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Effect of *Azolla pinnata* on Seed Germination, Vigour Index, Biomass and Yield of French Bean (*Phaseolus vulgaris*)

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ABSTRACT

A study was conducted to know the effect of *Azolla pinnata* collected from Hampapura of K.R. Nagar in Mysore district on seed germination, vigour index, biomass and yield of French bean. A total of four different treatments of earthen pots containing soil: sand: farmyard manure (2:1:1) amended with 10g (T1), 25g (T2), 50g (T3) and 100g (T4) of fresh *Azolla* biomass and pots amended with 10g (T_0) of Factamphos served as positive control. Pots without any supplement served as negative control. 10 seeds of French bean variety 'S 9' were sown in each pot and normal agronomic practices were followed. Seed germination was determined after eight days of sowing, and after every 15, 30, 60 and 90 days of sowing, vigour index, biomass and pod yield were determined. The results of the study showed that, high percentage seed germination (81.66%) was recorded with the treatment incorporated with 25g (T2) of *Azolla* biomass followed by 10g (76.33%), 100g (76.00%) and 50g (72.66%) of *Azolla* biomass respectively. Minimum seed germination percentage of 64.66% and 65.66 % were recorded in respective negative and positive controls. Significant increase in seedling vigour (VI) was recorded in all the treatments compared to controls. Pod yield increased in T2 followed by T0, T1, T3, T4 and untreated respectively after 90 days of sowing. The study showed that *Azolla* could be used as a supplement for other pulse crops for increased biomass and overall yield of French bean.

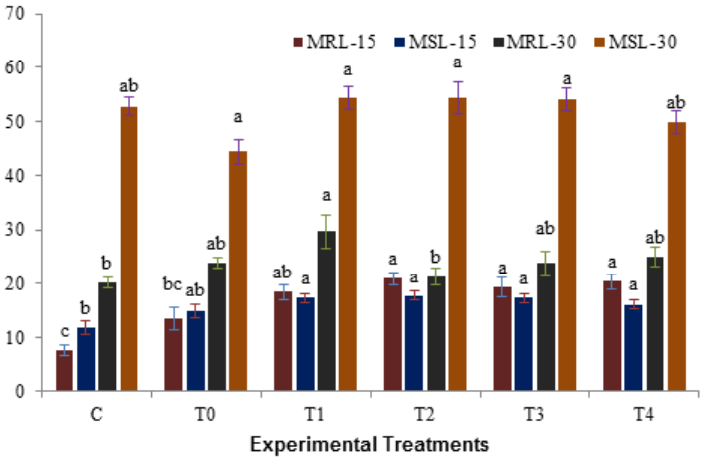
Key words: *Azolla*, Vigour index, Germination percentage, Biomass, French Bean.

INTRODUCTION

French bean (*Phaseolus vulgaris* L.) is an important leguminous, short duration pulse and vegetable crop used as main source of protein in human diet (Anon., 2010). The negative impact of chemical fertilizers on the soil fertility necessitated the need for an alternative supplements to replace the usage of chemical fertilizers (Anon.,1982). *Azolla* (*Azolla pinnata R.Br.*) contains high nitrogen content and has been used as a green manure for wetland rice cultivation and also for their capacity to retain large amounts of minerals (Arora and Singh, 2002; Kumar and Rao, 2012). It is one of the supplementary sources of compost used in integration with other bio-fertilizers. As a potential green manure it increases the soil organic matter and nitrogen fixing ability in standing water. *Azolla* in symbiosis with cyanobacterium (*Anabaena azollae*) can fix 2-4 kg N ha^{-1}day (Lumpkin and Plucknett, 1982) releasing the nutrients like gibberellins, cytokinins, auxins, abscisic acid, vitamins, antibiotics and amino acids into soil in an easily available form to plants (Bohlool et al., 1992 ; Wagner, 1997). It is extensively used as nitrogen supplement not only for rice cultivation but also for other crops such as Tomato, Wheat, and Taro (Anon, 1982; Kolhe and Mittra, 1990; Marwaha et al., 1992; Milica and Favilli, 1992; Tekle-Haimanot and Doku, 1994). The present study was conducted to know the effects of *Azolla* on seed germination, vigour index, biomass, and pod yield of French bean.

MATERIALS AND METHODS

An experiment was conducted in the greenhouse at the Department of Studies in Botany, University of Mysore. Seeds of French bean (variety S-9) obtained from Agro Seed Traders, Mysore. Fresh *Azolla* biomass was collected from a lake near Hampapura, K. R Nagar, Mysore district and was mass multiplied in the green house conditions. The species of *Azolla* was identified as per the key characteristics (Carrapico et al., 2000). A total of 120 pots filled with sterilized soil (2kg), sand (1kg) and organic compost (1kg) (2:1:1), incorporated with 10 (T1), 25 (T2), 50 (T3) and 100g (T4) of fresh *Azolla* biomass. The pots without *Azolla* biomass served as negative control. Pots with 10 g of Factamfos (20% N and 20% P2O5, 13% of Sulphur) served as positive control.Ten (10) seeds of French bean were sown in each pot and after eight days of sowing, seed germination was determined as per ISTA guidelines. At every 15, 30 and 60 days of sowing, vigour index (VI), biomass and pod yield after 90 days were determined. After 15 days of sowing, seedlings were uprooted, roots were washed in running tap water, shade dried, the mean root length (MRL) and mean shoot length (MSL) were determined and vigour index (VI) was calculated (Anon., 1996). Further the shoots and roots were separated and weighed and biomass was determined. Dry weight was also determined by heating for 16 to18 hours



Values are means of five independent replicates; Means followed by same letter(s) within the column is not significantly different according to Tukey's HSD.

Fig. 1: Effect of *Azolla* amendment on root length, shoot length of French bean after 15 and 30 days of sowing.

at 105 ± 2 °C in an oven,cooled in a desiccator for 20 min (Anon.,1996). The matured pods were harvested after 90 days of sowing and the yield was calculated.

Data analysis

The data was statistically analyzed by Analysis of variance (ANOVA) using SPSS Inc. 14.0. Means were separated by Tukey's HSD (honestly significant difference) test. Significant effects were determined by magnitude of F values (P<0.001).

RESULTS AND DISCUSSION

Azolla species collected from K.R. Nagar was identified as *Azolla pinnata* R.Br. The plant body appeared pinkish green and turned dark green as it matured. The rhizome was horizontal, deltoid, with minute papilla, up to 2 cm long and 0.2 mm in diameter. Hairy roots were 1-3 up to 3.5 cm long. Leaves were bilobed with the upper lobe imbricate, up to 1.1 mm long with papilla, central portion was chlorophyllous, border with 3-4 cells layers which

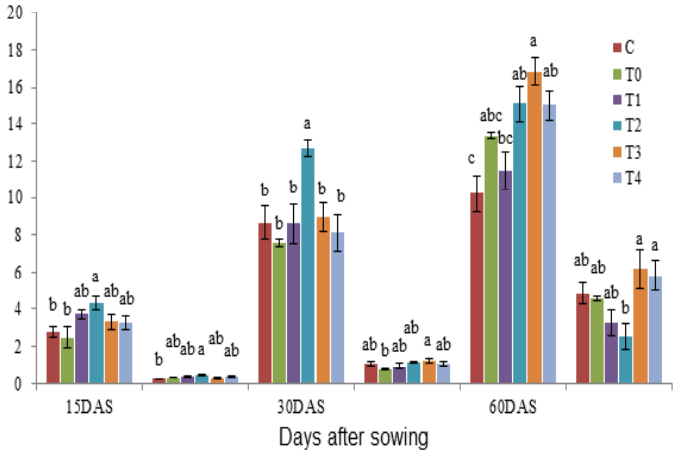


Fig. 2: Effect of *Azolla* amendments on the biomass of French bean after 15, 30 and 60 days of sowing.

Values are means of five independent replicates; Mean followed by same superscript letter(s) within the column is not significantly different according to Tukey's HSD at P<0.05.FW-Fresh weight, DW-Dry weight.

were hyaline, apex acute; lower lobe similar in size to the upper lobe, but hyaline (Caparrico *et al.,*2000). The results of seed germination and seedling vigour (VI) are presented in Table-I. Seed germination was ranged from 65-82% and there were significant differences in the seed germination among different *Azolla* treatments. Maximum seed germination of 81.66% was recorded in the pots incorporated with 25g of *Azolla* followed by 10g (76.33%), 100g (76.00%) and 50g (72.66%) of *Azolla* respectively. Similar studies conducted on *Pisum sativum* seeds soaked in lower concentration of aqueous *Azolla* extract showed maximum seed germination. A high seed germination rate of 99%, 97%, 97% was recorded in aqueous *Azolla* extract of 20%, 10% and 5% respectively (Bhindu, 2013). In contrast to seed germination, significant increase in vigour index was observed in all the treatments compared to controls.

Table: 1. Effect of *Azolla pinnata* on seed germination of French bean after 8 days of sowing and seedling vigour after 15days of sowing.

Treatments	Seed Germination (%)	Seedling vigour (VI)
C- Negative control	64.66 ± 1.76^{c}	1229.39± 3.79^{e}
T0- positive control	65.66 ± 0.33^{c}	1880.88±9.01^{d}
T 1 (2:1:1+10g *Azolla*)	76.33 ± 0.88^{b}	2714.82±8.05^{b}
T 2 (2:1:1+25g *Azolla*)	81.66 ± 0.33^{a}	3146.81± 5.96^{a}
T 3 (2:1:1+50g *Azolla*)	72.66 ± 0.33^{b}	2687.08±6.28^{bc}
T 4 (2:1:1+ 100g *Azolla*)	76.00 ± 0.57^{b}	2677.45± 2.84^{c}
Degree of Freedom	29	29
P Value	<0.001	<0.001
F value	5.52	121.04

Values are means of five independent replicates; Mean followed by same superscript letter(s) within the column is not significantly different according to Tukey's HSD.

Table: 2. effect of *Azolla* on the pod yield of French bean

Treatments	Pod yield(g) Number of days	
	60	90
C	118.0±1.37^{e}	120.0±0.80^{e}
T0	161.6±2.24^{a}	163.0±1.54^{a}
T1	144.4±1.28^{b}	143.8±1.35b
T2	164.6±1.32^{a}	166.2±1.01^{a}
T3	136.0±1.00^{c}	135.6±0.74^{c}
T4	125.0±3.76^{d}	126.2±1.46^{d}
DF	29	29
P value	<0.05	
F value	150.69	249.7

Values are means of five independent replicates; Mean followed by same superscript letter(s) within the column is not significantly different according to Tukey's HSD.FW-Fresh weight, DW-Dry weight.

The seedling vigour of French bean was maximum in T2 (3146.81) followed by T1 (2714.82), T3 (2687.08) and T4 (2677.45) respectively.

Effect of *Azolla* on biomass of French bean in terms of fresh weight and dry weight are presented in Figure 2. An increase in French bean biomass was observed in *Azolla* treatments compared to controls. Maximum biomass was recorded in T2 (25 g) after 15, 30 and 60 days of sowing compared to the controls. Similar results were observed in *Pisum sativum* (Bhindu, 2013). The effect of *Azolla* on pod yield after 60 and 90 days of sowing is presented in Table 2. The overall pod yield ranged from 125g-164.6 g and 126.2-166.2g after 60 and 90 days of sowing respectively. Pots containing soil amendment with 25g of *Azolla* recorded highest yield of 164g after 60 days and 166.2g after 90 days of sowing. Similar results were observed by Marwaha *et al.* 1992 with the application fresh fronds of *Azolla* for increased grain yield of Wheat. Incorporation of 200 kg/ha of *Azolla* biomass also increased the yield of Tomatoes by 21.2% (Milica and Favilli, 1992). Effect of *Azolla* on the yield of Mung bean in terms of increased number of pods and yield per plant after the incorporation of 12 tonnes of *Azolla*/ha was studied (Ram et al., 1994). The corm yield in Taro (*Colocasia esculenta*) was increased with the incorporation of 20 tonnes ha^{-1} *Azolla* into mud and soil application of 0.5 kg m^{-2} Tekle-Haimanot and Doku, 1994). Iron enriched *Azolla* used as a slow biofetilizer increased the yield of cucumber grown in the hydrophonic system (Plessner et al., 1998). *Azolla* as a biofertilizer is well known especially in rice fields (Nyalemegbe et al.,1996 ; Sanati et al., 2001; Singh et al., 2011). French bean is an important pulse crop in India with an annual production of 3989 Kg/ha. The application of nitrogen fertilizers positively increases the yield of French bean. However the amendment of *Azolla* as a supplement certainly reduces the application of nitrogenous fertilizers and increases the soil fertility. The result of the present study showed that the incorporation of 25 g of *Azolla*/ 4 kg of soil increased the seed germination, seedling vigour, biomass and yield of French bean. Further work need to be conducted to establish the same results and applications methods in field condition.

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