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Arbuscular Mycorrhizal Fungi in Chickpea across Different Agro-Ecological Regions of Punjab, India

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A B S T R A C T

Arbuscular Mycorrhizal Fungi (AMF) play a pivotal role in enhancing plant growth and soil health through symbiotic associations with crops, particularly cereals and legumes. Although AMF are widespread, their colonization is highly sensitive to environmental and soil conditions, which can affect their persistence. To evaluate the impact of various agro-ecological conditions on AMF colonization in chickpea, a comprehensive survey was conducted across major chickpea-growing districts of Punjab, India, during the Winter seasons of 2019-20 and 2020-21. AMF colonization and spore density were assessed in rhizosphere soil samples. In Winter 2019-20, the highest colonization was observed in Ludhiana (71.4%), followed by Moga (65.3%) and Hoshiarpur (57.4%), while Barnala recorded the lowest (48.2%). In Winter 2020-21, Faridkot led with 74.5% colonization, followed by Patiala (72.0%) and Sangrur (69.5%), while Bathinda showed the lowest (38.4%). Spore population trends mirrored colonization patterns, with *Glomus* spp. predominating across all regions. Variations in AMF colonization and spore density reflect differences in soil and climatic conditions, highlighting the importance of local environmental factors in shaping AMF symbiosis with chickpea. These survey findings underscore the need for region-specific management strategies to enhance AMF activity and optimize chickpea production.

Introduction

Arbuscular mycorrhizal fungi (AMF) are essential components of the soil microbiome (Askar and Rashad 2010), enhancing nutrient uptake, improving soil structure, and promoting plant tolerance to biotic and abiotic stresses. By forming symbiotic associations with plant roots, AMF facilitate the acquisition of essential nutrients, particularly phosphorus, while contributing to soil health through nutrient cycling and organic matter stabilization (Sasode and Singh 2008; Jamal *et al.,* 2002). These benefits make AMF key contributors to sustainable agriculture, enhancing crop productivity and reducing reliance on chemical fertilizers.

Chickpea (*Cicer arietinum* L.) is a major legume crop in India, known for its protein content, beneficial fatty acids, and micronutrients (Merga 2019). In addition to its nutritional significance, chickpea contributes to sustainable farming through nitrogen fixation and crop rotation with cereals (Fergusan *et al.,* 2010). India ranks among the top global producers of chickpea, with Punjab being a key cultivation region (Anonymous 2020). The state encompasses diverse agroecological zones (AEZs), making it ideal for studying spatial variability in AMF colonization. Soil properties, temperature, and rainfall across districts significantly influence AMF colonization and spore density. Despite the importance of AMF for crop productivity, limited information exists on their distribution in Punjab's chickpea-growing regions.

This study aimed to assess the distribution and abundance of AMF in chickpea across Punjab, evaluating root colonization, spore density, and variation across agroecological zones. The findings provide insights into region-specific AMF dynamics, informing strategies to improve soil health, enhance chickpea productivity, and promote sustainable agriculture.

Materials and Methods

Selection of sites and crop sampling

Surveys were conducted across seven districts in Winter 2019 and eleven districts in Winter 2020, all recognized for chickpea cultivation, representing diverse AEZs in Punjab (Figure 1). Districts selected were Barnala, Bathinda, Faridkot, Gurdaspur, Hoshiarpur, Jalandhar, Ludhiana, Mansa, Moga, Patiala, and Sangrur. AEZs were chosen randomly, focusing on areas with optimal chickpea production to assess AMF associations.

Procedure for root clearing and staining of mycorrhizal fungi

Collected root samples were transported to the Fungal Biocontrol Laboratory, Department of Plant Pathology, PAU, Ludhiana. Roots were washed, blotted dry, and cut into 1 cm tertiary segments. Colonization was assessed using the protocol of Phillips and Hayman (1970). Roots were cleared in 2.5% KOH at 90 °C for 1 hour, acidified in 1% HCl, and stained with 0.05% trypan blue in lactophenol for 5 minutes. Slides were observed under a microscope for mycorrhizal structures, including mycelium, vesicles, and arbuscules.

Determination of root mycorrhizal colonization

AMF colonization was quantified following Biermann and Linderman (1981) and Singh *et al.,* (2019). Root segments were categorized visually as nil (−), scanty (+), moderate (++), or abundant (+++). The percentage of root length colonized by AMF was calculated using frequency data.

$$\text{Percent colonization} = \frac{\text{Number of root segments colonized x 100}}{\text{Total number of root segments observed}}$$

Extraction of AM spores from the soil

AM spores were extracted using wet sieving and decanting (Gerdemann and Nicolson, 1963). 100 g of air-dried soil was suspended in 1 L of water, shaken, and settled. Supernatant was passed through sieves (710–44 µm), and the material retained on the 44 µm sieve containing spores was collected.

Purification employed sucrose density gradient centrifugation, and spores were recovered and counted microscopically.

Counting of the spore population

Spores were quantified in a counting dish with 64 squares. 1 mL of a 25 mL spore suspension was distributed across the dish, observed under a stereomicroscope, and counted square by square. The number of spores per mL was multiplied by 25 to estimate total spores (Singh *et al.,* 2019).

Results and Discussion

AMF colonization differs based on agro-ecological conditions

AMF colonization varied significantly across districts and seasons, reflecting the dynamic interaction between host plants and AM fungi under differing environmental conditions. During the 2019–20 Winter season, five districts were surveyed. The percentage of root colonization ranged from a minimum of 48.2% in Barnala to a maximum of 71.4% in Ludhiana (Table 1, Figure 2A). Intermediate colonization levels were observed in Jalandhar (51.5%), Hoshiarpur (57.4%), and Moga (65.3%), reflecting differences in local environmental conditions that may influence AMF associations. In the 2020–21 Winter season, 11 districts were surveyed. The highest colonization was recorded in Faridkot (74.5%), followed by Patiala (72.0%) and Sangrur (69.5%), while Bathinda showed the lowest colonization (38.4%) (Table 2, Figure 2B).

Because the districts surveyed in 2019 and 2020 were not completely overlapping, direct comparison of colonization patterns across seasons is limited. However, for districts included in both surveys (Barnala, Ludhiana, Jalandhar, Hoshiarpur, Moga), colonization levels were generally in the same range, indicating certain areas consistently support higher or lower AMF activity.

Abundance of mycelium, vesicles, and arbuscules

Different mycorrhizal structures—mycelium, arbuscules, and vesicles—were observed at various chickpea growth stages. At the initial stages, mycelium was abundant in all samples, whereas arbuscules were present in moderate numbers, primarily in finer rootlets. Vesicles were absent at early stages. As the crop matured, vesicle formation increased, reaching the highest abundance in later stages. These vesicles, often elongated and intercellular, serve as storage structures within the host tissue.

In Winter 2019-20, among the 5 districts sampled, mycelium was abundant (+++) in Ludhiana and Moga, and moderate (++) in Barnala, Jalandhar, and Hoshiarpur, consistent with the overall colonization percentages.

Vesicle formation was abundant (+++) in Ludhiana, moderate (++) in Moga and Hoshiarpur, and scanty (+) in Barnala and Jalandhar. Arbuscule formation was abundant (+++) in Ludhiana, moderate (++) in Moga and Hoshiarpur, and scanty (+) in Barnala and Jalandhar (Table 1).

In Winter 2020-21, across the 11 districts sampled, mycelium was abundant (+++) in Patiala, Faridkot, Ludhiana, and Sangrur, and moderate (++) in Barnala, Bathinda, Gurdaspur, Hoshiarpur, Mansa, Moga, and Jalandhar.

Vesicle formation was abundant (+++) in Patiala, Faridkot, and Ludhiana, moderate (++) in most other districts, and scanty (+) in Barnala and Bathinda. Arbuscules were abundant (+++) in Patiala, Faridkot, and Sangrur, moderate (++) in most other districts, and scanty (+) in Barnala and Bathinda (Table 2).

These observations demonstrate district-specific differences in AMF structural abundance, with Ludhiana consistently exhibiting strong networks, while Barnala and Bathinda showed weaker establishment.

Spore populations of *Glomus* spp. and other types across different regions

The spore populations of *Glomus* spp. and other arbuscular mycorrhizal fungi (AMF) were measured to assess fungal propagule density in the rhizosphere soils of different districts during two Winter seasons.

In Winter 2019-20, the total spore population of AM fungi in the chickpea rhizosphere varied among the surveyed districts. Barnala recorded the lowest population (408.4 spores), while Ludhiana had the highest (576.5 spores). Hoshiarpur (472.6 spores), Jalandhar (410 spores), and Moga (551 spores) showed intermediate spore densities, reflecting differences in soil properties and environmental conditions (Table 1, Figure 3A).

During Winter 2020-21, *Glomus* spp. spores ranged from 142.5 in Bathinda to 348.2 in Faridkot, with Patiala (336.8) and Sangrur (269) also showing high counts. Other AMF types ranged from 175.3 in Mansa to 392.8 in Faridkot. Total spore populations followed a similar trend, with Bathinda recording the lowest (336.3 spores) and Faridkot the highest (741 spores). The consistently high spore populations in Patiala, Faridkot, and Sangrur suggest these districts provide favorable conditions for AMF proliferation, whereas Bathinda and Barnala appear less suitable for fungal establishment (Table 2, Figure 3B).

These findings align with previous studies demonstrating regional variability in AMF colonization and spore abundance. Numerous reports have noted arbuscules at early growth stages and vesicles at later crop stages. This pattern reflects the crop's developmental dynamics, where arbuscules dominate young cortical cells during primary vegetative growth, followed by vesicle formation for carbon storage as the crop matures (Hayman 1970; Saif and Khan 1975; Singh 2016). Singh (2016) specifically observed abundant mycelial growth, moderate arbuscules in early chickpea growth, and abundant vesicle formation in later stages. Location-specific differences in colonization have also been documented in the literature. Hasan (2002) reported colonization levels of 18–98% in pulse crops in eastern Uttar Pradesh, India. In Bangladesh, Khanam *et al.,* (2006) observed 30–48% colonization across crops, while Valsalakumar *et al.,* (2007) reported 29–95% in Tamil Nadu and Karnataka.

Table.1 Arbuscular Mycorrhizal Fungi Colonization Across Punjab Districts During the Winter 2019–20 Season

Districts	Colonization (%)	Mycelium	Arbuscules	Vesicles	Total spore population
Barnala	48.2	+	+	+	408.398
Hoshiarpur	57.4	++	++	++	472.6
Jalandhar	51.5	+	+	+	410
Ludhiana	71.4	+++	+++	+++	576.5
Moga	65.3	++	++	++	551

Table.2 Arbuscular Mycorrhizal Fungi Colonization Across Punjab Districts During the Winter 2020–21 Season

Districts	Colonization (%)	Mycelium	Arbuscules	Vesicles	Total spore population
Barnala	44.3 (41.71)	+ +	+	+	353.6
Bathinda	38.4 (38.27)	++	+	+	336.3
Faridkot	74.5 (60.20)	+ + +	+++	+ + +	741.0
Gurdaspur	51.5 (45.84)	++	++	+ +	378.7
Hoshiarpur	50.2 (45.09)	+ +	++	+ +	418.9
Jalandhar	54.3 (47.45)	+ + +	++	+ +	482.6
Ludhiana	68.8 (56.07)	+ +	+ ++	+ + +	579.9
Mansa	47.0 (43.26)	+ +	++	+ +	375.8
Moga	60.5 (51.06)	+ +	+ +	+ +	525.4
Patiala	72.0 (58.04)	+ + +	+ + +	+ + +	715.2
Sangrur	69.5 (56.47)	+ + +	+ +	+ +	594.2

Figure.1 Geographic distribution of selected districts in Punjab, India, used for data collection during the 2019–20 and 2020–21 chickpea growing seasons.

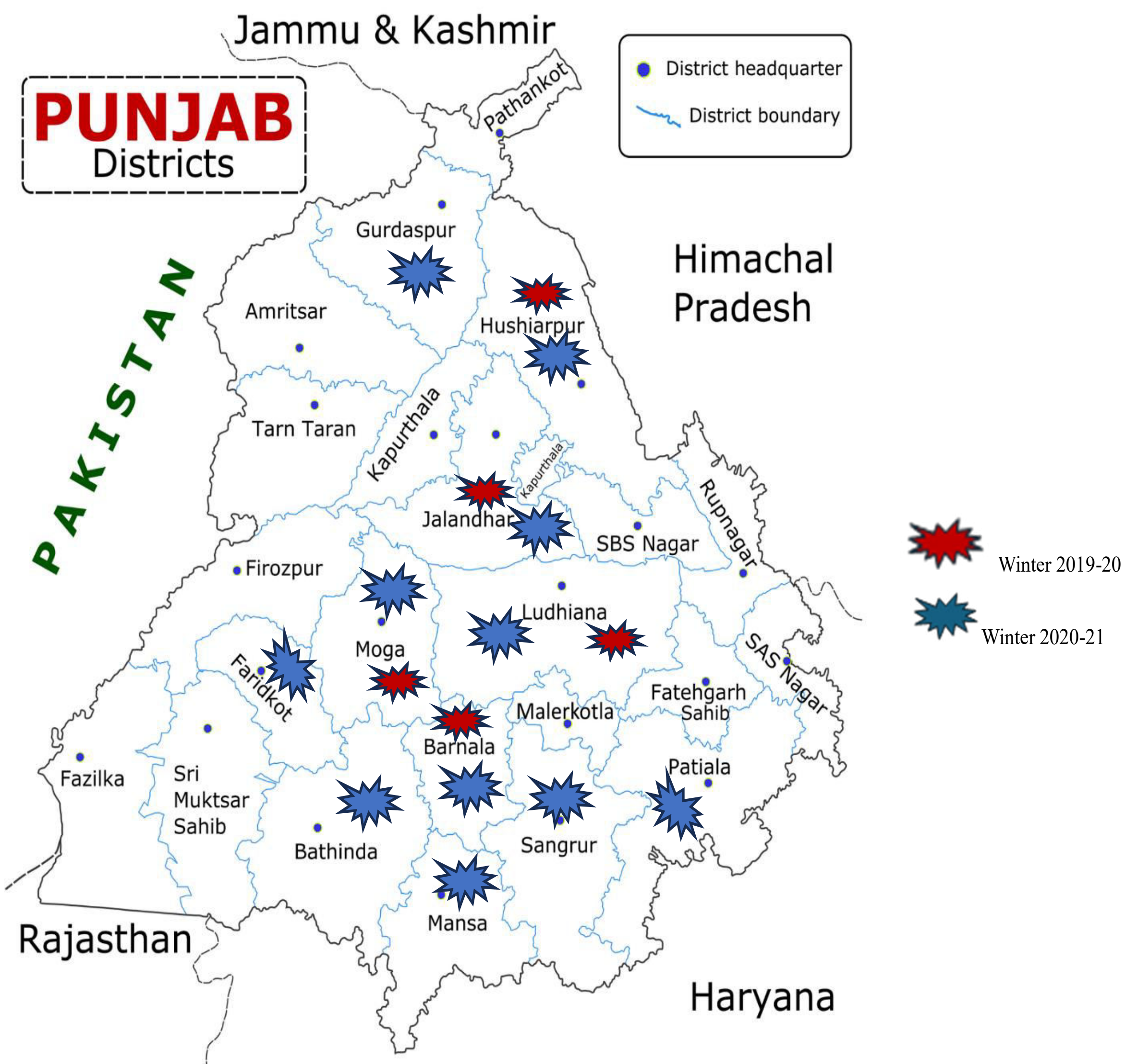
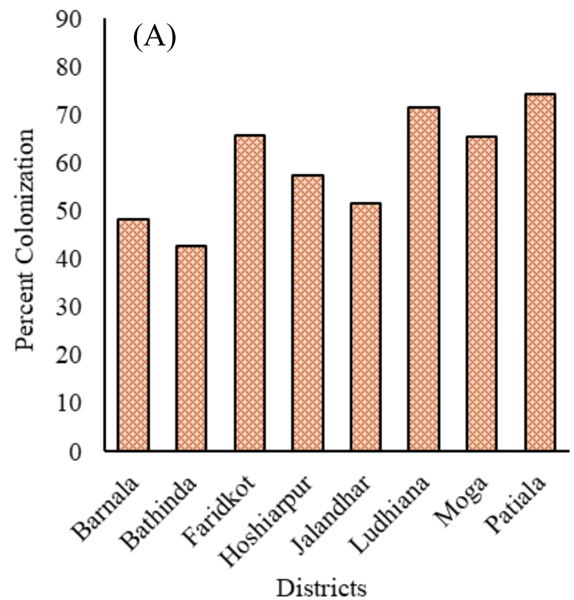


Figure.2 Percentage of mycorrhizal colonization in chickpea roots across different districts of Punjab during (A) Winter 2019–20 and (B) Winter 2020–21.



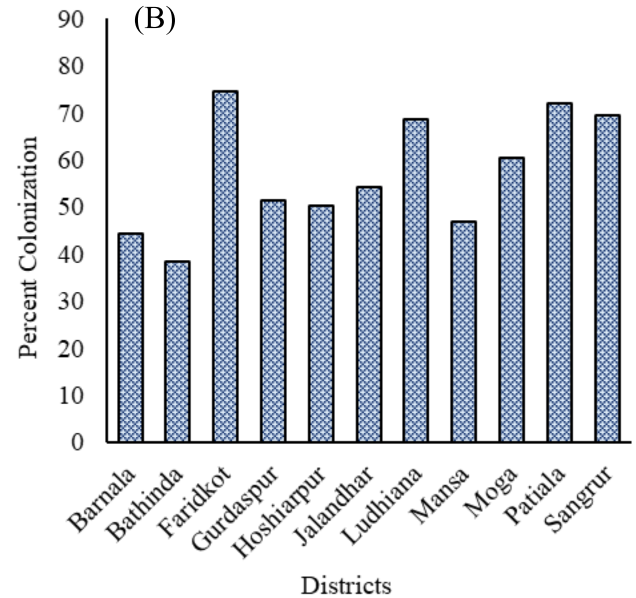
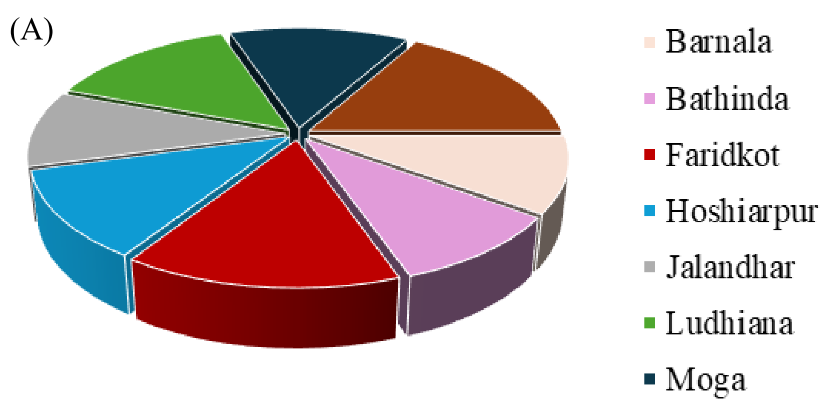
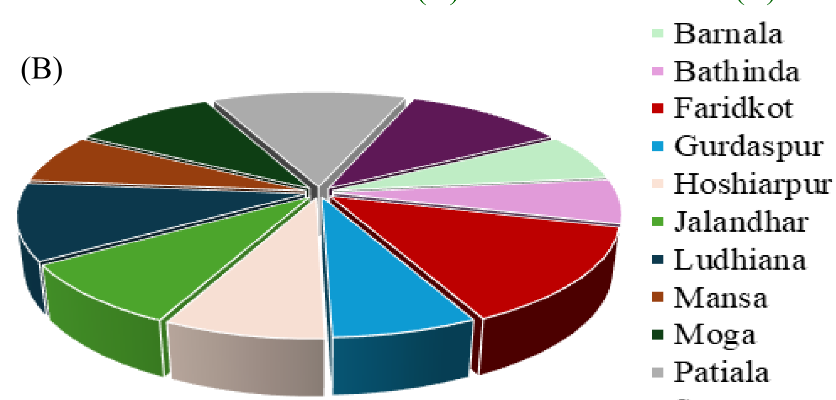


Figure.3 Abundance of AMF total spore population across different districts in Winter 2019-20 (A) and Winter 2020-21 (B).





In Punjab, Singh (2016) found maximum colonization of 76.5% in Patiala, followed by 74.4% in Faridkot and 71.1% in Sangrur. These results support the current findings, emphasizing the influence of local environmental factors and cropping practices on AMF associations. Additionally, Ludhiana showed higher colonization and spore counts than Hoshiarpur in chickpea treated with *Glomus bagyarajii*.

Leguminous crops are particularly favorable hosts for AM fungi (Manjunath and Bagyaraj 1986; Singh *et al.,* 1991). Singh and Singh (2001) recorded the highest spore count of 485 spores per 50 g of soil in Ludhiana and a minimum of 360 spores per 50 g in Hoshiarpur. Among the AMF, *Glomus* spp. were consistently the most abundant across all districts. Similar observations have been made internationally: Khanam *et al.,* (2006) reported 77–187 spores per 100 g soil in Bangladesh, Valsalakumar *et al.,* (2007) identified *Glomus*, *Gigaspora*, *and Scutellospora* in India (with *Glomus* being most prominent), and El-Hazzat *et al.,* (2018) found *Glomus* spp. dominated soil samples in six regions of Morocco, with populations ranging from 41 to 74 spores per 100 g soil.

Taken together, this study demonstrates that AMF in chickpea-growing districts of Punjab exhibit spore abundance and root colonization patterns strongly influenced by local soil and climatic conditions. The observed district-specific variations underscore the need for targeted management practices to enhance AMF activity and support sustainable chickpea production.

Author Contributions

Gagandeep Kaur: Investigation, formal analysis, writing—original draft. Daljeet Singh Buttar: Validation, methodology, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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