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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. 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. In current study, taxonomic description, macrographs of basidiomata, illustrations and light micrographs of microscopic features are given. Morpho-anatomical comparison with similar species is also provided.*

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 region 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 so as 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 & 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).

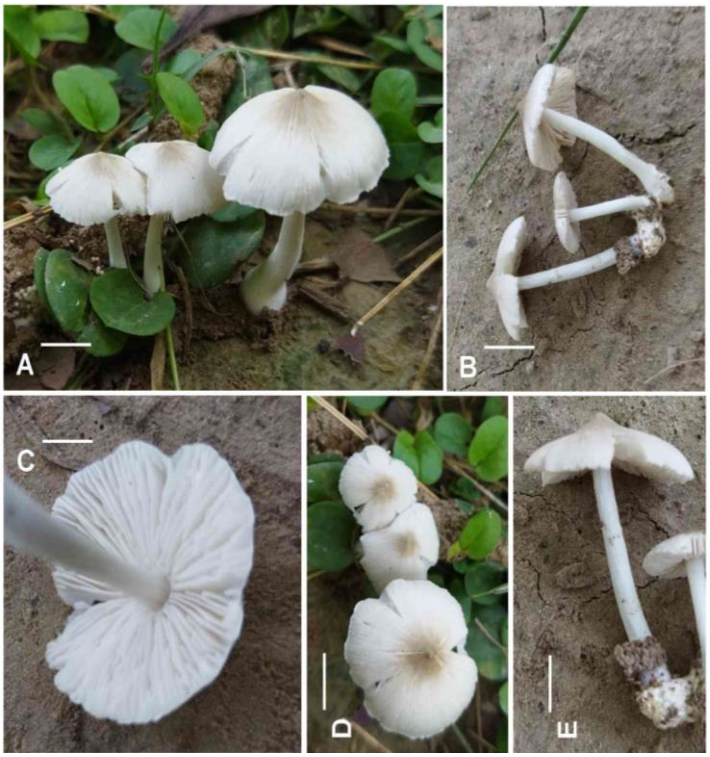

Fig. 1: A–E. Morphology of Termitomyces umkowaan (MC–67). A & B. Basidiomata. C. Gills. D. Pileus. E. Stipe. Scale bar: A = 1.5 cm, B = 6.5 cm, C = 1 cm, D & E = 2 cm

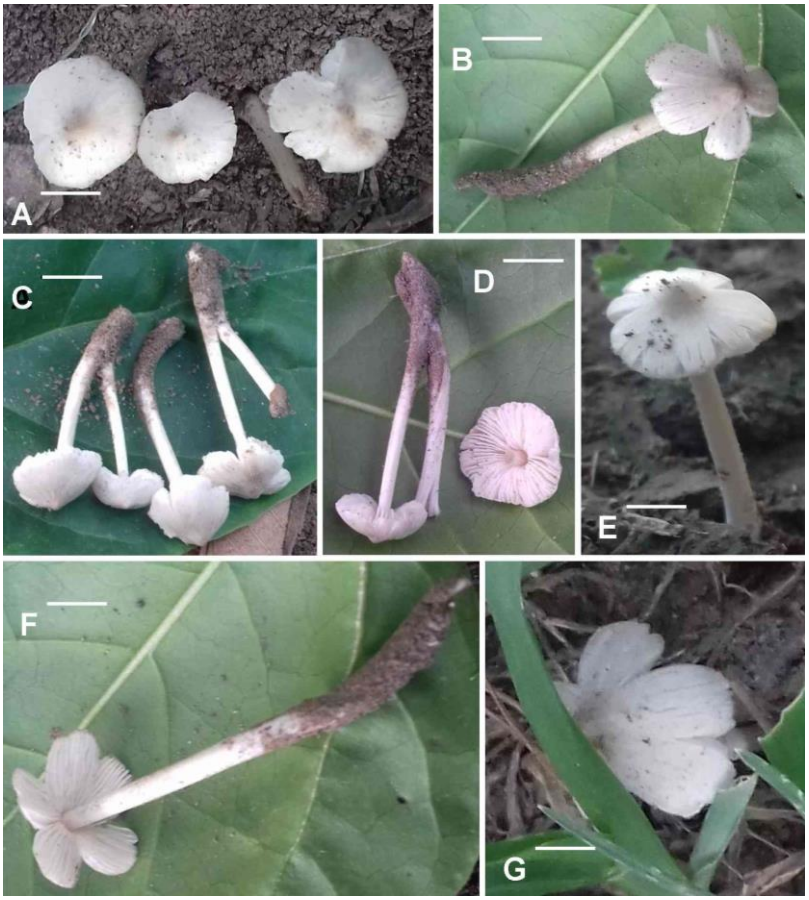

Fig. 2: A–G. Morphology of *Termitomyces umkowaan*. A, C & D. AC–4. B, E–G. AC–5. Scale bar: A & B = 2 cm, C & D = 4 cm, E = 1.5 cm, F = 2.5 cm, G = 1 cm

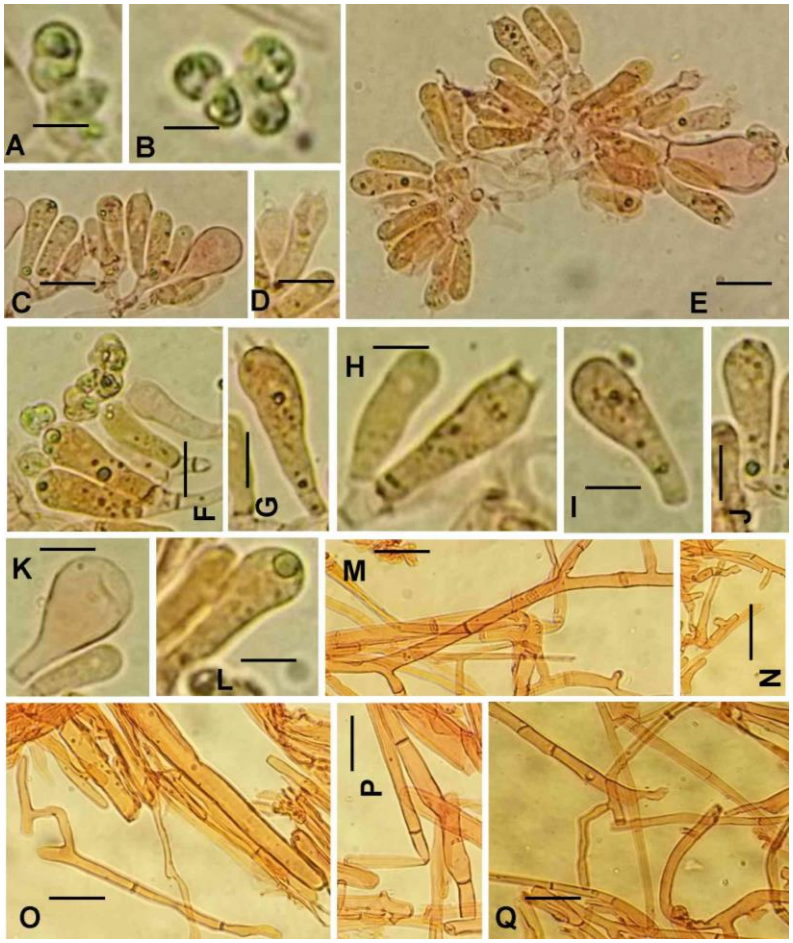

Fig. 3: A–Q: Light micrographs of microscopic features of *Termitomyces umkowaan.* A & B. Basidiospores. C & E. Portion of hymenium showing Basidia and cistidia. D, F–H, J. Basidia. I & L. Pleurocystidia K. Cheilocystidia. O & P. Pileipellis. M, N & Q. Stipitipellis. Scale bars: A = 8.5 µm, B = 8 µm, 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

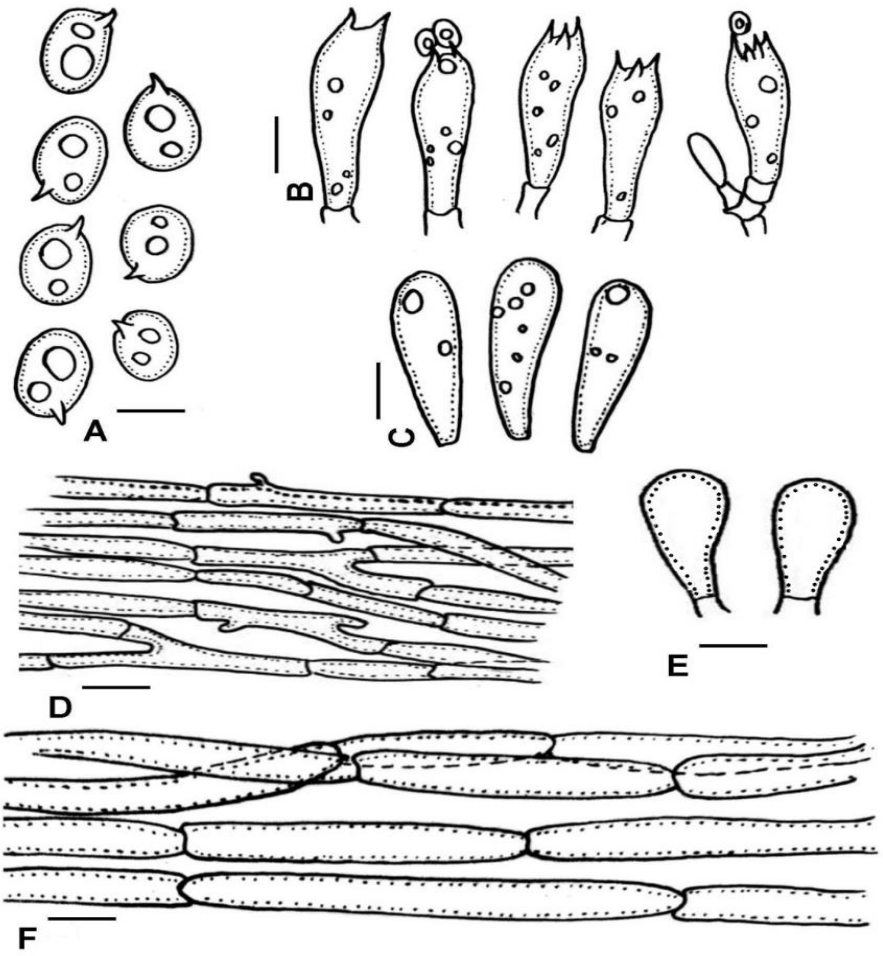

Fig. 4: A–F: Illustrations of microscopic features of *Termitomyces umkowaan.* A. Basidiospores. B. Basidia. C. Pleurocystidia. D. Stipitipellis. E. Cheilocystidia. F. Pileipellis. Scale bars: A = 7 µm, B = 11.7 µm, C = 10 µm, D = 36 µm, E = 23 µm, F = 20 µm

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 acrium bonatus	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 with varied regions.

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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

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