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Comparison of Three Green Manures for Growth and VA Mycorrhizal Colonization in Maize (*Zea mays* L.)

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Abstract: The influence of three leguminous green manures viz. *Trifolium alexandrianum, Medicago polymorpha* and *Melilotus parviflora* on growth, yield and vesicular arbuscular mycorrhizal (VAM) colonization in maize (*Zea mays* L.) was examined and compared with recommended NPK fertilizers. The three green manuring plants contained 4.44, 2.45 and 3.17% nitrogen; 0.065, 0.089 and -0.122% phosphorus and 1.24, 1.89 and 0.8% potassium, respectively. NPK fertilizers suppressed VAM colonization whereas green manures generally favoured it. The study of correlation coefficient revealed that there was a variable pattern of correlation between N, P and K contents of green manures and various parameters of VAM colonization, at different growth stages. All the soil amendments enhanced shoot growth in terms of length and biomass, at all the three growth stages. Effect of *Trifolium* was similar to that of NPK fertilizers while *Medicago* and *Melilotus* were proved significantly superior than NPK fertilizers in increasing shoot biomass. Negative and significant correlation of shoot length and biomass with N contents of green manures was observed whereas with P content the correlation was positive and highly significant. Root length and biomass was increased by NPK fertilizers and either of the green manure amendment. Pattern of correlation of root length and biomass with N and P contents of green manures was similar to that of shoot. Highest cob biomass was recorded in mixed green manure followed by *Medicago* and *Melilotus*, respectively. Difference was significant as compared with control, NPK fertilizers and *Trifolium*. A highly significant and negative correlation was recorded between cob biomass and N content of the green manures. Pattern of correlation of root and shoot growth and cob biomass with various parameters of VAM colonization was variable and insignificant at different growth stages.

Key words: Green manures, *Trifolium alexandrianum, Medicago polymorpha, Melilotus parviflora,* Arbuscular mycorrhizae

Introduction

Green manuring is the practice of turning into the soil unrecompensed green tissues. The potential benefits of green manures are many. Apart from increasing soil nitrogen (Pushpavalli *et al.*, 1994), the soil organic matter is maintained and renewed, and the physical and chemical characteristics of the soil are improved (Tiwari *et al.*, 1980). Generally legumes are used as green manure crops. Allen and Allen (1981) have listed nine genera of legumes viz. *Aeschnomene, Astragalus, Crotolaria, Indegofera, Lathyrus, Pongamia, Tephrosia, Trifolium* and *Sesbania* as best green manuring crops. Besides legumes, other symbiotic nitrogen fixing plants like *Azolla* are also used as green manures (Agarwal, 1998). The legumes used as green manures have high amount of nutrients and low C/N ratios. One thousand kilogram of fresh matter contains about 5 Kg N, 0.44 Kg P and 3.3 Kg of K (Lizhi, 1988). It is estimated that legumes fix 80 million tons of N annually from the vast free supply in the air. In contrast, fertilizer manufacturers produce at high cost only 50–60 million tons of N annually (FAO, 1984).

Vesicular arbuscular mycorrhizal (VAM) symbiosis and fungi occur in almost all habitats and climates (Barea *et al.*, 1997), including disturbed soils (Tonin *et al.*, 2001). Many of the reactions and interactions of microflora and fauna that occur in the soil around roots are mediated by VAM fungi that function to deliver mineral nutrients to the host plant in return for a sustained carbon supply. They also impart other benefits to plants including stimulation of growth regulating substances, increased rate of photosynthesis, osmotic adjustment under drought stress, enhancement of nitrogen fixation by symbiotic or associative N_2-fixing bacteria, and improved soil aggregation and thus improved soil physical properties and stability (Bethlenfalvay and Linderman, 1992). Fertilizers have varied effects on the VAM symbiosis and on the fungi themselves. Many field experiments showed that fertilizers decrease quantities of VAM fungi (Jensen and Jakobson, 1980; Vivekanandan and Fixen, 1991), however, other field experiments revealed that fertilizers increased quantities of mycorrhizae (Dehne, 1987; Gryndler *et al.*, 1990). Several studies indicated that the ratio of nutrients within the fertilizers influences mycorrhizal responses (Saif, 1986; Gryndler *et al.*, 1990). Addition of organic matter has been found to stimulate mycorrhizal growth (Joner and Jakobsen, 1995).

Melilotus parviflora, Medicago polymorpha and *Trifolium alexandrianum* are commonly available legumes in Pakistan. *M. parviflora* and *M. polymorpha* are wide spread annual winter weeds in wheat fields, grassy lawns and on wastelands. *T. alexandrianum* is an annual herb and is a common fodder crop, grown on a large scale in winter. All these legumes produce a huge amount of crop biomass and fix atmospheric nitrogen in their root nodules. The present study was designed to evaluate the usefulness of these species as green manure by studying their effects on crop growth and VAM colonization in maize.

Materials and Methods

This pot experiment was conducted in Botany Department, University of the Punjab, Lahore, Pakistan during 2000. Clay pots of 30cm diameter were filled with air-dried and sieved field soil @ 5 Kg per pot. Fresh shoot materials of three leguminous plant species viz. *Trifolium alexandrianum, Medicago polymorpha* and *Melilotus parviflora* were cut into 2 cm pieces and mixed in the pot soil separately @ 10 g/100 g of soil. In the mixed treatment a 1:1:1 mixture of the three species was added at the same rate as in individual treatments. In NPK fertilizer treatment, N, P_2O_5 and K_2O were applied equivalent to 70-35-25 kg/acre in the form of urea, triple super phosphate and potassium sulphate, respectively. Whole P_2O_5 and K_2O and half N dose were applied as basal while the remaining half N was top dressed just prior to initiation of flowers. Pots without any supplementation were used as control. Six maize seeds, surface sterilized with 1:1 mixture of H_2O_2 and spirit, were sown in each pot. There were three replicate pots of each treatment. After germination plants were thinned to three uniform seedlings per pot. Pots were kept in a wire netting enclosure under normal conditions of light and temperature. Three harvests were designed at 40, 80 and 110 day after sowing, corresponding to three growth stages viz. vegetative, flowering and maturity, respectively. At the time of each harvest, plants were carefully uprooted and washed under tap water to remove the soil. Root and shoot length and fresh weight were recorded. Shoot and root materials were oven dried at 70 °C for 48 h after separating a sub sample of roots of each treatment for VAM study. Yield in terms of number, fresh and dry weight of cobs

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was recorded at final harvest.

For VAM study, roots were cut into 1 cm segments, transferred into test tubes, added 10 % KOH and cleared them by autoclaving for five minutes, followed by staining with 0.05% trypan blue (Phillips and Hayman, 1970). The stained root pieces were observed under compound microscope and extent of VAM colonization was estimated. Arbuscular and vesicular infections were quantified by counting these structures per cm of root length. All the data were analyzed statistically by applying Duncan's Multiple Range Test (Steel and Torrie, 1980).

Phosphorus and potassium in the shoot of three green manuring plant species was estimated according to Jackson (1971) and nitrogen by the macro-Kjeladhal method (Bremner, 1965).

Results and Discussion

NPK analysis of green manures: Shoot N content of *T. alexandrianum* was highest and significantly different (P = 0.05) from the other two green manuring plant species (Table 1). This difference in N content may be attributed to different N_2-fixing efficiency of different legumes (Sheikh *et al.*, 1994). P content of shoot was significantly different from each other in all the three green manuring species, being highest in *Melilotus* and lowest in *Trifolium*. Like P, K content was also different significantly in the three leguminous green manures, being highest in *Medicago* and lowest in *Melilotus* (Table 1). The highest N:P ratio (68:1) was recorded in *Trifolium*. The N:P ratios in the other two green manuring plant species were comparatively lower and close to each other. Similarly P:K ratio in *Trifolium* and *Medicago* and N:K ratios in *Trifolium* and *Melilotus* were close to each other (Table 1).

Table 1: NPK analysis of the three green manures

Species	% N	% P	% K	N:P:K
Trifolium alexandrianum	4.44a	0.065a	1.24a	68:1:19
Medicago denticulate	2.45b	0.089b	1.89b	27:1:21
Melilotus parviflora	3.17b	0.122c	0.81c	26:1:7

In each column, means followed by different letters are significantly (P = 0.05) different

Effect of NPK and three green manures on VAM colonization in maize: Extent of VAM colonization was either suppressed or remained unaffected due to NPK fertilizers at various growth stages (Fig. 4). However, arbuscular and vesicular infections in terms of number of these structures per l00 cm of root length was significantly reduced in NPK treated plants (Fig. 5,6). Inorganic nutrients such as phosphorus (Arnijee *et al.*, 1989) and nitrogen (Johnson *et al.*, 1984) are known to reduce VAM colonization if present at high levels.

Addition of individual green manures enhanced extent of mycorrhizal infection significantly at all the three growth stages except in *Medicago* amended soil at 40 days growth stage. Addition of mixed green manures favoured extent of VAM infection significantly only at final growth stage (Fig. 4). This enhanced mycorrhizal growth in green manure amended treatments could possibly be attributed to the enhanced organic matter in the soil. St. John *et al.* (1983) showed that VAM hyphae were longer in treatments containing organic matter than in those containing only sand. Similarly Saif (1986) has reported that returning plant residues to soil increased mycorrhizal infection in tropical forage systems. Organic matter may indirectly affect mycorrhiza through its influence on soil structure, nutrient mineralization and water holding capacity (Johnson and Pfleger, 1992).

Arbuscular and vesicular infections were suppressed invariably and significantly at all the three growth stages, due to addition of mixed green manures. However, addition of individual green manures had variable effects on number of these structures in the root cortex (Fig. 5, 6). Addition of *Medicago* significantly increased number of arbuscules at 80 days growth stage while that of *Melilotus* both at 40 and 80 days growth stages. Addition of *Trifolium* did not prove beneficial for arbuscular infection at any of the three growth stages probably because of high N content.

Table 2: Correlation between shoot NPK of the three green manuring crops and various growth, yield and vesicular buscular mycorrhizal (VAM) parameters in green manure amended treatments

	Fertilizers		
Parameters	N	P	K
40 days after sowing			
Shoot length	-0.95*	0.77	0.17
Shoot fresh wt.	-0.98*	0.69	0.30
Shoot dry wt.	-0.95*	0.81	0.11
Root length	-0.76	0.96*	-0.22
Root fresh wt.	-0.54	0.99**	-0.49
Root dry wt.	-0.62	0.99**	-0.40
Extent of VAM	0.19	0.70	-0.96*
No. of arbuscules	0.64	0.27	-0.97*
No. of vesicles	-0.66	0.99**	-0.36
80 days after sowing			
Shoot length	-0.60	0.99**	-0.42
Shoot fresh wt.	-0.86	0.90	-0.05
Shoot dry wt.	-0.88	0.87	-0.01
Root length	-0.99**	0.57	0.44
Root fresh wt.	-0.70	0.95*	0.76
Root dry wt.	-0.86	0.80	0.83
Extent of VAM	0.99**	-0.50	0.98
No. of arbuscules	-0.96*	0.31	0.68
No. of vesicles	0.87	-0.88	0.02
110 days after sowing			
Shoot length	-0.83	0.95	-0.10
Shoot fresh wt.	-0.64	0.99**	-0.37
Shoot dry wt.	-0.83	0.99**	-0.38
Root length	-0.95*	0.83	0.07
Root fresh wt.	-0.81	0.95*	-0.14
Root dry wt.	-0.60	0.99**	-0.43
Number of cobs	-0.62	0.99**	-0.40
Cob fresh wt.	-0.99**	0.63	0.37
Cob dry wt.	-0.98*	0.70	0.27
Extent of VAM	0.19	0.98*	-0.60
No. of arbuscules	0.82	0.01	-0.87
No. of vesicles	-0.35	-0.57	0.99**

*, **, significant at 5 and 1 % level, respectively.

Table 3: Correlation between VA mycorrhizal colonization and various growth and yield parameters of maize

Traits	Extent of VAM	No. of arbuscules	No. of vesicles
40 days after sowing			
Shoot length	0.001	0.14	0.36
Shoot fresh wt.	0.05	0.22	0.56
Shoot dry wt.	0.15	0.27	0.59
Root length	-0.45	-0.50	-0.37
Root fresh wt.	0.54	0.51	0.72
Root dry wt.	0.41	0.47	0.64
80 days after sowing			
Shoot length	-0.20	0.18	-0.66
Shoot fresh wt.	-0.25	0.19	-0.70
Shoot dry wt.	-0.04	0.37	-0.50
Root length	-0.05	-0.01	-0.70
Root fresh wt.	0.13	0.07	-0.32
Root dry wt.	0.05	0.32	-0.25
110 days after sowing			
Shoot length	0.61	-0.08	0.10
Shoot fresh wt.	0.63	-0.06	0.11
Shoot dry wt.	0.62	-0.07	0.09
Root length	0.43	-0.17	0.17
Root fresh wt.	0.20	-0.25	-0.25
Root dry wt.	0.27	-0.10	-0.34
Number of cobs	0.38	-0.41	0.01
Cob fresh wt.	0.34	-0.58	-0.25
Cob dry wt.	0.43	-0.47	-0.14

All the correlations are insignificant.

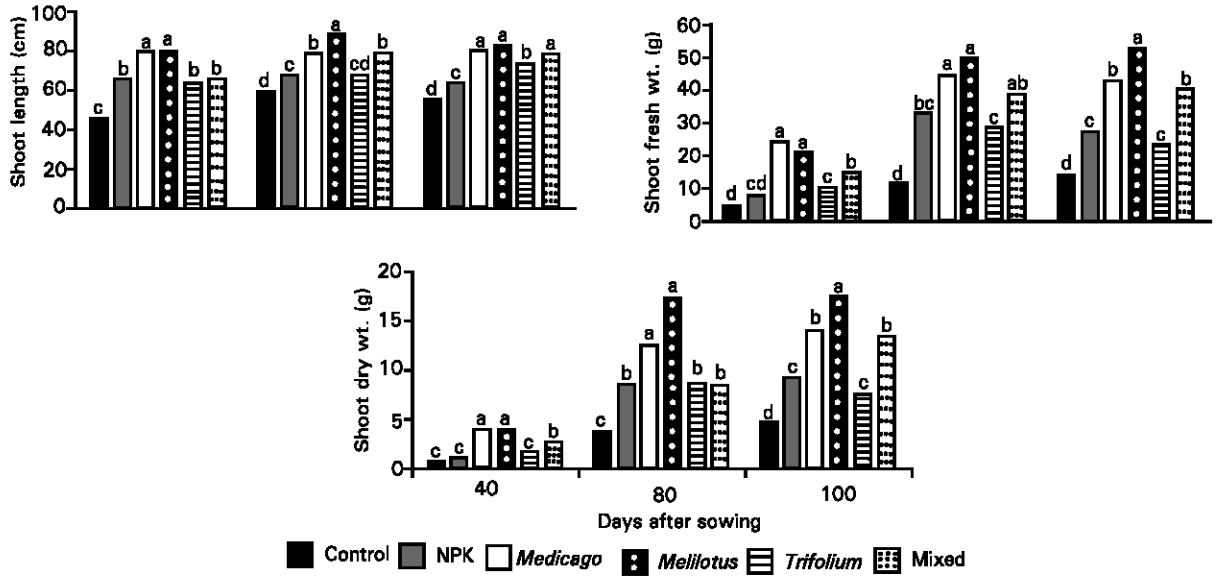

Fig. 1 (a-c): Effect of NPK fertilizers and three leguminous green manures on shoot growth of maize. Values with different letters at their tops show significant difference at 5% level of significance

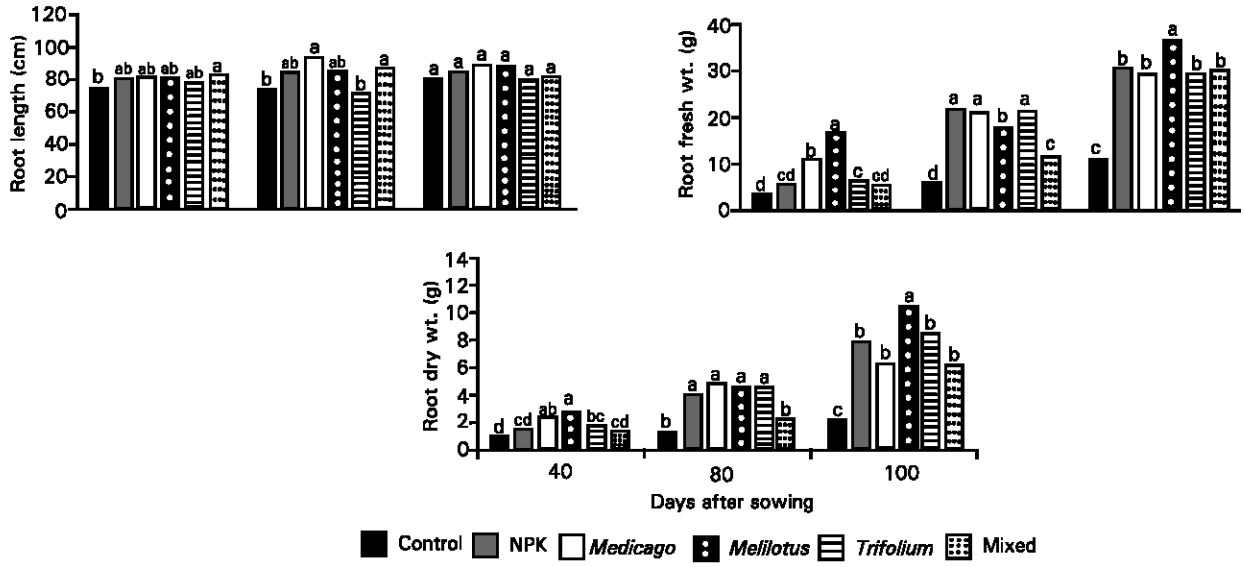

Fig. 2(a-c): Effect of NPK fertilizers and three leguminous green manures on root growth in maize. Vertical bars show standard error. Values with different letters at their tops show significant difference at 5% level of significance

Vesicular infection was generally suppressed by addition of any of the three green manures. Adverse impact was more pronounced at later growth stages as compared to the early phase (Fig. 6).

Correlation of different VAM parameters such as extent of infection and number of arbuscules and vesicles, with N, P and K of the three green manures are given in Table 2. Generally there was not any consistency in correlation between different characters at different growth stages. Correlation of extent of VAM was highly significant ($P = 0.01$) with N at 80 days growth stage but insignificant at the other two growth stages. Arbuscular infection showed significantly ($P = 0.05$) negative correlation with N at 80 days growth but insignificant and positive correlation at 40 and 120 days growth stages. The pattern of correlation of various VAM parameters with P of the green manures was also highly variable at different growth stages. Extent of VAM was significantly correlated with P at 120 days growth stage while arbuscular number showed insignificant correlation at all the three growth stages. Vesicle's number was highly significantly correlated with P of the green manures at 40 days growth but showed an insignificant negative correlation at the two later growth stages. All the mycorrhizal parameters exhibited negative correlation with K of green manures at 40 days growth. However, there was a great variation in correlation at the two later growth stages. This inconsistency in correlation between various VAM parameters and N, P and K of the green manures at different growth stages of the test maize crop may be attributed to the release of different quantities of N, P and K and other products of decomposition at different times.

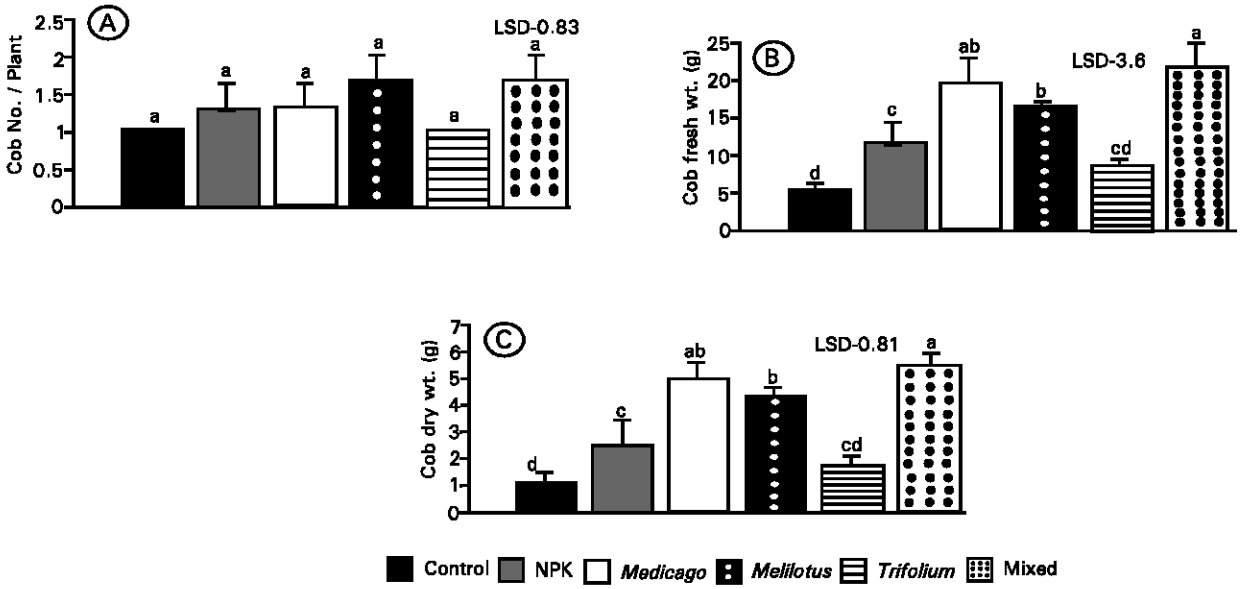

Fig. 3(a-c): Effect of NPK fertilizers and three leguminous green manures on number and biomass of cobs in maize. Values with different letters at their tops show significant difference at 5% level of significance. Vertical bars show standard error

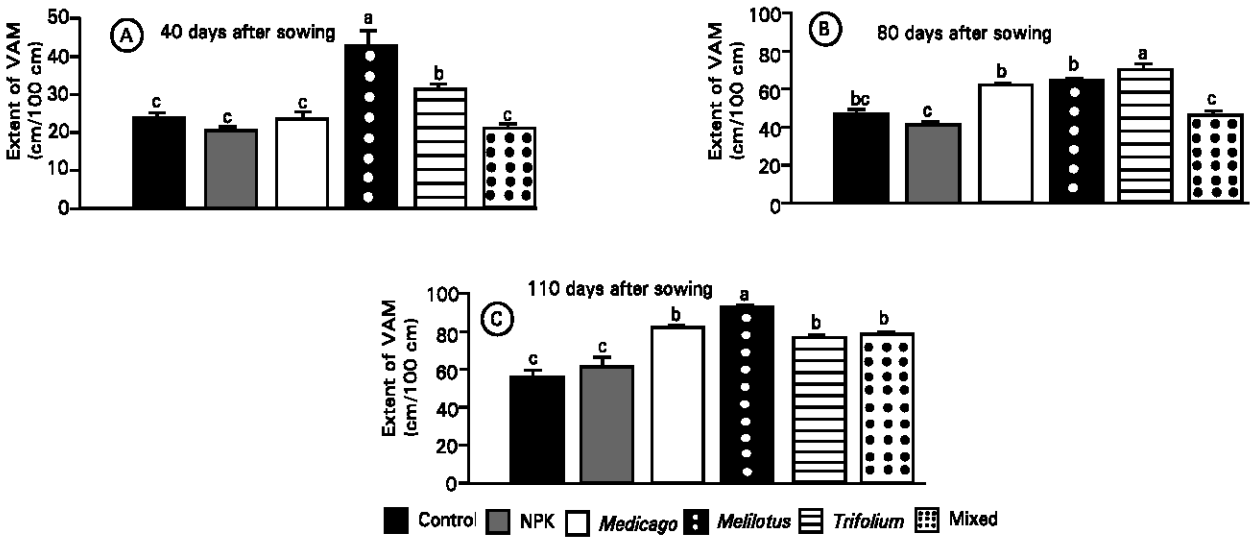

Fig. 4(a-c): Effect of NPK fertilizers and three leguminous green manures on extent of (VAM) infection in maize. Vertical bars show standard error. Values with different letters at their tops show significant difference at 5% level of significance

Effect of NPK and three green manures on shoot growth of maize: The results of effects of NPK fertilizers and three leguminous green manures on shoot growth of maize are presented in Fig. 1. All the soil amendments significantly increased shoot length as compared to un-amended control. In *Trifolium* amended treatment the increase in shoot length was similar to that of NPK fertilizers. The highest shoot length was observed in *Medicago* and *Melilotus* followed by mixed green manure amended treatment. Shoot length in these treatments was significantly greater than control and NPK fertilizer treated plants (Fig. 1A). The effect of NPK fertilizers and the three green manures on shoot fresh and dry weight was similar to that of shoot length (Fig. 1 B & C). Similar shoot growth enhancements have also been reported earlier due to other green manures (Hussain *et al.*, 1992). Among the three green manures, *Trifolium* contained the highest percentage of N but proved least beneficial for shoot growth. It seems probable that low N to P ratio is more beneficial for shoot growth than that of high N to P ratio. There was a negative correlation between different shoot growth parameters and percent N content of green manures. Negative correlation was significant ($P = 0.05$) at 40 days growth stage. Correlation between percent P of green manures and shoot growth in maize was positive at all the three growth stages and was highly significant at final growth stage (Table 2). Correlation between percent K content of green manures and shoot growth parameters was insignificantly positive at 40 days growth stage

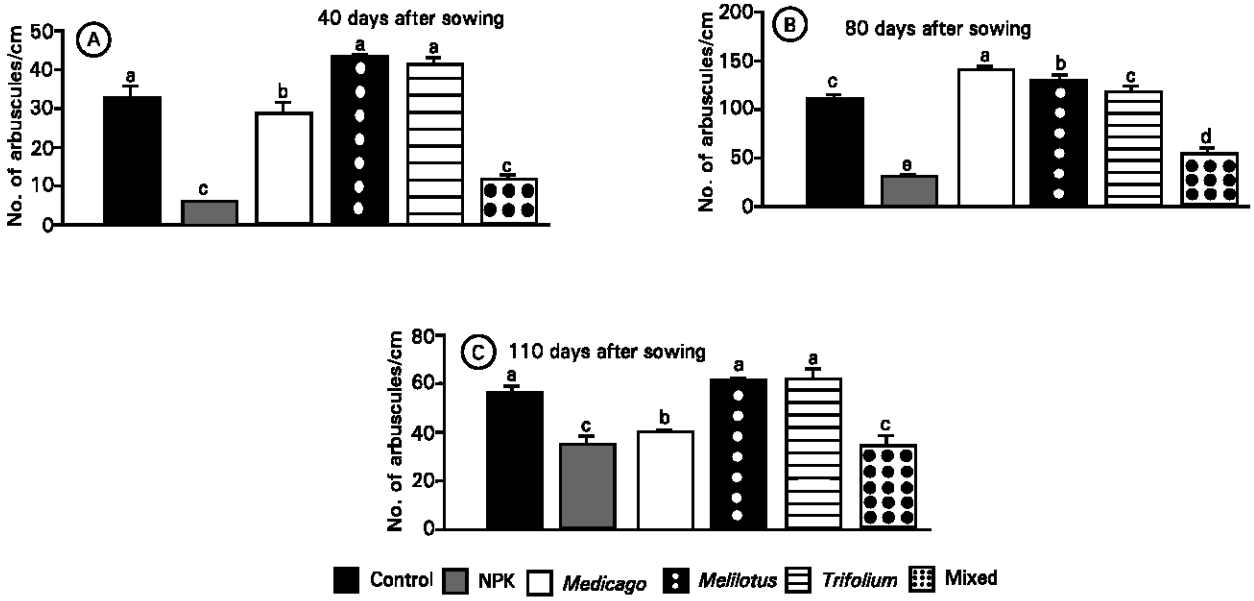

Fig. 5(a-c): Effect of NPK fertilizers and three types of green manures on number of arbuscules in maize roots. Vertical bars show standard error. Values with different letters at their tops show significant difference at 5% level of significance

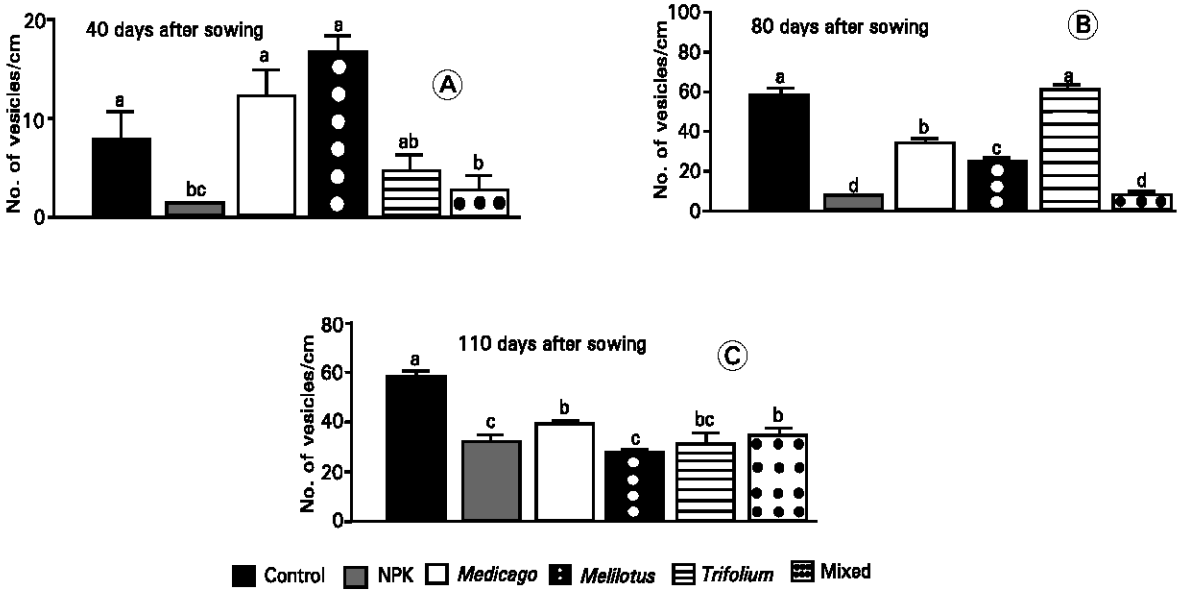

Fig. 6(a-c): Effect of NPK fertilizers and three types of green manures on number of varieties in maize roots. Vertical bars show standard error. Values with different letters at their tops show significant difference at 5% level of significance

and insignificantly negative at the later two growth stages (Table 2).

There was a positive correlation between extent of VAM and various shoot growth parameters at 40 and 120 days growth while negative correlation at 80 days growth stage (Table 3). However, the correlations at all the three growth stages were insignificant. The pattern of correlation between vesicle number and shoot growth was similar to that of extent of VAM. However, arbuscule's number was non-significant and positively correlated with shoot length and biomass at 40 and 80 days growth stage, and negative with very low value of coefficient of correlation at final growth stage. The negative correlation at final growth stage may be attributed to the disintegration of arbuscules at this growth stage.

Effect of NPK and three green manures on root growth of maize: NPK fertilizers and green manures except *Trifolium* amendment increased root length. Effect was significant only due to mixed green manure at 40 days growth stage (DGS) and due to *Medicago* at 80 DGS (Fig. 2A). In green manure amended treatments, root length was negatively correlated with N content of the green manures at all the growth stages. Effect was significant at later growth stages. There was a positive and significant correlation between root length and P content of green

manures. The correlation between K content of green manures and root biomass was insignificant and variable (Table 2). Root length was insignificantly and negatively correlated with various VAM parameters (Table 3). Earlier some workers have also reported an inverse relationship between root length and VAM colonization (Schweiger *et al.*, 1995; Tawaraya *et al.*,1999).

Root fresh and dry biomass was enhanced by NPK and either of the green manures at all the three growth stages. Effect was more pronounced and significant at 80 and 110 DGS (Fig. 2). Like root length, root biomass was also negatively correlated with N content of green manures. Correlation between P content of green manures and root biomass was positive and significant at all the growth stages. Pattern of correlation between root biomass and K content of green manures was insignificant and variable at different growth stages (Table 2). Root biomass was positively correlated with extent of VAM at all the growth stages while with number of arbuscules and vesicles, there was a positive correlation initially and negative at later growth stages. However, all the correlations were insignificant (Table 3).

Effect of NPK and three green manures on yield of maize: There was an insignificant difference in cob number among the treatments. However, a significant increase in cob fresh and dry biomass was observed in NPK and green manure amended treatments except *Trifoliu*m amendment, as compared with control. Highest cob biomass was recorded in mixed green manure treatment followed by *Medicag*o and *Melilotus* amendments, respectively. Difference was significant as compared with control, NPK and *Trifoliu*m (Fig. 3). Cob biomass was highly significant and negatively correlated with N content of the green manures (Table 2). Correlation of cob biomass with extent of VAM was positive while with number of arbuscules and vesicles it was negative. However, all the correlations were insignificant (Table 3).

This study concludes that higher N content of the green manures should not be the only criteria for its effectiveness in agricultural production but the N to P ratio is also very important.

Furthermore, the relationships among plant growth, nutrients of the green manures and symbiotic microbes like VAM fungi also need to be considered. Further field studies are required to evaluate the quantities of these green manures which are suitable not only for plant growth but also favourable for a significant and positive correlation among crop growth, nutrients of the organic manures and VAM colonization. Such work would be beneficial in reducing fertilizer needs while maintaining good soil health and better plant production and food quality.

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