Compatibility of most efficient *Rhizobium* strain with different seed dressing fungicides in Mung bean crop

ABSTRACT

Symbiotic nitrogen fixation is considered as an important source of nitrogen on earth. Numerous leguminouscrop fix about 200 – 300 kg N/ha.Some microorganisms especially bacteria are able to colonise and fix nitrogen biologically in the soil through symbiotic process. A pot culture experiment was conducted to examine the compatibility of *Rhizobium* with nine fungicides and their combination used for seed treatment. The combination effects of inoculation with fungicide growth and number of nodules, dry weight of nodules, dry matter parameters etc. were studied. The seed treatment of mungbean seed with copper oxychloride was found most effective among all the seed treatments. Seed treatment with different seed dressing fungicide had no adverse effect on germination, nodulation, yield-attributing characters and grain yield when applied alone or in combination with *Rhizobium* inoculant, thus, indicating the compatibility amongst all the two components, *viz.* fungicide and *Rhizobium* inoculant for seed treatment in (mungbean).Laboratory studies have also shown fungicides containing high levels of metalaxyl or metalaxyl-M have an inhibitory effect on rhizobia and nodulation, and so applying a granular inoculant or liquid or slurry inoculant in furrow is recommended when these fungicides are applied to seed.Treating seed with fungicide before sowing is a common method of protecting seedlings from fungal pathogens. While some fungicides are toxic to rhizobia, if these toxic effects are avoided, seed applied fungicides can increase plant health and root growth which in turn can benefit nodulation and N fixation. However, where the inoculants applied to seed come in direct contact with the fungicide, toxicity issues can occur.

Keywords: *Compatibility, Copper oxychloride, Fungicide,Nodulation, Rhizobium, Seed treatment*

INTRODUCTION

The green gram *(Vigna radiata*) is commonly known as mungbean or mung. In India, green gram is one of the most important pulsecrop, belongs to family Leguminaceae. It is generally cultivated in the tropical and subtropical countries. It requires soils from a black cotton soil to light sandy loam soil.Pulse although fix atmospheric nitrogen, there is evident that application of nitrogenous fertilizers increase yield(Habete & Buraka, 2016), (Otieno *et al*., 2009). Symbiotic nitrogen fixation is an important source of nitrogen, and various legume crops fixes about 200-300 kg nitrogen per hectare(Qureshi *et al*., 2019). Some microorganism specially bacteria called rhizobia are able to colonise the rhizosphere, and which infect the legume crops roots and fix nitrogen biologically in the soil through symbiotic process (Khan *et al*.,2023),(Singh, N. 2022).

The survival and inoculation efficiency of root nodules bacteria are highly affected by several soil and plant factors *viz*. soil constitution, soil physical properties and the chemical seed treatments. Seed treatment with chemicals may reduce microbial activity subsequently and attention towards this point has often been drawn in the rhizobia literature. Contradictory confirmation has been given on the compatibility between the rhizobia and seed dressing chemicals.

On the other hand, fungicide and insecticides may adversely affect the legume *Rhizobium* symbiosis, by inhibition of survival of the applied rhizobia on seed at sowing time(Rathjen *et al*.

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2020). This may lead to slow establishment of rhizobia in the rhizosphere, which may reduce nodulation and fixation. Several workers reported that at recommended rate of application, the fungicides have no suppressive effects on the growth parameters of legumes (Ghosh *et al.,* 2003, Guene *et al.,* 2004).Treating seed with fungicide before sowing is a common method of protecting seedlings from fungal pathogens. While some fungicides are toxic to rhizobia, if these toxic effects are avoided, seed applied fungicides can increase plant health and root growth which in turn can benefit nodulation and N fixation. However, where the inoculants applied to seed come in direct contact with thefungicide, toxicity issues can occur. Rhizobial survival and the subsequent success of the legume crop will depend on the toxicity of the applied fungicide to rhizobia and the duration the rhizobia spend in contact with the chemical.

MATERIALS AND METHODS

Nodulation Ability and Selection of Efficient Strain

Plastic Cup Experiment (Nodulation ability of *Rhizobium* isolates)

In these method transparent plastic cups were used for sowing of green gram seeds. Instead of soil, small pebbles of maximum 2-3 mm diameter were used as medium for sowing of seeds. Sieve was used for selection of pebbles. Plastic cups were filled two third with sterilized pebblesFerguson *et al.,* (2010) and Mangala (2013). Pebbles were sterilized by acid wash and 2-3 wash of water, to remove trace of acid. 3-4 seeds of variety (BM 2003-2) were sown in the plastic cup at depth of 1-2 cm and inoculated with 2 ml of *Rhizobium* suspension and one control was maintained without inoculation. Later the observations on nodule count and height of plant were taken at 15 days after sowing (DAS).

Pot Experiment (Growth promotion potential of *Rhizobium* isolates)

The clay pots were used for pot culture studiesFerguson *et al.,* (2010) and Rajpoot and Panwar (2013). Pots were sterilized with 5% formaldehyde solution. Medium type of soil sterilized in autoclave at 30 psi for 2 hours and used in this experiment. The pots were filled with sterilized soil and farm yard manure (FYM) mixed in proportion of 1:1. Mungbean seeds of variety BM 2003-2 were surface sterilized by dipping in 0.1% mercuric chloride. Five healthy seeds per pots were sown. Seeds were treated with *Rhizobium* suspension before sowing 10 ml /kg *Rhizobium* culture were given as seed treatment and remaining one pot was kept without treatment as control. For nodule count selected plants from each pot were uprooted on alternate days. The plants were selected randomly and uprooted carefully by digging with help of 'khurpi'. All possible precautions were taken to reduce the nodule loss. Later the observations on nodule count and plant height were taken at 30 days after sowing (DAS).

Compatibility of *Rhizobium* with Different Seed Dressing Fungicides

To study the compatibility of *Rhizobium* with different seed dressing fungicides a pot culture experiment was conducted over period of six months (June to November) in 2022 in the growing season of green gram at Agricultural Research Station, Badnapur, Maharashtra, India. The most efficient and superior strain were used for further studies. Initially the seeds of green gram variety (BM 2003-2) were treated with different fungicides at recommended rate and then these seeds were further inoculated with *Rhizobium* suspension (10^8 cells/ml), seeds dried in shade and sown. The experiment was laid out in CRD (Completely Randomized Design). The

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observation on nodule was recorded at 30 and 45 days after sowing (DAS). Whereas, plant height and dry matter was recorded at 45 days after sowing (DAS).

Details of experiment:

Design : CRD

Replication : Three

Variety : BM 2003-2

Treatments : Ten

Date of sowing : 8 June 2022

Date of observation : 23 July 2022

List 1 : Details of the treatment are as below

Treat. No.	**Treatment details**	**Method of application**
T_1	*Rhizobium* inoculation + Carbendazim 50% WP	Seed treatment
T_2	*Rhizobium* inoculation + Mancozeb 50 % WP	Seed treatment
T_3	*Rhizobium* inoculation + Propineb 70 % WP	Seed treatment
T_4	*Rhizobium* inoculation + Captan 50% WP	Seed treatment
T_5	*Rhizobium* inoculation + Sulphur 80% WP	Seed treatment
T_6	*Rhizobium* inoculation + Copper oxychloride 50% WP	Seed treatment
T_7	*Rhizobium* inoculation + Carbendazim 12% + Mancozeb 63 % WP	Seed treatment
T_8	*Rhizobium* inoculation + Carboxin 37.5% + Thiram 37.5% WP	Seed treatment
T_9	*Rhizobium* inoculation + Captan 70%+ hexaconazole 5% WP	Seed treatment
T_{10}	*Rhizobium* alone	Control untreated

Details of Observation

In all the pot culture experiment five plants per pot were used for recording the observations and averages were worked out for the purpose of recording the data. The details of observation as under.

Height of the plant:

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Height of the plant was measured from ground level up to terminal bud using a meter scaleat 45 days after sowing (DAS).

Number of nodules:

The plant was uprooted at the time of observation from the field by gentle washingoff the soil with running water and physically counted the total number of nodules per plant at 30 days after sowing (DAS) and 45 days after sowing (DAS). observation were recorded.

Dry matter content:

Entire plants were oven dried at 60^0C ± 2□ temperature till the constant weight was obtained. Later constant weight was recorded as dry matter weight.

RESULT AND DISCUSSION

Authentication of *Rhizobium* isolates / Testing the efficacy of selected *Rhizobium* isolate.

The *Rhizobium* isolates demonstrated on the growth and nodulation of green gram plants compared to the uninoculated control (Table 1). Rh_3, Rh_4 and Rh_2 isolates were found better among tested isolates for maximum nodule formation.

Brahmaprakash *et al.,* (2007) reported that nodule number per plant and grain yield were improved due to inoculation of liquid *Rhizobium* inoculants compared to carrier based *Rhizobium* inoculants. Plant growth promoting parameters such as plant height, nodule number, root volume, root surface and branch number recorded as per the methodology of Hiscox and Israelstam (1979).

Plastic Cup Experiment

Nodulation ability test was conducted on mungbean as the bacteria were isolated from mungbean plants. Isolated and identified *Rhizobium* isolates during the present investigation were tested for ability of nodulation test in plastic cup. Treated seeds of mungbean were sown in disposable paper cups and observed to produce nodule. It was observed that all the *Rhizobium* isolates formed nodules, however Rh_3 produced the greatest number of nodules (10) with a plant height of 25 cm, as recorded at 15 DAS (Table 1, Fig.1 and Fig. 2). Rh_1, Rh_2, Rh_4, Rh_5, Rh_6, Rh_7, Rh_8 control or uninoculated recorded 5, 8, 9, 2, 4, 6, 5, 2 nodules per plant respectively. The height of plant was maximum (25 cm) of isolate Rh_3 and the minimum i.e., 10 cm of isolate Rh_7. Results obtained from this study consistently support the previous findings of Ferguson *et al.,* (2010) and Mangala (2013)

Table 1: Authentication of *Rhizobium* isolates in plastic cup experiment at 15 DAS

Treatment No.	Number of nodules/ Plant	Height of plant (cm)
Rh_1	5	19
Rh_2	8	22
Rh_3	10	25
Rh_4	9	20
Rh_5	2	14
Rh_6	4	17
Rh_7	6	10

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Rh_8	5	21
Control (uninoculated)	2	7

Fig. 1 Authentication of *Rhizobium* in plastic cup method

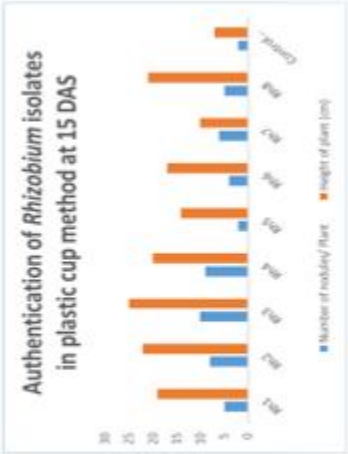

Fig. 2: Authentication of *Rhizobium* isolates in plastic cup method at 15 DAS

Pot experiment

The pot experiment was conducted to evaluate the growth promotion potential of the selected *Rhizobium* isolates under controlled conditions. The results of the pot experiment are expressed in Table 2.

Number of nodules produced by different *Rhizobium* isolates were studied in the pots under greenhouse condition. All isolates formed nodules, however, Rh_3 produced greater number of nodules (35) which were recorded at 30 DAS (Table 2,Fig.3, Fig.4). Rh_1, Rh_2, Rh_4, Rh_5, Rh_6, Rh_7, Rh_8 recorded 31, 20, 28, 20, 19, 32, 30 nodules per plant, respectively. The height of plant was also recorded. The maximum height of the isolate Rh_3 was 39cm and minimum height of the isolate Rh_6 was 15cm. These results are similar with the findings of Ferguson *et al.*, (2010) and Mangala (2013)

Table 2: Authentication of *Rhizobium* isolates in pot culture method at 30 DAS

Treatment No.	Number of nodules /Plant	Height of plant (cm)
Rh_1	31	19
Rh_2	20	28
Rh_3	35	39
Rh_4	28	21
Rh_5	20	27
Rh_6	19	15
Rh_7	32	30
Rh_8	30	32

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Control (uninoculated)	14	10

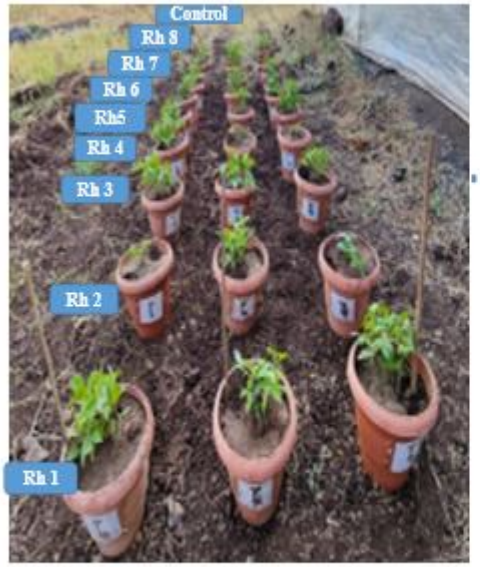

Fig. 3: Authentication of *Rhizobium* in pot culture method

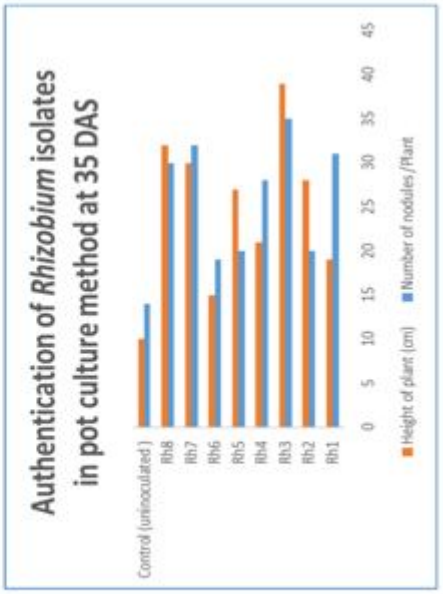

Fig. 4: Authentication of *Rhizobium* isolates in pot culture method at 35 DAS

Compatibility of *Rhizobium* with Different Fungicides

The compatibility of *Rhizobium* with different seed dressing fungicides was studied in a pot culture experiment. The most efficient *Rhizobium* strain Rh_3 was used for compatibility with nine seed dressing fungicides and mungbean variety BM 2003-2. The number of nodules, plant height and dry matter accumulation per plant were the criteria used to evaluate the compatibility (Table 3).

Height of the Plant:

The height of plant was recorded as highest (22.8 cm) in seed treatment with copper oxychloride (T_6), while lowest height (6.5 cm) of plant was observed in seed treatment with carbendazim (T_1). The height of plant in treatments T_2, T_3, T_4, T_5, T_7, T_8, T_9, T_{10} were recorded as 16, 14, 8.9, 10.8, 11.5, 10, 15.4, 17.9 cm, respectively(Fig.6).

Number of nodules:

Among the highest number of nodules up to 38.19 were observed in treatment of Copper oxychloride 50% WP per plant after 30 days and found most effective over the control. Captan 50% WP ranked second and recorded 30.62 number of nodules. Propineb 70 % WP and Sulphur 80 % WP recorded 28.30 and 23.82 number of nodules., respectively. *Rhizobium* alone recorded least number of nodules which is 11 nodules per plant. Seed treatment of Carbendazim 50% WP, Mancozeb 50 % WP, Carbendazim 12% WP+ Mancozeb 63 % WP, Carboxin 37.5% WP + Thiram 37.5% WP, Captan 70% + hexaconazole 5% WP recorded 17.21, 15.42, 21.30, 18.06 and 20.17 nodules per plant, respectively.

Observation on number of nodules were recorded at 45 days indicates that highest number of nodules 57.04 were observed in Copper oxychloride 50% WP and less number of nodules 23.26 were recorded in *Rhizobium* alone. Seed treatment of Carbendazim 50% WP, Mancozeb 50 % WP, Propineb 70 % WP, Captan 50% WP, Sulphur 80 % WP, Carbendazim 12% WP+ Mancozeb 63 % WP, Carboxin 37.5% WP + Thiram 37.5% WP and Captan 70% + hexaconazole 5% WP recorded 21.40, 49.16, 53.37, 31.61, 36.27, 50.02, 29.05 and 37.43 number of nodules per plant, respectively.

Least number of nodules were observed in seed treatment with *Rhizobium* alone and highest number of nodules were recorded in seed treatment with copper oxychloride (57.04), captan (31.61) and carbendazim (21.40).

Dry matter content:

The dry matter content of the plants in ten treatments (e.g. T_1, T_2, T_3, T_4, T_5, T_7, T_8 and $T_{9)}$ was recorded as 0.37, 0.51, 0.89, 0.68, 0.76, 1.23, 0.95 and 0.72 in grams respectively. The maximum (1.70 g) dry weight was found in treatment T_6, while lowest dry weight (0.31g) was found in the treatment T_{10}(Fig.5).

Findings of this study were highly consistent with the previous findings of Muthomi *et al.,* (2007). A similar study was carried out byMuthomi *et al.,* (2007) reported that the fungicide copper oxychloride was used for seed treatment along with Rhizobial inoculation, was beneficial in enhancement of nodulation in food legumes. Another study executed by Paraskar *et al.,* (2010) suggested that effect of *Rhizobium* spp. on soybean plant height, number of nodules per plant, root length and number of pods per plant by seed treatment and reported significant increase in these parameters over uninoculated control. Moreover, Panwar *et al.,* (2015) reported that thiram had no adverse effect on viable rhizobia or taproot nodulation.

Table 3 : Compatibility of *Rhizobium* with different seed dressing fungicides.

Treat.	Fungicides	Plant height (cm)	No. of Nodules/ plant 30 DAS	No. of Nodules/ plant 45 DAS	Dry weight of plant (g)
T_1	Carbendazim 50% WP	6.5	17.21	21.40	0.37
T_2	Mancozeb 50% WP	16	15.42	49.16	0.51
T_3	Propineb 70% WP	14	28.30	53.37	0.89
T_4	Captan 50% WP	8.9	30.62	31.61	0.68
T_5	Sulphur 80% WP	10.8	23.82	36.27	0.76
T_6	Copper oxychloride 50% WP	22.8	38.19	57.04	1.70
T_7	Carbendazim 12% + Mancozeb 63% WP	11.5	21.04	50.02	1.23
T_8	Carboxin 37.5% + Thiram 37.5% WP	10	18.06	29.05	0.95
T_9	Captan 70% + Hexaconazole 5% WP	15.4	20.17	37.43	0.72
T_{10}	*Rhizobium* alone	17.9	11.26	23.26	0.31

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	SE±m	0.70	1.15	1.67	0.07
	CD@1%	2.08	3.43	4.96	0.22

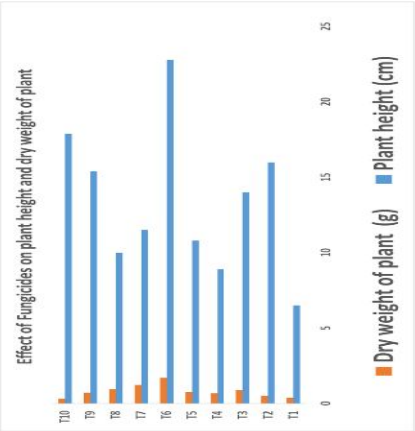

Fig. 5 Effect of fungicides on plant height and dry weight of plant

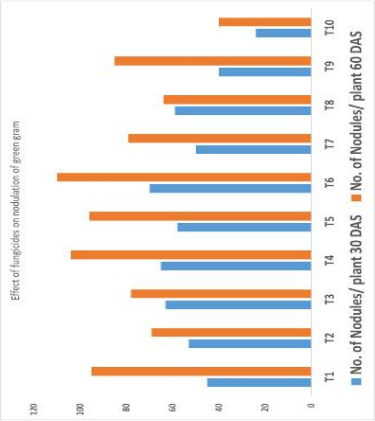

Fig 6: Effect of Fungicides on Nodulation of Green gram

CONCLUSION

The study revealed that fungicide copper oxychloride found to be effective as compare to other seed dressing fungicides. The height of plant was highest 22.8 cm in seed treatment with copper oxychloride (T_6), while lowest 6.5 cm height of plant was observed in seed treatment with carbendazim (T_1). Among the highest number of nodules up to 38.19 was observed in treatment of copper oxychloride 50% WP per plant and found effective over the control at 30 days. Observation on number of nodules were recorded at 45 days indicated that highest number of nodules 57.04 were observed in copper oxychloride 50% WP and less number of nodules 23.26 were recorded in *Rhizobium* alone. The highest dry weight 1.70 g found in treatment T_6, while lowest dry weight 0.31g was found in $T_{10.}$Carbendazim and copper oxychloride are commonly used in control of many fungal diseases in agriculture. The study was undertaken to assess the effects of carbendazim and copper oxychloride on seed germination, seedling growth, vigour index and biomass.

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