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Effect of Fertilizer on Sporulation and Root Colonization of Mycorrhizal Fungi Associated with Tea Plants

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ABSTRACT: Continuous application of synthetic fertilizer has shown undesirable effect on tea quality as well as reduction in beneficial microbial composition of the soil responsible for sustainable soil productivity. This study has been proposed to analyze the effect of different concentrations of nitrogen (20, 40 and 60 kg N per 1000 m^2), phosphorus (5, 10 and 15 kg P per 1000 m^2) and potassium (25, 50 and 100 kg K per 1000 m^2) fertilizer on sporulation and percent root colonization (PRC) of Arbuscular Mycorrhizal (AM) fungi in the tea-planted soil. Enumeration of sporulation and PRC was performed by collecting rhizospheric soils during the month of March, June, September and December. Study suggested the maximum sporulation and PRC with tea-planted rhizospheric soils fertilized with 40 kg of N, 10 kg of P and 75 kg of K fertilizer. However, doses of 60, 15 and 100 kg of N, P and K respectively showed reduced sporulation and PRC indicating the negative impact of higher doses of fertilizer. Seasonal evaluation showed that sporulation was prominent during the month of December and March whereas optimum percent root colonization was obtained for the month of June and September. Study concluded that colonization of AM fungi under field condition is the function of climatic condition and nutrient status of the soil. Further, this study clearly suggests that quality of the tea and beneficial bacteria composition particularly AM fungi can be maintained by limiting the application of chemical NPK fertilizer under field condition.

Keywords: AM Fungi, NPK Fertilizer, Sporulation, Root colonization, Tea plants.

INTRODUCTION

Tea (*Camellia sinensis*) is most widely consumed non-alcoholic beverages around the world and its consumption is growing steadily due to presence of beneficial antioxidants, vitamins, amino acids, tea polyphenols and caffeine (Lin *et al*., 2019; Wang *et al*., 2022). Furthermore, presence of bioactive compounds such as carotenoids, phenolic acids, flavonoids, coumarins, alkaloids, saponins, terpenoids etc. maintains the flavour and taste of the tea and plays significant roles in human health (Jin *et al*., 2014; Zafar *et al*., 2016; Wang *et al*., 2022). It has been also reported that consumption of tea also supports human health by protecting from chronic diseases, reducing body fat, preventing from cancer, improving oral health, maintaining the beneficial gut bacteria etc. (Sun and Yan 2014; Liu *et al*., 2021). Due to higher demand around the world, farmers are using synthetic chemical fertilizer for large scale production for monetary benefit. Such practices of application of higher doses of chemical fertilizer are reducing the quality of tea, soil productivity and beneficial soil bacteria (Li *et al*., 2016; Li *et al*., 2017; Lin *et al*., 2019).

Continuous degradation in fertility and productivity of agriculturally cultivated land is receiving worldwide attention for their immediate restoration through bioagents for sustainable agricultural practices of beneficial crop plants. Among several agriculturally beneficial bioagents/biosystems, arbuscular mycorrhizal fungi (AM fungi) are efficient scavengers of nutrients and play an important role in restoration and re-establishment of degraded land (Saito and Marumoto 2002; Yeasmin *et al*., 2007; Asmelash *et al*., 2016). Depending on the physico-chemical properties of soil and climatic conditions, AM fungi exhibits two morphologically and physiologically distinct stages *viz*., growing hyphae or root colonization (growing phase) and dormant spore (resting stage). Spore formation in AM fungi is triggered with unfavorable conditions such as alteration in pH, temperature, moisture and develops from extraradical hyphae (Bago and Bécard 2002; Nagahashi, 2000). AM fungi forms various structure such vesicles, arbuscules, hyphae in association with other plants. AM fungi association supports the plants for optimal nutrient transport by forming extensive network of intracellular hyphae ultimately increasing surface area for absorption of nutrients (Bowles *et al*.,

2016; Begum *et al*., 2019). AM fungi absorbs the nitrogen, phosphorus, potassium, calcium, sulfur, ferric, manganese, copper and zinc from the soil and translocate them to plants. Effect of AM fungi on growth and physiology of different plants have been variously studied (Abdullahi and Sheriff 2013; Parial *et al*., 2014; Sharma *et al*., 2021; Anupama and Chamola 2023).

AM fungi not only enhances the absorption but also enhances the resistant against various stresses such as drought, salinity, temperature, metals and diseases by forming symbiotic associations with plants (Begum *et al*., 2019). This group of organisms supports vigorous growth of plants under stressful conditions by enhancing the photosynthetic rates and other gaseous exchange by communicating between fungi and host plants (Begum *et al*., 2019). AM fungi forms symbiotic association with various groups of plants such as angiosperms, gymnosperms, pteridophytes and bryophytes. It has been reported that AM fungi forms endomycorrhizal relationship with 80% of angiosperms and two-third with other group of plants (Mondal *et al*., 2022). AM fungi is receiving much more attention for their significant ability to enhance the fertility of soil by maintaining the nutrient status and by providing stress tolerance to plants. The effect of fertilizer on distribution of rhizosphere microbial communities in tea orchard have been studied variously and is available in literature (Treseder, 2004; Li *et al*., 2016; Li *et al*., 2017; Lin *et al*., 2019). However, information on effect of fertilizer on AM colonization and sporulation in tea planted rhizospheric soil is lacking. The present study has been proposed to demonstrate the effect of different concentrations of fertilizers on AM colonization and sporulation in tea planted rhizosphere soils for future management of chemical fertilizer for better sustainable practices.

MATERIALS AND METHODS

Study was conducted at Good Rich site located at the foot hills of the Himalayas in the Doon Valley of Dehradun, Uttarakhand, India. Geographically, Doon Valley lies between 29° 58' and 31°2'30"N latitudes and 77°34'45" and 78°18'30"E longitudes. During the summers, the temperature ranges between 36°C and 16.7°C. In winters, the temperature lies in between 23.4°C and 5.2°C.

Experimental set up. Site cultivated with local edible cultivars of tea plants was divided into 40 subplots (5 m × 4 m) with a buffer zone of 2 m^2 between each plot to avoid the interaction among treatments. Twelve (12) subplots were used for N, P and K fertilizer treatments separately and control without fertilizer in 4 replicates. Tea plants were equidistantly planted in each subplot. Nitrogen (Urea) was used at the rate of 20, 40 and 60 kg, Phosphorus (P_2O_5) at the rate of 5, 10 and 15 kg and K (K_2O) at the rate of 25, 50 and 100 kg per 1000 sq meters (per hectare). The fertilizer was applied in the first week of the May at the concentration described above.

Rhizospheric soils from every treatment were collected randomly at the regular interval of three months for one year using a core borer of 15 cm in depth and 5 cm in diameter. Six soil samples per plot were collected and were placed in appropriately labeled polyethylene bags. Colonization of AM fungi was documented by collecting the root samples randomly at the depth of 6 to 8 cm below the soil surface from the 'root zones' of the tea plants during 1st week of the June, September, December and March. Roots were collected in conical flask and taken to the laboratory. Roots were washed in running tap water for half an hour followed by washing with sterile double distilled water thrice to avoid the presence of other microbes. Study of the root segments for AM colonization was done on very next day of the collection of the root segments. Root subsamples were removed and placed in 50% ethanol and stored at 4°C for % root colonization study.

Isolation of AM spores was performed using wet sieving and decanting procedure of Gerdemann and Nicolson (1963) and was enumerated using the method of Gaur and Adholeya (1994). Root colonization study was performed using the clearing and staining technique of Phillips and Hayman (1970).

RESULTS

Effect of nitrogen on sporulation and % root colonization (PRC) of VAM fungi. Study showed that various concentration of nitrogen had prominent effect on sporulation and root colonization compared to control at a given time. It was observed that an increase in sporulation was associated with decrease in root colonization and vice-versa (Table 1). Sporulation was higher for the month of December and lower for the month of September. However, PRC was higher for the month of September and lower for the month of December under various concentration of N including control. Further analysis in terms of effect of various concentration of nitrogen showed maximum sporulation and PRC for 40 kg of N ha^{-1} and minimum with 60 kg N ha^{-1}. It suggested that the application of 60 kg N ha^{-1} didn't support the association of tea plants with AM fungi.

Table 1: AM fungal spore and % root colonization under different level of N treatment.

Parameters	Time (Months)	N-treatment (kg / 1000 m^2)			
		0	20	40	60
AM fungal spore/10 g soil	March	86 ± 4	95 ± 6	105 ± 5	118 ± 6
	June	66 ± 4	78 ± 5	89 ± 3	108 ± 5
	September	48 ±3	65 ± 4	85 ± 7	93 ± 4
	December	106 ± 5	117 ± 3	124 ± 5	116 ± 7
% root colonization	March	38 ± 3	45 ± 6	60 ± 8	40 ± 5
	June	46 ± 2	65 ± 4	72 ± 5	54 ± 3
	September	61 ± 4	79 ± 5	93 ± 4	86 ± 5
	December	28 ± 3	39 ± 3	48 ± 4	34 ± 3

Effect of Phosphorus on sporulation and % root colonization (PRC) of VAM fungi. Study revealed that application of phosphorus has a profound effect on sporulation and percent root colonization over control up to the 10 kg phosphorus at a given time (Table 2). However, a marked decrease in sporulation and PRC was observed with 15 kg phosphorus compared to 5 and 10 kg P application. Sporulation and PRC showed similar trend of maximum and minimum during the month of December and September respectively under all concentrations of P used as observed for N described above.

Effect of Potassium on sporulation and % root colonization (PRC) of VAM fungi. No marked differences were observed for sporulation and PRC for different concentration of phosphorus used in experimental study (Table 3) at a given time compared to N and P. However, sporulation was higher for the month of December and lower for the month of September whereas PRC was higher for the month of September and lower for the month of December.

Table 2: AM fungal spore and % root colonization under different level of P treatment.

Parameters	Time (Months)	P-treatment (kg / 1000 m^2)			
		0	5	10	15
AM fungal spore/10 g soil	March	81 ± 4	94 ± 5	102 ± 4	92 ± 3
	June	63 ± 4	74 ± 3	93 ± 2	68 ± 5
	September	45 ± 5	58 ± 4	87 ± 5	52 ± 3
	December	99 ± 5	112 ± 4	132 ± 6	103 ± 4
% Root colonization	March	30 ± 3	42 ± 4	52 ± 3	53 ± 4
	June	44 ± 4	59 ±5	66 ± 3	53 ± 5
	September	62 ± 4	78 ± 6	87 ± 4	65 ± 3
	December	28 ± 3	36 ± 3	48 ± 4	31 ± 3

Table 3: AM fungal spore and % root colonization under different level of K treatment.

Parameters	Time (Months)	K-treatment (kg / 1000 m^2)			
		0	25	50	100
AM fungal spore/10 g soil	March	89 ± 5	102 ± 4	112 ± 3	95 ± 5
	June	69 ± 4	84 ± 4	101 ± 5	76 ± 4
	September	42 ± 3	62 ± 6	92 ± 4	54 ± 3
	December	110 ± 4	127 ± 7	132 ± 6	116 ± 7
% root colonization	March	38 ± 4	65 ± 6	72 ± 4	43 ± 6
	June	48 ± 4	86 ± 5	88 ± 6	68 ± 6
	September	65 ± 7	91 ± 5	93 ± 3	80 ± 5
	December	33 ± 3	59 ± 5	69 ± 4	38 ± 3

DISCUSSION

Application of fertilizer up to 40 kg of N, 10 kg of P and 50 kg of K under field condition has profound effect on sporulation and PRC compared to control. However, fertilizer application such as 60 kg of N, 15 kg of P and 100 kg of K reduced the sporulation and PRC. It was assumed that increase or decrease in sporulation and PRC is governed by nutrient status of the soil and climatic conditions. Lower doses of fertilizer promote plant root proliferation making a bigger contact with microbes and enhance the interaction between the microbes and plants resulting into the higher PRC during favourable condition of growth. Higher doses of fertilizer application inhibited the plant-microbes interaction resulting into the lower sporulation and PRC for easily available nutrient supply resulting into the sporulation. Lin *et al.* (2012) have also demonstrated that higher doses of long-term fertilization with phosphorus and nitrogen (NP and NPK) resulted into reduction in AM fungi diversity and richness in arable land. Hayman (1983); Tinker (1980) have also reported that high levels of phosphate may reduce the establishment of VAM-plant symbiotic associations. Arnold and Kapustka (1987) obtained similar result of reduction in spore population and percent colonization in the soil treated with urea-phosphate chemical fertilizer. However, they further reported no significant change in mycorrhizal colonization or spore population of a field treated with milorganite or sludge (538 kg N ha^{-1} yr^{-1}, 179 kg P ha^{-1} yr^{-1} and no K) with similar amount of nitrogen and phosphorus. It was assumed that no change in sporulation or root colonization may be attributed to slower release of phosphate ions present in milorganite or sludge. Further, it has been reported that in certain cases of gramineous plants, sporulation is reduced whereas under another condition, sporulation is increased with mycorhhiza *Miscanthus sinensis* under the application of exogenous N (Saito *et al.*, 2011).

Present investigation of decrease in sporulation and colonization compared to high fertilizer level is comparable with the earlier report on VAM infection (Huat *et al.*, 2002). They reported negative relationship between mycorrhizal infection and fertilizer level. Further investigation with respect to level of phosphorus represented no definite correlation between phosphorus and sporulation and colonization (Muthukumar *et al.*, 1994). They reported positive influence of phosphorus on sporulation and AM colonization in case of plant *Indigofera linnaei* while it was negative in *Alysicarpus monilifer*. It might be concluded that effect of fertilizers on sporulation and root colonization is the function of different factors like nutrient status and physicochemical properties of soil and climate.

Further investigation analyzed in terms of percent root colonization indicated higher active mycorrhizal infection for application N over 40 Kg, P over 10 kg and K over 50 kg per hectare during rainy season of

study. Present study is in accordance with the earlier literature that showed negative effect of high level of nitrogen and phosphorus on VAM colonization (Azcón *et al*., 2003; Xu *et al*., 2000). Azcón *et al*. (2003) demonstrated that VAM colonization was more prominent at 0.1mM phosphorus in comparison to comparatively higher doses of 0.5mM of phosphorus applied in tea planted soil. This study anticipated that use of low level of fertilizer would result in a better root colonization and can function like a slow-release fertilizer by establishing a symbiotic relationship with host plants to support the growth. Moreover, under sufficient nutrient supply, host plants absorb all essential nutrients by roots and don't allow the fungal partner to form symbiotic relationship thereby indicating possible role of soil nutrients in development of sporulation and AM infection.

Significant variation in sporulation and PRC with respect to time and concentration indicated the influence of seasonal variation in climatic condition and concentration of fertilizer used. Effect of seasonal variation and spatial heterogeneity on sporulation and colonization are already in literature (Anderson *et al*., 1983; Uhlmann *et al*., 2004). Maximum percent root colonization during rainy season might be attributed to favorable growth conditions in terms of temperature and rainfall. Similar results of seasonal variation in sporulation and colonization have been reported by Uhlmann *et al*. (2006). They compared the species diversity and sporulation during different season and reported low value for summer rainfall and higher for winter rainfall.

CONCLUSIONS

Study concludes that the season (time) and concentration (fertilizers) is crucial factor in establishing AM fungi to get maximum benefit for proper host plants under field condition. Under similar growth condition and host, no significant variation in AM population can be achieved. Moreover, study reflected that higher fertilizer level might reduce the AM population under field condition. Finally, it might be concluded that indigenous AM fungi can be effectively introduced as bioinoculant with lower fertilizer level for sustainable agriculture practices and for a rehabilitation of an overburdened soil with reduced fertilizers.

FUTURE SCOPE

Findings of this study provide platform for future research directed towards evaluation of effect of chemical fertilizer in combination with AM fungi on qualitative and quantitative production of tea and diversity of beneficial bacteria in the soil for sustainable practices.

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Conflict of Interest. None.

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