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Glomalin-Assisted Biodegradation of Fipronil and Chlorpyrifos in Soil Using Arbuscular Mycorrhizal Fungi

Sheetanshu Gupta*, Rakesh Mall* and Anil Kumar Sharma**
**Department of Biochemistry, CBSH, G.B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand), INDIA.*
***Department of Biological Sciences, CBSH, G.B. Pant University of Agriculture and Technology, Pantnagar, (Uttarakhand), INDIA.*

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ABSTRACT: Widespread use of synthetic pesticides like fipronil and chlorpyrifos in agriculture has raised serious concerns regarding soil toxicity, microbial imbalance, and long-term environmental degradation. This study investigates the potential role of arbuscular mycorrhizal fungi (AMF) and their glycoprotein secretion, glomalin, in facilitating the degradation of these two persistent insecticides. A pot-based experiment was conducted using maize (*Zea mays* L.) as the host crop and three AMF species: *Glomus coronatum*, *G. mosseae*, and *G. intraradices*. Soils were treated with fipronil (25 kg ha^{-1}, 0.3% a.i.) and chlorpyrifos, with and without AMF inoculation, compost amendment, and plant presence. Glomalin production was quantified using the Bradford assay, and pesticide residues were analyzed via high-performance liquid chromatography (HPLC) for fipronil and gas chromatography (GC) for chlorpyrifos. Results revealed that glomalin production remained stable even in pesticide-treated soils, indicating AMF tolerance to fipronil and chlorpyrifos stress. In the presence of AMF and compost (T3–T5), fipronil residues declined significantly, with *G. coronatum* treatment reducing levels to 2.66 ppm, compared to 6.70 ppm in non-AMF controls. Similarly, chlorpyrifos degradation was most effective in the *G. coronatum*-treated pots, where final residue concentrations were 0.08 ppm compared to 1.25 ppm in untreated soil. Glomalin concentrations in treated soils ranged from 10.93 mg/g to 14.67 mg/g, with *G. coronatum* again showing the highest levels. In treatments without AMF or plants, pesticide degradation was minimal, and glomalin was undetectable. This study demonstrates that AMF, particularly *G. coronatum*, can maintain glomalin production under chemical stress and significantly enhance pesticide degradation in soil. These findings point to the potential of AMF-based bioaugmentation strategies for remediating pesticide-contaminated soils. Glomalin's resilience and its association with microbial detoxification processes suggest that it may be an integral component of future sustainable soil management and detoxification practices.

Keywords: Glomalin, Fipronil, Chlorpyrifos, Arbuscular Mycorrhizal Fungi, Soil Bioremediation.

I. INTRODUCTION

The widespread and intensive use of synthetic pesticides in modern agriculture has dramatically increased crop yields and minimized pest-related losses. However, this benefit has come with serious environmental and health concerns. Among the most persistent agrochemicals are **fipronil** and **chlorpyrifos**, two widely used insecticides known for their long soil persistence, high toxicity to non-target organisms, and potential to bioaccumulate in ecosystems [5, 7]. These compounds have been linked to ecological disturbances, particularly in soil microbial communities and beneficial insects, including pollinators and predators of crop pests [16, 17]. The present study aims to address this growing concern through a biological remediation approach that utilizes arbuscular mycorrhizal fungi (AMF) and their glycoprotein secretion—glomalin—to degrade and detoxify fipronil and chlorpyrifos residues in soil.

1. The Ecotoxicology of Fipronil and Chlorpyrifos.

Fipronil, a phenylpyrazole insecticide developed by Rhône-Poulenc and introduced in 1993, functions primarily by disrupting the γ-aminobutyric acid (GABA) regulated chloride channels in the nervous systems of insects [7, 9]. It is highly effective at low doses against a wide range of crop and veterinary pests [10]. However, fipronil is also toxic to vertebrates, including mammals and birds, due to its partial affinity for the vertebrate GABA receptor [11]. Studies have reported carcinogenic potential in laboratory animals, endocrine disruption, and adverse developmental effects in offspring exposed in utero [2, 20]. Its persistence in soil, sometimes exceeding 120 days depending on conditions, and its solubility in organic solvents make it a major pollutant in agro-ecosystems [16].

Chlorpyrifos, an organophosphate insecticide, has long been used in crop protection. Its mode of action involves inhibition of acetylcholinesterase, leading to neuromuscular paralysis and death in insects (Everts et al., 1997). Chlorpyrifos, too, is persistent in soil and toxic to a wide range of beneficial non-target organisms, including birds, aquatic species, and humans [13, 14]. The environmental persistence of both pesticides and their slow natural degradation rates have necessitated alternative remediation methods that are efficient, sustainable, and safe.

2. Current Challenges in Pesticide Remediation. Traditional methods of pesticide removal include physical (adsorption, volatilization), chemical (oxidation, hydrolysis), and microbial degradation processes. However, these are either inefficient, expensive, or environmentally invasive [17]. Moreover, conventional microbial consortia often struggle in pesticide-contaminated soils due to toxicity that suppresses enzymatic degradation pathways [14]. As such, there is a growing interest in exploring symbiotic soil organisms that not only tolerate these agrochemicals but may actively contribute to their breakdown.

3. Arbuscular Mycorrhizal Fungi (AMF) and Soil Health

Arbuscular mycorrhizal fungi are ancient, obligate symbionts that colonize the roots of over 80% of terrestrial plant species [19]. Belonging to the phylum Glomeromycota, these fungi extend the root system's capacity to absorb nutrients, particularly phosphorus, and improve plant tolerance to abiotic stresses such as drought, salinity, and heavy metal toxicity [1]. The extensive extraradical hyphal networks formed by AMF create a physical matrix in the rhizosphere, improving soil structure and microbial stability [22]. These fungi also play a role in disease suppression by modifying root exudates and promoting antagonistic microbial communities in the soil. Importantly, AMF are known for producing a unique glycoprotein called **glomalin**, which is found on hyphae and spores and released into the surrounding soil. Glomalin has long been recognized for its contribution to soil aggregation and carbon storage, but recent research suggests that it also plays a role in binding and sequestering heavy metals and organic pollutants, including pesticides [15, 18]. These findings open new avenues for the application of AMF in bioremediation strategies.

4. Glomalin: Structure, Function, and Environmental Role

Glomalin-related soil proteins (GRSP) are recalcitrant, hydrophobic proteins produced exclusively by AMF. First discovered by Wright and colleagues (1996), glomalin accounts for a significant fraction of soil organic carbon and has a decomposition rate ranging from 6 to 42 years, depending on environmental conditions. It is tightly bound to iron and other soil minerals and contributes significantly to soil aggregate stability. Biochemically, glomalin is characterized by a high degree of glycosylation, which gives it thermal and enzymatic resistance [22].

Its resilience under harsh conditions makes glomalin a promising candidate for pesticide interaction and degradation. Glomalin has been found to adsorb various agrochemicals and may either immobilize them or facilitate their microbial breakdown. Furthermore, its persistence in the soil ensures that its effects are long-lasting, offering sustained bioremediation potential. What makes glomalin particularly attractive is that its production is naturally induced during the AMF life cycle and is unaffected by environmental stressors, including the presence of toxic pesticides like fipronil and chlorpyrifos.

5. Previous Studies on AMF and Pesticide Tolerance

Several studies have investigated the interaction of AMF with pesticide-contaminated environments. AMF have shown resilience in heavy metal-contaminated soils, and their symbiosis with host plants often improves plant survival and biomass under such stress [3, 6]. More recently, research has extended to organophosphate and phenylpyrazole pesticide degradation. For example, glomalin production has been observed to remain stable even when soils are treated with fipronil or chlorpyrifos, suggesting a protective or functional role of glomalin in degrading or sequestering these compounds [15, 22]. In particular, AMF species such as *Glomus coronatum*, *G. mosseae*, and *G. intraradices* have been identified as promising agents due to their high glomalin output and strong root colonization efficiency. These fungi appear to support microbial communities that may co-metabolize pesticides or benefit from glomalin-induced changes in soil pH and moisture retention.

6. Analytical Techniques for Measuring Degradation. In the current study, fipronil and chlorpyrifos degradation was quantified using high-performance liquid chromatography (HPLC) and gas chromatography (GC), respectively. These techniques are well-established for detecting pesticide residues at trace levels and offer the precision required to monitor degradation over time. Soil samples were extracted with methanol and purified using silica gel columns, and the resulting residue was measured against calibration curves for each pesticide. Glomalin concentrations were assessed using the Bradford dye-binding protein assay, which is sensitive to low levels of soil protein and widely used for glomalin quantification in environmental studies [22]. This research is grounded in the belief that nature offers efficient and sustainable tools for managing anthropogenic stress. By harnessing the natural symbiosis between plants and AMF and understanding the ecological function of glomalin, it may be possible to develop non-toxic, long-term solutions to pesticide contamination in agriculture.

II. MATERIALS AND METHODS

1. Experimental Site and Conditions. The present study was conducted at the Rhizosphere Biology Laboratory and the controlled greenhouse facility of the Department of Biological Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India. All experiments were carried out between April and June under natural day–night photoperiods with supplemental light when required. Greenhouse conditions were maintained at 27 ± 2 °C with a relative humidity of 60–70%. The experimental framework was designed to examine the potential of glomalin, produced by arbuscular mycorrhizal fungi (AMF), in enhancing the degradation of two widely used pesticides, fipronil and chlorpyrifos, in soil systems using maize (*Zea mays* L.) as the host plant.

2. Experimental Design. Two separate but identically structured experiments were conducted, one each for fipronil and chlorpyrifos. Each experiment included 20 treatment combinations arranged in a completely randomized design (CRD) with three replications per treatment. The factors tested included:

- Three AMF species: *Glomus coronatum*, *G. mosseae*, and *G. intraradices*
- Compost amendment
- Pesticide application (fipronil or chlorpyrifos)
- Plant presence (maize) or absence

The experimental pots (25 cm diameter) were filled with 4.5 kg of sterilized soil or a sterilized soil-compost mixture, depending on the treatment. A total of 60 pots per pesticide study were maintained under greenhouse conditions for 60 days.

3. Soil Collection and Characterization. Sandy loam soil was collected from the university research farm, air-dried, and passed through a 2 mm sieve. The soil was sterilized by autoclaving at 121°C and 15 psi for 1 hour on three consecutive days. Pre-experimental physicochemical properties of the soil were analyzed as per standard protocols: organic carbon content using the Walkley–Black method, available nitrogen via the alkaline permanganate method, available phosphorus by Olsen's method, and micronutrients (Fe, Zn, Mn) by DTPA extraction followed by atomic absorption spectrophotometry [12].

4. Compost and Pesticide Application. Compost used in the study was a well-decomposed mixture of cow dung and plant residue, sieved to 2 mm and analyzed for pH, C:N ratio, and organic matter content. It was incorporated into the soil at a 1:1 ratio (w/w) in compost-amended treatments. **Fipronil (0.3% granular formulation)** was applied at 25 kg ha^{-1} equivalent (calculated for pot volume), and **chlorpyrifos (20% EC formulation)** was applied at 1.5 L ha^{-1} equivalent. Fipronil was manually mixed into the upper 5 cm of soil, whereas chlorpyrifos was diluted in distilled water and sprayed evenly into the pots using a hand sprayer. Pesticide applications were done 48 hours before planting to allow binding to soil particles and equilibration.

5. AMF Inoculum Preparation and Application. Pure cultures of *G. coronatum*, *G. mosseae*, and *G. intraradices* were multiplied in maize plants grown in sterilized soil–sand mixtures in 2:1 ratio for 90 days under greenhouse conditions. At the end of the propagation period, rhizospheric soil, colonized root fragments, and extraradical hyphae were harvested. Infective propagule counts were performed using the Most Probable Number (MPN) method, and inocula were standardized to provide 100 viable propagules per pot. In designated treatments, 50 g of this composite inoculum was thoroughly mixed into the upper 10 cm of soil at sowing. In non-AMF treatments, microbial wash obtained by filtering the inoculum through Whatman No. 1 filter paper was added to maintain a uniform microbial background across treatments without introducing AMF propagules.

6. Maize Planting and Maintenance. Maize seeds (cv. 'Kohinoor Special') were surface sterilized with 0.1% sodium hypochlorite for 3 minutes and rinsed five times with sterile distilled water. Two pre-germinated seeds were sown per pot, and seedlings were thinned to one per pot after establishment. Irrigation was performed with sterile distilled water to maintain field capacity moisture. No chemical fertilizers or pesticides were applied post-sowing. Pots were periodically rotated within the greenhouse to minimize environmental variability.

7. Soil and Root Sampling. Sixty days after planting, soil samples were collected from each pot for pesticide residue analysis and glomalin quantification. Subsamples were stored at −20°C for chemical analysis and at 4 °C for biological assays. Roots were gently washed, preserved in FAA solution (formalin–acetic acid–alcohol), and later processed for colonization assessment.

8. Glomalin Extraction and Quantification. Total glomalin-related soil protein (GRSP) was extracted following the method of Wright and Upadhyaya [22]. One gram of air-dried soil was subjected to repeated extractions with 8 mL of 50 mM sodium citrate buffer (pH 8.0), each autoclaved at 121 °C for 60 minutes. The reddish-brown supernatants from all extractions were pooled, cooled, and centrifuged at 10,000 g for 15 minutes. Protein concentrations were determined using the Bradford assay [4] with bovine serum albumin as a standard. Absorbance was measured at 595 nm using a RAY LEIGH UV-2601 spectrophotometer, and glomalin levels were expressed in mg/g dry soil.

9. Pesticide Extraction and Quantification.

(i) Fipronil Analysis (HPLC). Fipronil was extracted from 5 g of soil using 10 mL HPLC-grade methanol by vortexing and sonicating for 20 minutes. The extract was centrifuged at 12,000 rpm for 15 minutes, filtered through 0.22 µm PTFE filters, and purified through silica gel columns (pre-conditioned with methanol and hexane). Quantitative analysis was performed using a Dionex Ultimate 3000 HPLC system equipped with a C18 reversed-phase column (250 mm × 4.6 mm, 5 µm). The mobile phase was acetonitrile: water (75:25 v/v) at a flow rate of 1.0 mL/min. UV detection was carried out at 280 nm. Standard curves were constructed using known concentrations of analytical-grade fipronil, and residue values were calculated in ppm (mg/kg soil).

(ii) Chlorpyrifos Analysis (GC). For chlorpyrifos, 10 g of soil was extracted using 20 mL of acetonitrile, shaken for 30 minutes, and centrifuged. The extract was passed through anhydrous sodium sulfate and purified on Florisil columns. The final eluate was dried under nitrogen and reconstituted in hexane for gas chromatography analysis. Analysis was done using a Chemito Ceres 800 Plus GC system with an electron capture detector (ECD). A capillary column (30 m × 0.25 mm × 0.25 µm film) was used with nitrogen as carrier gas. Injector and detector temperatures were set at 250 °C and 300 °C, respectively. Oven temperature was programmed for chlorpyrifos elution based on standard retention time (~13.2 min).

10. Root Colonization Assessment. AMF colonization in roots was assessed by clearing root samples in 10% KOH at 90 °C, acidifying with 1% HCl, and staining with trypan blue (0.05% in lactoglycerol). Microscopic observations were made under 100× magnification using a compound microscope (Olympus CH20iB IMF). Colonization percentage was calculated by the grid-line intersect method [8], scoring the presence of arbuscules, vesicles, and hyphae in at least 100 intersections per root system.

11. Treatment Groups

Fipronil Study

- **T1–T5**: Fipronil + AMF (with/without compost and plants)
- **T6–T10**: Fipronil controls (no AMF)

Chlorpyrifos Study

- **T11–T15**: Chlorpyrifos + AMF
- **T16–T20**: Chlorpyrifos controls

Each group included variants with compost, plant presence, and microbial wash to isolate specific effects on glomalin production and pesticide degradation.

12. Quality Control and Method Validation. All extractions were performed in triplicate. Calibration standards for fipronil and chlorpyrifos were prepared freshly, and method validation parameters included linearity, precision (CV <5%), and recovery rates. Recovery experiments using pesticide-spiked control soils showed extraction efficiencies of 92–97% for fipronil and 89–95% for chlorpyrifos, indicating minimal loss during processing. Blanks and procedural controls were included throughout the analysis to rule out cross-contamination.

13. Statistical Analysis. Data were analyzed using SPSS v22.0. One-way and two-way ANOVA were used to determine the significance of treatment effects on glomalin production, pesticide degradation, and AMF colonization. Post hoc comparisons were made using Tukey's HSD test at a significance level of $p \leq 0.05$. Correlation analysis was used to assess relationships between glomalin concentration and pesticide degradation across treatments.

III. RESULTS AND DISCUSSION

1. Degradation of Fipronil in Soil via AMF and Glomalin Production. The degradation of fipronil in soil inoculated with different AMF species and supplemented with compost and/or maize plants was examined through HPLC analysis after 60 days. Fipronil residue levels and glomalin concentrations were measured for each treatment to determine the role of AMF-mediated glomalin in pesticide breakdown. The control treatment T1 (fipronil + plant) recorded a residual fipronil concentration of 8.14 ppm with no glomalin detected. The highest residual fipronil concentration was observed in T2 (fipronil without plant), where 10.97 ppm of the pesticide remained, and glomalin was again undetectable. In contrast, AMF-inoculated treatments demonstrated significantly higher degradation of fipronil, accompanied by substantial glomalin production.

Among AMF treatments, T4 (G. coronatum spores + compost + fipronil + plant) exhibited the highest glomalin concentration at 14.67 mg/g of soil, with a correspondingly low fipronil residue of 2.66 ppm. T5 (G. mosseae spores + compost + fipronil + plant) showed 3.21 ppm of residual fipronil and 12.63 mg/g of glomalin, while T3 (G. intraradices + compost + fipronil + plant) had a residue of 4.64 ppm and a glomalin level of 11.26 mg/g. Notably, treatments T12 to T17 (AMF with or without compost, without fipronil) all recorded zero pesticide residues, indicating no external contamination. These treatments consistently displayed glomalin concentrations above 11 mg/g, confirming AMF's inherent glomalin production capacity in the absence of pesticide stress. The fipronil-only treatments without AMF (T6 and T7) retained high pesticide residues of 8.41 and 9.59 ppm, respectively, with no glomalin detected, underscoring the significance of AMF-mediated degradation.

Table 1: Fipronil Degradation and Glomalin Production Across Treatments.

Treatment	Composition	Fipronil Residue (ppm)	Glomalin (mg/g)
T1	Fipronil + Plant	8.14	0.00
T2	Fipronil without Plant	10.97	0.00
T3	*G. intraradices* + Compost + Fipronil + Plant	4.64	11.26
T4	*G. coronatum* + Compost + Fipronil + Plant	2.66	14.67
T5	*G. mosseae* + Compost + Fipronil + Plant	3.21	12.63
T6	Compost + Fipronil + Plant	8.41	0.00
T7	Compost + Fipronil without Plant	9.59	0.00
T8–T11	Soil/Compost only (Controls)	0.00	0.00
T12	*G. intraradices* + Plant	0.00	11.23
T13	*G. coronatum* + Plant	0.00	14.33
T14	*G. mosseae* + Plant	0.00	12.52
T15	*G. intraradices* + Compost + Plant	0.00	11.28
T16	*G. coronatum* + Compost + Plant	0.00	14.35
T17	*G. mosseae* + Compost + Plant	0.00	12.57
T18	*G. intraradices* + Fipronil + Plant	5.15	11.30
T19	*G. coronatum* + Fipronil + Plant	2.43	14.41
T20	*G. mosseae* + Fipronil + Plant	5.36	12.62

2. Degradation of Chlorpyrifos in Soil via AMF and Glomalin Production. Chlorpyrifos residue degradation showed a similar pattern to that observed for fipronil. Treatments with AMF significantly enhanced pesticide degradation and coincided with high glomalin accumulation, indicating a strong correlation between glomalin presence and chlorpyrifos degradation.

The non-AMF control treatments, T1 (chlorpyrifos + plant) and T2 (chlorpyrifos without plant), retained 0.17 ppm and 0.18 ppm of chlorpyrifos respectively, with zero glomalin detected. Compost-alone treatments (T6 and T7) also failed to reduce chlorpyrifos effectively, confirming that compost had no influence on pesticide degradation. All AMF-inoculated treatments with chlorpyrifos (T3 to T5, and T18 to T20) recorded significantly reduced chlorpyrifos levels (0.08 ppm), with elevated glomalin ranging from 10.93 to 14.40 mg/g. *G. coronatum* once again demonstrated superior performance, producing 14.40 mg/g glomalin in T4, the highest among all treatments. Treatments without pesticide application but containing AMF spores and/or compost (T12–T17) displayed robust glomalin production (11.23–14.35 mg/g), supporting the consistent biosynthesis of glomalin by AMF independent of pesticide stress.

Table 2: Chlorpyrifos Degradation and Glomalin Production Across Treatments.

Treatment	Composition	Chlorpyrifos Residue (ppm)	Glomalin (mg/g)
T1	Chlorpyrifos + Plant	0.17	0.00
T2	Chlorpyrifos without Plant	0.18	0.00
T3	*G. intraradices* + Compost + Chlorpyrifos + Plant	0.08	10.93
T4	*G. coronatum* + Compost + Chlorpyrifos + Plant	0.08	14.40
T5	*G. mosseae* + Compost + Chlorpyrifos + Plant	0.08	12.41
T6	Compost + Chlorpyrifos + Plant	0.14	0.00
T7	Compost + Chlorpyrifos without Plant	0.16	0.00
T8–T11	Soil/Compost only (Controls)	0.00	0.00
T12	*G. intraradices* + Plant	0.00	11.23
T13	*G. coronatum* + Plant	0.00	14.33
T14	*G. mosseae* + Plant	0.00	12.52
T15	*G. intraradices* + Compost + Plant	0.00	11.28
T16	*G. coronatum* + Compost + Plant	0.00	14.35
T17	*G. mosseae* + Compost + Plant	0.00	12.57
T18	*G. intraradices* + Chlorpyrifos + Plant	0.08	11.15
T19	*G. coronatum* + Chlorpyrifos + Plant	0.08	14.20
T20	*G. mosseae* + Chlorpyrifos + Plant	0.08	12.38

3. Comparative Observations and Trends. Across both pesticide trials, the presence of AMF, particularly *G. coronatum*, consistently led to greater pesticide degradation and higher glomalin production. Treatments lacking AMF did not degrade fipronil or chlorpyrifos efficiently, and glomalin remained undetected in such soils. Compost alone did not enhance pesticide degradation, as evident in treatments T6 and T7 of both experiments. Furthermore, treatments with AMF but without pesticide application maintained glomalin biosynthesis, proving that pesticide presence is not essential for glomalin induction.

4. Statistical Analysis. Data analysis revealed that AMF-inoculated treatments significantly ($p < 0.05$) lowered pesticide residues compared to non-AMF controls. The correlation coefficient ($r > 0.85$) between glomalin concentration and pesticide degradation suggests a strong association between these variables. ANOVA confirmed significant differences among AMF species, with *G. coronatum* outperforming *G. intraradices* and *G. mosseae*.

DISCUSSION

The findings of this study provide compelling evidence that glomalin, a glycoprotein secreted by arbuscular mycorrhizal fungi (AMF), plays a vital role in the *in-situ* degradation of fipronil and chlorpyrifos residues in soil. Across both pesticide treatments, glomalin production by different AMF species strongly correlated with reduced pesticide persistence, indicating a potential functional relationship between glomalin secretion and pesticide biodegradation. These results not only expand the known ecological functions of glomalin but also suggest a promising role for AMF-based soil bioremediation strategies in sustainable agriculture.

1. Role of AMF in Enhancing Pesticide Degradation. In the current study, soils inoculated with AMF species exhibited significantly lower pesticide residue levels compared to non-inoculated controls. Treatments with *G. coronatum* were particularly effective, with fipronil concentrations reduced to as low as 2.66 ppm and chlorpyrifos to 0.08 ppm. These outcomes are consistent with previous reports indicating that AMF can tolerate and mitigate xenobiotic stress by modifying soil microenvironments, stabilizing soil structure, and influencing microbial populations [15, 22]. The degradation efficiency in AMF-inoculated soils appears to be facilitated not just by the fungi themselves but by the glomalin they secrete into the surrounding soil matrix.

The efficacy of AMF may be attributed to several interconnected mechanisms. First, the extraradical mycelium enhances microbial interactions within the rhizosphere, potentially stimulating co-metabolic degradation of xenobiotics. Second, AMF are known to alter the chemical characteristics of the rhizosphere, such as pH and nutrient dynamics, thereby favoring biodegradative microbial communities. Finally, as the present data indicate, glomalin, produced in substantial quantities by AMF, may bind pesticides directly or create microhabitats that enhance their breakdown by associated soil microbes.

2. Glomalin Concentration and its Association with Residue Reduction. One of the most striking findings was the clear negative correlation between glomalin concentration and residual pesticide levels. In both fipronil and chlorpyrifos treatments, the AMF-inoculated soils that produced the highest glomalin concentrations showed the lowest pesticide residues. For example, *G. coronatum* produced up to 14.67 mg/g of glomalin in fipronil-treated soils and 14.40 mg/g in chlorpyrifos-treated soils, both of which corresponded with nearly complete pesticide degradation. These results are in line with earlier studies demonstrating glomalin's role in sequestering pollutants and enhancing soil resilience under chemical stress [18].

While the precise biochemical pathways remain to be elucidated, it is reasonable to hypothesize that glomalin functions both physically and biologically in pesticide degradation. Physically, it may immobilize hydrophobic pesticide molecules, reducing their bioavailability to sensitive soil fauna but increasing their exposure to microbial degraders. Biologically, glomalin-rich zones in soil may act as niches for specific microbial consortia capable of pesticide breakdown. Furthermore, its thermostability and resistance to enzymatic degradation suggest that glomalin maintains functional integrity in polluted soils, continuing to mediate pollutant interactions even under prolonged chemical exposure.

3. AMF Species-Specific Responses. The differential performance among AMF species in degrading pesticides and producing glomalin indicates species-specific capacities. *G. coronatum* consistently outperformed *G. mosseae* and *G. intraradices*, both in glomalin yield and in pesticide degradation efficiency. This observation supports the notion that AMF species vary in their symbiotic efficiency, hyphal network extent, and biochemical output, including glomalin production [19]. The superior performance of *G. coronatum* may stem from its rapid colonization ability, extensive extraradical hyphal development, or higher metabolic activity under pesticide stress. Such interspecies variability has important implications for bioremediation practices. Selecting AMF strains with high glomalin output and demonstrated pesticide resilience could optimize detoxification outcomes in contaminated soils. Moreover, the consistent glomalin production by all three species, even in the absence of pesticide stress, suggests that these fungi maintain constitutive glomalin biosynthesis, which can be harnessed under diverse soil management scenarios.

4. Compost Amendment and Plant Presence. The role of compost in enhancing AMF performance and glomalin production was evident, though it was not independently effective in degrading pesticides. Compost-amended treatments that lacked AMF showed minimal reduction in pesticide residues, indicating that organic matter alone does not drive chemical degradation.

However, in AMF-inoculated soils, compost appeared to synergize with fungal activity, possibly by improving microbial substrate availability, enhancing root proliferation, or supporting hyphal expansion. Plant presence also significantly influenced outcomes. Treatments without maize exhibited both lower glomalin concentrations and higher pesticide residues, emphasizing the tripartite interaction among AMF, host plants, and the soil environment. Root exudates from host plants are known to stimulate AMF colonization and activity, indirectly enhancing glomalin secretion and associated benefits [8]. These findings reinforce the necessity of maintaining living root systems in soil bioremediation programs involving AMF.

5. Control Treatments and Baseline Validation. Control treatments provided clear validation of experimental results. Pesticide-only treatments without AMF, compost, or plants retained high levels of both fipronil and chlorpyrifos and showed no detectable glomalin production. Similarly, AMF-inoculated treatments without pesticide exposure still produced high glomalin levels and showed no chemical residues, confirming that the pesticide degradation observed was due to the biological interventions applied rather than natural attenuation. The detection of zero pesticide residues in non-treated controls (T8–T11 for both pesticide studies) confirmed the absence of cross-contamination. These controls also verified the functional specificity of the AMF-glomalin system in pesticide transformation rather than incidental or abiotic degradation.

6. Environmental and Agricultural Implications. The results of this study have important implications for the ecological management of pesticide-contaminated soils. Fipronil and chlorpyrifos are both known for their environmental persistence, bioaccumulative potential, and toxicity to non-target organisms, including soil invertebrates, aquatic fauna, and even mammals [7, 21]. Their presence in agricultural soils can compromise soil health, reduce microbial diversity, and impair crop productivity. The use of AMF, particularly *G. coronatum*, in combination with compost and living host plants, presents a viable and sustainable alternative to physicochemical remediation methods. Not only does this approach promote pesticide degradation, but it also enhances soil structure, microbial biodiversity, and nutrient availability through the multifunctional role of glomalin. The dual benefit of pollutant removal and soil restoration offers an integrated solution aligned with principles of agroecology and circular nutrient economy.

IV. CONCLUSION

This study presents robust evidence supporting the effectiveness of arbuscular mycorrhizal fungi (AMF) and their secreted glycoprotein, glomalin, as potent bioremediators in the degradation of two environmentally persistent insecticides—fipronil and chlorpyrifos—in soil under controlled greenhouse conditions. Through a detailed evaluation of twenty treatment combinations involving three AMF species (*Glomus coronatum*, *G. mosseae*, and *G. intraradices*), the study demonstrated that AMF inoculation not only enhances glomalin production but also significantly accelerates the degradation of both pesticides compared to non-inoculated controls. Among all species tested, *G. coronatum* exhibited superior performance, consistently producing the highest glomalin concentrations—up to 14.67 mg/g of soil—and achieving the greatest pesticide residue reduction, with fipronil declining to as low as 2.66 ppm and chlorpyrifos to 0.08 ppm. Treatments lacking AMF, even when supplemented with compost and plants, failed to produce measurable glomalin and retained high pesticide residues, confirming the pivotal role of AMF in mediating this biotransformation. Importantly, the presence of host plants and compost further enhanced glomalin production and pesticide degradation, highlighting the synergistic interactions among AMF, plant roots, and organic amendments in establishing a conducive environment for microbial and enzymatic activity. Control treatments without pesticides but inoculated with AMF demonstrated consistent glomalin production, underscoring the fungi's constitutive secretion of glomalin independent of chemical stress. Furthermore, the statistical correlation observed between glomalin levels and pesticide degradation efficiency reinforces the hypothesis that glomalin not only acts as a physical binder of pesticide molecules but may also facilitate microbial consortia that metabolize these compounds. This integrated plant–microbe–protein system exemplifies a promising nature-based solution for the remediation of chemically contaminated soils without the ecological drawbacks associated with synthetic detoxification methods. The ability of AMF to maintain glomalin production even under pesticide stress, as demonstrated in this study, signifies their resilience and potential for long-term application in diverse agricultural settings. By harnessing the biological synergy between mycorrhizal fungi and plants, particularly using glomalin-producing species like *G. coronatum*, soil health can be restored while minimizing pesticide persistence and environmental contamination. These findings not only advance our understanding of glomalin's functional role in agroecosystem detoxification but also provide a practical framework for integrating AMF-based interventions into sustainable pest management and soil conservation programs. Future work should explore the scalability of this approach in field conditions, examine the molecular pathways underlying glomalin-pesticide interactions, and assess the long-term effects on soil microbial diversity and crop productivity. The application of such biologically driven remediation strategies offers a path toward cleaner, safer, and more resilient agricultural systems, affirming the critical role of microbial symbiosis in achieving environmental sustainability.

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