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EFFECT OF APPLICATION OF NPK FERTILIZERS ON THE NATURAL INCIDENCE OF VESICULAR ARBUSCULAR MYCORRHIZA IN THE RHIZOSPHERE SOILS AND FEEDER ROOTS OF TEA (*CAMELLIA SINENSIS*).

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This study was undertaken to establish the levels of VAM in feeder roots and rhizosphere soils of tea plants from a NPK fertilizer trial estalished in 1961, with clone TRI 2024 at St.Coombs Estate, Talawa-kelle.

N-fertilizer at 336 kg ha^{-1} yr^{-1} and/or soil available N of 104 mg kg^{-1} soil significantly reduced the % root infections (RI) by VAM while P-fertilizer reduced both %RI and viable spore count (SC) to a lower level. The highest %RI of 17.8 and viable SC of 816 was observed with no P-fertilizer application and/or available soil-P of 8.2 mg kg^{-1} soil. Application of K-fertilizer up to 116 kg ha^{-1} yr^{-1} K had no effect.

Improvement of soil C from 3.0 - 9.0 % significantly increased the viable SC but decreased the %RI to a very low level.

INTRODUCTION

Fungi forming mycorrhizal associations with roots of higher plants are widespread in nature; no perennial plants including tea are free from this interaction. Mycorrhizal colonization is more common than its absence in higher green plants (Wastie, 1965; Webster, 1953; Fang-Ming Thseng and Jee-Song Chen, 1984; Balasuriya, Arulpragasam and Ratnayake, 1991). Nicolson (1967), referred to this as the `Universal Symbiosis',

Some of the well documented attributes of these mycorrhizal associations are increased absorption of relatively immobile soil nutrients such as P, Cu, Zn, etc. (Kleinschmidt and Gerdemann, 1972) and greater tolerance to toxic heavy metals such as Mn, Ni, Cr, Cd, etc. (Tinker, 1975; Rhodes, 1980; Kothari, Marschner and Romheld, 1990). It is also to be noted that the role of Vesicular Arbuscular Mycorrhiza (VAM) on P nutrition of crop plants has been abundantly proved (Cress, Thorneberry and Lindsay, 1979; Jayachandran, Schwab and Hetrick, 1992).

The present investigation was carried out to establish the levels of VAM in feeder roots and rhizosphere soils of tea plants from a long term NPK fertilizer trial conducted at St.Coombs Estate, Talawakele.

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MATERIALS AND METHODS

Trial design

An NPK fertilizer trial established in 1961, with clone TRI 2024 at St.Coombs Estate (Field No.8, elevation 1370 m amsl) was selected and representative soil and root samples of the rhizosp-here-zone of tea plants were collected in May 1991. The samples were obtained from plots applied with 112, 224 and 336 kg ha^{-1} yr^{-1} N (sulphate of ammonia, 21.0% N) 0, 25 and 50 kg ha^{-1} yr^{-1} P (Rock Phosphate, 27.5-32.5% P_2O_5), and 0, 58 and 116 kg ha^{-1} yr^{-1} K (Muriate of Potash; 60.0% K_2O) in a factorial combination replicated twice, in a randomized complete block design. Dolomite fertilizer (18-20% MgO; 30-35% CaO) was applied to the experimental plots (24 bushes per plot) once a pruning cycle at the rate of 1000 kg ha^{-1} $cycle^{-1}$, at the time of pruning. Each pruning cycle was of 5 years duration.

Rhizosphere soils and feeder roots

Soil samples were collected about 15 cm from the base of the bush after removing the surface leaf litter and debris etc., down to a depth of 15 cm. They were refrigerated at 4EC in polythene bags, for the VAM spore assessments.

Sub-samples of feeder roots from each rhizosphere root/soil sample, were separated immediately. The soil contaminated root samples were washed free of soil, in running tap water and preserved (Kormanic and McGrow, 1984) in a mixture of Formalin: Acetic Acid (glacial): 50% Alcohol (FAA) at 13 : 5: 200 for VAM infection assessments.

The rest of the soil/root mass was air-dried for about 48 h and passed through a 2 mm sieve for the determination of soil C, pH, Borax extractable P, ammonium-N, nitrate-N and exchangeable K (Page, Miller and Keeney, 1982).

Separation of VAM Spores from soil

A sample of 120 g moist soil (equivalent to 100 g oven dried soil), was used for the separation of VAM spores, by the sucrose centrifugation & sieving technique (Daniels and Skipper, 1984). The VAM spores thus separated were transferred onto a No.541, 9 cm diameter, Whatman filter paper for counting under a dissecting microscope (Ernst Leitz Wetzler) at 4 x magnification.

Infection counts of Root samples

Feeder root samples were cleared with 10% KOH and stained with cotton-blue in lactophenol (Kormanik and McGrow, 1984). The number of intercepts with infections were counted on a grid of 1.3x1.3 cm, under the dissecting microscope x 4, magnification. The root samples were re-positioned three times on the same grid, and the mean of such three counts were taken as the final value. The percent root infections (RI) was calculated as follows (Giovannetti and Mosse, 1980);

$$\% \text{ Root Infections(RI)} = \frac{\text{Number of intercepts with infections} \times 100}{\text{Total number of intercepts}}$$

RESULTS AND DISCUSSION

Soil application of N, P and K fertilizers on yield of tea, viable spore counts (SC) and root infections (RI) by VAM, and effects of soil %C are discussed.

Fertilizer effects on yield

Application of increasing levels of N fertilizer from 112 to 336 kg ha^{-1} yr^{-1} at 112 kg N increments and of K fertilizer from 0 to 58 kg ha^{-1} yr^{-1} increased the yield of made-tea significantly. However, no crop response was observed with the increasing levels of P fertilizer at all three levels tested (Anon, 1991).

N fertilizer effects on SC and RI

Table 1 shows that only soil ammonium-N concentrations were significantly increased with increasing levels of N fertilizer, while no significant changes were observed with nitrate-N concentrations. Thus the total available soil-N, ie. ammonium-N + nitrate-N, was increased from 81 to 104 mg kg^{-1} soil.

The viable spore counts (SC) have not been affected with increasing levels of N fertilizer and/or available soil N of 81-104 mg kg^{-1} soil, while the root infections (RI) has been significantly reduced only at the highest level of N fertilizer and/or available soil N of 104 mg kg^{-1} of soil. This is in agreement with Slankis (1974) and Parameswaran and Augustine (1988), who have shown that available soil-N of 35-60 ppm, helped to maintain a good number of spores and infections.

TABLE 1: *Effect of soil application of N fertilizer, on viable VAM spore counts (in 100g of soil) and root infection*

N level (kg $ha^{-1}yr^{-1}$)	Soil N (mg kg^{-1} soil)		Spore count	% Root infection
	NH_4-N	NO_3-N		
112	15.2 a	65.4 a	629 a	15.9 ab
224	20.4 a	58.7 a	665 a	17.5 a
336	34.9 b	69.2 a	711 a	9.0 b

Note: Means followed by same letter in any column are not significantly different from each other.

P fertilizer effects on SC and RI

The soil exchangeable-P concentrations significantly increased with increasing levels of P fertilizer (Table 2).

The highest viable spore count of 816 and % root infections of 17.8 were observed with no P fertilizer at 8.2 mg kg^{-1} soil exchangeable-P. Slankis (1974) and Parameswaran and Augustine (1988), have shown that the highest response of viable spore counts and root infections are possible when soil exchangeable-P levels are in the range of 0.2 to 25 mg kg^{-1} soil. Nirmala, Mohankumar and Mahadevan (1988), using onion roots in rice soils reported a rapid development of root infections at soil-P level of 12 mg kg^{-1}.

Application of P fertilizer significantly reduced the number of viable spores. Similar results were obtained by Vyas and Srivastava (1988) with moth bean.

TABLE 2: *Effect of soil application of P fertilizer, on viable VAM spore counts (in 100 g of soil) and root infections*

Level of P (kg ha^{-1} yr^{-1} P)	Soil Exch .p (mg kg^{-1} soil)	Spore count	% Root infections
0	8.2a	816 a	17.8 a
25	63.6b	578 b	12.2 a
50	189.0c	610 b	12.4 a

Note: Means followed by same letter in any column are not significantly different from each other.

K fertilizer effects on SC and RI

The soil exchangeable-K concentrations were significantly increased with increasing levels of K fertilizer (Table 3).

TABLE 3: *Effect of soil application of K fertilizer, on viable VAM spore counts (in 100 g of soil) and root infections*

Level of K (kg ha^{-1} yr^{-1} K)	Soil Exch. K (mg kg^{-1} soil)	Spore count	% Root infections
0	18 a	613 a	14.7 a
58	33 b	739 a	14.7 a
116	50 c	653 a	13.0 a

Note: Means followed by same letter in any column are not significantly different from each other.

However, this soil exchangeable-K did not significantly affect SC or RI in rhizosphere-soil and in tea feeder roots. Slankis (1974) and Parameswaran and Augustine (1988) reported that the effective range of soil exchangeable-K for the maintenance of optimum levels of the above parameters in 44 plant species in a scrub jungle, ranged from 45 to 90 mg kg^{-1} soil. In this experiment at the highest level of K fertilizer, 50 mg kg^{-1} soil K, was recorded, thus approaching the optimum level for mycorrhizal activity. It is possible that the response of VAM to N and P were overriding the effect of K.

Effect of per cent Soil C and pH on SC and RI

TABLE 4: *Regression coefficients of soil C on viable VAM spore counts (in 100 g of soil) and root infections*

%C in soil	Spore count	% Root infections
3 - 4	430	22.5
4 - 5	635	17.6
5 - 6	669	17.7
6 - 7	750	10.1
7 - 8	778	11.1
8 - 9	762	8.7
r =	0.89*	0.94**

* significant at P <0.05
** significant at P <0.01

Increasing levels of soil C has significantly increased the viable SC and decreased %RI (Table 4). This is further supported by the findings of Reddy and Goud (1988), that the mycorrhizal colonization was extensive at a low soil C level of 1.23 % in loamy sand soils.

Soil pH of the 30 samples tested ranged from 3.35 to 5.05. For the comparisons these were grouped in to two, namely 3-4 and 4-5 (Table 5). High variabilities were found in both spore counts (350-1868) and the % root infections (1.6-34.2) in the 30 test samples. At lower pH (3-4) a higher range of VAM spores (444-1868) were encountered with a relatively lower range (1.6-29.2) in root infections. The root infections have shown an increase with the increase in pH up to 5. Viable spore counts have responded different to this, with reduced numbers at the higher pH.

TABLE 5 *Effect of soil pH, on viable VAM spore counts (in 100 g of soil) and root infections*

pH	Spore count %		Root infections	
	Mean	Range	Mean	Range
3-4	821 ± 361	444 - 1868	16.21± 6.91	1.6 - 29.2
4-5	746 ± 196	350 - 1084	17.81 ± 8.60	7.7 - 34.2
Mean	789 ± 303		16.90 ± 7.73	

The observations made here are consistent with the findings of Menge; Jarrel, Labanauskas, Ojala, Huszar, Johnson and Sibert (1982), where they have established that the mycorrhizal depen-dency of *Poncirus trifoliata* L., was positively correlated with soil pH and inversely correlated with extractable P and %C. Zao Bin (1988) using two *Glomus* species demonstrated that there was extensive infection on cotton roots at high soil pH while *Acaulospora laevis* reduced infection to lower than 16%, at a higher pH of 7.8. Balasuriya, Arulpragasam and Ratnayake (1991), observed both these species of VAM in St.Coombs soil of which, a very high proportion was of *Glomus* species. Therefore, we can accept the relatively low level of root infections (17%) in tea feeder roots under St.Coombs conditions. However, the higher individual infection values (34.2) obtained in this experiment, proves there is good potential to improve them.

CONCLUSIONS

Application of N fertilizer at 336 kg ha^{-1} yr^{-1} and/or soil available N (NO_3-N + NH_4-N) of 104 mg kg^{-1} soil significantly reduced the %RI by VAM, confirming the negative effect of soil application of higher doses of N-fertilizer on mycorrhiza in tea.

P fertilizer at or above 25 kg ha^{-1} yr^{-1} P and/or soil available P of 63.6 mg kg^{-1} soil, reduced both %RI and viable SC to a lower level, while the highest %RI of 17.8 and viable SC of 816 was observed with no P fertilizer application and/or available soil-P of 8.2 mg kg^{-1} soil, confirming the antagonism of P fertilizer and the VAM in the tea root/soil interface. Application of K fertilizer up to 116 kg ha^{-1} yr^{-1} K had little or no effect on %RI and SC.

High level of soil C (9.0 %) significantly increased the viable SC while decreasing the %RI to a very low level. Therefore, at higher elevations (1500 m), moderate soil C levels will be beneficial in maintaining VAM activity at a higher profile. Generally, low root infection levels may be due to the low pH ranges (3-5) present in the trial area. In view of this, for better performance we may have to think of introducing VAM species which can tolerate low pH.

This study shows the importance of judicious application of NPK fertilizers and maintenance of optimum levels of soil C along with plant available soil-nutrients to encourage the activity of VAM in tea soils. Studies in relation to the soil/plant factors which will directly affect the activity of VAM and establishment of beneficial levels of VAM, soil C and pH in tea soils under different elevations could prove very useful.

REFERENCES

ANON, (1991). Report of the Officer-in-charge for Agronomy. *Tech. Rep. Tea Res. Inst. Sri Lanka, 35-52.*

BALASURIYA, A., ARULPRAGASAM, P.V., and RATNAYAKE, A.(1991). Mycorrhiza in Tea, *Tea Bull.* **11**(1/2), 3-12.

CRESS, W.A., THORNEBERRY, G.O. and LINDSAY, D.L. (1979). Kinetics of phosphorous absorption by mycorrhizal and non mycorrhizal tomato roots. *Plant Physiol.* **64**, 484-487.

DANIELS, B.A. and SKIPPER, H.D. (1984). Methods for the recovery and quantitativanalysis of propagules from soil. Methods and Principles of Mycorrhizal Research, 29-35.

FANG-MING THSENG and JEE-SONG CHEN (1984). The preliminary investigations of tea mycorrhizal fungi. *Taiwan Tea Res. Bull.* 110-112.

GIOVANNETTI, M. and MOSSE, B. (1980). An evaluation of techniques for measuring vesicular-arbuscular micorrhizal infection in roots. *New Phytol.* **64**, 489-500.

HAYMAN, D.S. (1983). The physiology of Vesicular Arbuscular Endomycorrhizal symbiosis. *Canad. J. Bot.* **61**, 944-963.

JAYACHANDRAN, K., SCHWAB, A.P. and HETRICK, B.A.D. (1992). Mineralization of organic phosphorus by Vesicular-Arbuscular Mycorrhizal fungi. *Soil Biol.Biochem.* **24**(9), 897-903.

KLEINSCHMIDT, G.D. and GERDEMANN, J.W. (1972) Stunting of citrus seedlings in fumigated nursery soils related to the absence of endomycorrhizae. *Phytopath.* **62**, 1447-145

KORMANIK, P.P. and MCGROW, A.-C. (1984). Quantification of Vesicula Arbuscular Mycorrhizae in plant roots. Methods and Principles of Mycorrhizal Research, 37-45.

KOTHARI, S.K., MARSCHNER, H. and ROMHELD, V. (1990). Direct and indirect effects of VA mycorrhizal fungi and rhizosphere microorganisms on acquisition of mineral nutrients by maize (Zea maize L.) in a calcareous soil. *New Phytol.* **116**, 637-645.

MENGE,J.A., JARREL, W.M., LABANAUSKAS, C.K., OJALA, J.C., HUSZAR, C., JOHNSON, E.L.V. AND SIBERT, D. (1982). Predicting mycorrhizal dependency of Troyer citrange on Glomus fasciculatus in California citrus soils and nursery mixes. *Soil Sc. Soc. Amer. J.*, **46**(4), 762-768.

NICOLSON, T.H. (1967). Vesicular-arbuscular mycorrhiza - a Universal plant symbiosis. *Sci Prog.* **55,** 561-581.

NIRMALA, C.B., MOHANKUMAR, V. and MAHADEVAN, A. (1988). Viability of VAM spores in the fallow fields of Maduravoyal, Tamil Nadu. Mycorrhizae for Green Asia, 89-90.

PAGE, A.L., MILLER, R.H., and KEENEY, D.R. (1982). (Eds.) Methods of Soil Analysis Part 2. Chemical and Microbiological properties. Second edition.

PARAMESWARAN, P. and AUGUSTINE, B. (1988). Distribution and ecology of a VAM in a scrub jungle. Mycorrhizae for Green Asia, 91-94.

REDDY, C.N. and GOUD, G.R. (1988). Occurrence of VAM association with *Cassia occidentalis,* Mycorrhizae for Green Asia, 85-88.

RHODES, L.H. (1980). The use of mycorrhizae in crop production systems. *Outlook on Agriculture,* **10,** 275-281.

RUSSEL, E.W. (1973). Soil Conditions and Plant Growth (10th edition). William Clowers, Lond. pp 260.

SCHENCK, N.C. (ed); (1984). Methods and Principles of Mycorrhizal Research. Amer. *Phytopath. Soc.,* Minnesota. 244.

SLANKIS, V. (1974). *Ann. Rev. Phytopath.* **12,** 437-457.

TINKER, P.B.H. (1975). Effects of vesicular arbuscular mycorrhiza on higher plants. Symp. Proc., *Soc. Exp. Biol.* **29,** 325-349.

VYAS, A. and SRIVASTAVA, H.P. (1988). Studies on VAM of moth bean:ecological implications. Mycorrhizae for Green Asia, 118-119.

WEBSTER, B.N. (1953). Mycorrhiza. *T. Quart.* **24,** 26-30.

WASTIE, R.L. (1965). The occurrence of an endogone type of endotrop-hic m-ycor-rhiza in *Hevea brasiliensis. Trans. Brit. Mycol. Soc.,* **48,** 167-168.

ZHAO BIN (1988). The effect of soil pH on the production of external hyphae by three VAM fungi. Mycorrhizae for Green Asia, 189-191.