Density of Arbuscular Mycorrhizal Fungal Spores and Root Colonization in Weeds Found in Garlic Fields at Niphad Tahsil, Maharashtra

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Abstract-Arbuscular mycorrhizal fungi establish symbiotic relationships with plants through their roots, creating multi-colonization systems that benefit the host plants. This study aimed to assess the presence and distribution of Arbuscular mycorrhizal fungi in the rhizospheric soil of significant weed species in Niphad tahsil, Maharashtra. We collected roots and rhizospheric soil samples from 15 weed species across 12 families in a garlic field in Niphad tahsil, focusing on sporulation and types of root infections. The results indicated that *Melilotus indica* exhibited the highest spore density, with 276 spores, followed by *Malva neglecta* and *Sonchus asper*, which had 244 and 214 spores, respectively. The lowest spore density was observed in *Pao annua*, with only 35 spores. The highest density of *Glomus* was also found in *Melilotus indica*, with a mean value of 83.67±1.65. In contrast, the maximum spore density for *Acaulospora* and *Sclerocystis* was recorded in the rhizospheric soil of *Sonchus asper* and *Fumaria indica*, respectively. Root infection rates ranged from 20% to 85%, with the highest colonization observed in *Parthenium hysterophorus* (39.67±11.02) and the lowest in *Fumaria indica* and *Taraxacum officinale* (6.33±5.51 and 8.00±4.00, respectively). It is important to note that a high spore density does not necessarily correlate with high root colonization, as the growth stage of the host plant significantly influences the diversity and population of Arbuscular mycorrhizal fungi (AMF). Our findings indicate a close relationship between AMF spore density, root infection, and the physicochemical properties of the soil.

***Keywords*- Arbuscular Mycorrhizal Fungi (AMF), Garlic (*Allium sativum L.*), Density, Root Colonization**

INTRODUCTION

The extensive symbiotic relationships formed by arbuscular mycorrhiza result from the co- evolutionary interactions between plants and fungi, benefiting both parties through nutrient exchange (Sharma, 2003; Bonfant and Genre, 2008). This type of symbiosis is notably prevalent, with approximately 80% of higher plant species exhibiting colonization by these fungi (Koltai, 2010). In natural ecosystems, arbuscular mycorrhizal fungi (AMF) are integral components of the rhizospheric microflora, essential for a sustainable plant-soil system as they establish symbiotic relationships with terrestrial plants to form mycorrhiza (Sharma et al., 2009). The fungi enhance the plants' ability to absorb nutrients from the soil and offer protection against diseases, while the plants supply the fungi obtain carbon in a soluble form, which is essential for their growth (Auge, 2001; Entry et al., 2002; Wardle and Van der Putten, 2002; Gosling et al., 2006; Smith and Read, 2008; Sikes et al., 2009). The formation of new secondary metabolites can occur as a result of mycorrhizal relationships between fungi and plants (Venkateswarlu et al., 2008). These associations are crucial for the restoration of vegetation, as they enhance and stabilize soil structure, thereby facilitating mineral uptake by host plants and influencing population dynamics while preserving species diversity (Bothe et al., 2010). Arbuscular mycorrhizal fungi (AMF) are also known to help plants withstand heavy metals found in their rhizospheric soil (Jamal et al., 2002; Turnau et al., 2005). Research indicates that plants colonized by mycorrhizae exhibit improved root-to-shoot transport and better uptake of heavy metals (Dodd, 2000). Furthermore, AMF contribute to the reduction of heavy metals in the soil through phytostabilization (Khan, 2005). They also offer additional advantages to host plants, such as enhanced tolerance to salinity and drought, as well as increased resistance to diseases (Auge, 2001, 2004; Ruiz-Lozano, 2003). AMF are considered ecologically significant for many vascular plants and have the potential to influence the ecology of weed species, promoting their growth without adversely affecting major crops (Jordan et al., 2000). However, it is important to note that while AMF provide numerous benefits, they can also have detrimental effects on the growth of host plants (Van der Heijden, 2002; Klironomos, 2003).

The phylum Glomeromycota, which includes arbuscular mycorrhizal (AM) fungi, is distinguished by the formation of specialized intracellular structures known as arbuscules and vesicles. These fungi are among the most prevalent in soil, surpassed only by ectomycorrhizal fungi (Koide and Mosse, 2004). In natural ecosystems, they are integral components of the rhizospheric microflora, establishing symbiotic relationships with plants that are essential for a sustainable plant-soil system. However, the function of mycorrhizae within multispecies communities and natural plant populations remains inadequately understood (Sharma et al., 2009). Various studies have been conducted in Khyber Pakhtunkhwa, including those by Burni and Ilahi (2004), Zainab and Burni (2005), Sharief et al. (2005), and Nasrullah et al. (2010), focusing on the distribution and prevalence of arbuscular mycorrhizal fungi. Despite this, there is still a lack of comprehensive information, prompting the current study to assess the occurrence and distribution of AM fungi in the rhizospheric soil of significant weed species in Niphad Tahsil.

MATERIALS AND METHODS

Roots of fifteen weed species, along with their corresponding soil samples, were gathered from four distinct locations in Tehsil Niphad during the 2023-2024 period. The plant samples represented various families, including Asteraceae, Brassicaceae, Cannabaceae, Convolvulaceae, Euphorbiaceae, Fabaceae, Fumariaceae, Malvaceae, Plantaginaceae, Poaceae, Ranunculaceae, and Zygophyllaceae. The plants were carefully excavated along with their rhizospheric soil in triplicate and transported to the laboratory in polyethylene bags. The roots were gently rinsed under running water to eliminate soil particles, while the rhizospheric soil was shade-dried. The roots were then preserved in a formalin-acetic acid solution.

Extraction of AM spores

Rhizospheric soil samples of maize crop roots was collected at different stages. 100 gm of the fine soil was taken and remove the debris and other larger particles and dissolved in the water and kept for about 24 hours' duration when the soil was completely settled down in the bottom of the beaker the water was passed through 2mm sieve to remove the remaining residues. The cleaned water then passed from the three different sizes of sieves i.e. 140, 170 and 400 µm one by one. The remaining residues above the sieves were collected by rubbing the filter paper on the sieves and studied under the compound microscope for the fungal species diversity and density. The spores were collected through needle and kept on the drop of canda balsam and the slides were made then the picture was capture for identification. For the identifications the manual of Hall and Fish (1979), Trappe (1982) were and density of spores were calculated.

Study of Roots infection

For the observations related to root infections, the established protocols by Giovannetti and Mosse (1980) and Kormanik (1982) were adhered to. Initially, the plant roots were rinsed with tap water to eliminate any formalin and acetic acid residues. Subsequently, the roots were cut into smaller pieces and subjected to heating in a 10% KOH solution for 10 to 15 minutes. To bleach the pigmented areas of the roots, the segments were immersed in an alkaline hydrogen peroxide solution for approximately 10 to 15 minutes. Following this bleaching process, the root segments were washed again with tap water to remove any remaining hydrogen peroxide. Finally, the segments were treated with a 1% hydrochloric acid solution for about 1 to 2 minutes to ensure an acidic environment conducive to effective staining. Root segments were immersed in a 0.025% solution of acidic fuchsine and heated for a duration of 2 to 3 minutes. A total of ten segments, each approximately 1 cm in length, were randomly selected and examined under a microscope for morphological analysis of the amfa endophyte, with microphotographs captured during the process. The infection percentage was determined using the specified formula.

$$\text{Mycorrhizal Infection (\%)} = \frac{\text{No. of Infected segment}}{\text{Total no. of segment}} \times 100$$

Physiochemical Properties analysis of rhizospheric soil sample

The physicochemical characteristics encompass pH, electrical conductivity, soil texture, and the analysis of organic matter, specifically phosphorus, potassium, and nitrogen. The pH and electrical conductivity of the soil samples were measured using a pH meter and an EC bridge, adhering to the established protocols outlined by Jackson (1967) and Black (1965), respectively. The assessment of organic matter was conducted in accordance with the methodology proposed by Neloson and Sommer (1982), utilizing the formula provided below.

$$\% \text{ O.M.} = \frac{(\text{meq. of } K2\ Cr2\ O2 \text{ meq of } FeSO2) \times 0.05}{\text{Wt. of soil sample}} \times 100$$

RESULTS AND DISCUSSION

Arbuscular Mycorrhizal Fungal Spore Density

Roots and rhizospheric soil samples from fifteen weed species were collected from a carrot field in Niphad Tahsil, Maharashtra. The soil was analyzed to assess the presence and distribution of mycorrhizal spores. Three genera of spores were identified: *Glomus*, *Acaulospora*, and *Sclerocystis*. The highest spore density was observed in the rhizospheric soil of Fabaceae family species, with a count of 276 spores, surpassing all other families. In contrast, the Poaceae family exhibited the lowest spore density, with only 35 spores recorded.

This data aligns with findings from Khakpour and Khara (2012) and Surtiningsih et al. (2017), who reported similar sporulation results for these families. Among the identified spore types, Glomus was the most prevalent, followed by *Acaulospora* and *Sclerocystis*. These results corroborate the studies conducted by Chen et al. (2001) and Pande and Tarafdar (2004). The widespread distribution of the Glomus genus in soils globally is further supported by Minal and Anil (2012). The genera *Glomus* and *Acaulospora* are known for their rapid production of small spores, especially when compared to other genera like *Gigaspora* and *Scutellospora*, which produce larger spores (Sarkar et al., 2014). The widespread presence of Glomus can be linked to their reproductive strategies, specifically sporocarp formation, as well as their lower host specificity and ability to thrive across a broad pH range. Soil characteristics, including pH, can limit the distribution of certain taxa (Abbott and Robson, 1991). In our research, we observed a soil pH of 6.1 (Figure 2), which is conducive to *Glomus* growth. This observation aligns with findings by Costa et al. (2013), who noted that *Glomus* decipiens exhibited the highest spore counts at a pH of 6.50.

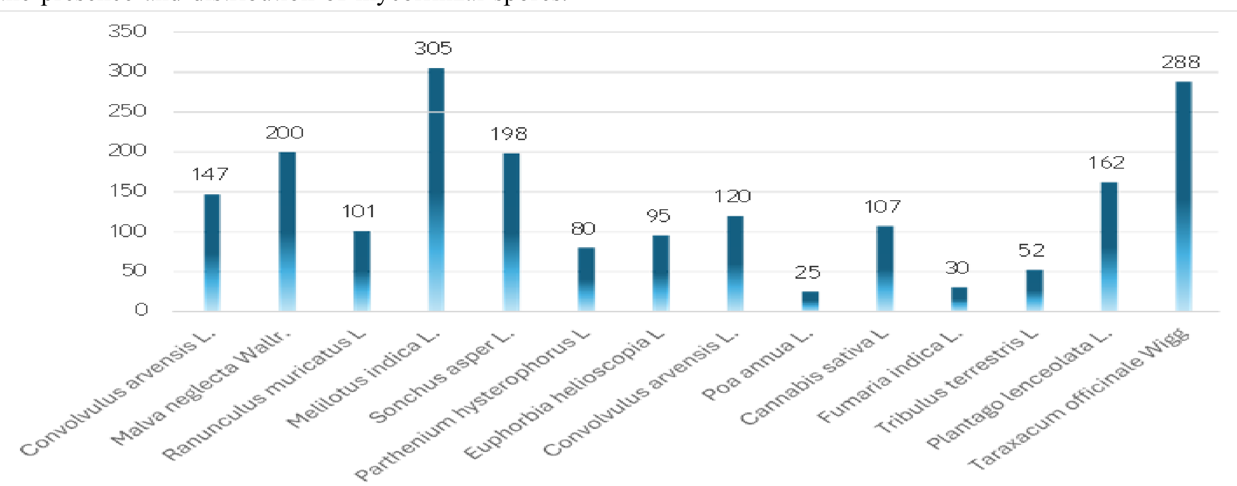

Figure 1: Total AMF spore density in selected weeds of garlic field

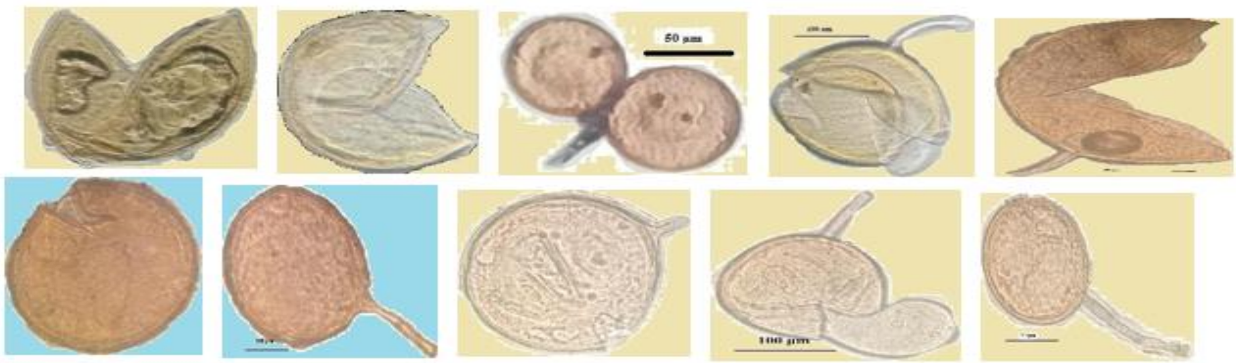

Figure 2: Different types of spores isolated from weeds of garlic field.

Root colonization

An examination was conducted on all weed species to assess root infection. All species exhibited Mycorrhizal infection in their roots, with the exception of Brassicaceae; however, the extent of colonization differed among the various plant species. Characteristic fungal structures were identified, including Arbuscules, Vesicles, External Hyphae, and Internal Hyphae. The level of root infection ranged from a minimum of 25% to a maximum of 75%. The highest level of root colonization was observed in P. hysterophorus, with an average percentage of 39.67±11.02, followed closely by R. muricatus at 34.00±15.39. This finding aligns with the research conducted by Hemavani and Thippeswamy (2013), Rodriguez-Rodriguez et al. (2013), and Wang and Jiang (2015), all of whom reported similar root infections within the same plant families. Moderate colonization rates were noted in S. asper at 23.67±10.60 and P. annua at 20.00±16.37. This observation is further supported by Khakpour and Khara (2012), who documented a colonization rate of 62.7% in the Poaceae family. The study found that P. hysterophorus exhibited the highest level of vesicular infection, while R. muricatus and S. asper showed the most significant arbuscular infection. These results align with the findings of Birhane et al. (2017), who reported the highest vesicular infection in the Rosaceae and Milaceae families, as well as the highest arbuscular infection in Boraginaceae. Additionally, the current research indicated that the two species, C. didymus and B. campestris, from the Brassicaceae family, displayed no colonization by arbuscular mycorrhizal fungi (AMF), a conclusion that is corroborated by the work of Harikumar et al. (2014).

Table 1: Shows mean values of different chemical constituents of soil.

Parameters	Values
pH	*05.14±0.45*
EC ds/m	*00.24±1.12*
OM %	*01.37±0.40*
N%	*00.27±0.08*
P mg/kg	*04.01±1.10*
K mg/kg	*121.73±20.10*

CONCLUSIONS

The study's findings indicate a significant prevalence of arbuscular mycorrhizal fungi (AMF) associations among the weed species in the examined area. All selected weed species, with the exception of those in the Brassicaceae family, were found to be colonized by AMF. The research identified three genera—*Glomus*, *Sclerocystis*, and *Acaulospora*—based on spore analysis from the sampled sites. The predominance of the Glomus genus is attributed to a deficiency of phosphorus (P) in the region, which also exhibits a neutral pH; in contrast, Acaulospora tends to thrive in slightly acidic environments. The study also documented the presence of vesicles, arbuscules, external hyphae, and internal hyphae. These results suggest to the agricultural sector that areas with lower microflora may experience mineral deficiencies, while mycorrhizal associations could enhance nutrient efficiency. The impact on plant communities can vary, potentially leading to either beneficial or detrimental effects.

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