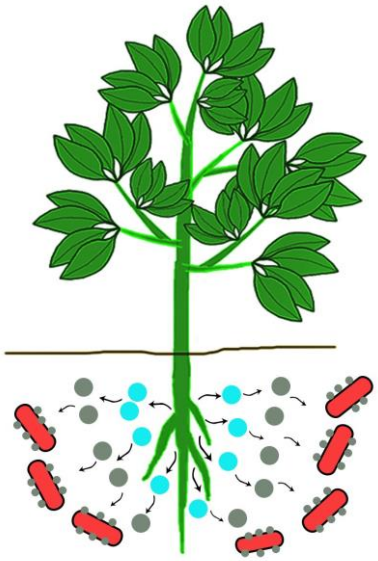

Fig. 3. Effects of Phytostabilization on microbial biosorption of heavy metals in the rhizosphere. Many plants can stabilize the heavy metals present in the soil through different means. These heavy metals are then efficiently adsorped / absorbed by the microorganisms present there, thus decreasing further their bioavailability

c) Phytovolatilization

Phytovolatilization is the conversion of heavy metals into volatile compounds through transpiration by plants. These volatile compounds are less toxic and are easily diluted by dispersion through the air. Many heavy metals such as mercury and selenium are volatilized by different plant species (Chandra et al., 2015). Volatilization may occur directly through stems and leaves, or indirectly by interactions between plant roots and the soil (Limmer and Burken, 2016). High rates of volatilization of heavy metals can be achieved by searching for new plant species with exceptional transpiration rates, as well as through genetic modification of already known plants (Guignardi and Schiavon, 2017). The main advantage of phytovolatilization is that, once the plantation is established, it seldom needs additional maintenance (Cristaldi et al., 2017).

d) Phytoextraction

Through their roots, plants draw toxins from the soil, water, or sediments and move them to the biomass above ground, where they build up in areas like shoots and other portions of the plant that may be harvested. This process is called as phytoextraction (Singh, N and Santal, AR, 2015). Plants that hyperaccumulate heavy metals (HMs) in above-ground portions and plants that produce a lot of above-ground biomass are used in phytoextraction as these

two traits are crucial in defining the phytoextraction efficieny of plant species (Robinson et al., 1998). There are around 450-500 plants that are known to be hyperaccumulators (Chaudhary et al., 2018). Once the plants have been grown on contaminated areas, the metal-enriched biomass that grows above ground is removed and disposed. This ultimately removes the heavy metals from the polluted site. The transfer of the metals to the shoots is very important since it is far easier to collect the shoots as compared to collecting the roots. Phytoextraction is the best phytoremediation technique to remove heavy metals from contaminated soils. In addition, it is also the most financially achievable option of remediation. The capacity of plants to uptake and retain metals, as well as soil properties, heavy metal speciation, and heavy metal bioavailability all influence how successful phytoextraction might be (Yan et al., 2020).

3. BIOREMEDIATION OF HEAVY METALS THROUGH PLANT MICROBE INTERACTIONS

The key components needed to remediate industrial wastes like heavy metals, pesticides, and toxic chemical fertilizers are microorganisms (which include bacteria, yeast, fungi, and even archaeon) and plants. This is because they function as biological catalysts in a bioremediation system that is set up by appropriate elements to fix contaminated environments, meaning that appropriate plants can remove and restrain metals from the ground and microorganisms that can take up and transform heavy metals. Although heavy metals may look like hard-to-manage pollutants, bioremediation is a preferred technique to repair damaged soils; moreover, combining plants and microbes is a strategy to ensure a more thorough cleanup. Thus, using plants and the bacteria they are associated with to clean up heavy metal-contaminated settings is a growing field that has benefits for the environment, sustainability, and cost. A well-liked and effective remediation method involves mixing a variety of microbes and plants. Synergistically using many plant and microbe species can enhance remediation capacity overall and address a broader range of

contaminants (Arantza et al., 2022).

3.1. Synergistic Effect of Microbial Biotransformation and Phytoextraction

A synergistic effect can be achieved by combining microbial biotransformation and phytoextraction. By complementing the capabilities of these two techniques, the effectiveness of metal bioremediation can be enhanced. The process involves the selection of hyperaccumulating plants in combination with metal resistant microorganisms in the rhizosphere. The metal-resistant microorganisms mediate the mobilization of the heavy metals in the rhizosphere, facilitating the uptake of the heavy metals by the plants. This leads to enhanced uptake of metals by the plants (Lebeau et al., 2011). However, selection of appropriate and compatible plants and microbial species as well as the optimization for optimal efficacy is very important. To combine both techniques, we first need to optimize metal-resistant bacteria living in the rhizosphere of hyper accumulator plants. Metals can be oxidized, reduced, and solubilized by these bacteria, increasing the metals' bioavailability in the rhizosphere. The rhizosphere is home to bacterial activity that alter soil properties and create a microenvironment that is favourable to the bacteria as well as the plants. Enhanced metal uptake environment is also influenced by pH, organic matter content, and nutrient availability changes. Metal release from soil matrices is also facilitated by bacterially generated organic acids and chelating agents. The transformed or bioavailable metals can be efficiently absorbed by hyperaccumulator plants through their roots. The xylem play role in transporting metals that are taken up by plant roots to tissues that are above ground. The plant's ability to remove metals is maintained or improved by metal-resistant microorganisms in the rhizosphere. Metal concentrations are decreased overall when harvested plants are removed from the site (Syranidou et al., 2016).

After correct selection of the plants, microorganisms that are resistant to metals and have active mechanisms of oxidizing / reducing the metals are selected. We can also use indigenous microorganisms which are already acclimated to the site or can go for

bioaugmentation which is the addition of specific strains at the site. The rhizosphere can be inoculated with metal-resistant bacteria by techniques such as root dipping, soil amendment, or seed coating. However, inoculation methods must be optimized to make sure that the added bacteria are effectively colonized and established (Syranidou et al., 2016).

For example, Arbuscular mycorrhizal fungi (AMF) form symbiotic relationships with plant roots. We can combine it with hyper accumulator plants. In this synergistic approach, AMF enhances nutrient uptake by plants as well as improve metal uptake by sunflower shoots by increasing the mobilization of the metals in the rhizosphere. Moreover, it was also reported that AMF protects the roots of the plants whereas promotes metal uptake by the shoots. As a result of this, there occur enhanced metal uptake by sunflowers and improved nutrient cycling. However, effectiveness of this combination may vary depending on soil conditions and AMF species compatibility (Zhang et al., 2018).

Metal-resistant bacteria, such as *Cupriavidus metallidurans* can be combined with Willow trees, which are frequently employed in the phytoextraction of metals, such as nickel and copper. In this combination, metal-resistant bacteria improve metal solubilization and availability while willow trees accumulate metals. As a result, there will be increased copper and nickel removal from the soil, potential for improved soil quality. However, there may occur phytotoxicity in the presence of high metal concentrations, which can cause slow growth of willows (Manzoor et al., 2019).

3.2. Synergistic Effect of Microbial Biotransformation and Phytovolatilization

Microbial transformation and phytovolatilization are two different methods for removing heavy metals from polluted environments. The two techniques can have effective results when combined, speeding up the remediation process. Microbial transformation mostly includes oxidation / reduction of heavy metals, whereas phytovolatilization is a process in which plants absorb heavy metals, translocate them, and then release the volatile metals into the atmosphere (Ma et al., 2016).

Through microbial transformation, heavy metals get converted into more soluble and bioavailable forms. The mechanisms during transformation may involve oxidation, reduction or methylation. This helps enhance bioavailability of metals for plants which take these solubilized forms of heavy metals from soil through roots and transport it to upper parts of plants. Moreover, the microbial transformation also reduces the toxicity of the heavy metals. These metals are then volatilized into the environment through leaves by the process of phytovolatilization.

Mutualistic interactions, in which bacteria facilitate the uptake or transformation of metals and plants supply organic molecules to their microbial partners, can be fostered by symbiotic associations. Addition of specific microbial strains with improved metal transformation capacities can increase the effectiveness of metal removal overall (Sharma et al., 2023). For increased volatilization process, genetically modified plants with enhanced metal absorption capacities or altered metabolic pathways can also be considered.

The combination of phytovolatilization and microbial transformation provides a clear benefit in transforming pollutants into less harmful forms. But a thorough assessment of the environment is required since arguments may arise regarding volatile chemical emissions during phytovolatilization.

3.3. Synergistic Effect of Phytostabilization and Microbial Biosorption

It is feasible to work on the bioremediation of metals by combining Phytostabilization with biosorption, especially in circumstances when the two techniques function admirably together. Certain plants are employed in Phytostabilization to either immobilize or lessen the mobility of heavy metals in the soil. The selection of the plants for this purpose depends on their ability to grow in such polluted environments without up taking the metals in large concentrations. The plants used for such combination should also be able to promote the development of microbes involved in biosorption in the rhizosphere, the latter of which can be bioaugmented as well. Microbial biosorption involves the absorption and

adsorption of heavy metals by microbes. The combination of Phytostabilization and microbial biosorption can enhance the bioremediation of heavy metals. The Phyto stabilized heavy metals can be biosorped by the microbial population in the rhizosphere in large amounts. The overall effectiveness on this synergistical approach depends on the physiochemical properties of the site as well as the climate conditions (Bingöl et al., 2017). Genetically modified plants microbes can also be considered for this synergistical approach.

CONCLUSION

Both plants and microorganisms are important for the remediation of heavy metals. Both phytoremediation and bioremediation involve different mechanisms to reduce the toxicity of the heavy metals. These plants and microbes - based mechanisms can be combined in a synergistical manner to increase the effectiveness of heavy metal remediation.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

CONTRIBUTION OF AUTHORS

Iqra Arshad and Hifza Iqbal contributed equally to drafting of the manuscript. Syeda Saira Iqbal and Muhammad Afzal assisted in the draft preparation. Yasir Rehman proposed the concept and provided a detailed outline, assisted in the draft preparation and edited the final draft.

REFERENCES

1. Ankit, Bauddh K, Korstad J (2022). Phycoremediation: Use of algae to sequester heavy metals. Hydrobiol. 1(3): 288-303.
2. Arantza SJ, Hiram MR, Erika K, Chávez-Avilés MN, Valiente-Banuet JI, Fierros-Romero G (2022). Bio-and phytoremediation: Plants and microbes to the rescue of heavy metal polluted soils. SN Appl. Sci. 4(2): 59.
3. Azubuike CC, Chikere CB, Okpokwasili GC (2016). Bioremediation techniques–classification based on site of application: principles, advantages, limitations and prospects. World J. Microbiol. Biotechnol. 32: 1-18.
4. Berti WR, Cunningham SD (2000). Phytostabilization of metals. Phytoremediation of

toxic metals: Using plants to clean up the environment. Wiley, New York. 71-88.
5. Bingöl NA, Özmal F, Akın B (2017). Phytoremediation and biosorption potential of *Lythrum salicaria* l. for nickel removal from aqueous solutions. Pol. J. Environ. Stud. 26(6): 2479-2485.
6. Chandra R, Saxena G, Kumar V (2015). Phytoremediation of environmental pollutants: an eco-sustainable green technology to environmental management, *In* Advances in biodegradation and bioremediation of industrial waste. 1-29.
7. Chaudhary K, Agarwal S, Khan S (2018). Role of phytochelatins (PCs), metallothioneins (MTs), and heavy metal ATPase (HMA) genes in heavy metal tolerance, *In* Mycoremediation and Environmental Sustainability. Volume 2: 39-60.
8. Choudhary M, Kumar R, Datta A, Nehra V, Garg N (2017). Bioremediation of heavy metals by microbes, *In* Bioremediation of salt affected soils: an Indian perspective. 233-255.
9. Chugh M, Kumar L, Shah MP, Bharadvaja N (2022). Algal bioremediation of heavy metals: An insight into removal mechanisms, recovery of by-products, challenges, and future opportunities. Energy Nexus. 7:100129.
10. Congeevaram S, Dhanarani S, Park J, Dexilin M, Thamaraiselvi K (2007). Biosorption of chromium and nickel by heavy metal resistant fungal and bacterial isolates. J. Hazard. Mat. 146(1-2): 270-277.
11. Cristaldi A, Conti GO, Jho EH, Zuccarello P, Grasso A, Copat C, Ferrante M (2017). Phytoremediation of contaminated soils by heavy metals and PAHs. A brief review. Environ. Technol. Inno. 8: 309-326.
12. Crusberg T, Mark S. (2000). Heavy metal remediation of wastewaters by microbial biotraps, *In* Bioremediation. Springer. 123-137.
13. Emenike CU, Jayanthi B, Agamuthu P, Fauziah S (2018). Biotransformation and removal of heavy metals: a review of phytoremediation and microbial remediation assessment on contaminated

soil. Environ. Rev. 26(2): 156-168.
14. Ghosh M, Singh S (2005). A review on phytoremediation of heavy metals and utilization of it's by products. Asian J. Energy Environ. 6(4): 18.
15. Guignardi Z, Schiavon M (2017). Biochemistry of plant selenium uptake and metabolism, *In* Selenium in plants: molecular, physiological, ecological and evolutionary aspects. 21-34.
16. Hong-Bo S, Li-Ye C, Cheng-Jiang R, Hua L, Dong-Gang G, Wei-Xiang L (2010). Understanding molecular mechanisms for improving phytoremediation of heavy metal-contaminated soils. Crit. Rev. Biotechnol. 30(1): 23-30.
17. Igiri BE, Okoduwa SI, Idoko GO, Akabuogu EP, Adeyi AO, Ejiogu IK (2018). Toxicity and bioremediation of heavy metals contaminated ecosystem from tannery wastewater: a review. J. Toxicol. 2018.
18. Jabeen R, Ahmad A, Iqbal M (2009). Phytoremediation of heavy metals: physiological and molecular mechanisms. Bot. Rev. 75: 339-364.
19. Joshi P, Swarup A, Maheshwari S, Kumar R, Singh N (2011). Bioremediation of heavy metals in liquid media through fungi isolated from contaminated sources. Indian J. Microbiol. 51: 482-487.
20. Junaid M, Hashmi MZ, Tang YM, Malik RN, Pei,DS (2017). Potential health risk of heavy metals in the leather manufacturing industries in Sialkot, Pakistan. Sci. Rep. 7(1): 8848.
21. Kapahi M, Sachdeva S (2019). Bioremediation options for heavy metal pollution. J. Health Pollut. 9(24): 191203.
22. Lebeau T, Jézéquel K, Braud A (2011). Bioaugmentation-assisted phytoextraction applied to metal-contaminated soils: state of the art and future prospects, *In* Microbes and Microbial Technology: Agricultural and Environmental Applications. 229-266.
23. Leong YK, Chang JS (2020). Bioremediation of heavy metals using microalgae: Recent advances and mechanisms. Bioresour.Technol. 303: 122886.

24. Limmer M, Burken J (2016). Phytovolatilization of organic contaminants. Environ. Sci. Technol. 50(13): 6632-6643.
25. Ma Y, Oliveira RS, Freitas H, Zhang C (2016). Biochemical and molecular mechanisms of plant-microbe-metal interactions: relevance for phytoremediation. Front. Plant Sci. 7: 918.
26. Manzoor M, Gul I, Ahmed I, Zeeshan M, Hashmi I, Amin BAZ, Kallerhoff J, Arshad M (2019). Metal tolerant bacteria enhanced phytoextraction of lead by two accumulator ornamental species. Chemosphere. 227: 561-569.
27. Mueller B, Rock S, Gowswami D, Ensley D (1999). Phytoremediation decision tree. Prepared by-Interstate Technology and Regulatory Cooperation Work Group. 1-36.
28. Nies DH (1999). Microbial heavy-metal resistance. Appl. Microbiol. Biotechnol. 51: 730-750.
29. Nies DH, Silver S (1995). Ion efflux systems involved in bacterial metal resistances. J. Ind. Microbiol. 14: 186-199.
30. Pande V, Pandey SC, Sati D, Bhatt P, Samant M (2022). Microbial interventions in bioremediation of heavy metal contaminants in agroecosystem. Front. Microbiol. 13: 824084.
31. Pandey VC, Bajpai O (2019). Phytoremediation: from theory toward practice, *In* Phytomanagement of polluted sites. Elsevier. 1-49.
32. Robinson BH, Leblanc M, Petit D, Brooks RR, Kirkman JH, Gregg PE (1998). The potential of *Thlaspi caerulescens* for phytoremediation of contaminated soils. Plant Soil. 203: 47-56.
33. Romantschuk M, Lahti-Leikas K, Kontro M, Allen JA, Sinkkonen A (2023). Bioremediation of contaminated soil and groundwater by *in situ* biostimulation. Front. Microbiol. 14: 1258148.
34. Sabreena, Hassan S, Bhat SA, Kumar V, Ganai BA, Ameen F (2022). Phytoremediation of heavy metals: An indispensable contrivance in green remediation technology. Plants. 11(9): 1255.
35. Saha L, Tiwari J, Bauddh K, Ma Y (2021). Recent developments in microbe–

plant-based bioremediation for tackling heavy metal-polluted soils. Front. Microbiol. 12: 731723.

36. Sharma I. (2020). Bioremediation techniques for polluted environment: concept, advantages, limitations, and prospects, *In* Trace metals in the environment-new approaches and recent advances. IntechOpen.
37. Sharma JK, Kumar N, Singh NP, Santal, AR (2023). Phytoremediation technologies and their mechanism for removal of heavy metal from contaminated soil: An approach for a sustainable environment. Front. Plant Sci. 14: 1076876.
38. Shen X, Dai M, Yang J, Sun L, Tan X, Peng C, Ali I, and Naz I (2022). A critical review on the phytoremediation of heavy metals from environment: Performance and challenges. Chemosphere. 291: 132979.
39. Silver S (2011). BioMetals: a historical and personal perspective. Biometals. 24(3): 379-390.
40. Silver S, Phung LT (2005). A bacterial view of the periodic table: genes and proteins for toxic inorganic ions. J. Ind. Microbiol. Biotechnol. 32: 587-605.
41. Singh N, Santal AR (2015). Phytoremediation of heavy metals: the use of green approaches to clean the environment, *In* Phytoremediation: Management of Environmental Contaminants. Volume 2: 115-129.
42. Strong PJ, Burgess JE (2008). Treatment methods for wine-related and distillery wastewaters: a review. Bioremediation J. 12(2): 70-87.
43. Syranidou E, Christofilopoulos S, Gkavrou G, Thijs S, Weyens N, Vangronsveld J, Kalogerakis N (2016). Exploitation of endophytic bacteria to enhance the phytoremediation potential of the wetland helophyte *Juncus acutus*. Front. Microbiol. 7: 1016.
44. Umrania VV (2006). Bioremediation of toxic heavy metals using acidothermophilic autotrophes. Bioresour. Technol. 97(10): 1237-1242.
45. Valls M, De Lorenzo V (2002). Exploiting the genetic and biochemical capacities of bacteria for the remediation of heavy metal pollution. FEMS

Microbiol. Rev. 26(4): 327-338.

46. Verma P, George K, Singh H, Singh S, Juwarkar A, Singh R (2006). Modeling rhizofiltration: heavy-metal uptake by plant roots. Environ. Model. Assess. 11: 387-394.
47. Wu Y, Li Z, Yang Y, Purchase D, Lu Y, Dai Z (2021). Extracellular polymeric substances facilitate the adsorption and migration of Cu^{2+} and Cd^{2+} in saturated porous media. Biomolecules. 11(11): 1715.
48. Wuana RA, Okieimen FE (2011). Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. International Scholarly Research Notices. 2011.
49. Yan A, Wang Y, Tan SN, Mohd Yusof ML, Ghosh S, Chen Z (2020). Phytoremediation: a promising approach for revegetation of heavy metal-polluted land. Front. Plant Sci. 11: 359.
50. Zhang Y, Hu J, Bai J, Wang J, Yin R, Wang J, and Lin X (2018). Arbuscular mycorrhizal fungi alleviate the heavy metal toxicity on sunflower (*Helianthus annuus* L.) plants cultivated on a heavily contaminated field soil at a WEEE-recycling site. Sci. Total Environ. 628: 282-290.

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Epidemiology and Transmission of Foot and Mouth Disease among Small Ruminants – A Review

Abdul Kabir[1]*, Asghar Ali Kamboh[1], Midrar Ullah[2], Muhammad Owais Iqbal[3], Zuhaib Akhtar[3], Irfan Haider[3], Naveed Khan[3]

1. Department of Veterinary Microbiology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam, Pakistan
2. Livestock and Dairy Development Department Government of Khyber Pakhtunkhwa
3. College of veterinary sciences, The University of Agriculture Peshawar

Corresponding Author's Email: kabirvet32@gmail.com

ABSTRACT: *Foot-and-mouth disease (FMD) is a highly contagious viral infection that affects cloven-hoofed animals, including small ruminants such as sheep and goats. FMD causes severe economic losses due to reduced productivity, trade restrictions, and control measures. The epidemiology and transmission of FMD among small ruminants are influenced by various factors, such as the virus strain, the host species, the environmental conditions, and the animal management practices. This paper reviews the current knowledge on the epidemiology and transmission of foot-and-mouth disease (FMD) among small ruminants. It also examines the global distribution and prevalence of FMD in small ruminants, the clinical signs and lesions of FMD in small ruminants, the role of small ruminants in the maintenance and spread of FMD, the transmission routes and risk factors of FMD in small ruminants, and the implications of FMD in small ruminants for disease control and eradication. The paper also identifies the knowledge gaps and research priorities for improving the understanding and management of FMD in small ruminants.*

Keywords: *Foot-and-mouth disease, Sheep, Goats, Epidemiology, Transmission*

INTRODUCTION

Foot and mouth disease is the virus that causes FMD in animals. It has seven different types (O, A, C, Asia 1, SAT 1, SAT 2, and SAT 3) and many subtypes that can vary in spread and affecting the animals (Azeem et al., 2020). FMDV infects the skin cells of animals and makes blisters on their feet, mouth, and mammary glands (Arzt et al., 2011). The virus can also damage other organs like the heart, pancreas, and milk glands, causing problems like heart inflammation, pancreas inflammation, and mastitis (Arzt et al., 2011). The symptoms of FMD in sheep and goats are not the same for all animals. They depend on many things, such as the type of virus, the genes of the animal, the weather, and other infections (Abas et al., 2021). Usually, sheep and goats have less severe symptoms than cows and pigs, so they are harder to notice and report (Law et al., 2011). But some types of viruses can cause very bad disease in sheep and goats, especially in young animals or those with weak immune systems. Also, sheep and goats can carry the virus without showing any symptoms for a long time, and they can pass the virus to other animals that can get sick (Clemmons et al., 2021). FMD can spread among sheep and goats in different ways. They can get the virus by close contact with sick animals or their saliva, milk, urine, or poop, or through indirect contact with contaminated fomites or aerosols (Fig. 1) (Paton et al., 2018).

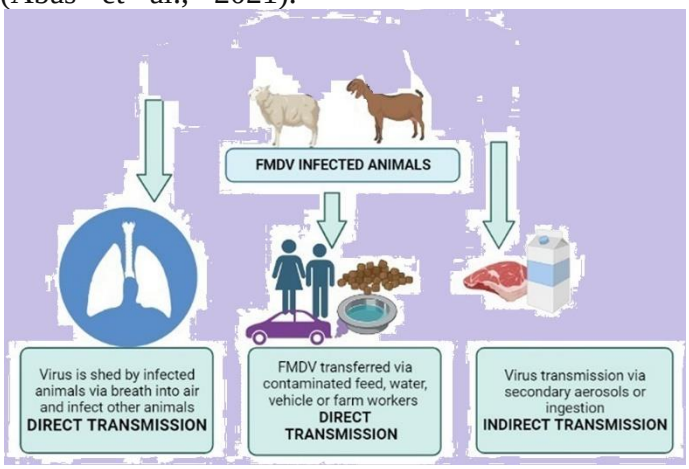

Fig. 1. Transmission routes of FMDV (Aslam and Alkheraije, 2023)

The viruses spread depend on many things, such as the weather, the type of virus, and the type of animals. For example, the viruses can spread more easily in the air when it is humid and windy, or

when the virus is very infectious or stable. Also, the virus can spread more easily by touching when there are many animals or different kinds of animals together, or when the animal is very sensitive or sheds a lot of viruses (Brown et al., 2022). There are many things that can make sheep and goats more likely to get or spread FMDV. These things include how old, what sex, and what breed they are, what kind of production they are used for, where they live, how they move or trade, what the climate and plants are like, and what their immune system and other infections are like. Many studies have tried to find out and measure using different methods, such as blood tests (Chepkwony et al., 2021), outbreak studies (Wood et al., 2020), virus tests (Yousif et al., 2017), math models (Chepkwony et al., 2021), and so on. To control FMD in sheep and goats, we need to use different ways that can stop the virus from coming in, stop the virus from going out, and get rid of the virus completely. These ways include watching and reporting sick animals, limiting and checking animal movement, keeping things clean and safe, giving vaccines and having vaccine plans, killing sick animals and paying farmers, and teaching and telling people about FMD. How well these ways work depends on how much money, how easy, how acceptable, and how followed they are (Blacksell et al., 2019).

By addressing these gaps in our knowledge of FMD epidemiology and transmission among small ruminants, we can enhance our comprehension of FMD dynamics, refine control strategies, and ultimately contribute to minimizing the impact of this economically devastating disease. Closing these gaps will empower us to protect small ruminant populations and uphold the broader stability of agricultural and economic systems. This requires collaborative efforts from researchers, veterinary experts, and policymakers, who can share their expertise, resources, and experiences. By delving into these unexplored areas, we can advance the science and practice of FMD control and prevention.

1. Epidemiology

Animals with split hooves, like cows, pigs, sheep, and goats, can get FMD, a viral infection. It leads to huge economic losses for people who farmed or trade these animals globally. Sheep and goats are very important for how FMD spreads among animals (Dabasa et al., 2021). FMD in these animals is hard to study and control, especially in places like Asia, where many people depend on livestock for their income. The prevalence of FMD in Asia and Pakistan is high due to the large number of livestock, and the

disease poses many challenges for these regions. FMD in Pakistan is affected by many factors, such as the weather, the way people trade animals, animal movement, and the type of the virus (Rashid et al., 2020). FMD is endemic and persistent in Pakistan. Researchers are investigating the epidemiology and pathogenesis of FMD and developing effective vaccines and biosecurity measures. FMD can spread among sheep and goats in different ways. They can get the virus from other sick animals, from the air, or from fomites that have the virus on them. Sheep and goats may not look very sick, but they can still have the virus and spread it to other animals (Jena et al., 2022). Sometimes, they may not show any signs of the disease, but they can still carry the virus for a long time. This makes it hard to find and stop the disease, and it makes these animals possible sources of the virus Trade and Movement in Pakistan: Animals move around a lot for different reasons, such as food (e.g., seasonal grazing), money (livestock markets), and travel (cross-border migration), for religion, and for fun. This helps FMD spread among animals. Sometimes, animals move across countries, and they can bring new types of the virus with them. This means that countries need to work together and have the same rules to stop FMD from spreading (Apolloni et al., 2019). Animals move around in Pakistan for many reasons, such as trade, grazing, migration, religious festivals, and social events. For example, every year, there is a big festival called Eid-ul-Adha, where people buy and slaughter millions of animals. This is a time when FMD can spread easily (Sherman et al., 2011). Also, Pakistan has borders with other countries, like Afghanistan, Iran, India, and China, where animals can move in and out. This can It bring new types of the virus into Pakistan (Osmani et al., 2019).

Research and Challenges to fight FMD well, we need to know how the disease works in sheep and goats. We need to use good tools and methods to study how the virus moves and changes. We need to look at the genes of the virus to see how they are different in different places (Clemmons et al., 2021). The weather also affects how the virus lives and spreads. The virus can live longer in cold and wet weather than in hot and dry weather. So, FMD is more likely to happen in winter and spring than in summer (Björnham, 2020). Also, the virus can travel far in the air when the wind is strong and in the right direction. Wild animals can also have the virus and give it to farm animals. Some wild animals that can get FMD are wild pigs, deer, antelopes, gazelles, yaks, camels, and buffaloes (Gortázar et al., 2022; Admassu et al.,2015). Farm

and wild animals can meet and share the virus when they used the same land, water, fences, or hunting (Knight-Jones et al., 2016). FMD affects the health of humans, animals, and the environment. There is need to work together with animal doctors, disease experts, government people, and communities to stop FMD by making good plans (Calistri et al., 2013). In Pakistan, people keep their sheep and goats in different ways. Some people keep them in small places and do not let them meet other animals. Some people move them around a lot to find food and water. This is more common in dry areas of Pakistan, where sheep and goats are very important for people's lives and food (Chepkwony et al., 2022).

2. Virus Excretion in Small Ruminants and Its Implications in FMD Transmission

FMD is an infection that impacts animals with split hooves, like sheep and goats. They can transmit the virus, even when they are less ill than cows. We need to understand how these animals release the virus to prevent FMD. Research shows that these animals can give out the virus in different ways, such as through their breath, spit, nose, and poop (De Rueda et al., 2014). They can give out a lot of viruses in the air, which can make other animals sick (Alexandersen et al., 2003). Some of these animals may not look sick, but they can still have the virus and pass it on. This can make FMD stay longer and come back again (Casey et al., 2014). It is hard to control FMD when the virus is hidden in these animals (Sutmoller et al., 2003). There is need to know how long the viruses stays in these animals. Studies showed that these animals give out the most viruses right after they get sick. Sellers et al. (1977) found two main times when this happens: from half an hour to a day after they get sick (virus stuck in their wool) and from two to seven days after they get sick (virus growing in their throat). This matches with high virus levels in their throat fluids (Nishi, 2021), which are important for FMD to spread. There is a lot of debate and research about how the virus stays in these animals. Some research says that the virus levels in their throat fluids are too low to make other animals sick (Sutmoller, 2003), but sometimes the viruses can be found there. This means that the virus may still grow a little in these animals. So, we need to know how likely these animals are to infect other animals.

3. Species Adaptation and Virulence of FMD Viruses in Small Ruminants

The way Foot-and-Mouth Disease Virus (FMDV) spreads among small animals like sheep and goats

is very important to understand. It helps us to know how different types of FMDV can affect different animals and change over time (Arzt et al., 2011). Sheep and goats can pass FMDV to other animals, so we need to study how easily they get infected and how much they can spread the disease (Knowles et al., 2003). Some FMDV types are more suited to sheep and goats than others, and they may cause different levels of sickness in other animals, such as cows and pigs. The genes and history of FMDV types from small animals affect how they can grow and cause disease in other animals (Klein et al., 2009).

Some experiments have shown that FMDV types from small animals may have different features and effects when they infect other animals (Bauer et al., 1977). Some real-life situations have also shown interesting things, such as sheep and goats having FMDV antibodies without getting sick recently, even living with cows that do not have antibodies (Mackay, 1994; Mackay and Rendle, 1996). This shows the complicated relationships among animals as possible sources or carriers of FMDV. Khukhorov et al. (1973) pointed out the role of sheep and goats in FMDV spread, showing their ability to keep and maintain the virus within their groups. The genetic variety of FMDV types affects how they interact with different animals. Anahory (2016) stressed that genetic changes influence FMD patterns and show the possibility of FMDV types to change and adapt to new animal environments. The genetic features of FMDV types that infect small animals in places like Asia, especially Pakistan, tell us about their potential to cross animal boundaries and adjust to different animal reactions (Knight-Jones et al., 2016).

4. Vaccination of Small Ruminants against Foot-and-Mouth Disease

Foot-and-mouth disease (FMD) is a very contagious disease that affects animals with split hooves, like goats and sheep. FMD can cause a lot of problems for farmers and rural people, as it can make animals produce less, die more, face trade restrictions, and cost more to control. FMD is common in many parts of the world, especially in Africa, the Middle East and Asia, where it is a big challenge for the livestock sector and rural development (Choudhury et al., 2021) Giving vaccines to small animals is a keyway to prevent and control FMD. However, there are many difficulties and limitations in vaccinating small animals against FMD. These include not enough vaccines and not good distribution of vaccines, especially in poor countries where FMD is always

present. The need for FMD vaccines is often more than the supply, and the delivery of vaccines is difficult because of bad roads, lack of cold storage, and not enough money (Lombard et al., 2007). Bad quality and effectiveness of vaccines are also big difficulties. The quality and effectiveness of FMD vaccines depend on many things, such as how well the vaccine matches the virus, how strong and clean the vaccine is, how stable and stored the vaccine is, how and how much the vaccine is given, and how healthy the animal is (Chepkwony et al., 2021). Many FMD vaccines are killed or weakened, which need more than one dose and booster to make enough immunity. Also, some FMD vaccines may not stop the carrier state of FMD virus (FMDV) in small animals. This state can last for several months after infection and recovery, and carrier animals may infect other animals that can get sick under good conditions (Mansoo et al., 2018). Low awareness and compliance among farmers are important difficulties in FMD vaccination for small animals. Many farmers in areas where FMD is always present do not know the benefits and importance of FMD vaccination, and some may have wrong ideas or distrust about the safety and effectiveness of FMD vaccines. Also, some farmers may not follow the recommended vaccination times or ways because of various reasons, such as no access, no money, or no convenience (Win et al., 2021). The high cost of vaccination is also a big concern. The cost of FMD vaccination for small animals includes the cost of the vaccine itself, as well as transportation, storage, delivery, labour, and equipment costs. These costs may be too high for small farmers with little resources and income. Also, the cost-effectiveness of FMD vaccination may depend on many things, such as how common and frequent FMD is, how the animals are raised and sold, and how available and reachable other control measures are (Choudhury et al., 2021). New generation vaccines have many benefits over old vaccines. They make wider and longer-lasting immunity, stop the carrier state, reduce the need for more than one dose and booster, improve the stability and storage of vaccines, make it easier to tell the difference between infected and vaccinated animals (DIVA), and simplify the production and quality control of vaccines (Olsen et al., 2011)

A good way to stop FMD from spreading is to give vaccines to the small animals that are most likely to get sick or are most important for farmers. This way is called risk-based vaccination strategies. It helps to use less vaccines but more effectively,

lower the chance and spread of FMD, protect important farming areas or markets, and control or get rid of FMD. To use this way, you need to know well how FMD spreads in different places and situations. You also need a good system to watch and find high-risk areas or groups (Wada et al., 2022). Teaching people is important. By having campaigns, farmers can learn about FMD in their animals, like what causes it, what happens, how to prevent it, and how to control it. Education programs can teach farmers how to store, use, give, and throw away FMD vaccines properly. Not only farmers, but also animal doctors, workers, policymakers, and buyers, can help and support FMD vaccination for small animals (Sieng et al., 2022). It's also important to work together across borders. By working together, information, resources, and experiences can be shared among different groups, like national authorities, international organizations, research institutions, vaccine makers, and civil society groups. Working together can also help stop FMDV from crossing borders and support the regional or global control or eradication of FMD (Gongal et al., 2022).

CONCLUSIONS

Research is needed to understand the role of sheep and goats in spreading foot-and-mouth disease (FMD) and their potential to transmit the virus. Regular vaccination programs should be implemented in endemic and high-risk areas, and sufficient vaccine supplies maintained in FMD-free regions. These measures will help reduce the risk and impact of FMD outbreaks.

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Conflict of Interest

Authors declare there is no conflict of interest.

REFERENCES

1. Abas O, Abd-Elrahman A, Saleh A, Bessat M (2021): Prevalence of tick-borne haemoparasites and their perceived co-occurrences with viral outbreaks of FMD and LSD and their associated factors. Heliyon, 7(3).
2. Admassu B, Getnet K, Shite A, Mohammed S (2015): Review on foot and mouth disease: Distribution and economic significance.
3. Alexandersen S, Zhang Z, Donaldson AI, Garland AJM (2003). The pathogenesis and diagnosis of foot-and-mouth disease. J. Comp. Pathol. 129(1): 1-36.
4. Anahory IV (2016). Improvement of a liquid phase blocking ELISA for enhanced detection and measurement of antibodies against the SAT3 serotype of

FMDV (Doctoral dissertation, University of Pretoria).
5. Apolloni A, Corniaux C, Coste C, Lancelot R, Touré I (2019). Livestock mobility in West Africa and Sahel and transboundary animal diseases. Transboundary animal diseases in Sahelian Africa and connected regions, 31-52.
6. Arzt J, Baxt B, Grubman MJ, Jackson T, Juleff N, Rhyan J, Rodriguez LL (2011). The pathogenesis of foot-and-mouth disease II: viral pathways in swine, small ruminants, and wildlife; myotropism, chronic syndromes, and molecular virus–host interactions. Transbound Emerg. Dis. 58(4): 305-326.
7. Arzt J, Fish IH, Bertram MR, Smoliga GR, Hartwig EJ, Pauszek SJ, Stenfeldt C (2021). Simultaneous and staggered foot-and-mouth disease virus coinfection of cattle. Virol. J. 95(24): 10-1128.
8. Arzt J, Juleff N, Zhang Z, Rodriguez LL (2011). The pathogenesis of foot-and-mouth disease I: viral pathways in cattle. Transbound. Emerg. Dis. 58(4): 291-304.
9. Aslam M, Alkheraije KA (2023). The prevalence of foot-and-mouth disease in Asia. Front. Vet. Sci. 10:1201578.
10. Azeem A, Rashid I, Hassan MM, Asad M, Kaukab G, Tehseen A, Aamir S (2020). 10. A review on foot and mouth disease in dairy animals, etiology, pathogenesis and clinical findings. PAB. 9(1):821-832.
11. Bauer K, Sanders R, Oleksiewicz MB (1977). Characteristics and effects of foot-and-mouth disease virus in different animal hosts. Vet. Microbiol. 23(1), 15-25.
12. Björnham O, Sigg R, Burman J (2020). Multilevel model for airborne transmission of foot-and-mouth disease applied to Swedish livestock. PLoS One. 15(5): 0232489.
13. Blacksell SD, Siengsanan-Lamont J, Kamolsiripichaiporn S, Gleeson LJ, Windsor PA (2019). A history of FMD research and control programmes in Southeast Asia: lessons from the past informing the future. Epidemiol. Infect. *1*47.
14. Brown E, Nelson N, Gubbins S, Colenutt C (2022). Airborne transmission of foot-and-mouth disease virus: a review of past and present perspectives. Viruses. 14(5): 1009.
15. Calistri P, Iannetti S, L. Danzetta M, Narcisi V, Cito F, Di Sabatino D, Giovannini A

(2013). The components of 'one world–one health' approach. Transbound. Emerg. Dis. 60: 4-13.
16. Casey MB, Lembo T, Knowles NJ, Fyumagwa R, Kivaria F, Maliti H, Cleaveland S (2014). Patterns of foot-and-mouth disease virus distribution in Africa: The role of livestock and wildlife in virus emergence. Role Anim. 21-38.
17. Chepkwony EC, Gitao GC, Muchemi GM, Sangula AK, Kairu-Wanyoike SW (2021). Epidemiological study on foot-and-mouth disease in small ruminants: Sero-prevalence and risk factor assessment in Kenya. PloS One. 16(8) :0234286.
18. Choudhury SM, Ma X, Dang W, Li Y, Zheng H (2021) Recent development of ruminant vaccine against viral diseases. Front. Vet. Sci. 8: 697194.
19. Clemmons EA, Alfson KJ, Dutton Iii, JW (2021). Transboundary animal diseases, an overview of 17 diseases with potential for global spread and serious consequences. Anim. 11(7): 2039.
20. Dabasa G, Abunna F (2021). Review on Epidemiology of Foot and Mouth Disease (FMD) in Ethiopia. J. Trop. Dis. 9: 269.
21. De Rueda CB, Dekker A, Eblé PL, De Jong MC (2014). Identification of factors associated with increased excretion of foot-and-mouth disease virus. Prev. Vet. Med. 113(1): 23-33.
22. Elahi E, Zhang L, Abid M, Javed MT, Xinru H (2017). Direct and indirect effects of wastewater use and herd environment on the occurrence of animal diseases and animal health in Pakistan. Environ. Sci. Pollut. Res. 24: 6819-6832.
23. Gongal G, Rahman H, Thakuri KC, Vijayalakshmy K (2022). An Overview of Transboundary Animal Diseases of Viral Origin in South Asia: What Needs to Be Done? Vet. Sci. 9(11): 586.
24. Gortázar C, Barroso P, Nova R, Cáceres G (2022). The role of wildlife in the epidemiology and control of Foot-and-mouth-disease and Similar Transboundary (FAST) animal diseases: a review. Transbound Emerg. Dis. 69(5): 2462-2473.
25. Jena BR, Patra RC, Biswal JK, Rath PK, Dash A, Sahoo R, Gupta R, Senapati SK, Panda SK (2022). Foot-and-Mouth Disease (FMD) Carrier State in Livestock Population and its Diagnosis. J. Anim. Res. 12(6): 807-823.

26. Khukhorov BS, Abdullaeva KA, Kamilov AB (1973). Role of sheep and goats in the epidemiology of foot-and-mouth disease. J. Hyg. Epidemiol. Microbiol. Immunol. 17(4): 431-439.
27. Klein J (2009): Understanding the molecular epidemiology of foot-and-mouth-disease virus. Infect. Genet. Evol. 9(2): 153-161.
28. Knight-Jones TJ, Robinson L, Charleston B, Rodriguez LL, Gay CG, Sumption KJ, Vosloo W (2016). Global foot-and-mouth disease research update and gap analysis: 2–epidemiology, wildlife and economics. Transbound. Emerg. Dis. 63: 14-29.
29. Knowles NJ, Samuel AR (2003). Molecular epidemiology of foot-and-mouth disease virus. Virus Res. 91(1): 65-80.
30. LAW J, MOL A (2011). Veterinary realities: what is foot and mouth disease? Soc. Rural. 51(1)*: 1-16.*
31. Lombard M, Pastoret PP, Moulin AM (2007). A brief history of vaccines and vaccination. Rev. Sci. Tech. 26(1): 29-48.
32. Mansoor MK, Al-Rawahi AH, El-Tahir HA, Al-Faraei B, Hussain MH, Asi MN, Al-Hussani I, Sabar S (2018). Concurrent vaccination of goats with foot and mouth disease (FMD) and peste des petits ruminants (PPR) booster vaccines. Trop. Anim. Health Prod. 50: 1-3.
33. Nishi T, Morioka K, Kawaguchi R, Yamada M, Ikezawa M, Fukai K (2021). Quantitative analysis of infection dynamics of foot-and-mouth disease virus strain O/CATHAY in pigs and cattle. Plos One. 16(1):0245781.
34. Olsen L, Choffnes ER, Relman DA, Pray L (2011). Fungal diseases: an emerging threat to human, animal and plant health. Workshop summary. In Fungal diseases: an emerging threat to human, animal and plant health. Workshop summary. Nat. Acad. Press.
35. Osmani A, Robertson ID, Habib I, Aslami AA (2019). History and epidemiology of foot-and-mouth disease in Afghanistan: A retrospective study. BMC Vet. Res. 15(1):1-12.
36. Paton DJ, Gubbins S, King D P (2018) Understanding the transmission of foot-and-mouth disease virus at different scales. Curr. Opin. Virol. 28: 85-91.
37. Sherman DM (2011). The spread of pathogens through trade in small ruminants and their products. Revue. Scientifiq. ET Technique-OIE. 30(1): 207.

38. Sieng S, Patrick IW, Windsor PA, Walkden-Brown SW, Sar C, Smith RGB, Kong R (2022). Knowledge, attitudes and practices of smallholder farmers on foot and mouth disease control in two Cambodian provinces. Transbound Emerg. Dis. 69(4): 1983-1998.
39. Sutmoller P, Barteling SS, Olascoaga RC, Sumption KJ (2003). Control and eradication of foot-and-mouth disease. Virus Res. 91(1): 101-144.
40. Torsson E, Berg M, Misinzo G, Herbe I, Kgotlele T, Päärni M, Johansson Wensman J (2017). Seroprevalence and risk factors for peste des petits ruminants and selected differential diagnosis in sheep and goats in Tanzania. Infect. Ecol. Epidemiol. 7(1) :1368336.
41. Wada M, Subharat S, Sutar A, Abila R, Khounsy S, Heuer C (2022). Socioeconomic impacts of clinical foot-and-mouth disease and a risk-based partial vaccination campaign for smallholders in Lao People's Democratic Republic. Transbound Emerg. Dis. 69(5):1825-e1838.
42. Win TTZ, Campbell A, Magalhaes RJS, Oo KN, Henning J (2021). What drives small-scale farmers to vaccinate their multiple livestock species animals against common infectious diseases in Myanmar? Plos one. 16(10): e0258765.
43. Wood BA, Mioulet V, Henry E, Gray A, Azhar M, Thapa B Eschbaumer M (2020): Inactivation of foot-and-mouth disease virus A/IRN/8/2015 with commercially available lysis buffers. J. Virol. Meth. 278: 113835.
44. Yousif H, Almutlab AA, Hassen AA, Al-Majali A, Tibbo M (2017). Role of small ruminants in the epidemiology of foot-and-mouth disease in Sudan. (BAHPA). 65(1): 145-156.