



First Report and Distribution of a Common Edible Mushroom *Agaricus campestris* (Basidiomycota: Agaricaceae) across Different Districts of Punjab, Pakistan

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Abstract

Current study presents first reports of common edible field mushroom viz., *Agaricus campestris* from 6 districts (Gujranwala, Jehlum, Khanewal, Nankana Sahib, Rajanpur and Vehari) and a second report from district Kasur of Punjab province, Pakistan. During extensive field surveys for macrofungal collection, 17 collections of this species were made from 2019-2022 to explore its distribution across Punjab. The taxon is morphologically described and anatomically characterized along with microphotographs and illustrations. Distribution map of *Agaricus campestris* in Punjab province is given. Comparison of *A. campestris* with morpho-anatomically similar species of genus *Agaricus* is also presented in this paper.

Keywords: *Agaricus*; Basidiomycota; Edible Mushroom; Taxonomy

1. Introduction:

Linnaeus was the first who described the family Agaricaceae in 1753 with *Agaricus campestris* L. as the type species (Donk, 1962). Members of family Agaricaceae are unique in morphology and characterized by great variations in size of basidiomaata, white to brown pileus, central stipe, presence of annular ring, free lamellae which turn pink to chocolate brown at maturity and dark basidiospores. About 500 species belong to genus *Agaricus* L. (Heinemann, 1974; Stoichev & Lacheva, 2002; Lacheva & Stoichev, 2004; Bashir et al., 2021; Jaichaliaw et al., 2021). Around 6,000 records of *Agaricus* are presented by Index Fungorum (accessed in December 2022) with some errors of misidentification, and some are not well-documented species (Jaichaliaw et al., 2021). So far, 31 species of genus *Agaricus* have been identified

from Pakistan (Aman et al., 2022). This genus includes seven subgenera and twenty-seven sections (Zhao et al., 2016; Chen et al., 2017; Callac & Chen, 2018; He et al., 2018). comprised *Agaricus* is a wide genus that consists of large number of saprotrophic fungi. These various edible and some toxic species (Parra, 2008; Zhao et al., 2012; Thongklang et al., 2014; Chen et al., 2015). Section *Agaricus* of genus *Agaricus* is monophyletic and 26 species had been reported worldwide from this section (Geml et al., 2004; Parra, 2008; Kerrigan 2016). The habitat for *Agaricus* species ranges from pastures, grass to mixed forests (Bas, 1991). The name Meadow mushroom commonly belongs to *Agaricus campestris*, a common edible mushroom in the whole world (Manzi et al., 2001; Kuo, 2018). Common habitats of this field mushroom

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are mostly meadows, fields, lawns and grassy areas, often forming fairy rings (Eira et al., 2002; Ratkowsky & Gates, 2002; Leonard, 2017). *A. campestris* has high protein contents mainly Glutamic acid 3.08 (g/100 g) as an essential amino acid and it is of equitable interest in growing population of world and its protein shortages (Wahid et al., 1992). *A. campestris* is also of great importance as traditionally used for diabetes treatment due to its insulin like activity (Gray & Flatt, 1998).

1.1. Objectives:

- To find out the distribution and abundance of *Agaricus campestris* across different districts of Punjab, Pakistan.

2. Materials and Methods:

2.1. Sampling Sites and Morphological Observations:

Different field visits during rainy season were done from the years 2019-2022 to explore fungal diversity of different areas of Punjab province. During the surveys, 17 specimens of *Agaricus campestris* were collected from 9 sampling sites of 7 districts (Gujranwala, Jehlum, Kasur, Khanewal, Nankana Sahib, Rajanpur and Vehari). These sites mainly include plain areas having variation in climatic conditions from subtropical desert climate of Rajanpur to hot semi-arid climate of Gujranwala. Labeling and photography of each specimen was done in the field. Specimens were excavated carefully to avoid any harm to basidiocarps. Morphological features of fruiting bodies were recorded. Under fan heater samples were processed to dry out the moisture and safeguarded in slider storage bags. From fresh specimens, macroscopic characterization of features such as pileus, lamellae, stipe, annulus, spore print, odor, habitat and habit were prepared.

2.2. Microscopic Analysis:

Slides preparation was done by using 5% aqueous KOH as the mounting media and 1 % Congo red as staining agent for anatomical investigation. Details of microscopic examination were recorded at 40× magnification. Basidiospores, pileal hyphae, stipe hyphae, cystidia, and basidia were observed. By using ocular micrometre twenty readings of basidiospores, and ten measurements for other microscopic characters were done. For basidiospores, average and quotient values were calculated and documented.

3. Results:

3.1. Taxonomy:

Agaricus campestris sensu Cooke [Ill. Brit. Fung. 527 Vol. 4 (1885)] (Fig. 1-4).

3.2. Morphological Description:

Basidiocarp 3.6-8.3 cm, off-white to milky white, fleshy, dull, scattered too gregarious, epigeous. Pileus 4.2-4.6 cm, surface creamy white, silky, unwrinkled, smooth or sometime minor patches covering the cap, spongy, fleshy, strongly convex to plano-convex, smooth or entire margins first and finally cracked at maturity. Lamellae free, pink when young to chocolate brown when fully mature, thick, crowded with numerous long gills, notched, broadly ventricose, even edges and non-deliquescent. Stipe 3.0-4.3 × 1.7-3.5 cm, white to off-white, dry surface, rough, fibrous, firm, same consistency above or below annulus, cylindric and centrally attached with pileus. Annular ring or partial veil white, present on superior part, thin, pendant, uneven margins and same whitish smooth surface above and below annulus. Odor pleasant smell. Taste unrecorded. Habitat sloppy or filthy soil. Habit scattered. Spore prints light brown to dark brown.

3.3. Microscopic Characterization:

Basidiospores 5.4-8.5 × 4.3-5.7(6.3) µm, Q= 1.1-1.7, Q_{avg} = 1.4, greenish to light brown, hyaline in 5 % KOH, ellipsoid or broadly ellipsoid to ovoid, smooth to thick-walled, oil droplets present. Basidia 25.7-28.5 × 7-12 µm, hyaline in 5 % KOH, clavate to slightly clavate, thick-walled, abundant. Sterigmata 2-4. Cheilocystidia 17-27 × 5.7-9.0 µm, hyaline, thin walled, clavate. Pleurocystidia absent. Sub hymenium 2.9-5.1 µm wide, hyphae rounded and thin walled. Pileipellis 8.5-11.4 µm, branched, thin-walled, septate, irregular, wavy and hyaline in 5 % KOH. Stipitipellis 9-12 µm, thick-walled, branched, regularly septate and hyaline in 5 % KOH. Clamp connections not observed.

3.4. Material Examined:

Collections 1 & 2: PAKISTAN: Punjab province, District Gujranwala, Tehsil Gujranwala. (Collector: Saliha Ruksar).

- Solitary, along the road at the base of the tree, at 226 m a.s.l., 7th September 2019, (Coupon # MS-36).

- Yusufwala, one pair, at the base of the tree along the roadside, at 226 m a.s.l., 7th September 2019, (Coupon # MS-37).

Collection 3 & 4: District Gujranwala. (Collector: Samra Saeed).

- Village Nur Pur, along the wall growing in litter ground area, 4th August 2021, (Coupon # GC-05).
- Village Uggo Chak, near field area, growing among grasses, 5th August 2022, (Coupon # GC-61).

Collection 5: District Jhelum. (Collector: Marriam Dilawaiz Khan)

- Nograan village (25.97 °N, 39.47 °E), partially buried in humid soil, along the roadside on grassy land, 234 m a.s.l., 29th September 2019, (Coupon # MP-20).

Collection 6: District Kasur, Kotla Sheikh Natha. (Collector: Saba Ibrahim)

- Solitary on ground, 9th September 2022, (Coupon # SK-5).

Collections 7-9: District Khanewal, Tehsil Khanewal, Chak # 16/v. (Collector: Muhammad Usman)

- Growing singly, under *Acacia nilotica* on ground, 29th June 2019, (Coupon # KU-11).
- On ground, under *Ziziphus jujuba* Mill., 20th July 2019, (Coupon # KU-21).

- On ground, 11th August 2019, (Coupon # KU-40).

Collections 10-12: District Khanewal, Tehsil Mian Channu. (Collector: Ukasha Iqbal)

- Near Lahore Mian Channu road, on grassy soil, 29th August 2022, (Coupon # MC-17).
- Jinnah hall Municipal committee, on ground, under *Acacia* sp., 29th August 2022, (Coupon # MC-18).
- On grassy ground, 29th August 2022, (Coupon # MC-21).

Collections 13 & 14: District Nankana Sahib, Sangla Hill, Ghakhar. (Collector: Ayesha Noor Awan)

- Morar Chak 42, under *Acacia* sp. on ground, 194 m (636 ft.) a.s.l., 18th July 2019, (Coupon # SH-19).
- Faradon, on grassy soil, 194 m (636 ft.) a.s.l., 30th July 2019, (Coupon # FAR-1).

Collection 15: District Rajanpur, Tehsil Jampur. (Collector: Sadaqat Ali)

- Near the eastern bypass of Jampur, on ground growing in the field of *Brassica campestris*, 7th February 2022, (Coupon # SA-26).

Collections 16 & 17: District Vehari, Tehsil Burewala, Chak # 47/v, solitary. (Collector: Ayesha Ghafoor)

- On the ground, in grass, 13th September 2021, (Coupon # B247-1A).
- On soft mud, 29th September 2021, (Coupon # B247-1B)



Figure 1: **A-I.** Morphology of basidiomata of *Agaricus campestris* (MC-17). **A-D.** Different views of basidioma. **E.** Upper side of pileus. **F & G.** Arrangement of gills. **H & I.** Stipe with annular ring. **Scale bars:** **A-C & G** = 1 cm, **D-F** = 1.4 cm, **H** = 1.7 cm, **I** = 2 cm.

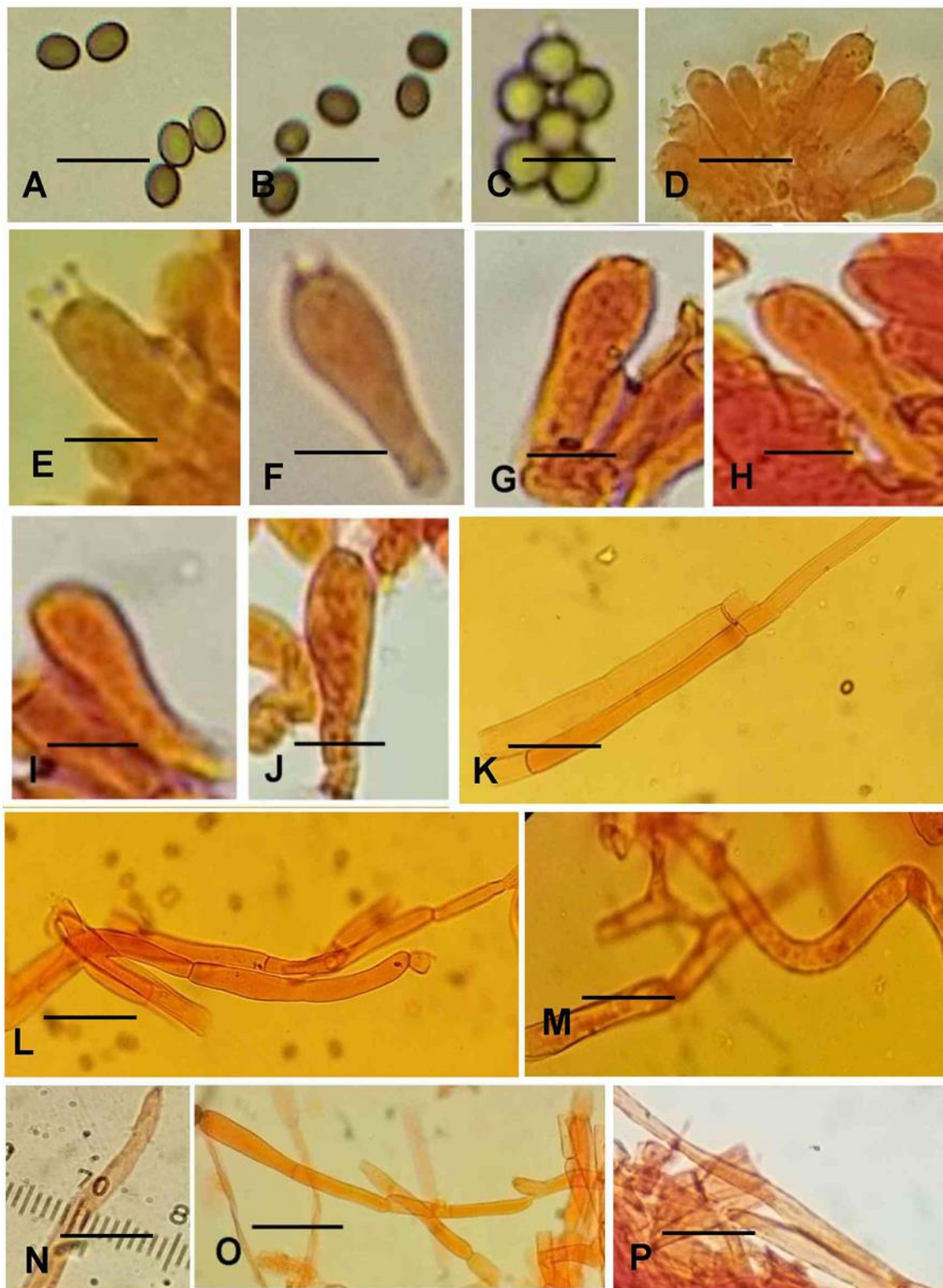


Figure 2: **A-P.** Light micrographs of microscopic features of *Agaricus campestris* (MC-17). **A-C.** Basidiospores. **D.** Basidia and cistidia. **E-H.** Basidia. **I & J.** Cistidia. **K-M.** Pileipellis. **N-P.** Stipeipellis. **Scale bars:** **A & B=** 10 μ m, **C=** 4.5 μ m, **D=** 17 μ m, **E-H=** 27 μ m, **I=** 7 μ m, **J=** 2.5 μ m, **K-M=** 33 μ m, **N-P=** 36 μ m

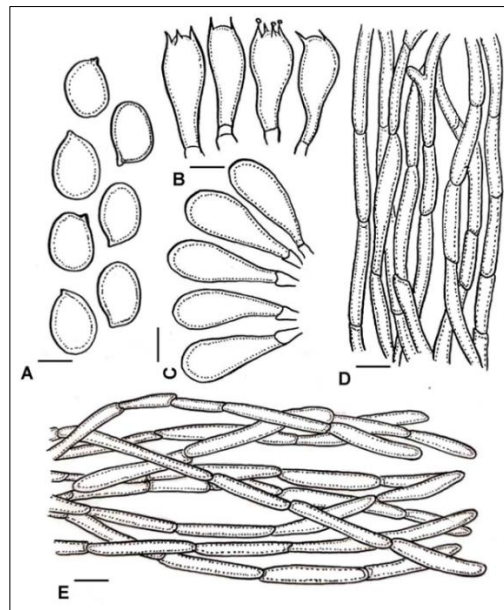


Figure 3: **A-E**. Illustration of microscopic features of *Agaricus campestris* (MC-17). **A**. Basidiospores. **B**. Basidia. **C**. Cystidia. **D**. Pileipellis. **E**. Stipitipellis. **Scale bars**: **A**= 6.5 µm, **B**= 12 µm, **C**= 3.5 µm, **D**= 33 µm, **E**= 36 µm.



Figure 4: **A1-I6**. Morphology of basidiomata of *Agaricus campestris* collected from different districts of Punjab, Pakistan. **Scale bars**: **A1**, **A3**, **D1-D3**, **G5** & **I6** = 6 cm, **A2**, **A4** & **F2** = 7.3 cm, **B1**, **B4**, **B5**, **H2**, **H5** & **H6** = 3.2cm,

B2 & H4= 4.8 cm, B3= 13.3 cm, B6 & I5= 10.5 cm, B7, B8, B10, C1-C3, G1 & G3= 2.5 cm, B9= 26.6 cm, C4, C5 & F1= 3.8 cm, E1-E3, E5, E6 & F3= 4.1 cm, E4= 9.1 cm, G2= 1.4 cm, G4, H1, H3, I1-I3= 5.5 cm, I4= 8.4 cm

Detailed comparison of *A. campestris* with some other species of genus *Agaricus* is given in Table 1.

Table 1: A comparison of *Agaricus campestris* with closely related species of section *Agaricus*.

Macroscopic characteristics	<i>A. campestris</i> L. (MC-17)	<i>A. andrewii</i> A.E. Freeman	<i>A. aristocratus</i> Gulden	<i>A. cupreobrunneus</i> (Jul. Schäff. & Steer) Pilát	<i>A. jilinensis</i> R.L. Zhao & A.Q. Liu	<i>A. zhangyensis</i> R.L. Zhao & A.Q. Liu
Size of pileus	4.2–4.6 cm	6.2–7.5 cm	8–13.4 cm in diam., 10–14 mm thick at disc	6.4–6.9 cm	8–10 cm in diam., 6–8 mm thick at disc	6–9 cm in diam., 7–9.6 mm thick at disc
Colour of pileus	Creamy white	Creamy white to white with light orange	Whitish to light brown with grey scales when mature	Rusty white or reddish brown	Reddish brown with dirty white background and a dark brown centre	White to light brown
Shape of pileus	Strongly convex to plano-convex	Broad	When young, convex with plane centre, then plano-convex at maturity.	Convex when young to applanate at maturity	Subspherical to plane	Convex when young to broadly convex at maturity
Colour of gills	Pink when young to chocolate brown when fully mature	Dark brown	Pinkish to dark brown at maturity	Dark chocolate	Chocolate brown	Pink when young to chocolate brown when fully mature
Size of stipe	3.0–4.3 × 1.7–3.5 cm	6.2 × 1.4 cm	4.5–6.7 × 1.8–2.3 cm	8.1 × 1.1 cm	8.6–16 × 1.2–1.6 cm	4.0–6.8 × 1.2–2.0 cm
Microscopic characteristics						
Size of basidiospores	5.4–8.5 × 4.3–5.7(6.3) µm	6.96–8.45 × 5.0–5.8 µm	6.4–8.3 × 5.8–6.7 µm	6.7–9.1 × 3.3–5.1 µm	6.4–7.7 × 4.1–4.3 µm	6.4–9.4 × 5.6–7.0 µm
Shape of basidiospores	Ellipsoid or broadly ellipsoid to ovoid	Ellipsoid	Subglobose to ellipsoid	Ovoid	Ellipsoid to elongate	Broadly ellipsoid
Basidia	25.7–28.5 × 7–12 µm	20.28–32.11 × 8.45–10.14 µm	20.8–32.2 × 7.7–12.5 µm	16.9–18.7 × 6.4–7.6 µm	20.3–26.2 × 7.3–10.1 µm	18.4–32.4 × 7.4–12.5 µm
Cheilocystidia	17–27 × 5.7–9.0 µm, clavate	21.97–33.80 × 7.6–10.98 µm, clavate, abundant	Absent	Absent	15.7–24.2 × 8.2–11.5 µm, clavate	9.0–22.9 × 7.8–8.9 µm, clavate
References	Present study	Kaur et al. (2017)	Zhao, (2020)	Amandeep et al. (2015)	Zhao, (2020)	Zhao, (2020)

4. Discussion:

Agaricus campestris belongs to family Agaricaceae, first described and named in 1753 by Carl

Linnaeus. *A. campestris* is the type species of the genus (Donk, 1962). Genus *Agaricus* is widely distributed in both temperate and tropical areas (Kerrigan et al., 2005; Chen et al., 2015; Zhao et al., 2016; He et al., 2017, 2018; Callac & Chen, 2018). In Pakistan, there is great

diversity of fungi because of presence of wide variety of habitats (Yousaf *et al.*, 2012). From last six years, a lot of work has been done on many specimens of *Agaricus*, collected from different areas of the country with varying ecological and climatic conditions (Bashir *et al.*, 2021).

From Pakistan, *A. campestris* has only been reported from two sites, i.e., Changa Manga forest of district Kasur (Sabir *et al.*, 2002) and Soon Valley of district Khushab in Punjab province (Farooq *et al.*, 2013). Punjab province of Pakistan consists of 36 districts, most of land consists of plain areas with variable climatic conditions. This paper reports *A. campestris* for the first time from 6 districts of Punjab. Distribution map of *A. campestris* across Punjab, Pakistan is given in Figure 5. Fleshy cap, chocolate brown lamellae at maturity, and chocolate brown spores are the characterizing feature of *Agaricus*. Basidia and cystidia of this genus are mostly clavate to claviform (Roehl, 2017).

A. campestris is mostly confused with following closely related species. *A. campestris* closely resembles with *A. bisporus* due to having similar morphological characters but both species are different in anatomical characters. Basidiospore color and shape is a noteworthy difference. *A. campestris* basidiospores are ellipsoid or broadly ellipsoid to ovoid and greenish to light brown in KOH. On the other hand, *A. bisporus* has ellipsoid spores and brown in KOH. *A. bitorquis* also closely resembles with *A. campestris* morphologically. Both have similar spore color but *A. bitorquis* has smaller spores than *A. campestris* which are $5.1-6.8 \times 4-5 \mu\text{m}$ and $5.4-8.5 \times 4.3-5.7(6.3) \mu\text{m}$, respectively. *A. campestris* is morphologically confused with *A. hondensis*. Both show negative result to chemical color reaction (Schaeffer's reaction). But both vary in spore size. *A. hondensis* has smaller spores $5.91-8.45 \times 5.07-5.91 \mu\text{m}$, then basidiospores of *A. campestris* $5.07-5.91 \times 3.38-4.22 \mu\text{m}$. (Smith, 1949; Lincoff, 1981; Kaur *et al.*, 2016).

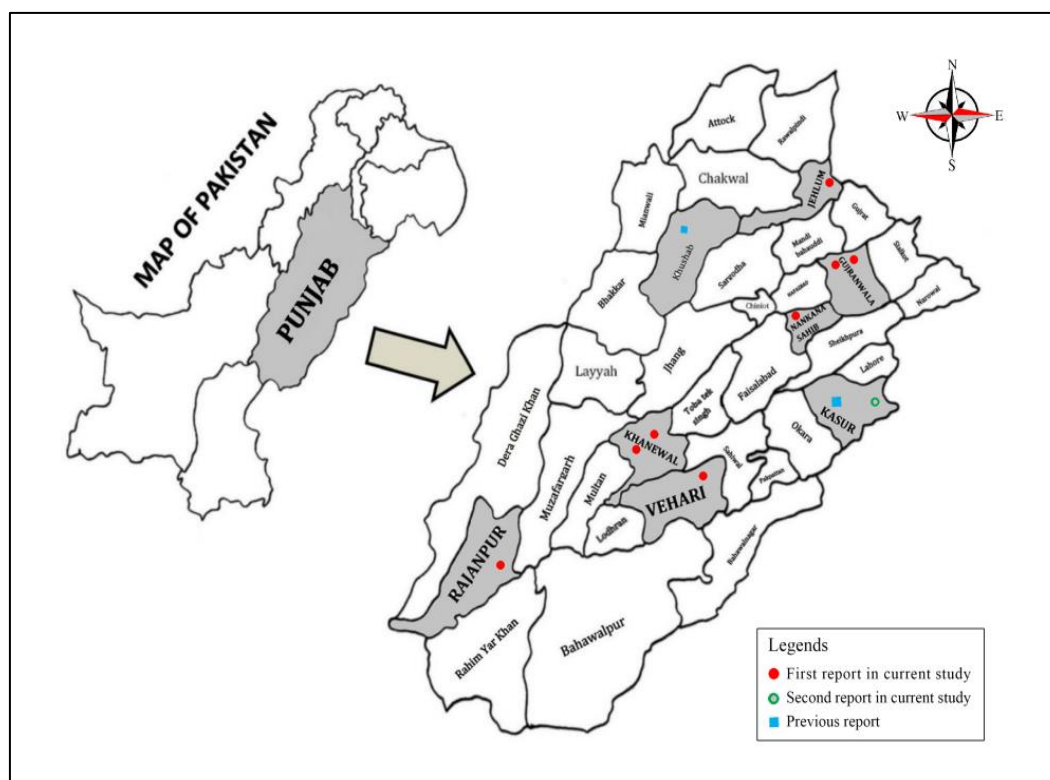


Figure 5: Distribution of *Agaricus campestris* across different districts of Punjab, Pakistan.

5. Conclusion:

It is concluded that identified specimens of *Agaricus campestris* were collected from 7 districts of

Punjab, with varying climatic conditions ranging from subtropical desert to hot semi-arid. Current study generated first records of this taxon from 6 districts of Punjab province, Pakistan.

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