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EFFECT OF MYCORRHIZA AND *RHIZOBIUM* INOCULATION ON THE GROWTH AND YIELD OF MUNG (*VIGNA RADIATA*) PLANT

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ABSTRACT

In this study, the effect of *Rhizobium* and mycorrhizal fungal spores were examined on *Vigna radiata* (Mung bean). Seeds of Mung bean var. PDM-139-SAMRAT were sown in plastic pots, in the mixture of half soil and half sand. *Rhizobium* and mycorrhizal fungal spores inoculated individually and in combination with mung seeds. After germination data have been reported for plant height, leaf area, number of root nodules and pods, chlorophyll amount and yield measured after 30,45,60,75,90 and 120 days of sowing. Results of these experiments showed that *Rhizobium* and mycorrhiza positively affected the growth and yield of mung plant. Combined inoculation of *Rhizobium* and mycorrhiza shows better results in comparison to their individual inoculation. Mixture of *Rhizobium* and mycorrhizal spores improve the growth, yield, number of root nodules and pods, leaf area and chlorophyll amount of mung plant.

Keywords: Mycorrhiza, *Rhizobium, Vigna radiata*.

INTRODUCTION

In present days it is difficult to fulfill the basic requirement of food for everyone. Because of increasing population there is limited land for cultivation. There are various types of chemical fertilizers are harming the quality of soil. There are two plant symbionts i.e. *Rhizobium* and mycorrhizal fungi, they improve the plant growth, plant nutrition and production. These both types of symbiosis also prevent the fungal plant pathogens (Demir and Akkopru, 2007).

The symbiosis between mycorrhizal fungi and most of the plants are very beneficial to both partners (Miransari, 2010). Mycorrhizal fungi produce a branch like hyphal structure within the plant cell. This structure increases the surface area of plant cells so that it can absorb nutrients from soils (Woolhouse, 1975).

Nitrogen fixation between the rhizobia and legumes and mycorrhizal association between fungi and roots of higher plants are most commonly studied symbiosis (Newman and Reddell, 1987). Legume crops are capable to form symbiotic relationship with soil micro-organisms so they can grow in low water and low nutrient soil (Kawaguchi *et al.,* 2010).

Mycorrhiza and *Rhizobium* benefits the host plant with their functions. *Rhizobium* plays an important role in biological nitrogen fixation whereas mycorrhiza provides various types of minerals like phosphorus, potassium, zinc etc. to the host (Schublin and Van der Heijden, 2006., Sundar *et al.,* 2010). Biofertilizers and organic wastes are another source to gain the nutrient requirements of crops (Arumugam *et al.,* 2010). Mung (*Vigna radiata*) crop is very important part of human food. Specially in India mung have a major place in people's diet. Seeds of mung high in protein as well as vitamin-A (Ghildiyal, 1992). There is a need to increase its productivity to fulfill the demands of increasing population.

MATERIALS AND METHODS

Sowing

In March 2019, seeds of *Vigna radiata* i.e. mung variety PDM-139 SAMRAT have been sown in 20 plastic pots which contained half soil and half sand in the ratio of 1:1. Each pot had 10 seeds.

Treatment of seedlings

After 10 days of sowing seedlings of mung treated with *Rhizobium*, mycorrhiza and dual inoculation of *Rhizobium* and mycorrhiza (R+M). To treat with *Rhizobium* mung seedlings of 5 pots were plugged out carefully and dipped in *Rhizobium* suspension (which were prepared early by the root nodules of mung plant) and planted again in pots. The left *Rhizobium* suspension poured over in pots. To treat with mycorrhiza spore suspension has been made and seedlings of other pots (besides *Rhizobium*) plugged out and dipped in spore suspension and planted again in same pots.

To treat with dual inoculation *Rhizobium* + mycorrhiza, seedlings of new 5 pots were plugged out and dipped in both suspensions (R+M) and planted again (Singha and Sharma, 2013). 5 left pots of mung seedlings were controlled.

Plants were observed daily and measured on 30, 45, 60, 75, 90 and 120th days after sowing. Growth parameters were plant height, chlorophyll amount, leaf area, number of root nodules, number of pods and yield.

1. **Measurement of plant height**

Plant height were measured with the help of scale and thread, 2 plants were randomly and carefully plugged out from each pots which were treated with *Rhizobium*, mycorrhiza and *Rhizobium* + mycorrhiza (R+M) and controlled plants. Root, shoot and total length of plants recorded.

2. **Leaf area**

Leaf area of mung plant leaves measured by the help of graph paper. Leaves were sampled from dual inoculated, single inoculated and controlled plants as well. Each leaf was spread over graph paper and the outline of leaf was drawn. The area of the graph paper covered by the outline of leaf had smallest squares which were counted (Pandey and Singh, 2011). The following equation was used to calculate the leaf area non-destructively-

Leaf area (cm^2) = x/y, where x is the weight of the graph paper covered by the leaf outline (g) and y is the weight (g), of the cm^2 area of the graph paper.

3. **Chlorophyll amount**

For measure the chlorophyll amount 1 gram green leaves of mung plants were weighed and crushed with the help of mortar and pestle and 10ml 80% acetone were added and then filtered with the help of filter paper. Filtered liquid centrifuged at 5000rpm for 5 minutes. After centrifugation optical density of chlorophyll were measured at 645nm and 663nm by using systronic UV-Visible spectrophotometer against 80% acetone.

Estimation of chlorophyll content-

Chlorophyll content was estimated by using Arnon's (Arnon, 1949) method.

For calculation following formula was used-

$$\text{Chl. a (mg/g tissue)} = 12.7\,(A_{663}) - 2.69\,(A_{645}) \times \frac{V}{1000 \times W}$$

$$\text{Chl. b (mg/g tissue)} = 22.9\,(A_{645}) - 4.68\,(A_{663}) \times \frac{V}{1000 \times W}$$

$$\text{Total chlorophyll (mg/g tissue)} = 20.2\,(A_{645}) + 8.02\,(A_{663}) \times \frac{V}{1000 \times W}$$

where,

V= final volume of chlorophyll extract
A= absorbance at specific wavelength
W= fresh weight of tissue extract

4. Number of root nodules and pods collected and counted on 30, 45, 60, 75, 90 and 120th days after sowing.

5. **Yield**

100 seeds of mung were collected from total pods and weighed.

RESULT AND DISCUSSION

Rhizobium and mycorrhiza positively affected the growth and yield of mung plant. Combined inoculation of *Rhizobium* and mycorrhiza shows better results in comparison to their individual inoculations.

Plant height

The plant treated either with *Rhizobium* and mycorrhiza and dual inoculation (*Rhizobium* + mycorrhiza) increased the shoot length and root length of plants, but maximum plant height (46.0 cm) was observed when dual inoculation (R+M) was given to plants. Minimum plant height was observed in control (40.0 cm). The result showed the effect of dual inoculation of *Rhizobium* and mycorrhiza enhancing the growth of *Vigna radiata* in comparison to single inoculation of both individually (Table -1).
Similar results were observed by Tiajini *et al.,* (2011), Havugimana *et al.,* (2015), Nida *et al.,* (2017) and Arumugam *et al.,* 2010.

Number of root nodules

Maximum root nodules (23) were observed in dual inoculated (*Rhizobium* + mycorrhiza) plants whereas in single inoculated plants number of nodules observed fewer than dual inoculated plants. Minimum nodule numbers (16) were observed in controlled plants (Table - 2). Similar results were observed by Nida *et al.,* (2017) and Arumugam *et al.,* 2010.

Leaf area

The results observed that dual inoculated (R+M) plants had broad leaf area than other plants (Table - 3).

Chlorophyll amount

Results of the experiments shows that maximum chlorophyll amount (29.41%) was observed in dual inoculated plants (R+M) and minimum chlorophyll amount (21.94 %) was observed in controlled plants (Table - 4).

Table 1: Effect of inoculants (Mycorrhiza, *Rhizobium and Rhizobium* + Mycorrhiza) on plant growth in terms of root height and shoot length

Control			T_1(*Rhizobium*)			T_2(Mycorrhiza)			T_3(*Rhizobium* + Mycorrhiza)			Days
Root	Shoot	Total	Root	Shoot	Total	Root	Shoot	Total	Root	Shoot	Total	
5	18	23.0	5.9	19	24.9	6	19	25.0	8.1	21	29.1	30
6.5	23	29.5	7	24	31.0	7.2	25	32.0	8.5	26	34.5	45
7	23.5	30.5	8	25	32.0	8.75	25	33.75	9	27.25	36.25	60
8	24	32.0	9	25	34.0	9.5	26	35.5	10	29.50	39.50	75
9	25	34.0	9	26	35.0	10	26.05	36.05	10	31	41.0	90
10	30	40.0	12	30.75	42.75	13	31.5	44.5	14	32	46.0	120

Table 2: Effect of inoculants (Mycorrhiza, *Rhizobium and Rhizobium* + Mycorrhiza) on plant growth in terms of root nodules

Control	T_1(*Rhizobium*)	T_2(Mycorrhiza)	T_3(*Rhizobium* + Mycorrhiza)	Days
3	5	3.5	6	30
3.5	7.5	6	9	45
5	10	8.5	11.5	60
9.5	13	10.5	14	75
11	15.5	13	17	90
16	19	17	23	120

Table 3: Effect of inoculants (Mycorrhiza, *Rhizobium and Rhizobium* + Mycorrhiza) on plant growth in terms of leaf area

Control	T_1(*Rhizobium*)	T_2(Mycorrhiza)	T_3(*Rhizobium* + Mycorrhiza)	Days
2.64	3.85	5.26	6.49	30
6.72	7.20	8.24	9.42	45
9.14	9.60	10.65	12.91	60
11.00	12.20	13.23	16.00	75
14.60	15.39	16.02	19.23	90
16.21	17.66	19.01	21.02	120

Table 4: Effect of inoculants (Mycorrhiza, *Rhizobium and Rhizobium* + Mycorrhiza) on plant growth in terms of chlorophyll amounts (mg)

Control			T_1(*Rhizobium*)			T_2(Mycorrhiza)			T_3(*Rhizobium* + Mycorrhiza)			Days
Chl. a	Chl. b	Total	Chl. a	Chl. b	Total	Chl. a	Chl. b	Total	Chl. a	Chl. b	Total	
0.1462	0.1246	0.2708	0.1660	0.1336	0.2996	0.1842	0.1617	0.3459	0.2169	0.1805	0.3974	30
0.1508	0.1304	0.2812	0.1960	0.1519	0.3479	0.2110	0.1703	0.3813	0.2330	0.1934	0.4264	45
0.1642	0.1446	0.3089	0.2156	0.1805	0.3961	0.2603	0.2306	0.4909	0.2815	0.2239	0.5054	60
0.1704	0.1540	0.3244	0.2317	0.1972	0.4289	0.2930	0.2624	0.5554	0.3113	0.2740	0.5853	75
0.1811	0.1710	0.3521	0.2702	0.2404	0.5106	0.3324	0.2956	0.6280	0.3548	0.3115	0.6663	90
0.2075	0.1925	0.3903	0.3363	0.2734	0.6097	0.3821	0.3342	0.7163	0.4017	0.3527	0.7544	120

Table 5: Effect of inoculants (Mycorrhiza, *Rhizobium and Rhizobium* + Mycorrhiza) on plant growth in terms of number of pods

Control	T_1(*Rhizobium*)	T_2(Mycorrhiza)	T_3(*Rhizobium* + Mycorrhiza)	Days
0	0	0	0	30
2.5	3	3.5	4.5	45
4.5	5	5.5	7.5	60
5.5	6.5	7	8.5	75
8	8.5	9	11	90
12	12.5	15	16	120

Table 6: Effect of inoculants (Mycorrhiza, *Rhizobium and Rhizobium* + Mycorrhiza) on plant growth in terms of yield

Control	T_1(*Rhizobium*)	T_2(Mycorrhiza)	T_3(*Rhizobium* + Mycorrhiza)
3.555	**3.793**	**3.948**	**4.198**

Graphs of plant height, number of root nodules, leaf area, chlorophyll amount, number of pods and yield

Plants inoculated with mycorrhiza, either alone or in combination with Rhizobium, brought about significant changes in chlorophyll a, b and total chlorophyll amount. There are several reports indicates that the chlorophyll may play a vital role in making plants resistant to pathogens (Bhavani, 1983., Rajasekaran and Nagarajan, 2005., Charitha and Reddy, 2001). It is also reported that mycorrhizal fungi serve as biocontrol agents against certain soil-borne plant pathogens and can survive under inhospitable soil environments. These results also have similarity with the results observed by Nida *et al.,* (2017) and Arumugam *et al.,* 2010.

Number of pods

Number of pods observed higher (16) in dual inoculated (R+M) plants whereas minimum (12) numbers of pods were observed in controlled plants. Plants which were treated with single inoculation (R or M) reported fewer numbers of pods than dual inoculation plants (Table - 5).

Yield (seeds)

The results observed that yield of mung plant higher (4.198g) in dual inoculated (R+M) plants and minimum (3.555g) in controlled plants. Co-inoculation of *Rhizobium* and mycorrhiza has positive impacts on yield (Table - 6).

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