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ROLE OF MYCORRHIZA IN MODULATING MICROPLASTIC EFFECTS ON PEA (PISUM SATIVUM)

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ABSTRACT
This study investigated the role of arbuscular mycorrhizal fungi (AMF) in modulating the effects of microplastics on the growth performance of peas (Pisum sativum). Pea plants were grown under controlled pot culture conditions with treatments including AMF inoculation, microplastic addition, combined AMF and microplastic, and a control group. Growth parameters, such as dry and fresh weights, shoot and root lengths, number of leaves, pea pods, and yield, were recorded at 15-day intervals. The results indicated that AMF inoculation significantly enhanced plant growth and yield, whereas microplastics negatively affected these parameters. However, co-inoculation of AMF with microplastics mitigated some of the adverse effects of microplastics, improving growth vigor and yield compared to microplastic treatment alone. These findings highlight the potential of AMF to support sustainable agriculture by alleviating microplastic-induced stress in pea plants.
KEYWORDS: Mycorrhiza, **Microplastic**

INTRODUCTION
Peas (Pisum sativum) are crops of high commercial significance. Peas belong to the Leguminosae family (Genus: Pisum, subfamily: Faboideae tribe: Fabaceae), which has an important ecological advantage owing to its contribution to the development of low-input farming systems by fixing free atmospheric nitrogen and serving as a break crop that further minimizes the need for external inputs. Peas (Pisum sativum) are important pulse crops. Pea cultivation requires a cool growing season with a moderate temperature range. The best temperature for germination is approximately 22°C and for plant growth and development13°C to 18°C. Peas (Pisum sativum) require less rain and low humidity for proper growth and development. Pea crops can be grown in various soil categories. Well-drained soil with ample moisture-retaining capacity, such as deep loam soil, is considered the best for its cultivation.
The term mycorrhizae comes from the Greek word ‘mykes’ and ‘rhiza’, meaning ‘fungus’ and ‘root, ’ respectively, which was first applied to the association of trees with fungal symbionts .3 Arbuscular mycorrhizal fungi (AMF) are the most common type of mycorrhizae and form symbiosis with over 80% of terrestrial plant species. AMF plays an active and important role in improving plant nutritional status (especially for the acquisition of nutrients with poor mobility in soils). The improved effects of AMF inoculation on plant biomass are more obvious in legumes than in non-legumes (**Wu et al., 2024a, 2024b).**
The use of mycorrhizal symbionts has proven to be effective and beneficial for the sustainable growth of agricultural cropping systems. These fungal symbionts solely rely on the host system to fulfill their carbon requirements, and in exchange, AMF provides numerous benefits to the plant system in terms of sustainable nutritional flow, improved plant development, productivity, yield stress tolerance, water uptake enzymatic antioxidants accumulation, and soil fertility **(Dhiman et al., 2022)**.
The use of beneficial microorganisms (e.g., plant growth-promoting bacteria (PGPB) and arbuscular mycorrhizal fungi (AMF)) can enhance agricultural production, as these microorganisms can improve soil fertility and plant tolerance to environmental stresses, thus enhancing crop yield in an eco-friendly manner (**Thoker & Patel, 2020).** Mycorrhization benefits plants by upregulating the catalytic activities of soil enzymes, assisting in the breakdown of complex organic compounds in the soil, and positively influencing other microbes present in the rhizosphere for improved nutrient uptake. Activation of these mechanisms, in turn, provides the ability to withstand drought stress, alleviate salinity, aid in micronutrient absorption and water absorption, and defend the plants (**Dhiman et al., 2022).** AMF-induced growth enhances the absorption of water and mineral nutrients from the surrounding soil while safeguarding plants against fungal infections (**Umer et al., 2025**). Therefore, AMFs are inevitable endosymbionts that influence plant productivity and ecosystem functioning. It is essential to improve crops sustainably (**Umer et al., 2025**).
AMF form a symbiotic association with almost two-thirds of terrestrial plants in ecosystems, providing essential nutrients and supporting their growth (**Umer et al., 2025**). AMF develop synergistic interactions with plants in the ecosystem by colonizing their root systems, contributing to enhanced water uptake and nutrient absorption , as well as increased resistance to various biotic and abiotic stresses (**Umer et al., 2025**). AM Fungi inoculation significantly increased the biochemical constituents, especially chlorophyll-a, chlorophyll-b and total chlorophyll.

Mycorrhizae are obligatory biotrophs that depend on their host plants for nutritional requirements. They act as key contributors to sustainable agro-ecological enforcement and have a global impact on eco-systemic processes. These soil inhabitants devote themselves to continuous nutrient flow and extemporize resistance against various environmental stresses, such as drought, flood, metal toxicity, and salinity. (**Dhiman et al., 2022).**
AM fungi are critically important endosymbionts that play an effective role in improving plant productivity and the sustainability of ecosystems, and AM fungal symbiosis also improves tolerance to different biotic and abiotic stresses. The strategy of using native AM fungal species from soil has always proven beneficial for plant nutrition, adaptation to stress conditions, and productivity (**Dhumal & Shinde, 2020).**
Microplastics are a complex group of compounds encompassing a wide range of properties in terms of size, shape, chemistry, state of weathering, and additives, both intentional and unintentional. They are either produced as micro-sized particles (< 5 mm), the so-called primary microplastics, or reach this size through fragmentation from larger pieces, the secondary microplastics. Sewage sludge is an important source of primary and secondary microplastics in agricultural soils. Wastewater treatment plants filter out solid particles originating from roads and households, such as tire abrasion particles and fibers from washing synthetic clothes. Filtered particles are retained in sewage sludge, which is used in large quantities as a fertilizer **(Lehmann et al., 2022).**
AM fungi help plants respond positively to microplastic fiber contamination of the soil, likely by supporting the plant in nutrient uptake **(Lehmann et al., 2022).** Data were recorded at the interval of 15 days to observe the combined effect of arbuscular mycorrhizal fungi and microplastics on the growth performance of pea plants. Data during the treatment of mycorrhizal plants, mycorrhizal plants with microplastics, plants with microplastics only, and control plants were recorded from all four types of setups. During the treatment, it was observed that growth performance and growth vigor index were much higher in plants treated with co-inoculation of mycorrhiza and microplastics, whereas plants treated with microplastics or mycorrhizal inoculation alone showed lower growth vigor index and growth performance.

1. To evaluate the effects of arbuscular mycorrhizal fungi (AMF) inoculation on the growth performance and yield of pea (Pisum sativum) plants under controlled pot culture conditions.
2. To assess the impact of microplastic contamination on vegetative growth, reproductive output, and overall productivity of pea plants.
3. To investigate the combined influence of AMF inoculation and microplastic addition on mitigating microplastic-induced stress in pea plants.
4. To compare key growth parameters—dry and fresh biomass, shoot and root length, leaf number, pod number, and seed yield—across treatments: control, AMF alone, microplastics alone, and combined AMF with microplastics.
5. To elucidate the role of AMF in enhancing nutrient uptake, water absorption, and rhizosphere microbial interactions under microplastic stress conditions.
6. To explore the feasibility of using AMF inoculation as a sustainable agricultural strategy to improve crop resilience and productivity in soils contaminated with microplastics.
7. To identify the limitations of AMF in fully overcoming microplastic-induced growth constraints and recommend further research on the underlying biochemical and physiological mechanisms, including field-level validation.
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20. To explore the feasibility of using AMF inoculation as a sustainable agricultural strategy to improve crop resilience and productivity in soils contaminated with microplastics.
21. To identify the limitations of AMF in fully overcoming microplastic-induced growth constraints and recommend further research on the underlying biochemical and physiological mechanisms, including field-level validation.

Highlights

• Pot experiment tested AMF, microplastics, AMF + microplastics and control in pea with 10 replicates each.
• AMF inoculum was a six-species mix (Acaulospora, Glomus and Scutellospora) at 260 propagules/100 g.
• Growth and yield traits were recorded every 15 days for 60 days, including biomass, height, leaves, pods, and seeds.
• AMF alone maximized performance, yielding 1.38 g dry mass, 6.1 pods, and 80 g seed yield, approximately fourfold over the control.
• Microplastics reduced reproductive output, with dry mass of 0.86 g and seed yield of 36 g, despite slightly taller plants than the control.
• AMF co-inoculation partly mitigated microplastic stress, increasing the dry mass to 1.12 g and seed yield to 42 g.
• Findings support the use of AMF to sustain pea productivity in microplastic-contaminated soils, although AMF did not outperform AMF-only.

MATERIALS AND METHODS

Preparation of experimental set up

Pea (Pisum sativum) seeds were sown in pots under controlled pot culture conditions with four treatment groups: (1) Control (no AMF or microplastics), (2) AMF inoculation only, (3) Microplastic addition only, and (4) Combined AMF inoculation and microplastic addition. Each treatment was replicated ten times to ensure statistical reliability. The AMF inoculum consisted of a mixture of species: Acaulospora denticulata, Acaulospora gerdemannii, Glomus macrocarpum, Glomus maculosum, Glomus fasciculatum, and Scutellospora minuta, with a propagule density of 260 AM propagules per 100 g soil. Ten grams of mycorrhizal soil were mixed into the pots at sowing for the AMF and combined treatments.

AM fungi have been shown to help retain soil moisture and facilitate the uptake of important nutrients during stressful conditions (**Thakur & Shinde;2020)** Plants were maintained under standardized environmental conditions optimal for pea growth (temperature range 13–22°C, well-drained loam soil with adequate moisture). Growth parameters were recorded every 15 days over a 60-day period, including the fresh and dry weights of shoots and roots, shoot and root lengths, number of leaves, number of pea pods, seed weight, and total seed yield (**Thakur & Shinde;2020)**. Measurements were taken at 15, 30, 45, and 60 d after sowing. Statistical analyses were conducted to compare the growth and yield parameters across the treatments, assessing the significance of AMF inoculation and microplastic effects, individually and in combination.

RESULTS

A study was conducted to examine the combined effect of arbuscular mycorrhizal (AM) fungi and microplastics on the growth performance of pea plants grown under pot culture conditions. Data were collected and interpreted at an interval of 15 days. Ten replicates of each set were maintained. The mixture of AM fungi used for current experiment included the species of Acaulospora denticulata, A. gerdemannii, Glomus macrocarpum, G. maculosum, G. fasciculatum and Scutellospora minuta.

Microplastic additions to soil have varying effects on the overall microbial community composition and activity, likely as a function of the concentration and chemical composition of the MP (**Leifheit et al., 2021).**The underlying mechanisms can be assumed to be in the change of soil properties, especially bulk density, which improves the aeration of the soil and could thus stimulate aerobic microorganisms; or more generally speaking MP induces a shift in the microbial community composition (**Leifheit et al., 2021**).

In addition to microbial community composition the microbial activity can be altered by MP (**Leifheit et al., 2021**). One of the mechanisms that can be responsible for this alteration is the addition of an energy resource through MP, as MP itself represents organic carbon (**Leifheit et al., 2021**). Although generally inert, MPs are mainly composed of carbon, which can partly leach as dissolved organic carbon before fragmentation occurs (**Leifheit et al., 2021**).

The MP-induced increase in microbial activity could have an indirect effect on AMF: it could trigger similar synergies between AMF and other soil microbes, leading to increased nutrient uptake by plants (**Leifheit et al., 2021).** AMF have repeatedly been proposed as an important contributor for sustainable agriculture, where they contribute to resistance and resilience against GCFs such as drought, salinity, but also against pathogens **(Leifheit et al., 2021)**). This role, however, can be challenged if AMF are affected by MP. If MP alters soil porosity and

Sr. No.	Treatment	Dry mass (g)	Number of Pea pods	Seed weight	Seed yield (g)
1.	Control	0.16	3.3	0.20	20
2.	AM fungi	1.38	6.1	0.50	80
3.	Microplastic	0.86	4.0	0.31	36
4.	Microplastic + AM fungi	1.12	4.3	0.36	42

water transport of the soil, AMF-mediated supply with water and nutrients to plants could be assigned a different relevance in both directions **(Leifheit et al., 2021).**

The association of a crop host with AMF can have positive effects on nutrient uptake and biomass yield, enhance drought-, metal-, and salinity tolerance, reduce nutrient leaching, improve soil structure, and increase plant biodiversity (**Wu et al., 2024a, 2024b).** AMF have been proposed to assist in stabilizing sustainable forest and agricultural productivity in the struggle with the increasing impact of GCFs, which now include MPs **(Leifheit et al., 2021).**

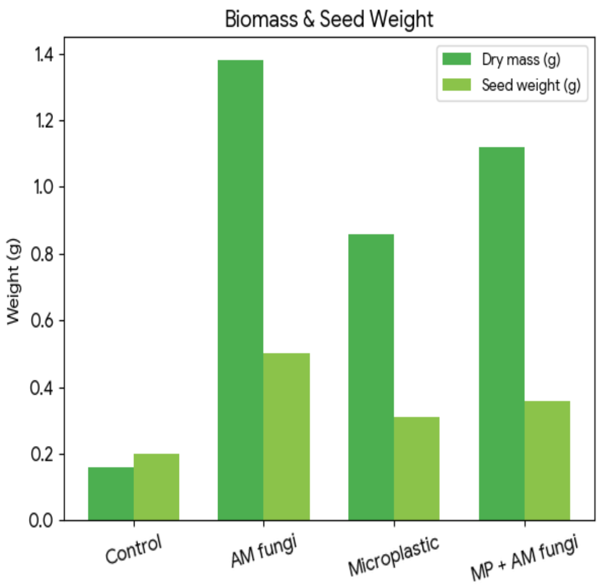
The experiment demonstrated the distinct effects of the treatments on pea growth and yield. AMF-treated plants showed the highest dry mass (1.38 g), number of pea pods (6.1), seed weight (0.50 g), and seed yield (80 g), outperforming all other groups in all parameters. Microplastic treatment alone resulted in reduced growth parameters compared to AMF, but was still better than the control, with a dry mass of 0.86 g and seed yield of 36 g. The combined treatment of microplastics and AMF improved these parameters relative to microplastic treatment alone, with a dry mass of 1.12 g and seed yield of 42 g, indicating partial mitigation of microplastic stress by AMF. Plant height and number of leaves were the highest in AMF-treated plants (20.5 cm and 32.2 leaves, respectively). Interestingly, microplastic treatment alone showed slightly higher plant height (20 cm) and number of leaves (31.7) than the combined treatment (18.7 cm and 28.8 leaves), although pod number and weight were greater in the combined treatment than in microplastic treatment alone. Overall, AMF inoculation enhanced growth vigor, nutrient uptake, and yield, whereas microplastics had a suppressive effect that was alleviated by AMF.

Table 1. Impact of Microplastic and AM fungi on plant height, number of leaves, and number of pea pods per plant, average of pea pod weight, pea pod length

Table 2. Impact of Microplastic and AM fungi on growth parameters and yield of Pea Plant

Sr. No.	treatment	Plant height (cm)	Number of leaves	Pea pod per plant	Average weight of pea pod	Pea pod weight
1.	Control	16.8	27.4	6	3.2	4.1
2.	AM fungi	20.5	32.2	14	5.0	6.6
3.	Microplastic	20	31.7	11	4.5	5.7
4.	Microplastic+ AM fungi	18.7	28.8	9	4.1	5.2

Fig.an Impact of AM Fungi and Microplastic Stress on Plant Growth Parameters 1. AM fungi, 2. AM fungi + microplastic (MPs), 3.Control and 4.Microplastic



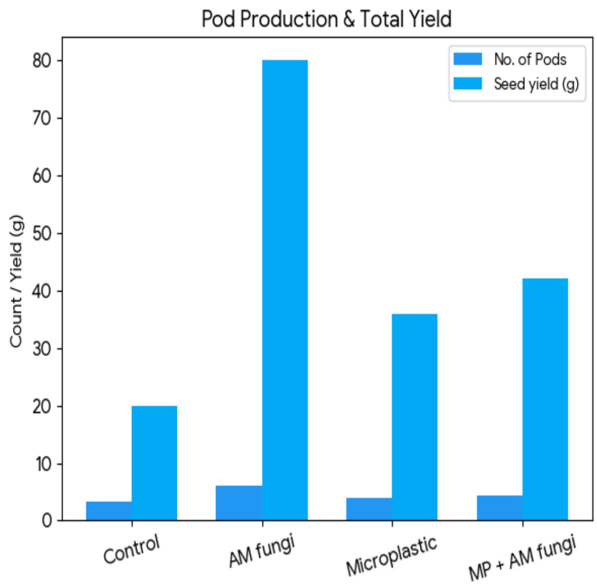
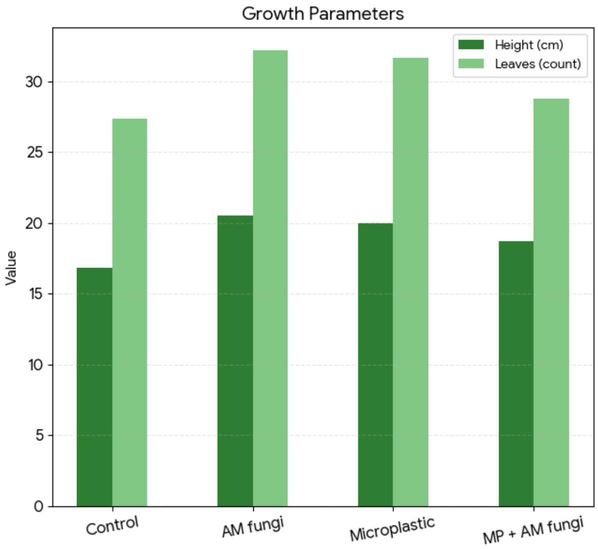


Chart a:- Effects of Arbuscular Mycorrhizal Fungi and Microplastic Stress on Biomass Allocation and Yield Components

Chart b:- Impact of AM Fungi and Microplastic Stress on plant production and yield



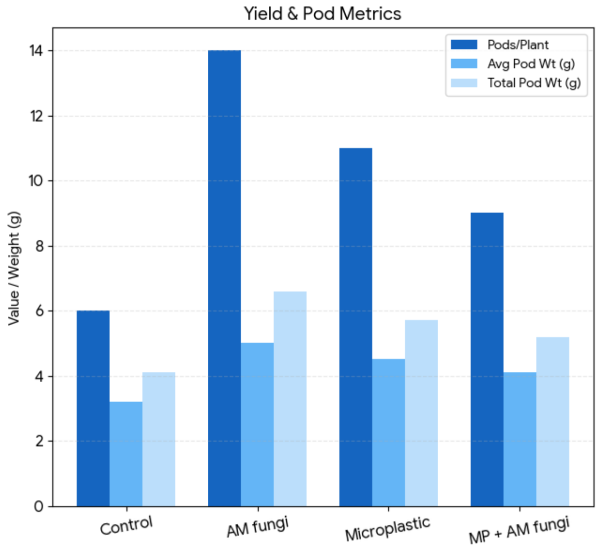


Chart c:- Impact of AM Fungi and Microplastic Stress on Plant Growth Parameters

Chart d:- Comparative Effects of AM Fungi and Microplastic on yield and Pod Metrics

DISCUSSION

- **AM Fungi (Arbuscular Mycorrhizal Fungi):** This treatment showed the highest performance across all parameters, with a **seed yield (80 g)** that was 4x higher than that of the control.
- **Microplastic Impact:** The presence of microplastics reduced dry mass and yield compared to the AM Fungi group but remained higher than the absolute control.
- **Synergy (MP + AM Fungi):** When AM fungi were added to microplastic-affected plants, growth parameters improved (e.g., seed yield increased from **36 g** to **42 g**), suggesting that AM fungi may help mitigate some of the negative stress caused by microplastics.
- **Peak Performance:** The **AM Fungi** group consistently outperformed all others, especially in **terms of pods per plant (14)**, which was more than double that of the control (6).
- **Microplastic Interaction:** Interestingly, in this specific dataset, the microplastic treatment alone showed higher growth (20 cm height, 31.7 leaves) than the Microplastic + AM Fungi combination (18.7 cm height, 28.8 leaves).
- **Yield Efficiency:** While Microplastics increased leaf and height counts over the control, the **total pod weight** was highest with pure AM Fungi (6.6 g), confirming that fungi provide the best boost for actual fruit production.
- Arbuscular mycorrhizal fungi significantly boosted the growth and yield of pea plants, confirming their role in improving plant nutrient acquisition, stress tolerance, and productivity of pea plants. AMF treatment resulted in a fourfold increase in seed yield compared to that in the control and doubled the number of pods per plant, demonstrating its strong positive impact. Microplastics, while increasing some vegetative growth parameters, such as plant height and leaf number, compared to the control, reduced reproductive outputs, such as pod number and seed yield, indicating a negative impact on overall plant productivity. The combined treatment of AMF and microplastics improved growth parameters relative to microplastics alone, suggesting that AMF can partially counteract microplastic-induced stress, possibly through enhanced nutrient uptake and improved soil microbial interactions. However, the combined treatment did not surpass AMF alone in performance, indicating that microplastics still impose a limiting effect, even in the presence of AMF. These results emphasize the importance of AMF in sustainable agriculture, especially in environments contaminated with microplastics, as they support plant resilience and productivity.
- The findings demonstrate that arbuscular mycorrhizal fungi (AMF) substantially enhance pea plant growth and productivity, confirming their pivotal roles in nutrient acquisition, stress tolerance, and overall plant vigor. AMF treatment resulted in a fourfold increase in seed yield and more than doubled the pod number compared to the control, underscoring the effectiveness of the fungi in promoting reproductive success.
- Microplastic contamination exhibited a complex influence: while it slightly increased vegetative growth parameters, such as plant height and leaf number, relative to the control, it significantly reduced reproductive outputs, including pod number and seed yield. This suggests that microplastics may induce a stress response that reallocates resources toward vegetative growth at the expense of reproduction, or possibly interfere with nutrient availability and uptake, which are critical for fruit development.
- The combined AMF and microplastic treatment improved growth and yield parameters relative to microplastic treatment alone, indicating that AMF can partially mitigate microplastic-induced stress. This mitigation may be attributed to AMF's enhancement of nutrient and water uptake by AMF, as well as its positive modulation of rhizosphere microbial communities, which can be disrupted by the presence of microplastics. However, the

combined treatment did not surpass the AMF-only group, implying that microplastic contamination imposes constraints that AMF alone cannot overcome.

- These results highlight the dual challenges posed by microplastics to agricultural productivity and the promising role of AMF as a sustainable intervention. AMF inoculation could be a viable strategy for maintaining crop resilience and yield in microplastic-contaminated soils, although further research is needed to elucidate the biochemical and physiological mechanisms underlying this interaction. Additionally, field-level studies are necessary to validate these pot culture findings and optimize AMF application protocols under variable environmental conditions.

CONCLUSION

The study concludes that arbuscular mycorrhizal fungi play a vital role in enhancing the growth, yield, and stress tolerance of pea plants, particularly under microplastic stress conditions. While microplastics negatively affect plant growth and yield, AMF inoculation mitigates some of these adverse effects by improving nutrient uptake, water absorption, and microbial synergy within the rhizosphere. Co-inoculation of AMF with microplastics led to better growth performance than microplastic treatment alone, although AMF alone remained the most effective treatment. These findings highlight the potential application of AMF as a sustainable agricultural tool for improving crop productivity and resilience in soils contaminated with microplastics. Further research is recommended to explore the mechanisms underlying AMF-mediated mitigation of microplastic stress and evaluate field-level implications.

Authors Contribution:

SW conceived and supervised this study. PC and NS performed the experiments. PC and SW contributed to writing and revising the manuscript. NS contributed to data analysis and interpretation. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement: The data supporting the findings of this study are available from the corresponding author upon reasonable requests.

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Conflicts of Interest : The authors declare no competing financial interests.

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