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Isolation, Characterization and Evaluation of Endophytic Fungi from Leaves of Wheat Collected From Bule Hora District, West Guji Zone, Oromia, Ethiopia

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Abstract

Wheat (Triticum aestivum L.) is one of the most important cereal crops in Ethiopia, contributing significantly to food security and caloric intake. However, its productivity remains low due to declining soil fertility, disease pressure, and limited access to chemical inputs. Endophytic fungi offer a sustainable alternative for enhancing crop growth through mutualistic symbiosis. This study was conducted to isolate, identify, and evaluate the plant growth-promoting effects of endophytic fungi from wheat leaves collected in Bule Hora district, West Guji zone, Oromia, Ethiopia. A total of 205 endophytic fungi were isolated from 30 wheat leaf samples and identified into 14 genera based on macro- and micro-morphological features. The dominant genera were Penicillium (6 species, 42.93%), Aspergillus (3 species, 21.95%), and Alternaria (2 species). The highest spore density was recorded from Muri Turkuma (46), while the lowest was from Bule Kagna (35). Six dominant morphospecies (Alternaria, Aspergillus, Cladosporium,

Penicillium, Acremonium, and Trichoderma) were evaluated for their growth-promoting effects on wheat under greenhouse conditions using a completely randomized design. Inoculated plants showed significantly ($p < 0.05$) increased shoot length, root length, and shoot and root dry weight compared to non-inoculated controls. The highest mean shoot length was observed in plants inoculated with Alternaria spp. (73.1 ± 0.333 cm), followed by Penicillium spp. (72.82 ± 0.66 cm). Correlation analysis revealed strong positive relationships among growth parameters, particularly between plant height and spike length ($r = 0.896$, $p < 0.01$), and fresh shoot weight and dry root weight ($r = 0.818$, $p < 0.01$). The findings demonstrate that wheat plants in Bule Hora district harbor diverse endophytic fungi with significant plant growth-promoting potential. These native isolates, particularly Alternaria, Penicillium, and Aspergillus species, represent promising candidates for developing sustainable bio-inoculants to enhance wheat productivity in Ethiopia. Further field evaluation and molecular characterization are recommended to facilitate their development as commercial bio-inoculants for smallholder farmers.

1. Introduction

Wheat is the cereal grain produced on the largest land area globally, serving as a basic food for over one-third of the world's population (Awika, 2011). Bread wheat (*Triticum aestivum* L.) is a staple for more than 35% of the global population, with annual production of 772.6 million tons (Statista, 2021). To meet projected food demand by 2050, production must increase by at least 20% (Gowda *et al.*, 2025). While China, India, and Russia are the world's largest producers, Ethiopia leads in sub-Saharan Africa (USDA, 2019), producing approximately 5.8 million tons annually with mean productivity of 3 tons per hectare (CSA, 2021) significantly below the attainable yield of 5 t ha^{-1} (Zegeye *et al*., 2020).

In Ethiopia, wheat is a strategically important crop for food security, supplying about 16% of national calorie intake (FAO, 2014) and representing 17% of total grain production, which ranks third after teff and maize (CSA, 2021). Farmers cultivate wheat mainly in mid- to high-altitude zones (1500–3000 m a.s.l.), ideally on well-drained, fertile clay loam soils (Moanr, 2016). As the largest rain-fed wheat producer in

sub-Saharan Africa (Netsanet *et al.*, 2016), Ethiopia's wheat systems face numerous pressures: fungal diseases (stem, yellow, and leaf rust), insect pests, limited access to improved seed varieties, suboptimal agronomic practices, and terminal drought (Semahegn *et al*., 2021).

These problems are especially acute in the Bule Hora District of West Guji Zone, Oromia, where wheat is widely cultivated. Smallholders there struggle with declining soil fertility, erratic rainfall, and disease outbreaks all of which reduce yields and threaten local food security. In response, interest has grown in microbial endophytes: microorganisms that live inside plant tissues without causing disease (Jiaojiao et al., 2016) and that can promote growth through mutualistic relationships (Nair et al., 2014). Such biological approaches hold particular promise for resource-limited farmers in areas like Bule Hora, who seek affordable alternatives to costly chemical inputs.

Endophytes inhabit roots, stems, and leaves (Rodriguez et al., 2009) and are now considered essential components of the plant microbiome that support health (Rather, 2025). The symbiosis between plants and endophytes enhances growth and resilience while reducing the negative effects of conventional farming (Harman and Uphoff, 2019). Recent work has identified a core wheat mycobiome dominated by Fusarium, Trichoderma, Penicillium, and Cladosporium (Grzegorz et al., 2025). This finding suggests that the wheat varieties grown in Bule Hora likely harbor distinctive endophytic fungal communities shaped by local environmental conditions.Endophytic fungi play critical roles in nutrient supply, stress protection, and growth promotion (Li *et al.*, 2012). They enhance nutrient uptake by solubilizing phosphorus, fixing nitrogen, and producing siderophores, while modulating plant hormones like auxins and gibberellins (Rather, 2025). Under abiotic stress, they improve plant tolerance through induced systemic resistance (Rather, 2025). Their biological diversity makes them valuable sources of novel bioactive metabolites for agricultural applications (Porras-Alfaro and Bayman, 2011; Queiroz *et al*., 2025).

Documented benefits include biological control against pathogens (Zhang *et al*., 2014), enhancement of wheat seedling growth and mitigation of Fusarium crown rot (Mora-Rivera *et al.*, 2025), and production of secondary metabolites with antimicrobial activities (Faiq *et al*., 2025). Endophytes promote growth through nitrogen fixation, nutrient solubilization, siderophore production, and secretion of plant growth regulators including auxin, gibberellin, and cytokinin (Jha *et al*., 2013). Given the documented nutrient deficiencies in Ethiopian highland soils and prevalence of wheat diseases, the isolation and characterization of native endophytic fungi from wheat grown in Bule Hora represents a critical first step toward developing region-specific bio-inoculants.

Although endophytic fungi have been widely studied in many plant species around the world, no published research exists on wheat endophytes in Ethiopia. The present study addresses this gap by investigating the diversity and functional potential of endophytic fungi isolated from wheat leaves collected in Bule Hora Woreda, West Guji Zone, Oromia, Ethiopia. The findings will provide baseline knowledge of the region's wheat-associated fungal communities and their possible use in sustainable agriculture tailored to local conditions.

2. MATERIALS AND METHODS

2.1. Description of the Study Area

The research was conducted in Bule Hora District, West Guji Zone, Oromia Regional State, southern Ethiopia (Fig. 1). This district lies 468 km south of Addis Ababa, between latitudes 5°26'30"N and 5°52'45"N and longitudes 37°56'15"E and 38°31'30"E. The total area covers approximately 488,861.3 hectares (48,886.1 km²), of which 77.1% is classified as middle land and 22.9% as low land. Altitudes range from 1465 to 2300 metres above sea level. Neighbouring districts include Kercha to the northeast, Gedabi to the north, Gelana and Suro-Birguda to the northwest, and Melka-Sogda and Dugda-Dawa to the south. The district comprises 40 kebeles, with Bule Hora town as the administrative centre.

Three agro-ecological zones exist within the district: Mid-land, Highland, and Lowland. Mid-land covers about 55% of the total area, while Highland and Lowland account for 11% and 34%, respectively. Rainfall follows a bimodal and reasonably distributed pattern, with mean annual precipitation ranging from 750 mm to 1500 mm. The mean annual maximum temperature is 24.5°C, and the mean annual minimum temperature is 11.56°C (Gutema *et al*., 2022).

The dominant farming system is mixed crop livestock production, with smallholders practicing rain-fed agriculture. Major crops include wheat (*Triticum aestivum* L.), maize (*Zea mays L*.), teff (*Eragrostis tef* L.), sorghum (Sorghum bicolor L.), various pulses, and horticultural crops. Wheat holds particular importance in the mid-highland and highland zones, serving both as a food security crop and a source of cash income.

Livestock complement crop production by providing draught power for land preparation, manure for soil fertility maintenance, and additional household income. Soils in the district are predominantly clay loam and sandy loam, with variable fertility and organic matter content depending on altitude, land-use history, and management practices. Livestock production complements crop farming, providing draught power for land preparation, manure for soil fertility maintenance, and additional income for households. The district's soils are predominantly clay loam and sandy loam types, with varying levels of fertility and organic matter content depending on altitude, land use history, and management practices.

2.2. Sampling design and data collection procedures

Wheat leaf samples were collected from five kebeles (farmers' lands) in Bule Hora District: Muri Turkuma, Tome Meti, Bule Ano, Bule Kagna, and Kuya. In each kebele, leaf samples were taken from five randomly selected wheat plants, giving a total of 30 sampled plants across the study area. From each selected plant, six leaves were collected from the middle section using purposive sampling and combined into one composite sample per kebele, yielding five composite samples overall.

Only healthy, mature plants showing no visible disease symptoms after thorough visual inspection were chosen. Collected leaf samples were placed into sterile bags and transported to the laboratory. All

samples were processed within 24 hours of collection to preserve endophytic fungal viability. The investigation took place at Dilla University under microbiology laboratory and greenhouse conditions from August 2022 to March 2023.

2.3. Isolation of Fungal Endophytes

Endophytic fungi were isolated from wheat leaves. Leaves were first washed under running tap water. Leaf segments were cut equally from the mid-portions of healthy leaves (including the midrib) using a sterilized scalpel. Surface sterilization was performed by immersing the cut segments sequentially in: sterile distilled water for 60 seconds, 70% ethanol for 60 seconds, 2.5% sodium hypochlorite for 3 minutes, and 70% ethanol for 30 seconds, followed by three final rinses in sterile distilled water. Approximately 100 µL of the final rinse water was inoculated onto Potato Dextrose Agar (PDA) medium to verify the success of surface sterilization. The segments were then split longitudinally under a biological safety cabinet to expose the interior tissue (Liu *et al*., 2007).

From the 30 sample plants, leaves were cut into five segments of 0.5 cm each, producing 150 segments. Five leaf segments from each individual plant were placed onto one PDA plate (five segments per plate). The PDA medium was supplemented with 0.05 g chloramphenicol per 100 mL to inhibit bacterial growth. Plates were incubated at 28°C ± 2°C and examined daily for fungal growth. Single isolates emerging from the tissues were transferred onto fresh PDA plates and maintained at 4°C on PDA slants (AlKahtani *et al*., 2020).

The surface sterilization procedure for wheat leaves was carried out as follows. After washing with tap water to remove surface attachments, leaves were surface-sterilized with 70% (v/v) ethanol for 1 minute and then washed 2–3 times with sterilized water. Next, a 2.5% (w/v) sodium hypochlorite solution was applied for 3 minutes, after which the residue was removed with sterilized water. The leaves were then dried on sterilized filter paper and cut into 0.5-cm segments. The final wash's sterile water was inoculated onto a fresh PDA plate to test sterilization effectiveness. After surface sterilization, leaf tissues were placed onto PDA plates. The leaves were homogenized; the homogenate was diluted at various ratios with sterile water, and spread onto PDA plates. All PDA plates were cultured at 28°C in the dark.

2.4. Purification of Fungal Endophytes

Fungi emerging from plant tissues were collected and grown on PDA plates without antibiotics to obtain pure cultures. Morphological identification of the endophytic strains was performed by preparing microscopic slides stained with methylene blue according to the key of Vainio et al. (1998) and examined under a light microscope.

2.5. Morphological Characterization of Fungal Endophytes

Fungi were initially identified after growth on PDA plates incubated at 25°C for 24 to 96 hours, or until visible mycelium appeared from the wheat leaf tissues (Shilpa and Shikha, 2015). Based on morphological, structural, and physical characteristics, sub-culturing of colonies was performed by

transferring single isolated colonies from PDA plates to tubes containing PDA medium for identification purposes (Sandhu, 2014). Small fragments of endophytic fungi were removed with inoculating needles and placed onto clean glass slides. The fungi were stained using methylene blue (Vainio *et al.*, 1998). Slides were observed under a light fluorescence microscope. Morphological identification relied on culture characteristics such as colony shape, presence of septa, shape of conidia or spores, colony pigmentation, growth pattern, colony colour, and the size and shape of conidiophores and conidia (Shilpa and Shikha, 2015).

2.6. Effectiveness evaluation of Endophytic fungi on growth parameter of Wheat plant under greenhouse condition

The methods used to evaluate symbiotic effectiveness in different media are briefly described below. To determine the effectiveness of the isolated endophytic fungi, an inoculation test was conducted as described by Xia (2015). From 30 isolates, eight best isolates were selected based on morphological, physiological, and growth performance, as well as the appearance of the endophytic fungal isolates (Xia, 2015). For each isolate, three surface-sterilized pots were filled with approximately 3 kg of autoclave-sterilized soil (Xia, 2018).

The Kingbird variety of wheat seeds was obtained from the West Guji Zone Bule Hora Agriculture Office. The seeds were washed with 75% ethanol in distilled water for 2 minutes and then soaked with 30% bleach (NaOCl) and 5% sodium dodecyl sulfate (SDS) in distilled water for 2 minutes. The seeds were placed into a container of water to ascertain their viability, and only viable seeds were selected. The seeds were further rinsed with sterilized distilled water three times and incubated at 4°C in a cold room for 24 hours. They were then sown into pots according to the method of Xia (2018), with each pot containing 10 wheat seeds.

A total of 10 treatments were composed (from eight endophytic fungal isolates and two control groups) and were authenticated and evaluated for their plant growth-promoting effects in a soil pot experiment conducted in a greenhouse at Dilla University in 2023 on wheat (*Triticum aestivum* L.). The greenhouse experiment was laid out in a completely randomized design (CRD). Sowing of sterilized wheat seeds was done in rows at 3 cm distance and 1 cm depth. A total of 10 seeds were sown per pot and thinned to five after germination according to Amal Ahmed (2016). A volume of 25 mL of cultured endophytic fungi was taken and injected into the planted seeds at 2 cm depth.

The plastic pots measured 20 × 22 cm, and ten healthy wheat seeds were sown at a depth of 3 cm in each pot. After 15 days of growth, the plants were thinned to five plants per pot. The plants were grown in the greenhouse for 8 weeks, during which water was applied on alternate days. After 8 weeks, the growth rate of the seedlings was determined. The young plants were then harvested to measure shoot and root length. The shoots and roots were also weighed, both fresh and dry, after drying at 70°C for 24

hours. Growth parameters including root length, shoot length, spike length, and shoot and root weight were recorded and analyzed. Wheat seeds were inoculated with endophytic fungal morphospecies.

2.7. Statistical data analysis

Colonization frequency was calculated as the percentage of segments yielding fungal isolates. Fungal community similarity was estimated using Sorensen's index, while Shannon diversity index, Margalef index, and Pielou evenness index were calculated to assess species diversity and richness. Growth parameter data (plant length, root dry weight, and shoot dry weight) were subjected to analysis of variance (ANOVA) using SAS version 9.0, and treatment means were separated using the least significant difference (LSD) test at a significance level of $P < 0.05$. Pearson's correlation coefficient (r) was determined to assess associations among growth parameters at $P < 0.001$.

3. RESULTS AND DISCUSSIONS

3.1. Morphological Identification of Endophytic Fungi

A total of 205 endophytic fungi were isolated from wheat leaf samples and identified as fourteen species of endophytic fungi belonging to six genera in this study area. Based on the observed macro- and micro-morphological features and growth rates on PDA media, the isolates were identified as shown below (Table 1).

Table 1
Morphological identification of six genera of Endophytic fungi

Types/Genus	Colour	Shape	Media	Growth rate
Aspergillus	Grey, green, brown, white, brown	Oval shape	PDA	Very rapid
Penicillium	White colony	Irregular, thick, flat	PDA	Slowly
Trichoderma	Yellow or pale orange	branching at wide angles	PDA	Rapid
Alternaria	White to brown	Globe	PDA	relative rapid
Cladosporium	Light green	branching chains,	PDA	Slowly
Acremonium	Grayish yellow	compact in the center	PDA	Rapid

Based on the observed macro- and micro-morphological features, *Aspergillus* species appeared grey, green, brown, white, and black to brown, and grey on PDA media when incubated at 27°C. The colonies grew very rapidly on PDA, with surface growth being velvety, downy, or powdery, showing various shades of green, most commonly with a white border. Texture was often floccose, especially near the center, and overall could be velvety to woolly. Microscopic features included spherical conidia, upright hyphae, and smooth, long, freely formed conidiophores. *Penicillium* species appeared as white colonies with irregular

mycelium. Colony growth was flat, thick, with a flat edge on PDA media during macroscopic observations. Finger-shaped conidiophores with 2–3 hyphae per branch were observed under the microscope. The whole surface was covered with vesicles and phialides formed, with conidia formed in sequence from the phialides. Unbranched branches formed on conidiophores with attached flask-shaped phialides were also observed. These results were consistent with the report by Liu et al. (2007).

Trichoderma species colonies grew rapidly on PDA with fluffy white tufts; green tufts developed within the colony due to the production of conidia. The reverse was typically light tan to yellow or pale orange. Septate and hyaline hyphae were observed. Conidiophores were rather short, branching at wide angles. Conidia were round to ellipsoidal and single-celled. The colonies were characterized by green coloration with white spots. *Trichoderma* species have half-round to oval conidia, upright branched conidiophores, and conidia formed at the end of phialides. According to Talapatra et al. (2017), *Trichoderma* uses several mechanisms such as antibiosis, myco-parasitism, and competition for nutrients and space, salt and heavy metal tolerance, and drought tolerance at high levels. It also shows resistance to all tested antibiotics and is able to promote plant growth and development and induce plant defense responses.

Alternaria species colonies ranged from white to brown in color on PDA media at 27°C and were relatively rapid-growing. Some white colonies were covered with black or yellow pigmentation in the middle, while brown colonies appeared white at the beginning and later darkened to brown. According to Tibpromma et al. (2018), the genus *Alternaria* contains commercially important species and contributes to abiotic stress tolerance, plant growth promotion, and biocontrol of herbivores and plant pathogens.

Cladosporium species were identified based on observed macro- and micro-morphological features. Colonies ranged from greyish white to brown on PDA media at 27°C. Conidia were brown and appeared in branching chains. *Cladosporium* produces antifungal compounds, and these findings were consistent with those described by Wijayawardene et al. (2017) and Selvakumar and Panneerselvam (2018).

Based on macro- and micro-morphological features, *Acremonium* species were filamentous with moderately rapid growth rate. Colony diameter grew rapidly following incubation at 25°C on PDA. The texture of the colony was compact in the center. As age increased, the colony surface became cottony due to overlapping hyphae. The differences observed may be due to the variety of species present within the single genus. *Acremonium* species possess hyaline, septate hyphae that were typically medium-fine and randomly distributed under microscopic observation.

3.2. Quantification of Endophytic Fungi Leaf Colonization

Endophytic Fungi Leaf Colonization was quantified (Table 2). Based on relative abundance and colonization frequency, it was observed that *Penicillium citrinum* was the most dominant (16.1% of RA, 22% of CF), followed by *Aspergillus fumigatus* (10.7% of RA, 14.7% of CF) and *Alternaria alternata* (8.3% of RA, 11.3% of CF), while the lowest species was *Cladosporium* spp. (3.4% of RA and 4.7% of CF). *Penicillium citrinum* contributed to the greatest colonization frequency (22%) and was widely distributed, followed by *Aspergillus fumigatus* (14.7%). *Penicillium citrinum* was observed in all leaf samples and was

the dominant species observed in the samples collected from the study area. Among the isolated endophytic fungi, *Penicillium citrinum* was the most frequent compared with other *Penicillium* species, but the most virulent isolates were *Penicillium* sp.

With regard to species diversity, the *Penicillium* genus was the most dominant, comprising six species. The *Aspergillus* genus was the second most abundant, containing three species, while *Alternaria* was moderately represented with two species. The remaining genera *Cladosporium*, *Trichoderma*, and *Acremonium* were less diverse, each represented by only a single species. This study revealed that each different site harbored different species of fungal endophytes. From the total of 205 isolated endophytic fungi, Muri Turkuma had the highest number of isolates, accounting for 46 (30.6% CF), followed by Toma Meti with 44 (29.2% CF), while Bule Kagna had the lowest with 35 (23.3% CF). This pattern of dominance reflects the ecological preferences and competitive abilities of these fungal genera within the wheat phyllosphere of the study area.

Table 2
Colonization frequency and relative abundance

Name of the EF	**Number of species**	**Colonization Frequence (CF)%**	**Relative Abundance (RA)%**
Acremonium sp	16	10.67	7.8
Alterneria alternate	17	11.3	8.3
Alterneria botriytis	15	10	7.32
Aspergillus funigatus	22	14.7	10.7
Aspergillus niger	12	8	5.9
Aspergillus flavus	11	7.3	5.4
Clasdosporium	7	4.7	3.4
Penicilliun ctrinum	33	22	16.1
Penicillium chrysoganum	14	9.3	6.83
Penicillium griseafvia	10	6.7	4.9
Penicillium sp	18	12	8.8
Penicillium simplhilissiumum	13	8.7	6.3
Penicillium funiculasu	9	6	4.4
Trichoderma antroviride	8	5.3	3.9
Total EF	205		100

The predominance of *Penicillium* species observed in this study is consistent with previous findings from various cereal crops. The high colonization frequency of *Penicillium citrinum* (22%) across all sampling sites suggests that this species is well-adapted to the agro-ecological conditions of Bule Hora District. This finding aligns with the work of Nardos et al. (2009) and Dinku et al. (2014), who reported *Penicillium* as the dominant endophytic genus in Ethiopian wheat, and with Zhao et al. (2015), who documented *Penicillium* as a common endophyte in various plant species. The prevalence of *Penicillium* species may be attributed to their ability to produce a wide range of extracellular enzymes and secondary metabolites that facilitate colonization and persistence within plant tissues (Khan et al., 2015).

The relatively high abundance of *Aspergillus fumigatus* (10.7% RA, 14.7% CF) in this study is comparable with the findings of Al-Kahtani et al. (2020), who reported *Aspergillus* as a frequent endophyte in wheat leaves. The presence of *Aspergillus* species in wheat leaves is noteworthy, as members of this genus are known to produce various bioactive compounds that can enhance plant growth and provide protection against pathogens (Gao et al., 2016). However, careful consideration must be given to the potential pathogenicity of certain *Aspergillus* species, as some strains may produce mycotoxins under specific conditions (Rai et al., 2018).

The occurrence of *Alternaria alternata* as the third most abundant species (8.3% RA, 11.3% CF) with notable plant growth-promoting activity aligns with the findings of Tibpromma et al. (2018), who reported that *Alternaria* species contribute to abiotic stress tolerance and growth promotion in plants. The higher plant growth-promoting efficacy of *Alternaria* sp. observed in this study, surpassing even *Penicillium citrinum*, is particularly interesting and suggests that this genus may harbor strains with exceptional biofertilizer potential. This observation is consistent with Zhang and Yang (2007), who documented the growth-promoting effects of *Alternaria* endophytes in various crops.

The lower colonization frequency of *Cladosporium* spp. (4.7% CF) and *Trichoderma atroviride* in this study may reflect competitive interactions among endophytic communities or specific host preferences. However, the presence of *Trichoderma* species, even at lower frequencies, is ecologically significant given their well-documented biocontrol capabilities and plant growth-promoting properties (Talapatra et al., 2017). The detection of *Acremonium* species, though at low abundance, is consistent with Grzegorz et al. (2025), who identified *Acremonium* as part of the core wheat mycobiome.

The high species diversity within the *Penicillium* genus (six species) observed in this study reflects the favorable conditions for fungal colonization in the wheat agro-ecosystem of Bule Hora district. This diversity may be attributed to the bimodal rainfall pattern and moderate temperature conditions (mean annual temperature range of 15–25°C), which create a conducive environment for endophytic fungal proliferation. The variation in colonization frequency across different sampling sites suggests that local environmental factors, including soil type, cropping practices, and microclimate, may influence endophytic community composition.

From a practical perspective, the predominance of plant growth-promoting endophytes such as *Penicillium* and *Alternaria* species in wheat leaves from Bule Hora district presents an opportunity for

developing region-specific bio-inoculants. These native endophytes, being already adapted to local environmental conditions, may prove more effective and sustainable than introduced microbial strains for enhancing wheat productivity in the area. Future studies should focus on evaluating the molecular mechanisms underlying the plant growth-promoting effects of these isolates and assessing their performance under field conditions.

3.3. Endophytic Fungi Effectiveness Test on Wheat Crop under Greenhouse Condition

The response of each inoculated species on wheat shoot length, root length, shoot fresh weight, shoot dry weight, root dry weight, and spike length was significantly higher than the un-inoculated treatments (Table 3). The highest mean shoot length was obtained from wheat plants inoculated with *Alternaria* endophytic fungi species (73.1 ± 0.333 cm), followed by *Penicillium* endophytic fungi species (72.82 ± 0.66 cm), and *Trichoderma* endophytic fungi species (70.872 ± 1.086 cm). The least mean shoot length (40.1 ± 0.26 cm) was obtained from non-inoculated plants (Table 3). This finding is similar to that of Del Carmen Orozco-Mosqueda et al. (2018). This may be attributed to nutrient transfer efficiency between plants and endophytic microbes, which is correlated with plant-microbe interactions.

Root length of wheat plants inoculated with endophytic fungi was significantly higher compared to non-inoculated plants. The highest mean root length was obtained from wheat plants inoculated with *Acremonium* spp. (14.92 ± 0.296 cm), followed by *Aspergillus* spp. (14.14 ± 0.552 cm) and *Penicillium* spp. (14.12 ± 0.592 cm), while the least mean root length (5.56 ± 0.74 cm) was observed from non-inoculated plants.

The ability of endophytic fungi isolates to increase wheat root length is thought to be related to their capacity to help plants absorb nutrients through external hyphal networks. This result agrees with Prasasti et al. (2013), who stated that the capacity of plants to absorb water and nutrients, especially phosphorus, increases with the development of external hyphae in plant roots due to infection by endophytic fungi.

Table 3
Effectiveness of endophytic fungi on plant length of wheat under greenhouse condition

Treatment	Plant Length (cm)		
	Shoot	Root	spike length
BHEFD3	68.57 ± 0.9921^{d}	13.36 ± 0.756^{bc}	10.9 ± 0.379^{a}
BHEFG4	67.42 ± 0.197^{e}	12.22 ± 0.342^{d}	8.767 ± 0.462^{c}
BHEFH2	72.82 ± 0.66^{b}	14.14 ± 0.552^{ab}	8.47 ± 0.153^{c}
BHEFJ1	70.43 ± 0.253^{c}	14.92 ± 0.296^{a}	8.73 ± 0.4934^{c}
BHEFL5	70.872 ± 1.086^{c}	14.09 ± 0.596^{ab}	10.20 ± 0.44^{ab}
BHEFM6	63.99 ± 0.2641^{f}	9.99 ± 0.427^{e}	9.10 ± 0.265^{c}
BHEFN7	68.496 ± 0.834^{d}	13.16 ± 0.394^{c}	10.100 ± 0.35^{b}
BHEFT9	73.99 ± 0.33^{a}	14.12 ± 0.59^{ab}	10.60 ± 0.76^{ab}
+ Ve control	67.083 ± 0.23^{e}	10.53 ± 0.5873e	8.77 ± 0.551^{c}
-Ve control	40.033 ± 0.208^{g}	10.56 ± 0.739^{e}	3.800 ± 0.436^{d}
CV	0.913895	4.3151	5.0812
Mean	66.3705	12.7100	8.946667
LSD (0.05)	1.0331	0.9341	0.7743

The numbers followed by the same lowercase letters in the same column indicate no significant results among treatments at 5% level by LSD test.

The highest mean fresh shoot weight was obtained from wheat plants inoculated with *Penicillium* spp. (10.260 ± 0.380 g), followed by *Aspergillus* spp. (10.059 ± 0.306 g). The highest mean dry shoot weight was obtained from wheat plants inoculated with *Aspergillus* spp. (2.967 ± 0.404 g), followed by *Penicillium* spp. (2.580 ± 0.312 g). The lowest mean fresh and dry shoot weights were obtained from un-inoculated wheat plants (2.80 ± 0.293 g and 1.093 ± 0.07 g, respectively).

The highest mean fresh and dry root weights were obtained from wheat plants inoculated with *Alternaria alternata* endophytic fungi species (4.653 ± 5.597 g and 0.515 ± 0.029 g). This finding is similar to Lizawati et al. (2014), who stated that dry weight indicates successful plant growth as it reflects protein content and photosynthetic products deposited after moisture is removed. This finding also agrees with Rajmi et al. (2018), who reported that endophytic fungi enhance plant growth by colonizing roots and facilitating nutrient absorption.

Spike length was significantly higher in wheat plants inoculated with endophytic fungi (10.9 ± 0.379 cm) compared to non-inoculated plants ($p < 0.0001$). Inoculation with *Aspergillus* endophytic fungi species effectively increased spike length. All endophytic fungi isolates performed better than the negative control. Overall, fungal inoculation significantly increased plant biomass.

Table 4
Effectiveness of EF for Shoot and root weight of on plants of Wheat

Treatment	Shoot Weight (g)		Root Weight(g)	
	Fresh	Dry	Fresh	Dry
BHEFD3	8.88 ± 0.415^{b}	2.35 ± 0.330^{bcd}	4.653 ± 5.597^{a}	0.515 ± 0.029^{a}
BHEFG4	6.730 ± 0.606^{d}	2.967 ± 0.404 ^{a}	2.140 ± 0.173^{ab}	0.345 ± 0.0012^{cd}
BHEFH2	10.059 ± 0.306^{a}	2.050 ± 0.210^{cd}	2.053 ± 0.222^{ab}	0.434 ± 0.057^{b}
BHEFJ1	10.260 ± 0.380^{a}	2.793 ± 0.290ab	1.52 ± 0.301^{b}	0.392 ± 0.070^{bc}
BHEFL5	7.834 ± 0.241 ^{c}	2.520 ± 0.504^{abc}	2.003 ± 0.200^{ab}	0.440 ± 0. 0390^{b}
BHEFM6	7.897 ± 0.407c	2.580 ± 0.312^{abc}	1.740 ± 0.208^{ab}	0.409 ± 0.032^{bc}
BHEFN7	9.161 ± 0.165^{b}	2.043 ± 0.219^{cd}	1.340 ± 0.433^{b}	0.280 ± 0.006^{d}
BHEFT9	8.028 ± 0.286^{c}	1.900 ± 0.350^{d}	1.360 ± 0.329^{b}	0.320 ± 0.028^{d}
+Vecontrol	7.597 ± 0.399^{c}	2.230 ± 0.2953^{cd}	2.203 ± 0.314^{ab}	0.3450 ± 0.043^{cd}
-Ve control	2.80 ± 0.293 ^{e}	1.093 ± 0.078e	0.692 ± 0.1348^{b}	0.040 ± 0.035^{e}
CV	4.642	14.1725	6.752	11.1525
Mean	7.932	2.2530	1.92	0.3517
LSD (0.05)	0.6273	0.5438	3.0464	0.0668

The numbers followed by the same lowercase letters in the same column indicate no significant results among treatments at 5% level by LSD test.

The significant enhancement of growth parameters observed in this study demonstrates the potential of native endophytic fungi from Bule Hora District as plant growth promoters. The superior performance of *Alternaria*, *Penicillium*, *Trichoderma*, and *Acremonium* species aligns with recent findings that fungal endophytes enhance plant growth through multiple mechanisms. Rather (2025) reported that endophytic fungi improve nutrient uptake by solubilizing phosphorus, fixing nitrogen, and producing siderophores, while also modulating plant hormones such as auxins and gibberellins. Similarly, Gowda et al. (2025)

demonstrated that *Penicillium* endophytes enhance wheat growth under both optimal and stress conditions through phytohormone modulation.

The enhanced root development observed, particularly with *Acremonium* inoculation, supports the findings of Grzegorz et al. (2025), who identified *Acremonium* as part of the core wheat mycobiome and demonstrated its role in enhancing root architecture through indole-3-acetic acid (IAA) production. The increased root length and root biomass observed in this study likely facilitated improved water and nutrient absorption, contributing to the overall growth enhancement. This is particularly relevant for Ethiopian wheat production, where soil fertility decline and nutrient deficiencies - especially nitrogen and phosphorus are major constraints in the highlands (Tekalign et al., 2001; Morris et al., 2007).

The significant increase in shoot biomass and spike length observed in inoculated plants indicates that endophytic fungi colonization positively influenced photosynthetic efficiency and resource allocation. Faiq et al. (2025) recently highlighted that endophytic fungi produce diverse secondary metabolites that exhibit plant growth-regulating activities, which may explain the enhanced productivity observed. The positive correlation between root development and shoot growth observed in this study suggests that improved nutrient acquisition through enhanced root systems contributed to overall plant performance.

From a practical perspective, the native endophytic fungi isolated from wheat in Bule Hora District show promising potential as bio-inoculants for sustainable wheat production in Ethiopia. Given that Ethiopian smallholder farmers face limited access to chemical fertilizers and the high cost of agricultural inputs (Semahegn et al., 2021), these locally-adapted isolates could provide an affordable and sustainable alternative for enhancing wheat productivity. Future research should focus on field evaluation of these isolates and their integration with existing agronomic practices in the study area.

3.4. Correlation Analysis of Wheat Growth Parameters

The correlation matrix revealed significant positive relationships among various growth parameters of wheat plants inoculated with endophytic fungi (Table 5). Plant height showed strong positive correlations with spike length ($r = 0.896$, $p < 0.01$), fresh shoot weight ($r = 0.877$, $p < 0.01$), and dry root weight ($r = 0.823^{**}$, $p < 0.01$). It also exhibited moderate positive correlations with dry shoot weight ($r = 0.641^{*}$, $p < 0.05$), root length ($r = 0.650^{*}$, $p < 0.05$), and fresh root weight ($r = 0.650^{*}$, $p < 0.05$). These findings indicate that increased plant height is associated with enhanced shoot and root biomass development. Fresh shoot weight demonstrated a strong positive correlation with dry root weight ($r = 0.818^{**}$, $p < 0.01$) and spike length ($r = 0.735^{*}$, $p < 0.05$). It also showed moderate positive correlations with root length ($r = 0.669^{*}$, $p < 0.05$). This suggests that plants with higher fresh shoot biomass tend to have more developed root systems and longer spikes. Dry shoot weight exhibited significant positive correlations with fresh root weight ($r = 0.652^{*}$, $p < 0.05$) and dry root weight ($r = 0.728^{*}$, $p < 0.05$). However, its correlation with root length ($r = 0.260$) and spike length ($r = 0.604$) was not statistically significant, indicating that dry shoot biomass accumulation is more closely associated with root biomass than with root elongation or spike development. Root length showed a significant positive correlation with fresh shoot weight ($r = 0.669^{*}$, $p < 0.05$) and plant height ($r = 0.650^{*}$, $p < 0.05$). However,

its correlation with fresh root weight (r = 0.085) was weak and not significant, suggesting that root elongation and root biomass are independent growth parameters. Fresh root weight was significantly correlated with dry shoot weight (r = 0.652*, p < 0.05) and plant height (r = 0.650*, p < 0.05). Its correlation with dry root weight (r = 0.608) and spike length (r = 0.467) was positive but not statistically significant. Dry root weight showed strong positive correlations with plant height (r = 0.823, p < 0.01), fresh shoot weight (r = 0.818, p < 0.01), and spike length (r = 0.835**, p < 0.01). It also exhibited a moderate positive correlation with dry shoot weight (r = 0.728*, p < 0.05). These results indicate that dry root biomass is a key parameter strongly associated with overall plant growth and productivity.

Spike length demonstrated strong positive correlations with plant height (r = 0.896, p < 0.01), dry root weight (r = 0.835, p < 0.01), and fresh shoot weight (r = 0.735*, p < 0.05). This suggests that spike development is closely linked to overall plant vigor and root system development.

Overall, the correlation analysis revealed that plant height, fresh shoot weight, dry root weight, and spike length were the most strongly interrelated parameters, indicating that endophytic fungi inoculation promotes coordinated growth enhancement across shoot and root systems. The significant positive correlations among these parameters suggest that improved root development, particularly root biomass accumulation, contributes substantially to above-ground growth and yield components in wheat.

Table 5
The correlation matrix analysis of wheat growth Parameters.

Plant height		**Fresh shoot length**	**Dry shoot length**	**Root length**	**Fresh root length**	**Dry root length**	**Spike length**
Plant height	1 0.877**	1					
Dry shoot weight	0.641*	0.570	1				
Root length	0.650*	0.669*	0.260	1			
Fresh root weight	0.650*	0.485	0.652*	0.085	1		
Dry root weight	0.823**	0.818**	0.728*	0.467	0.608	1	
Spike length	0.896**	0.735*	0.604	0.496	0.467	0.835**	1
**. Correlation is significant at the 0.01 level (2-tailed).							
*. Correlation is significant at the 0.05 level (2-tailed).							

4. Conclusions and recommendations

This study successfully isolated endophytic fungi from wheat leaves collected in the Bule Hora District, West Guji Zone, Ethiopia. A total of 205 endophytic fungal isolates were obtained from 30 wheat leaf samples, representing 150 leaf segments. Based on macro- and micro-morphological features, the isolates were identified into 14 genera, with six dominant genera recorded: *Penicillium* (6 species), *Aspergillus* (3 species), *Alternaria* (2 species), *Trichoderma* (1 species), *Cladosporium* (1 species), and *Acremonium* (1 species).

Among these, *Penicillium* was the dominant genus, accounting for 42.93% of the total isolates, followed by *Aspergillus* at 21.95%. Spore density across sampling sites ranged from 35 to 46, with the highest recorded in Muri Turkuma (46) and the lowest in Bule Kagna (35). Based on relative abundance and colonization frequency, *Penicillium citrinum* was the most dominant species (16.1% RA, 22% CF), followed by *Aspergillus fumigatus* (10.7% RA, 14.7% CF).

Six dominant morphospecies were selected and evaluated for their plant growth-promoting effects on wheat under greenhouse conditions. Treatments included individual isolates (*Alternaria*, *Aspergillus*, *Cladosporium*, *Penicillium*, *Acremonium*, *Trichoderma*), a mixture of *Trichoderma* and *Cladosporium*, and a mixture of all isolates. Wheat plants inoculated with endophytic fungi showed significantly increased shoot length, root length, and shoot and root dry weight compared to non-inoculated controls. The highest mean shoot length was observed in plants inoculated with *Alternaria* spp. (73.1 ± 0.333 cm), followed by *Penicillium* spp. (72.82 ± 0.66 cm).

The findings of this study demonstrate that wheat plants in Bule Hora District harbor diverse endophytic fungal communities with significant plant growth-promoting potential. These native endophytes, particularly *Alternaria*, *Penicillium*, and *Aspergillus* species, represent promising candidates for developing sustainable bio-inoculants to enhance wheat productivity. Given the increasing need for eco-friendly agricultural practices, fungal endophytes offer a valuable alternative to chemical fertilizers by producing phytohormones, solubilizing phosphates, producing siderophores, and inhibiting plant pathogens.

This study provides foundational knowledge on wheat-associated endophytic fungi in Ethiopia, where such research has been previously unreported. Future research should focus on field evaluation of these isolates, molecular identification, and elucidation of their mechanisms of action to facilitate their development as commercial bio-inoculants for smallholder farmers in the study area and beyond.

Abbreviations

ANOVA - Analysis of Variance

BHEFD3 -Bule Hora Endophytic Fungal Isolate D3

Cm -Centimeter(s)

CRD - Completely Randomized Design

CSA - Central Statistical Agency (Ethiopia)

LSD Least Significant Difference

m a.s.l. - Metres above sea level

P -Probability value

PDA - Potato Dextrose Agar

SAS - Statistical Analysis System (software)

NaOCl -Sodium hypochlorite

w/v - Weight per volume

+Ve control - Positive control

–Ve control - Negative control

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the research guidelines of Dilla University. No ethical approval for human or animal subjects was required, as the research exclusively used plant material (wheat leaves). Written permission for field sampling was provided by the Bule Hora District Agriculture Office. Verbal informed consent was obtained from all farmers whose land was sampled, following full disclosure of the study's purpose and methods.

Consent for publication

All authors consent to the publication of this manuscript. No personal, identifiable, or restricted data are included. Verbal consent from farmers regarding publication of aggregated, anonymized results was obtained during initial sampling permission.

Availability of data and materials

All data generated or analyzed during this study are included within this published article and its supplementary information files. Raw data on fungal isolation, morphological characterization, and greenhouse growth parameters (including root length, shoot length, spike length, fresh and dry weights, and colonization frequency) are available from the corresponding author upon reasonable request.

Fungal isolates are maintained at the Microbiology Laboratory, Dilla University, and can be accessed for further research with permission from the university.

Competing Interests

The authors declare no competing interests.

Funding Declaration

The Authors received no funding for publication costs."

Authors' contributions

Author1: Conceptualization, methodology, investigation, validation, supervision**,** project administrationformal analysis, writing – review & editing

Author2: Conceptualization, Investigation, data curation, laboratory work, writing – original draft.

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Figures

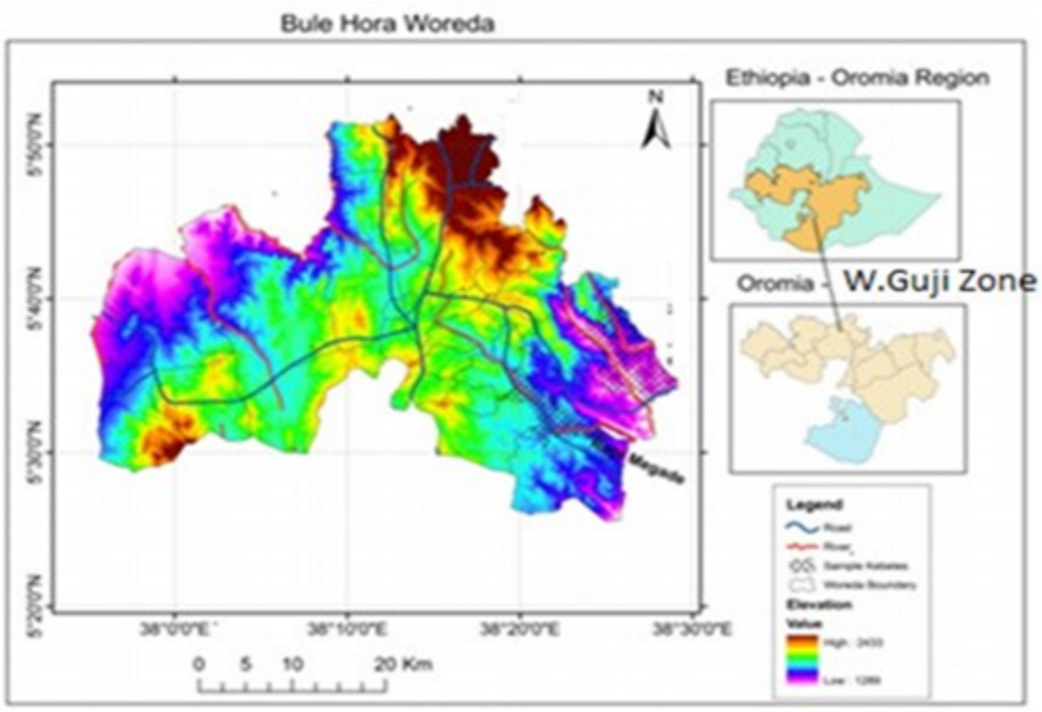


Figure 1

Map of the study area