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BIOINOCULATION OF RHIZOSPHERIC AND BULK SOIL FUNGI ENHANCE GROWTH, QUALITY, AND RESILIENCE OF MAIZE SEEDLINGS

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SUMMARY

Plant growth-promoting rhizospheric fungi (PGPRFs) are fungi mainly present in the soil rhizosphere. Through their mutual interaction with plants, these fungi provide a range of developmental benefits, yet some species of the fungi are harmful. The current study had nine fungi isolated, where five strains were from the rhizosphere and the remaining strains were from the bulk soil of maize (*Zea mays* L.). The research also tested maize seedlings against rhizospheric and bulk soil fungi. The Rhizo Brown and Bulk Gray have shown the highest growth rate compared to all other fungi. Association of isolates with host plants increased growth kinetics and biomass production, as measured by root length (36%), shoot length (37%), fresh weight (37%), dry weight (43%), and chlorophyll (67%) content. Besides, the association also promoted the biosynthesis of Indole Acetic Acid (46%) and Gibberellic acid (30%), improving the nutritional quality in maize. Results of the growth of the fungal strain on the agar plate indicated the absence of their antagonistic effect on each other's growth. It was evident that combining both fungi can serve as bioinoculants to promote plant growth. The interaction between seeds and fungi confirmed the roots of the seedlings move toward the fungus, suggesting a beneficial plant-microbe interaction. Study results revealed that the rhizospheric and bulk soil fungi were plant growth-promoting fungi, improving agricultural productivity and are marketable for wider use in farming practices in Pakistan.

Keywords: Rhizosphere, bulk soil, maize (*Z. mays* L.), IAA (Indole-3-acetic acid), GA (gibberellic acid), nutritional quality, plant-microbe interaction

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Key findings: The isolates of rhizospheric and bulk soil fungi promoted the growth of maize (*Z. mays* L.) seedlings by increasing root and shoot length, biomass, and chlorophyll content. These fungi also increased the production of plant hormones in maize and colonized its roots. The presented study identified these fungi as promising tools for promoting maize growth and potentially improving agricultural practices.

INTRODUCTION

Various microbial communities in soil thrive for plant-microbe interaction both under normal and harsh environmental conditions. Extreme environments support fascinating microbial species as unique ecosystems (Shu and Huang, 2022). Plant microbial communities from demanding environments, such as, species with high pH, salinity, water scarcity or deficiency, and high temperatures provide resources for understanding how microorganisms and plants adapt to adversity (Kumawat *et al*., 2022). The beneficial plant-microbe interaction promotes plant growth, agricultural productivity, and soil fertility. It is common knowledge that certain distinctive and effective microbial strains of plant microbial communities, called plant-growth stimulating (PGS) organisms, help maintain soil vitality under all environmental conditions by promoting and strengthening plant growth, protecting against pathogens (Sagar *et al*., 2021).

As beneficial endophytic microbiomes, plant microbes interact with each other as they invade the internal tissues of the plant (seeds, fruits, flowers, stems, or roots) vertically or horizontally (Negi *et al*., 2023). *Rhizophagus irregularis* CD1 is an arbuscular mycorrhizal fungus positively affecting plant improvement promotion and resistance to cotton diseases (Dowarah *et al*., 2021). Plant-associated microorganisms often support plant health and growth by utilizing several plant growth-promoting (PGP) processes, such as, improving mineral solubility, altering the signaling of phytohormones (auxin, cytokinin, and gibberellin), and directly providing nutrients, in addition to improving resistance to phytopathogens (Patz *et al*., 2021). Since they influence plant physiological parameters under abiotic stress conditions and promote plant development and tolerance to abiotic stress, plant-associated microorganisms are of interest to researchers for use in agriculture. Therefore, it is challenging to effectively administer and utilize PGPMs due to complex interactions arising between the crop, environment, and soil microorganisms, particularly in the rhizosphere (Ayaz *et al*., 2023).

In addition to environmental factors, such as, soil temperature and pH, the factors controlling these interactions may also depend on the genotype and the microbes already present in the soil (Dastogeer *et al*., 2020). Plant growth-promoting fungi are a diverse group of non-pathogenic fungi known to promote plant growth by acting on the rhizosphere. They can further comprise three groups: free-living PGPF, occurring throughout the rhizosphere; epiphytes, living freely on the surface of the roots; and endophytes, living within the roots in the inter- and intracellular spaces and directly exchange metabolites with the plants (Malgioglio *et al*., 2022). These fungi belong to the phyla Ascomycetes, Basidiomycetes, and Oomycetes and are soil-dwelling organisms. Some of the most isolated genera are *Aspergillus, Fusarium, Gliocladium, Penicillium, Phoma* and *Trichoderma*, the latter being the most separated genus from different soils (Malgioglio *et al*., 2022). Talking about PGPF refers to the fungi *ectomycorrhiza* and *endomycorrhiza*, which usually cause mutualism without necessarily being tied to the host plant (Malgioglio *et al*., 2022).

On the other hand, for these organisms to produce asexual spores throughout their life cycle, they must establish a mutualistic connection with their host plant (Giovannini *et al*., 2020). The positive effect of widespread rhizosphere fungi on plant growth, especially in agricultural plants, has gained extensive studies. Injection with rhizosphere fungi is a successful method for improving host plant establishment and resistance to stressors, such as, nutrient deficiency, drought, and soil disturbance (Orozco-Mosqueda *et al*., 2022). Some authors have suggested that mycorrhizal fungi could enhance seedling performance by increasing plant nutrient uptake, particularly nitrogen (N) and phosphorus (P), or promoting soil aggregation in degraded soils (Mohamed *et al*., 2022). Plant growth-promoting rhizospheric fungi participate in several essential ecosystem processes, including biological control of plant diseases, nutrient solubilization, and phytohormone production, and therefore, require special attention for sustainable agriculture.

Mortierella species with plant growth-promoting properties have emerged from agricultural soils (bulk soil). Several strains in this genus are plant growth-promoting fungi (PGPF) that can occur in soil, rhizosphere, and plant tissue (Ozimek and Hanaka, 2020). These microorganisms are also commonly existing in harsh environments, where they are responsible for improving soil access to bioavailable forms of phosphorus (P) and iron (Fe), synthesis of phytohormones and 1-aminocyclopropane-1-carboxylate (ACC) deaminase, and are responsible for protecting agricultural plants from pathogens (Ozimek and Hanaka, 2020). Hence, this study aimed to evaluate the biochemical and phytohormonal responses of maize (*Zea mays* L.) to rhizospheric and bulk fungi associations and determine the potential beneficial role of the isolated fungi on the growth and development of maize.

MATERIALS AND METHODS

Plant material and treatment conditions

Maize (*Zea mays* L.) plant samples' collection came from Turo Mardan, Khyber Pakhtunkhwa, Pakistan, to isolate the fungus living in their bulk soil and rhizosphere. The loosely attached soil to the roots easily fall by gently shaking off the fine roots is the bulk soil. Inversely, the soil tightly attached to fine roots is the rhizosphere. Serial dilutions proceeded to isolate rhizospheric and bulk fungi. Preparing a soil suspension for serial dilutions included 1 g of rhizospheric soil and 1 g of bulk soil added to separate test tubes containing 10 mL of distilled water. The first dilution (10-1) comprised 1 ml of the removed soil suspension and transferred to another test tube with 9 ml of autoclaved distilled water. Until achieving a dilution of 10-6, repetition of the process consisted of adding 1 ml of the successive dilutions to another test tube. The synthesis of PDA followed the stated protocol (Westphal *et al*., 2021). From the mixed culture that had formed on agar plates, various fungal colonies' recognition bore markings on the parent plates. The fresh PDA plate was central, with a small square section removed from each colony. Examining the purity of the colony continued after an incubation period of 3–5 days at 28 °C. Each isolated fungus was cultured individually in 500 mL capacity round-bottom flasks with a volume of 50 mL per flask. The stated process helped prepare the Czapek medium (Qadir *et al*., 2022). Maize seeds, sourced from the market, were surface-sterilized with 70% ethanol for 30 seconds and subsequently washed five times with distilled water. Thoroughly cleaned maize seeds were planted in plastic containers, each filled with 300 g of soil.. One gram of fungal biomass was added to 100 g of autoclaved soil. Three replicates of pots, each containing five seeds, were arranged in a completely randomized design.

Growth kinetics of *Zea mays* L

Seedlings harvested after 30 days had their growth parameters, such as, root length, fresh weight, biomass, and dry weight measured.

Biochemical determination in plant

The described procedure for the measurement of chlorophyll A, B, and carotenoids followed established conventions (Iosob *et al*., 2019). Standard protocols were applied for the measurement of IAA (Mehmood *et al*., 2020) and GA (Ismail *et al*., 2016)accordingly. The described technique for the Salkowski reagent was also performed (Hatamzadeh *et al*., 2023).

Root colonization assay, culturing, and microscopy of fungal morphology

Standardized protocols for root colonization (Kiheri *et al*., 2017) and the study of fungal morphology (Agu and Chidozie, 2021) were followed, accordingly.

Fungal interaction and their activities

Studying different fungal interactions followed the standard protocol, as described by Paludo *et al*. (2019). The study of the interaction between fungal spores and seeds used sterilized Petri dish plates with the prepared water-agar media, where the agar concentration was 0.6% to 0.8%. The medium poured into the Petri dish plates reached partial solidification. One-half of the plate was inoculation with the fungal spores using a sterilized inoculum loop. The sterilized corn seeds were placed at the center of the Petri plate.. Observations on the effect of rhizospheric, bulk, and Rhizoplane solution on the fungal growth were conducted, following the described protocol for this activity (Barillot *et al*., 2013).

Fungal filtrate and seed interaction activity

Corn seeds' sterilization, drying, and growing continued on 0.6% to 0.8% sterilized water-agar medium in sterilized Petri dish plates. Fungal culture filtrate was added to the walls of agar plates, which were then incubated to allow seed germination and growth. The plates were inspected daily for any effects on fungal inhibition and seed interaction activity. The collected sterilized Petri dishes and water agar maintained the agar concentration at 0.6% to 0.8%. The medium was poured into the Petri dishes and allowed to solidify. One-half of the plate was inoculated with a fungal block using a sterilized inoculation loop. Sterilized corn seeds were then placed in the center of the Petri dish.

Statistical analysis

The experiment had three repetitions under the same conditions. The derived data came entirely from random design experiments. Analysis of variation (ANOVA) followed the Duncan's multiple range test (DMRT) using the SPSS software (IBM SPASS statistic 21) to determine the significant level ($P < 0.05$). Graphs were generated using GraphPad Prism.

RESULTS

Isolation of rhizospheric and bulk fungi

Nine rhizospheric and bulk fungal strains came from the rhizospheric and bulk soil of maize grown in areas of the District Mardan, Pakistan for planned experiments. All strains obtained sustained subculturing onto PDA media plates to achieve purified strains and stored at 4 °C for further experiments (Figure 1).

Effect of rhizospheric and bulk fungus on growth kinetics of maize

Root length measurements of control and treated plants were taken 30 days after seed germination. All the isolated fungal strains enhanced the root growth of maize seedlings. The highest root length resulted in Bulk gray and Rhizo brown associated seedlings, which were 56% and 53% greater than the control. Similarly, the seedlings associated with Rhizo new (15%), Rhizo gray (26%), Rhizo black (35%), Rhizo white (48%), Bulk brown (27%), Bulk black (16%), and Bulk new (49%), significantly enhanced seedling root length, compared to the control, as shown in Figure 2a.

Shoot length measurements were taken after 30 days. Different strains of fungus showed positive effect on the shoot length. Among all the fungal strains, Bulk gray and Rhizo brown have significantly increased shoot

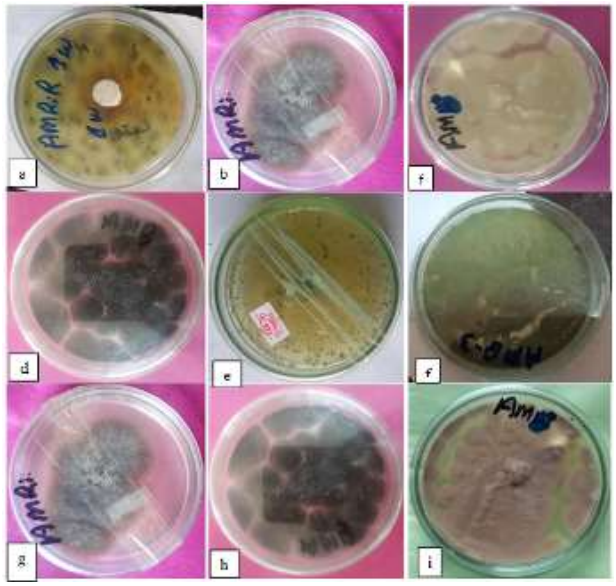

Figure 1. Colonies of the isolated fungi from the rhizosphere and bulk soil of maize plants on PDA. a, b, c, d, and e were rhizospheric, and f, g, h, and i were the bulk fungi.

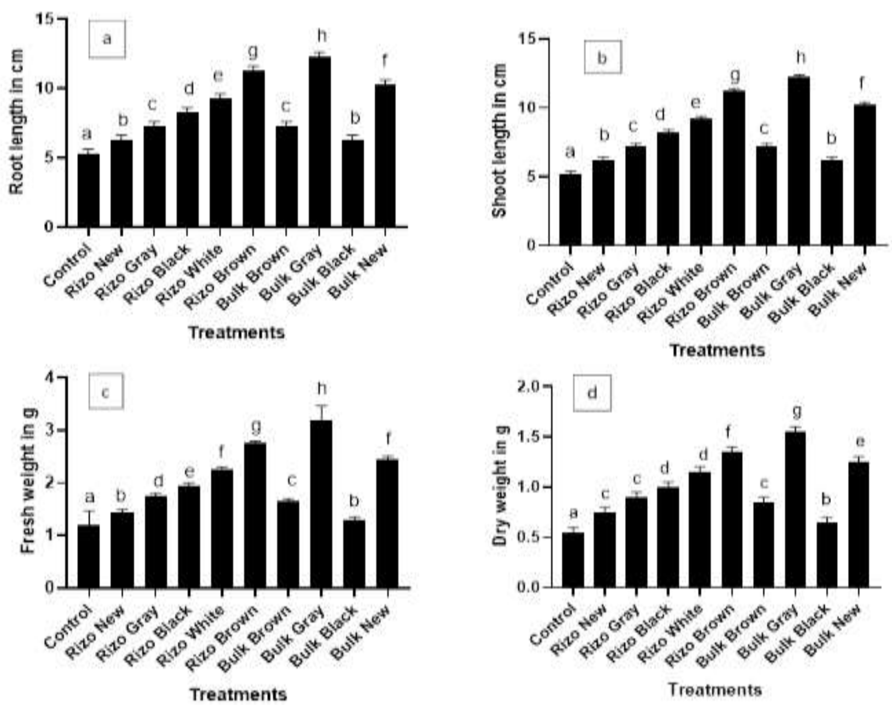

Figure 2. Effect of fungi isolated from the rhizospheric and bulk soil of maize on a) root length, b) shoot length, c) fresh weight, and d) dry weight of maize seedlings grown under laboratory conditions. Data calculations came from three replicates with their respective error bars.

length by 57% and 54%, respectively, as compared toto the control. Besides, all other strains have also increased the shoot length of maize (Rhizo new by 16%, Rhizo gray by 27%, Rhizo black by 36%, Rhizo white by 43%, Bulk brown by 28%, Bulk black by 17%, and Bulk new by 50%), versus the control, as presented in Figure 2b.

All the fungal strains exhibited various effects on the fresh weight of maize plants. The isolated fungal strains Bulk gray and Rhizo brown increased fresh weight in fungus-associated maize plants by 62% and 56%, respectively, as compared toto the control. Fungus Rhizo new (17%), Rhizo gray (31%), Rhizo black (38%), Rhizo white (46%), Bulk brown (27%), Bulk black (7%), and Bulk new (50%) also boosted fresh weight, as compared toto the control (Figure 2c).

The fungal strains isolated from rhizospheric and bulk soil of maize positively affected the dry weight of maize plants. The strains Bulk gray and Rhizo brown have more increased dry weight in fungus-associated maize plants by 64% and 59%, respectively. Meanwhile, other fungal strains—Rhizo new (26%), Rhizo gray (38%), Rhizo black (45%), Rhizo white (52%), Bulk brown (35%), Bulk black (15%), and Bulk new (56%)—increased the dry weight in fungus-associated maize plants, as compared toto the control (Figure 2d).

Effect of rhizospheric and bulk fungi on chlorophyll content and carotenoid

The concentration of chlorophyll A was measured in the leaves of fungi-associated maize seedlings. The fungal strains had favorably influenced maize plants in increasing chlorophyll A concentration. The highest chlorophyll A resulted in Rhizo brown and Bulk gray associated seedlings, which were 96% and 95%, respectively, greater than the control. The rhizospheric fungal strains Rhizo new (15%), Rhizo gray (84%), Rhizo black (66%), and Rhizo white (94%) increased the chlorophyll A level, as compared toto the control. The fungus isolated from bulk soil, such as, Bulk brown (75%), Bulk black (26%), and Bulk new (60%) also enhanced the chlorophyll A (Figure 3a).

The fungal strains had a positive effect on maize plants in raising the chlorophyll B concentration. The highest chlorophyll B was evident in Rhizo black and Bulk black associated seedlings that were (89% and 87%, respectively) superior to the control. The rhizospheric fungal strains Rhizo new (30%), Rhizo gray (65%), Rhizo white (85%), and Rhizo brown (77%) increased the chlorophyll B level, as compared toto the control. The fungus isolated from bulk soil, such as, Bulk brown (80%), Bulk gray (82%), and Bulk new (83%) also enhanced the chlorophyll B (Figure 3b).

The fungal strains had benefitted the maize plant by increasing carotenoid concentration. The highest carotenoids appeared in Bulk brown and Rhizo white associated seedlings, which were 74% and 73%, respectively, greater than the control. The rhizospheric fungal strains Rhizo new (40%), Rhizo gray (65%), Rhizo black (63%), and Rhizo brown (51%) increased the carotenoids level versus the control. The fungi isolated from bulk soil, such as, Bulk gray (69%), Bulk black (49%), and Bulk new (59%) also boosted carotenoids (Figure 3c).

Determination of indole acetic acid (IAA) and gibberellic acid (GA) in culture filtrates

Both the rhizospheric and bulk fungal strains demonstrated the production of IAA using the Salkowski reagent. The rhizosphere and bulk fungi produced IAA and GA in different concentrations. Among the selected strains, Rhizo gray and bulk black released the highest quantity of 26.41 and 17.51 μg/mL of IAA, while Bulk brown and Rhizo white released the highest quantity of 100.37 and 84.80 μg/mL of GA in the culture filtrate. All other fungal strains produced IAA and GA in different concentrations, as shown in Figures 4a and b.

Indole acetic and gibberellic acids' determination

Fresh leaves of one-month-old maize seedlings had 57.74 μg/g of IAA, while fresh roots of one-month-old maize seedlings had 40.78 μg/g

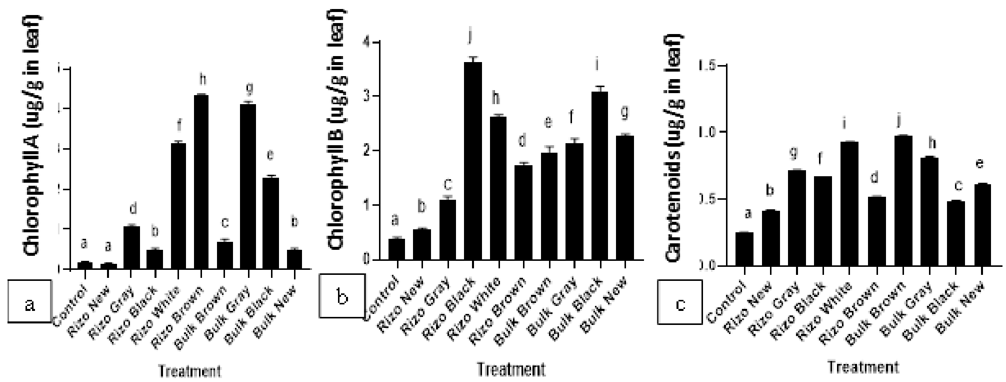

Figure 3. Effect of fungi isolated from the rhizospheric and bulk soil of maize on a) chlorophyll-A, b) chlorophyll-B, and c) carotenoids of maize seedlings grown under laboratory conditions. Data calculations came from three replicates with their respective error bars.

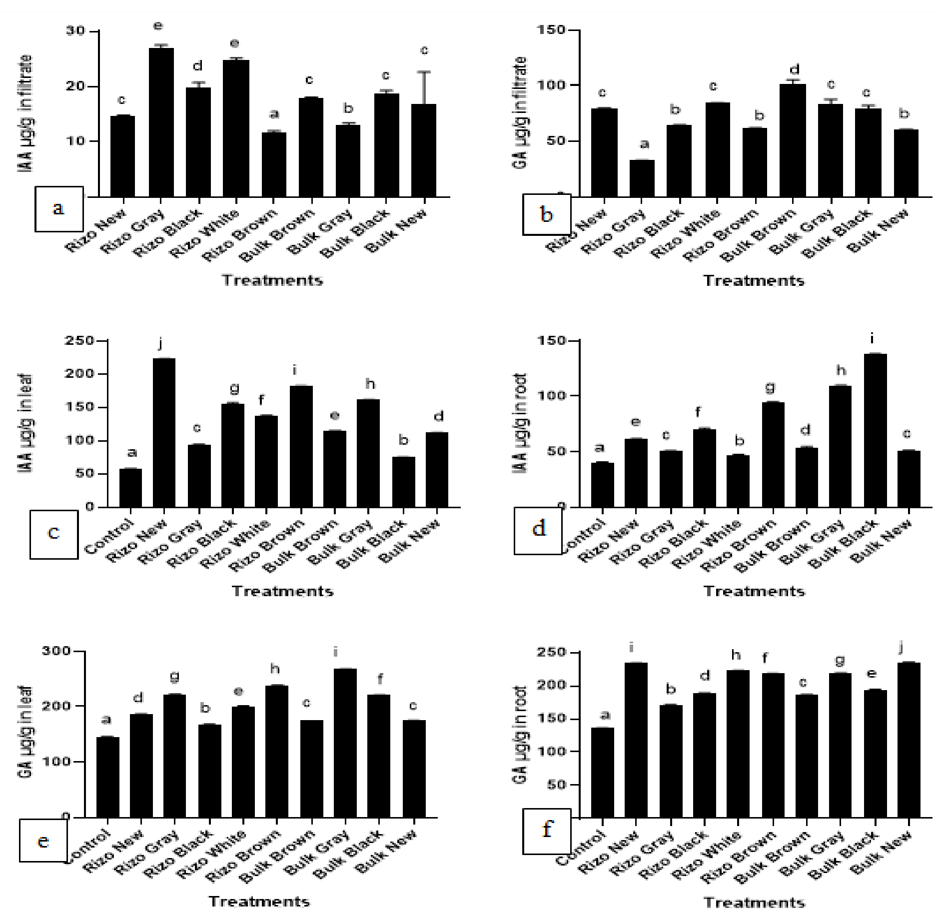

Figure 4. Exogenous secretions of a) IAA and b) GA by selected rhizospheric and bulk fungi in their culture filtrates grown in the Czapek medium in a shaking incubator at 28 °C and 120 rpm for seven days. Effect of fungi isolated from the rhizospheric and bulk soil of maize on c) IAA in the leaf, d) IAA in the root, e) GA in the leaf, and f) GA in the root of maize seedlings bred under laboratory conditions. Data calculations came from three replicates with their respective error bars. Seedling grew for 30 days at room temperature and a photoperiod of 18 hours.

of IAA. The endogenous level of IAA was highly superior in Rhizo new (74%) and Bulk gray (64%) in leaves, while Bulk black (70%) and Rhizo brown (57%) in roots, as compared to the control. All other fungal strains also increased IAA concentrations in leaves and roots of the maize plant in various concentrations (Figures 4c and d).

Fresh leaves of one-month-old maize seedlings had 145.67 µg/g of GA, and fresh roots of the seedlings had 136.11 µg/g of GA. The endogenous level of GA was greater with Rhizo brown (38%) and Bulk gray (45%) in leaves, while Rhizo new (41%) and Bulk new (42%) in roots than the control. All other fungal strains enhanced GA concentrations in leaves and roots variably, as shown in Figures 4e and f.

Root colonization and fungus microscopy

All rhizospheric and bulk fungi could colonize the cortical area and epidermis tissues of corn roots. The tribes colonized the division zone, the elongation zone, and the differentiation zone equally (Figure 5).

The microscopic images revealed that the sporangiophore of some strains was aseptate, but some strains were septate, and the sporangia had an almost spherical shape. The hyphae were almost unbranching and unicellular, filled with cytoplasm and a single nucleus, but some hyphal stems appeared branched. In some strains, the conidia carriers were also aseptic, yet in some strains, the conidia were septate. The conidia were star-shaped before dispersal but became teardrop-shaped when released into the depressed arms. The conidia size varied depending on the culture medium and substrate. Conidia production occurred within a cell, but reached exposure to the external environment upon maturity. In the Bulk New strain, the sporangia also appeared in the middle of the sporangiophores (Figure 6).

Fungi-fungi and seedling-fungi interaction

The brown and black fungal strains did not influence each other's growth when cultured side by side on an agar plate. After three days of growth, their hyphae mixed freely with each other, not inhibiting each other's growth. The study concluded that both fungi in combination could serve as a bioinoculant for plant growth and development.

The interaction between fungi and seeds of maize on Petri plates is explainable with a particular focus on the phenomenon of movement of seedling roots toward the fungus and subsequent root colonization. During this activity, we noticed the seedling roots moved toward the fungus and colonized it.

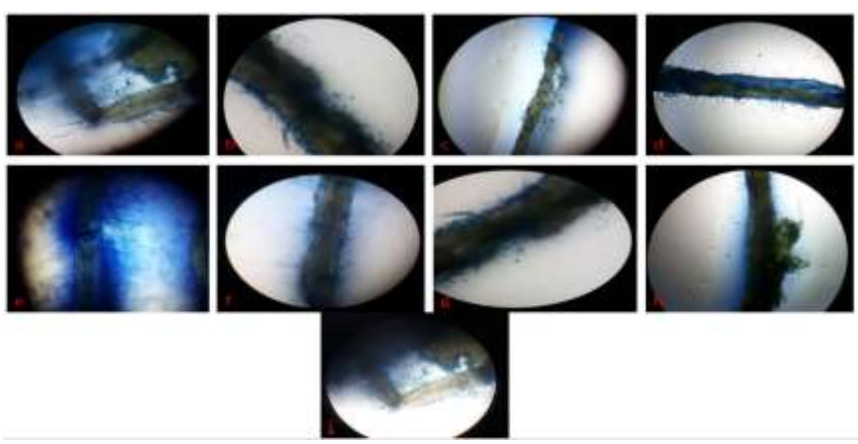

Figure 5. Root colonization of the fungi arising from the rhizospheric and bulk soil of maize a) Rhizo white, b) Rhizo new, c) Rhizo gray, d) Rhizo brown, e) Rhizo black, f) Bulk new, g) Bulk black, h) Bulk brown, and i) Bulk gray.

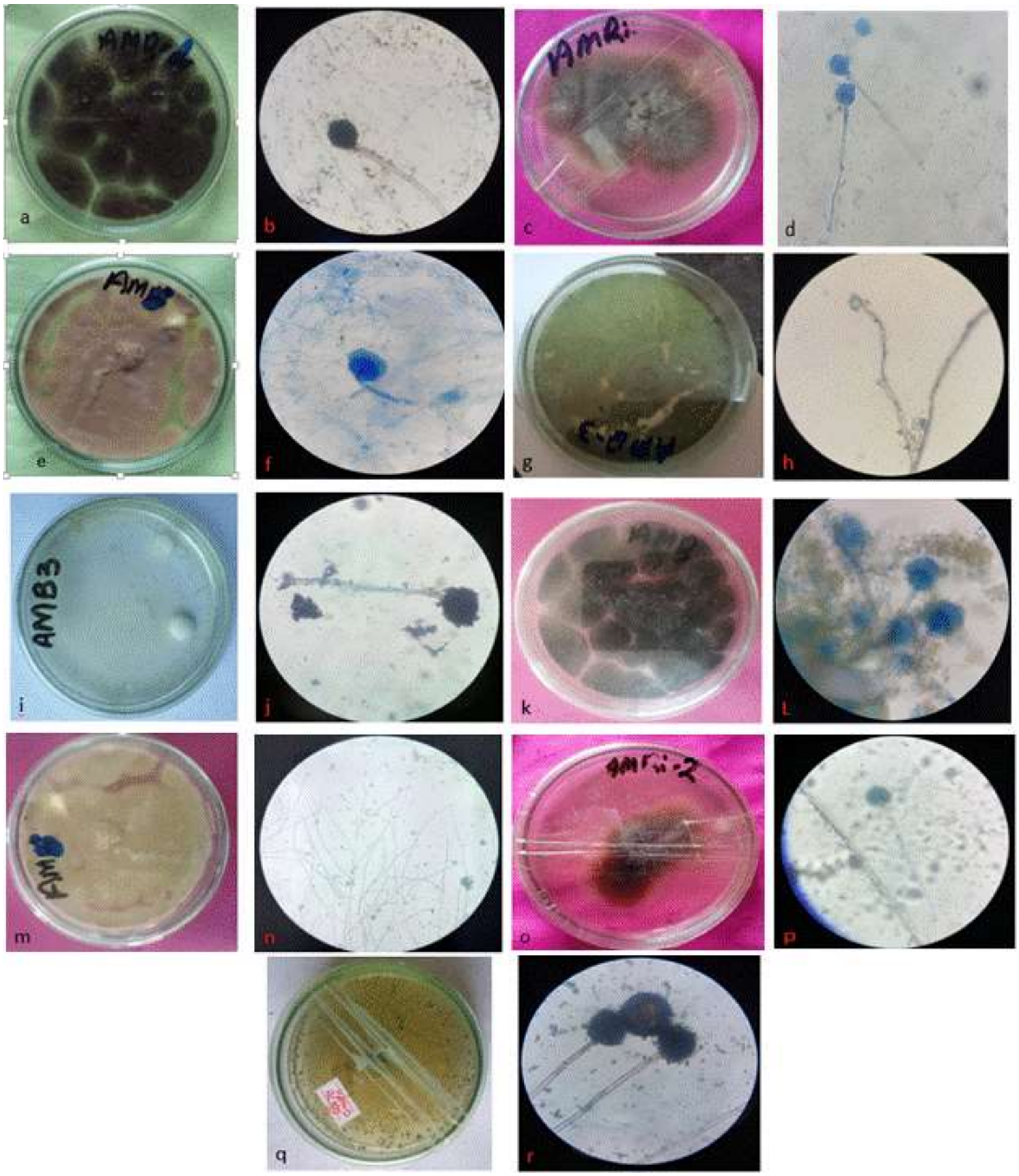

Figure 6. Colonies of the isolated fungus and microscopy: a,b) Rhizo black, c,d) Rhizo new, e,f) Rhizo brown, g,h) Rhizo gray, i,j) Rhizo white k,l) Bulk black, m,n) Bulk brown, o,p) Bulk new, and q,f) Bulk gray.

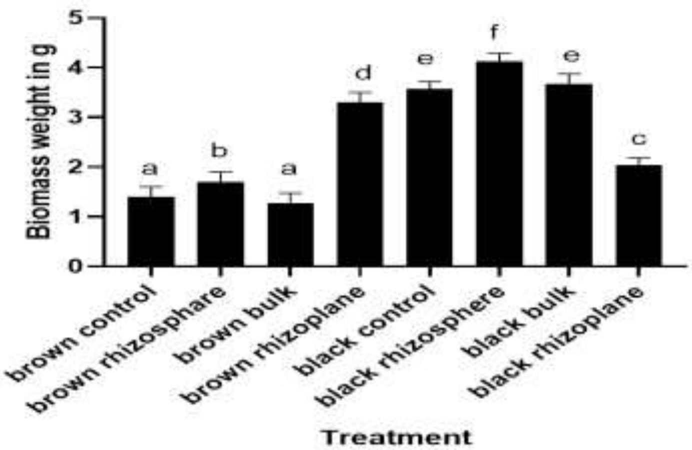

Figure 7. Effect of rhizosphere, rhizoplane, and bulk solution on fungal growth in a Czapic media.

Rhizospheric activity and root-microbe interaction

The biomass of the brown strain in the rhizosphere is 21% and that of the rhizoplanes is 57% greater than the control; however, the biomass bulk reduction is 14%, compared to the control. The biomass of the black strain in the rhizosphere is 17% and the bulk is 2.8% greater than the control; however, the biomass reduction of the rhizoplane is 42%, compared to the control (Figure 7).

During the experiment, we found that the roots of the seedling moved toward the fungal filtrate and colonized with it. The interaction between the fungal block and seeds of maize on Petri plates can refer to a particular focus on the phenomenon of movement of seedling roots toward the fungal block and subsequent root colonization. It was evident that the roots of the seedling were moving toward the fungal block.

DISCUSSION

The isolation of rhizospheric and bulk fungi contains different fungi collected from the soil surrounding the plants. Various studies showed differences in fungal communities between the rhizosphere and bulk soil (Qiao *et al*., 2019). A diversity of culturable fungi was available in the rhizosphere and bulk soil area of the maize plants collected from different locations in District Mardan, Pakistan. Our results showed the diversity of fungal phyla was comparable both in the rhizosphere and bulk soil. However, five strains reached isolation from the rhizosphere and another four from the bulk soil. Previous studies have shown bulk soils have greater fungal diversity than the rhizosphere region. This may be due to the rhizosphere effect, which is selective. However, the fungal operational taxonomic units (OUT) variety indices of the rhizosphere were lower than the bulk soil. Previous findings also revealed soil had a larger microbial library, which may help build the microbial community for the next season (Essel *et al*., 2019).

The role of rhizospheric and bulk fungi on growth attributes of maize showed how fungal communities in the rhizosphere and bulk soil enhanced plant growth and biochemical parameters. The current study highlighted fungal diversity and abundance are different in the rhizosphere and bulk soil, which has support from previous findings where the rhizosphere exhibits higher fungal diversity, especially during plant growth and flowering phases (Rüger *et al*., 2021). Plant growth strongly affects fungal alpha diversity within the rhizosphere compared to the bulk soil having soil enzyme activities and physical structure, thus, playing a role in microbial communities. The response of microbial groups toward factors like nitrogen treatment tends to be higher in maize rhizosphere than soybean, confirming plant-specific effects on fungal communities (Wang *et al*., 2017).

The effect of rhizospheric and bulk fungi on chlorophyll and carotenoid levels in plants is significant, enhancing plant health and growth. The presence of specific rhizo-fungus leads to variation in photosynthetic pigments, influencing photosynthesis and plant pigmentations, crucial for plant growth and nutrient uptake (Ahamad and Siddiqui, 2021). During the latest research plan, the isolated strains' screening for maize determined their interaction and plant-beneficial potential. Our data showed that all isolates were able to colonize within maize seedlings and promote their growth. They influenced the accumulation of pigments (chlorophyll and carotenoids) in the host seedlings. For example, inoculated seedlings had up to 95% more chlorophyll-A than non-inoculated seedlings. Chlorophyll production was beneficial as a measure of the plant's net physiological iron availability. These types of fungal strains seemed of greater interest to counteract iron deficiency in plants on demanding soils and increase agricultural yields (Prisa *et al*., 2023). Improved chlorophyll accumulation emerged with a significant increase in root/shoot length and seedling biomass in this research. Previous studies have also reported that most rhizosphere-dwelling fungi have shown to interact with host seedlings and promote their growth (Roberts, 2022).

The isolated fungus produced culture filtrates, which is evident from the results of quantitative tests. Previous research has also displayed that a diverse fungal population is capable of producing IAA, although the isolates from different sources showed different levels of their production (Lestari *et al*., 2021). We concluded that the isolated fungi increased phytohormone levels in the host seedlings. The phytohormones tested in our experiment were IAA and GAs, which are crucial for plant growth and development. The isolated fungi can produce Indole Acetic Acid (IAA) in culture filtrates, which significantly influenced plant growth and development. IAA is a crucial plant hormone that regulates various aspects of plant physiology. It confirms that these fungi have the potential to produce IAA, thereby, promoting plant growth and enhancing plant-microbe interactions (Asrul *et al*., 2020). Therefore, the higher values may have contributed to greater growth and pigment accumulation in our research. The ability of rhizospheric and bulk soil fungi to promote plant development may correlate to the synthesis of growth hormones, such as, IAA. The isolated rhizospheric fungus produces phytohormones in culture filtrates, which play an essential physiological role in the interaction between microbes and plants. Like other free-living fungi, the rhizospheric fungi are also capable to produce several types of phytoregulators, including IAA and gibberellic acid (GA). Furthermore, the rhizospheric fungi synthesized GA, which promotes plant cell development and elongation. Documentations proved that gibberellic acid is a key in alleviating salt stress by enhancing maize growth and increasing chlorophyll content, total protein levels, and potassium ion concentration. It also helps reduced oxidative stress and sodium ion accumulation under salinity conditions (Shahzad *et al*., 2021).

Interestingly, all isolates were able to colonize in the roots of the host seedlings in this study. As reported earlier, the fungus colonized the root cortex, endodermis, and even the vascular bundles, showing their ability to colonize extensively within the maize roots' system (Sukno *et al*., 2008). Their colonization was more dominant in the epidermal and cortical areas. The hyphae projected from the root surface would benefit the host seedling by increasing the surface area of the roots for efficient absorption. In arbuscular mycorrhiza, the hyphae of the fungus penetrate the root cells and create a branched structure known as an arbuscle, which significantly increases the roots' surface area for nutrient absorption (Ebbisa, 2022). Results have shown both the rhizospheric and bulk fungi make associations with seeds that support seedlings and the germination process at early stages of plant development (Nelson, 2018; Karabayev *et al*., 2024). Previous studies have also indicated the most common areas of fungal colonization are the epidermis and the cortex area. The hyphae of arbuscular mycorrhiza, the most popular type of mycorrhizal association, tend to colonize the epidermis and bark of the roots (Pujasatria *et al*., 2022). Root colonization is considerably a critical step in the beneficial connection between fungi and host plants and under these circumstances, both partners benefit. A conclusion could be the maize plant had several beneficial fungi in its rhizosphere, as well as, in the soil. These fungi appeared to have the same potential to promote the growth of host plants by modulating their endogenous phytohormone levels.

The results confirmed that rhizosphere and bulk soils are key zones where plant-microbe interactions occur, influencing root exudates, supporting a diverse microbial community, and nutrient cycling. Studies confirmed these interactions influence plant growth, nutrient uptake, and stress tolerance because of the plant-microbe symbiosis and ecosystem functioning (Rüger *et al*., 2021).

CONCLUSIONS

Maize (*Zea mays* L.) grown in the District Mardan, Pakistan, supported a diverse group of rhizospheric and bulk fungi. The current study suggested the rhizosphere and bulk fungi significantly increased host biomass, primary metabolites (chlorophyll A, B, and carotenoids), and phytohormones (IAA and GA) production, as well as, enhanced growth parameters (RL and SL) and plant development. Thus, the use of microorganisms, such as, rhizosphere and mass fungi, opens a new perspective for increasing plant productivity and protecting the agricultural sector from dangerous effects of chemical fertilizers.

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