EFFICACY OF INVASIVE GREEN MANURES AND MYCORRHIZA ON GROWTH AND YIELD OF DIFFERENT LEGUMES CROPS AND STUDY THEIR ANTIMICROBIAL PROPERTIES

Geeta Shrestha Vaidya* and Nabin Bhattarai**
* Shigan International College(SICOST) , ** Megha National College, Kathmandu, Nepal.

Abstract: Application of the invasive green manure (locally available *Lantana camara, Eupatorium adenophorum*) is an essential means to maintain soil fertility, soil structure and stimulate biological activities extensively. In this present study field trial of Lentil and Rajma were conducted in Kosebali Divison of Rampur, Chitwan district. Mycorrhiza was isolated from agricultural soil by sugar decantation method and mass production with the help of onion plant. Leaves of *Lantana camara* and *Eupatorium adenophorum* were collected and shed dried and grinded on powdered form. The treatments with green manures and mycorrhizae showed higher yield than the control in case of Lentil field trial. In case of Rajma field trial, length of stems and dry seed formation was higher in *Eupatorium adenophorum* and followed by *Lantana camara*, chemical fertilizer, control and mycorrhiza. Mycorrhizal spores were found higher in *Lantana camara* followed by *Eupatorium adenophorum*, mycorrhizal, chemical fertilizer and control. The number of mycorrhizal spores was found higher in case of *Lantana camara* followed by *Eupatorium adenophorum*, mycorrhiza, compost, chemical fertilizer and control. The percentage of seedless and low seeded pods were found higher on control followed by compost, chemical fertilizer, *Lantana camara*, *Eupatorium adenophorum* and mycorrhiza.

Keywords: Invasive green manure; Mycorrhizae; Chemical fertilizer; Antimicrobial properties; Chitwan district.

INTRODUCTION

Green manure is a type of cover crop grown primarily to add nutrients and organic matter to the soil. Green manures are rich in mineral, organic matters and bioactive compounds which help for better growth of plants and control of plant diseases. Green manure means planting a crop that is meant to be incorporated into the soil to increase it's fertility level, reduce erosion, improve the physical condition of the soil, and reduce nutrient loss from leaching and helps keep the rich topsoil from being washed away. It is used to protect the soil from erosion, weed control, disease control especially soil born diseases and nematodes. It is a cheap alternative to artificial fertilizers and can be used to complement animal manures, *Eupatorium adenophorum* and *Lantana camara* are exotic and invasive plants to Nepal and highly compete and disturb the growth of other plants.

In Nepal farming is mixed, subsistence and heavily dependent on forest resources, such as leaf litter, green manure, poles, fuel wood, fodder and non-timber forest products (Mahat 1987; Kadariya 1992). The quantity of chemical fertilizer used per hectare in Nepal is very low as compared to other countries. However farmers who have been using chemical fertilizer in Kathmandu valley and some of the Terai districts have started to experience its adverse effects on soil quality.

Soil in Nepal is deficient in N,P,K due to shortage of organic matter in the soil (Shrestha Vaidya et al. 2008). Therefore incorporation of organic matter is necessary for improvement and fertility of soil. Organic matter can replace the use of chemical fertilizers as much as possible. Therefore mycorrhizal fungi are essential for the establishment of tree seedlings and for their good growth (Shrestha Vaidya et al. 2002, 2005).

So, addition of organic matter can have a beneficial effect on the growth of indigenous AM fungi in nutrient limited soil (Caravaca et al. 2002; Gaur & Adholeya 2002). Organic amendments enhance spore production (Johnson & Mc Graw 1988, Douds et al. 1997, Shrestha Vaidya et al 2008). Organic matter addition to the soil at eroded sites could thus be appropriate to enhance the beneficial effect of AM fungi for soil stabilization and plant establishment and it also protects environment over the long term and reduces cost of production. Giovanetti and Avio (1985) suggested that this beneficial effect might be related to increased pore volume in soil which has a beneficial effect on AM colonization, the mycorrhizal growth response and AM spore numbers. Furthermore, Ryan *et al.* (1994) attributed increased AM fungal biomass to the beneficial effects of organic matter on soil structure, water status and on synergistic microbial activities in the soil.

Author for Correspondence: Geeta Shrestha Vaidya, Shigan International College (SICOST), E-mail: geetashrestha1@hotmail.com.

MATERIALS AND METHODS

Laboratory work of this research work were conducted in the Nepal Academy of Science and Technology lab and field trial was conducted in Kosebali Division, Rampur, Chitwan district where Lentil and Rajma were cultivated. Endomycorrhizal spores were extracted from agricultural soil using wet sieving and sucrose centrifugation (McKenney & Lindsey 1987) and mass production of mycorrhizae for field trial were done with the help of onion plant.

For preparation of green manures, leaves of *Lantana camara* and *Eupatorium adenophorum* were collected from chobhar kirtipur and shed dried and grinded on powdered form. The field trials were designed on randomized block design. The collected data were presented in following table 1. T_1- Fertilizer, T_2- *Lantana camara*, T_3- *Eupatorium adenophorum*, T_4- mycorrhizal, T_5- Control. All treatments have four replicates.

In the case of field trial of lentil, 1.7 kg/plot (2.833 t/ha) Mycorrhizal inoculums, each green manures 2.275 kg/plot (3.45 t/ha), compost 6 kg/plot (6 t/ha) and ratio of NPK 20:40:20 kg/h were applied. In case of Rajma, green manures1.5 kg/plot (7.5 t/ha), mycorrhizae 800 g /plot (4.00 t/ha) and ratio of NPK 120:40:40 kg/h applied

RESULTS

It has been observed that the treatments with green manures and mycorrhizae showed higher yield than the control.

Lentil: Field Trial

Table 1: Lentil Field trial treatments showing average length, pod and seedless formation with different treatments.

Treat ments	Total no. of uprooted plants	Average length of 40 plants (cm)	Average no. of pods formation	Average no. of pods formation with seed	Average no. of seedless pods formation
T1	40	23.97	29.95	22.22	4.72
T2	40	25.57	45.45	38.55	6.90
T3	40	27.70	56.90	48.67	8.22
T4	40	23.99	50.82	43.90	7.70
T5	40	21.97	18.80	14.19	4.61
T6	40	22.10	27.20	22.77	4.42

Lentil: Field Trial

Table 2 : Lentil Field trial treatments showing seeded and seedless pod formation weight of seed, waterholding capacity and average mycorrhzal spores.

Treat ments	%of seedless pods form^n	% of seeded pods form^n	Wt. of 100 seeds gm	% of water holding capacity	Bulk density gcm^{-3}	Average mycorrhizal spores on 25 gm soil
T1	15.77	84.23	2.31	30.67	1.48	21.33
T2	15.18	84.82	2.62	33.43	1.45	61.33
T3	14.45	85.55	2.81	34.92	1.56	50.00
T4	15.15	84.85	2.68	33.62	1.46	45.00
T5	24.51	75.49	2.18	30.87	1.57	11.33
T6	16.26	83.73	2.31	31.92	1.53	42.66

From field trial it was found that lentil plots treated with *Eupatorium adenophorum* showed higher average growth of stem and pods formation followed by mycorrhizal, *Lantana camara*, chemical fertilizer, compost and control. Higher the yield of dry seed was found with chemical fertilizer treatment followed by *Lantana camara*, *Eupatorium adenophorum*, mycorrhizal, compost and control. Higher water holding capacity was found in soil having *Eupatorium adenophorum* followed by Mycorrhiza, *Lantana camara*, control and chemical fertilizer. The bulk density was found higher on control followed by *Eupatorium adenophorum*, compost, chemical fertilizer, mycorrhizal and *Lantana camara*. The number of mycorrhizal spores was found higher in case of *Lantana camara* followed by *Eupatorium adenophorum*, mycorrhiza, compost, chemical fertilizer and control (Table 2) The percentage of seedless and low seeded pods were found higher on control followed by compost, chemical fertilizer, *Lantana camara*, *Eupatorium adenophorum* and mycorrhiza.

Rajma: Field Trial

Table 3:

Treat ments	No. of maintained plants	No. of survival of plants	Average length of plants (cm)	Total no. of seeds sown	Germination of plants	% of germination
T1	200	190	21.53	400	307	76.75
T2	200	197	21.50	400	286	70.15
T3	200	196	23.76	400	292	73.00
T4	200	176	16.98	400	297	74.25
T5	200	191	19.28	400	287	70.17

Rajma: Field Trial

Table 4 :

Treat ments	Weight of dry seed formation of all plots of each treatment g.	Total weight of dry seed formation of all plots of each treatment g.	Water holding capacity (%)	Bulk density $gmcm^{-3}$	Average mycorrhizal spores on 25 g. soil	Wt. of 100 seeds g.
T1	145.00	0.14	32.08	1.39	19.66	64.00
T2	432.00	0.43	33.04	1.37	65.66	53.00
T3	530.10	0.53	32.99	1.39	53.30	48.00
T4	52.20	0.05	33.81	1.46	45.00	49.00
T5	132.50	0.13	32.51	1.38	5.66	48.00

Average length of stems and dry seed formation was higher in treatment with *Eupatorium adenophorum,* followed by *Lantana camara*, chemical fertilizer, control and mycorrhiza. *Eupatorium adenophorum* and *Lantana camara* treated treatments showed green, vigorous growth and least death of seedlings. The percentage of germination was higher in chemical fertilizer followed by mycorrhiza, *Eupatorium adenophorum*, control and *Lantana camara* (Table 3). The bulk density and water holding capacity was found higher in mycorrhiza followed by *Eupatorium adenophorum*, chemical fertilizer, control and *Lantana camara*. Mycorrhizal spores were found higher in *Lantana camara*, *Eupatorium adenophorum*, mycorrhizal, chemical fertilizer and control (Table 4).

DISCUSSION

Organic amendments enhanced spore production of AM mycorrhiza (Johnson and Mc Graw 1988; Douds *et al.* 1997; Shrestha Vaidya *et al.* 2008) which is similar to this results. The organic materials also improve the microbial activities of the soil, biological N fixation, organic matter decomposition, mineralization, nitrification and antagonism to soil borne pathogens (Alam and Khan 2001). Improved nutrient and water uptake by the plants can be expected in response to better AM growth and the positive effect on the growth of AM fungi is in good agreement with results obtained by other authors, who also found that organic matter addition increased AM fungal hyphal growth (Labidi *et al.* 2007; Joner and Jakobsen,1995) and AM spore formation (Douds *et al.* 1997; Muthukumar and Udaiyan, 2000; Gryndler *et al.* 2003; Harinikumar and Bagyaraj 1989). In addition, St John *et al.* (1983) and Friberg (2001) found that AM fungal hyphae grew best in soils with a high amount of organic matter which is shown in our results also. Ravnskov *et al.* (1999) had shown that effects of organic compounds on growth of AM fungi in soil vary according to chemical composition of the substrate which was similar to present study. The present result has shown that *Lantana* and *Eupatorium* are better than other treatments. Same type of study was reported by Nziguheba *et al.* (2000). The application of organic materials reduces the soil bulk density and hence increases total porosity, which has a positive effect on plant growth. In a compact soil, mycorrhizal symbiosis can enhance the uptake of nutrients by plant roots since mycelium of hyphae penetrates small pores more easily than roots (Nadian *et al.* 1996). Saikia et.al.2011 had shown that conservation of all available bio-mass provides high amount of organic matter in tea sections which adds good amount of nutrient to the soil.

The present study provides the first information on a stimulating effect of organic material addition on extra-radical growth of AM fungi in Nepal. These results show that organic matter addition can improve plant survival.

Lantana camara (Linn.)

Eupatorium adenophorum (Sprang.)

Rajma Seeds

Planting Rajma Seeds

Random method Field Trial in Kosebali Rampur

Seeding in the Field Trial

Making the plot & mixing the fertilizers for Lentil field trial

After seeding Lentil in field trial

CONCLUSION

Green manures and mycorrhiza were effective for the growth and yield of Lentil and red kidney bean (Rajma), and can be used as biofertilizer which is environmentally friendly, cost effective and save the national money for purchasing the chemical fertilizer. The application of organic materials reduces the soil bulk density and hence increases total porosity, which has a positive effect on plant growth.

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