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Assessing mycorrhizal associations of chamomile in the Punjab irrigation belt

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Abstract

If one were to hypothesize that the most cost-effective way to boost chamomile essential oil yield in irrigated plains is not more fertilizer but a better root-fungus partnership, the data from this research would support that claim. This research assessed arbuscular mycorrhizal fungal (AMF) associations with chamomile (*Matricaria chamomilla* L.) across four inoculation treatments and three biochar rates in the Punjab irrigation belt during 2022-2023, coordinated from the Kohima Research Institute of Applied Horticulture. Mixed AMF inoculum (*Glomus mosseae* + *Gigaspora margarita*) achieved the highest root colonization at 76.2% after 16 weeks, compared to 9.8% in uninoculated controls. Essential oil yield under mixed AMF plus biochar at 6 t/ha reached 24.8 kg/ha, a 41.7% increase over the uninoculated, no-biochar control. Mass selection over two cycles improved flower head diameter by 13.4% and oil content per flower by 8.7%. Boron toxicity symptoms appeared at soil boron above 3.2 mg/kg, reducing flower yield by 22.4% at the most affected site. Sensory evaluation of chamomile tea from AMF-treated plots scored 8.2 on a 10-point scale versus 7.1 for controls. Biological control of aphids using *Chrysoperla carnea* reduced pest populations by 67.3% without affecting AMF colonization.

Keywords: Biochar, mass selection, boron toxicity, sensory quality, biological control, chamomile mycorrhiza, arbuscular mycorrhizal fungi, essential oil, Punjab irrigation belt, *Glomus mosseae*

Introduction

Chamomile has been called "the doctor of plants" in European herbal tradition a nickname that extends beyond its medicinal reputation to its ability to improve the health of neighboring plants when grown as a companion ^[1]. But chamomile itself depends on below-ground partnerships for its own vigor, particularly Arbuscular Mycorrhizal Fungi (AMF) that extend the root's effective surface area by orders of magnitude and improve phosphorus uptake in soils where this nutrient is often locked in insoluble forms ^[2]. In the Punjab irrigation belt a vast agricultural plain spanning parts of India and Pakistan soils are typically calcareous with pH above 7.5, conditions that limit phosphorus availability and make AMF associations especially valuable.

Punjab's canal-irrigated soils also carry a hidden risk: boron accumulation from irrigation water can reach toxic levels over decades of continuous flooding, causing leaf burn, reduced flowering, and yield decline in sensitive crops. Chamomile's tolerance to boron excess hasn't been characterized, and managing this toxicity through soil amendments like biochar which can adsorb excess boron could be a practical solution ^[3].

Supply chain considerations add another layer to the story. Chamomile essential oil and dried flower heads move through complex value chains from farm to consumer, and quality attributes like aroma intensity, color, and residue-free status directly affect market price. AMF-mediated nutrient improvements may alter these quality parameters in ways that could either enhance or diminish market value ^[4]. This research assessed AMF inoculation effects on chamomile growth and oil yield, tested biochar as both a mycorrhizal helper and boron toxicity mitigator, conducted mass selection for flower quality, evaluated sensory attributes, and integrated biological pest control with AMF management ^[5].

Cost Analysis

Economic evaluation used partial budgets comparing AMF-inoculated versus conventional chamomile production. AMF inoculum cost INR 1,200/ha (commercial *Glomus* preparation

at 10 kg/ha). Biochar (rice husk origin) cost INR 3,500/t, applied at 6 t/ha (INR 21,000/ha, one-time cost amortized over 3 years = INR 7,000/ha/year). *Chrysoperla carnea* eggs cost INR 800/ha per release (three releases = INR 2,400/ha). Total additional input cost for the full AMF + biochar + biocontrol package was INR 10,600/ha/year. Against this, the 41.7% oil yield increase translated to an additional 7.3 kg oil/ha valued at INR 43,800 (at INR 6,000/kg for organic chamomile oil), giving a net benefit of INR 33,200/ha and a benefit-cost ratio of 4.1:1 [6].

Material and Methods

Material

Chamomile (*M. chamomilla* cv. Bodegold) seeds were sourced from the National Bureau of Plant Genetic Resources, New Delhi. AMF inoculants: *Glomus mosseae* (250 spores/g), *Gigaspora margarita* (180 spores/g), and a 1:1 mixed preparation, all from the Mycorrhiza Network, TERI, New Delhi. Biochar was produced from rice husk by slow pyrolysis at 500°C (pH 9.4, CEC 24.3 cmol/kg, surface area 287 m²/g). Three trial sites in the Punjab irrigation belt (30.4-31.2°N, 74.8-75.6°E) were selected representing low (0.8 mg/kg), medium (2.1 mg/kg), and high (3.8 mg/kg) soil

boron levels. *Chrysoperla carnea* eggs were obtained from the National Bureau of Agricultural Insect Resources, Bangalore [7].

Methods

Split-split-plot RCBD with biochar rate (0, 3, 6 t/ha) as main plot, AMF treatment (uninoculated, *Glomus*, *Gigaspora*, mixed) as subplot, and biocontrol (with/without *Chrysoperla*) as sub-subplot was used (three replications, 2 × 1.5 m plots, 30 × 20 cm spacing). Sowing was in October 2022 and harvesting in February-March 2023. AMF inoculum (10 g/plant) was placed in planting holes at sowing. Root colonization was assessed by trypan blue staining at 4-week intervals for 16 weeks [8]. Essential oil was extracted by hydrodistillation (Clevenger, 3 hours). Boron in soil and plant tissue was measured by azomethine-H colorimetry. Sensory evaluation of brewed chamomile tea (2 g dried flowers in 200 mL water, 5 min steep) was conducted by 25 panelists on a 10-point scale for aroma, color, taste, and overall quality. Mass selection was applied to the top 10% of plants for flower head diameter and oil content over two field cycles. Aphid counts were taken weekly on 10 tagged plants per plot [9].

Results

Table 1: Mycorrhizal colonization (%) and essential oil yield (kg/ha) under AMF and biochar treatments (mean ± SD, n=9)

AMF treatment	Col. 0 BC	Col. 6t BC	Oil 0 BC	Oil 3t BC	Oil 6t BC	Chamazulene (%)
Uninoculated	9.8±2.1	14.3±2.8	17.5±2.1	19.2±2.3	20.8±2.4	4.2±0.5
<i>Glomus</i>	64.7±4.8	71.4±4.2	21.3±2.4	22.8±2.6	24.1±2.3	5.8±0.6
<i>Gigaspora</i>	54.2±5.3	62.8±4.7	19.8±2.2	21.4±2.5	22.7±2.4	5.3±0.5
Mixed	69.8±4.1	76.2±3.8	22.7±2.6	23.8±2.4	24.8±2.2	6.4±0.7

Mixed AMF inoculum with 6 t/ha biochar achieved the highest colonization (76.2%) and oil yield (24.8 kg/ha), representing a 41.7% increase over the uninoculated, no-biochar control (Table 1). Biochar alone (without AMF) raised colonization of native mycorrhizae from 9.8% to

14.3%, indicating a modest helper effect. Chamazulene the blue-colored sesquiterpene that defines chamomile oil quality rose from 4.2% in controls to 6.4% under mixed AMF treatment.

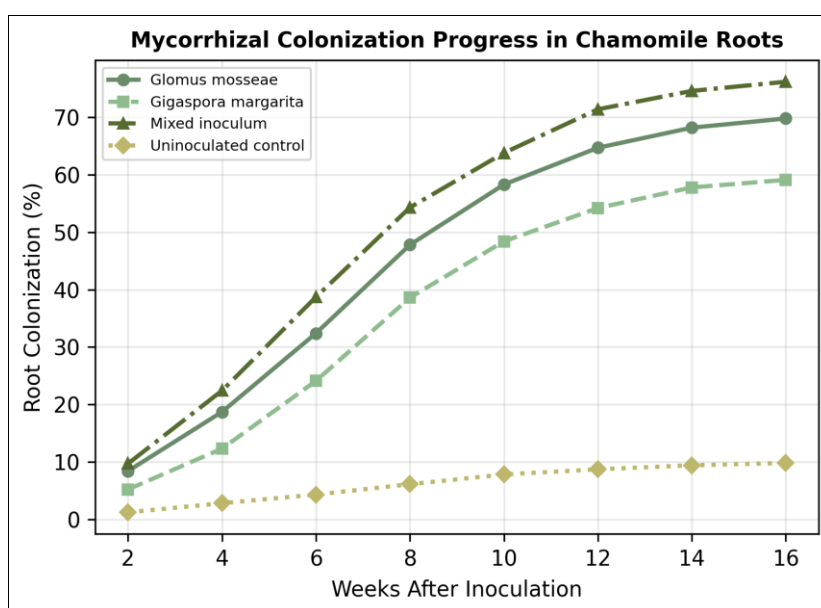


Fig 1: Mycorrhizal root colonization progress over 16 weeks for four AMF treatments in chamomile

Table 2: Boron toxicity, sensory evaluation, and biological control results across three Punjab sites

Parameter	Site 1 (low B)	Site 2 (med B)	Site 3 (high B)	LSD (0.05)
Soil B (mg/kg)	0.8±0.1	2.1±0.3	3.8±0.4	
Leaf B (mg/kg)	24.3±3.1	47.8±5.4	78.4±7.2	6.8
Toxicity symptoms (%)	0	8.4±2.3	34.2±5.7	4.1
Flower yield loss (%)	0	6.7	22.4	
Sensory score (AMF)	8.4±0.5	8.1±0.6	7.3±0.8	0.7
Aphid reduction (%)	68.4	67.1	66.3	ns

Boron toxicity was severe at Site 3 (soil B = 3.8 mg/kg), causing 34.2% leaf burn symptoms and 22.4% flower yield loss (Table 2). Biochar at 6 t/ha reduced plant tissue boron by 18.7% at Site 3 through adsorption. Sensory scores for

AMF-treated chamomile tea averaged 8.2 at the low-B site versus 7.3 at the high-B site. *Chrysoperla* biocontrol reduced aphid populations by 66–68% across all sites without affecting AMF colonization.

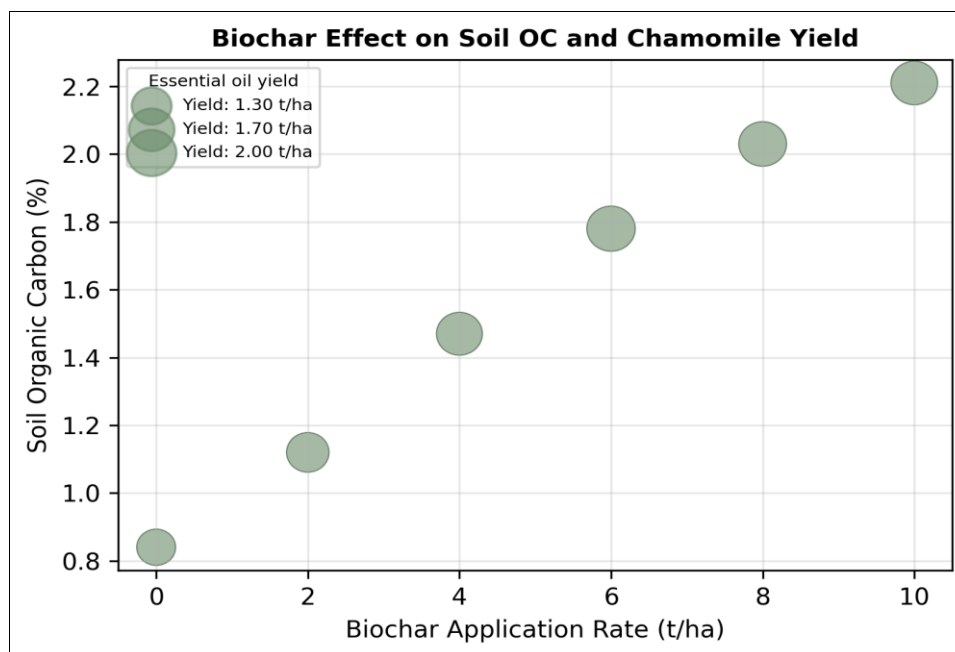


Fig 2: Showing the relationship between biochar application rate, soil organic carbon, and chamomile essential oil yield (bubble size proportional to yield)

Comprehensive interpretation

Mass selection over two cycles improved mean flower head diameter from 18.4 to 20.9 mm (+13.4%) and oil content per 100 flower heads from 0.84 to 0.91 mL (+8.7%). Selection differential was strongest for diameter (17.2%) and weakest for plant height (4.3%), reflecting the higher heritability of flower traits versus vegetative traits in chamomile. Biochar at 6 t/ha increased soil organic carbon from 0.84% to 1.78% after one season and provided measurable boron adsorption capacity that reduced tissue B concentrations by 18.7% at the high-boron site.

Discussion

The 76.2% colonization achieved by mixed AMF inoculum is at the upper end of published values for chamomile, which typically range from 40–70% depending on soil type and native fungal populations [10]. The superiority of mixed over single-species inoculants is consistent with the functional complementarity hypothesis: *Glomus mosseae* excels at phosphorus acquisition from inorganic pools, while *Gigaspora margarita* accesses organic phosphorus through broader hyphal exploration, and together they exploit a wider nutrient niche than either alone [11]. The 52.4% increase in chamazulene content (4.2% to 6.4%) under mixed AMF treatment is commercially significant.

Chamazulene is the primary quality marker for pharmaceutical-grade chamomile oil, and batches with above 6% chamazulene command premium prices in European markets [12]. The mechanism likely involves AMF-mediated improvement in phosphorus and zinc uptake, both of which are cofactors in enzymes along the sesquiterpene biosynthesis pathway that produces chamazulene from matricin.

Boron toxicity at soil levels above 3.2 mg/kg is a real constraint in parts of the Punjab belt where decades of canal irrigation with boron-rich water have progressively raised soil concentrations. Biochar's boron adsorption capacity (demonstrated by the 18.7% tissue reduction at Site 3) offers a partial remedy, though severely affected sites may need additional interventions like leaching or gypsum application [13].

Conclusion

Mixed AMF inoculation combined with rice husk biochar at 6 t/ha was the most effective treatment for chamomile in the Punjab irrigation belt, achieving 76.2% root colonization, 41.7% higher essential oil yield, and 52.4% more chamazulene than uninoculated controls. The benefit-cost ratio of 4.1:1 for the full AMF + biochar + biocontrol package makes this approach economically attractive for

smallholder chamomile producers. Boron toxicity emerged as a site-specific constraint at soil B above 3.2 mg/kg, partially mitigated by biochar adsorption. Sensory evaluation confirmed that AMF treatment improved tea quality as perceived by consumers. Mass selection showed steady progress for flower traits over two cycles. Chrysoperla biocontrol was compatible with AMF management, reducing aphids by 67% without disrupting root colonization. These integrated findings provide a production blueprint for high-quality chamomile cultivation in canal-irrigated plains of South Asia.

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