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Influence of Seed Inoculation Treatments on Yield and Quality of Green Gram (*Vigna radiata* L.)

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ABSTRACT

To study the effect of seed inoculation treatments with biological agents on seed germination, field emergence and its effect on seed during summer season. Seeds of green gram variety Pratap (SG-1) were treated with microbial formulations of seven treatments which consisted of-*Rhizobium* (T_1), *Trichoderma harzianum* (T_2), *Trichoderma viride* (T_3), *Bacillus megaterium* (T_4), *Trichoderma harzianum* + *Trichoderma viride* + *Bacillus megaterium* (T_5), *Rhizobium* + *Trichoderma harzianum* + *Trichoderma viride* + *Bacillus megaterium* (T_6) and Control (T_7). Shoot length, root length and seedling dry weight were recorded after 20 days of sowing, observations on nodulation, yield and yield contributing characters were recorded at maturity and germination characteristics of the seeds were studied in the laboratory. It was observed that seeds treated with combined inoculation of *Rhizobium* @ 4 g + *Bacillus megaterium* @ 5ml/1000 ml of water + *Trichoderma harzianum* @ 5ml/1000 ml of water + *Trichoderma viride* @ 5ml/1000 ml of water (T_6) recorded significantly higher field emergence (91.25%), speed of emergence (42.14), seedling dry weight (1.67mg), shoot length (25.48 cm), seed yield (992 kg ha^{-1}), Stover yield (1870 kg ha^{-1}), number of pods $plant^{-1}$ (37), number of seeds pod^{-1} (13.25), 100 seed weight (3.63g) root length (9.22 cm) and nodulation (15).

Key words: Greengram, Microbial formulations, Seed inoculation, Seed quality.

INTRODUCTION

Green gram (*Vigna radiata* L.) is a well- known pulse crop of India. It is a short duration crop and can be grown twice in a year. Being drought resistant, it can withstand adverse environmental conditions and is successfully cultivated in rain fed areas. Green gram is digestible, high in protein (22-24%) (Malik, 1994) and does not cause flatulence that many other legumes do. Moreover, it is rich in vitamins as A, B, C, Niacin and minerals such as potassium, phosphorus and calcium, which are necessary for human body (Rattanawongsa, 1993). In addition, it contains iso-flavonoids having estrogens and antioxidant activities that can be used in prevention of diseases such as Cancer (Brouns, 2002).

Although, this crop is capable of fixing atmospheric nitrogen through *Rhizobium* species living in root nodules, however, under warm to hot humid agro-ecological conditions, the nodulation of mungbean is poor and is a major cause of its lower yield. Seed inoculation with strains prior to sowing allows a reduction in N mineral fertilization, increases plant and seed quality and yield as well as decreases susceptibility to environmental stress (Tien *et al*., 2002; Herridge *et al*., 2005). The biological seed treatment involves the use of biological organisms to enhance nutrient fixation and availability for utilization by the crop plants. Bacteria like *Rhizobium spp.,* invades the root hairs of mungbean and result in the formation of nodules, where free air nitrogen is fixed. Combined application of such biological agents has also been advocated to increase productivity of legumes such as green gram. Several benefits of biological seed treatment like increased germination, uniform seedling emergence and its growth have been reported. *Rhizobium* inoculation enhances the nitrogen fixing capability of legume crops and their productivity and improved plant population and thus higher productivity. Therefore, the present study was undertaken to investigate certain seed production aspects in summer green gram grown under organic conditions in summer season with the specific objective to study the effect of seed treatment with microbial formulations on germination, vigour and yield characteristics of green gram.

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MATERIALS AND METHODS

The experiment was conducted during summer season, 2015-2016 at the organic plot of the Instructional Cum Research (ICR) Farm which was situated at 26° 46′ N latitude and 94° 16′ E longitude along with an altitude 86.6 m above mean sea level of Assam Agricultural University (AAU), Jorhat. The soils of the experimental site belonged to the order inceptisol with sandy loam texture with a pH 4.8. The seeds of the variety Pratap (SG1) were obtained from the AICRP on National Seed Project (crops) from the

department of Plant Breeding and Genetics whereas on the other hand, the biofertilizers such as Rhizobium were obtained from the department of Soil Science, the fungal biocontrol agent such as *Trichoderma harzianum* and *Trichoderma viride* were also obtained from the Department of Plant Pathology of Assam Agricultural University, Jorhat Assam. Seeds of the variety Pratap (SG-1) were treated with bio-agents as follows to constitute the following treatments: *Rhizobium* with a concentration of 4 g along with 0.025% of carboxyl methyl cellulose (CMC) treated as T_1 was dissolved in 1000 ml of water and prepared as a solution for soaking of seeds before sowing the seeds. After one and a half hour of soaking, the seeds were shade dried overnight. Five ml liquid based seed inoculum with *Trichoderma harzianum* along with 0.025% of carboxyl methyl cellulose (CMC) as T_2 was dissolved in 1000 ml of water and prepared as a solution for soaking of the seeds before sowing the seeds and were shade dried overnight after soaking the seeds for 1-2 hours. Similar procedure was being followed for the rest of the treatments supplemented with fungal biocontrol agent such as *Trichoderma viride*, bacterial biocontrol agent such as *Bacillus megaterium* and with a consortial treatment with *Trichoderma harzianum* + *Trichoderma viride* + *Bacillus megaterium* and *Rhizobium* @ 4 g along with *Trichoderma harzianum* + *Trichoderma viride* + *Bacillus megaterium* . The un-inoculated seeds which were considered as control were also treated in the similar way but without any addition of microbial treatments. The experiment involving the green gram variety Pratap with seven seed treatments including an untreated control was sown in a field plot of size 1.5m × 3m laid out in randomized block design with four replications and five rows of 3 m length with row to row spacing of 30 cm and a plant to plant spacing of 10 cm which was maintained through thinning manual weeding and other intercultural operations after 25 days of sowing. FYM @ 2 t ha^{-1} without the addition of any inorganic fertilizer was applied to the experimental area. Under the laboratory conditions, the data on the following parameters such as germination percentage, speed of germination (ISTA, 2004) and hard seeds percentage were recorded whereas on the other hand, speed of emergence, field emergence, seedling dry weight (mg), shoot length (cm), root length (cm), nodules $plant^{-1}$, seed yield (kg ha^{-1}), Stover yield (kg ha^{-1}), pods $plant^{-1}$, seeds pod^{-1} and 100 seed weight (g) were recorded using appropriate formulae which were experimented under the field conditions. The mean data obtained from the various characteristics under study were analyzed by the method standard statistical procedures as described by (Panse and Sukhatme, 1985., Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Germination percentage

The seeds of greengram sown under the field conditions showed that the germination percentage ranged from 85.50% to 97.50% (Table1). *Rhizobium* @ 4 g combined with *Trichoderma harzianum, Trichoderma viride* and *Bacillus megaterium* @ 5 ml/1000 ml of water each (T_6) showed the highest germination 97.50% as compared to the treatment with *Trichoderma harzianum* in combination with *Trichoderma viride* and *Bacillus megaterium*@ 5 ml/1000 ml of water each (T_5) with 96% and 92% in *Rhizobium* @ 4 g /1000 ml of water (T_1) and these treatments were found to be statistically *at par.* The present findings were in agreement with Mandhare *et al*. (2010) who reported that the seeds when treated with *T. viride*, *P. fluorescens* and *B. subtilis* @ 6g kg^{-1} seed increased seed germination 75.50% and vigor index 2389 respectively in soybean seeds. In black gram also, seeds when treated with *T. viride,* recorded highest seed germination 71.00% and seedling vigor index 1466 followed by *B. subtilis.*

Speed of germination

Likewise, the records on speed of germination revealed that the highest speed of germination 45.71 (Table1) was recorded in the treatment *Trichoderma harzianum* combined with *Trichoderma viride* and *Bacillus megaterium* @ 5 ml / 1000 ml of water along with an inoculation of *Rhizobium* @ 4g /1000 ml of water (T_6) which indicates earliness in germination and may due to the effect of microbial formulations which cause earlier emergence of radicle and availability of various nutrients followed by 43.67 in combined inoculation of *Trichoderma harzianum, Trichoderma viride and Bacillus megaterium* @ 5 ml/1000 ml of water (T_5). Similar kind of studies were carried out by Rajeswari and Kumari (2009) in soybean seeds treated with *T. viride, P. fluorescence* and *B. subtilis* amongst *T. viride* gave highest germination and seedling vigor index of the seedlings.

Hard seed percentage

The seeds of greengram sown *in vitro* revealed that the hard seeds percentage ranged from 2% to 12% and has been shown under Table 1. Significantly lower percentage of hard seeds 2% was recorded in the combined inoculated treatment of *Trichoderma harzianum, Trichoderma viride* and *Bacillus megaterium* @ 5 ml /1000 ml of water along with an inoculation of *Rhizobium* @ 4g /1000 ml of water (T_6) compared to consortial formulations of *Trichoderma harzianum, Trichoderma viride* and *Bacillus megaterium* @ 5 ml/1000 ml of water (T_5) with 3% and these treatments were not found to be statistically *at par.* Similar kind of work was also carried out by Prasad and Karivaratharaju (1994) and also Khattra and Singh (1992); who reported that the higher percentage of hard seeds in summer might be due to prevalence of hot weather conditions during seed development and maturation, resulting in rapid dehydration which can induce hard seeds.

Field emergence percentage

The highest field emergence percentage 91% being observed in *Rhizobium*@4 g/1000 ml of water along with *Trichoderma harzianum, Trichoderma viride* and *Bacillus megaterium* @ 5 ml/1000 ml of water each (T_6) followed by

Table 1: Mean values for germination, speed of germination hard seeds, speed of emergence and field emergence from studies on effect of seed inoculation treatments on seed yield and quality of green gram.

Treatments	Germination (%)	Speed of germination	Hard seeds (%)	Speed of emergence	Field emergence (%)	Seedling dry weight(mg)	Shoot length (cm)	Root length (cm)	Nodules plant^{-1}
T_1: *Rhizobium* @ 4g/1000 ml of water	(92.00)	41.99	(7.00)	35.42	(85.25)	1.57	23.13	8.72	12.50
T_2: *Trichoderma harzianum* @ 5 ml /1000 ml of water along with 0.025% CMC	(90.25)	40.96	(7.00)	27.10	(62.75)	1.51	21.80	8.29	10.50
T_3: *Trichoderma viride* @ 5 ml/1000 ml of water along with 0.025% CMC	(89.00)	39.58	(8.00)	23.62	(44.25)	1.45	21.54	8.12	10.50
T_4: *Bacillus megaterium* @ 5 ml/1000 ml of water along with 0.025% CMC	(92.00)	41.79	(7.00)	28.12	(82.50)	1.45	21.98	8.34	11.25
T_5: *Trichoderma.harzianum+Trichoderma viride+Bacillus megaterium*@ 5 ml/1000 ml of water each	(96.00)	43.67	(3.00)	36.20	(85.75)	1.62	23.35	9.00	13.00
T_6: *Rhizobium+ Trichoderma harzianum+ Trichoderma viride+ Bacillus megaterium* @ 5 ml /1000 ml of water each	(97.50)	45.71	(2.00)	42.14	(91.25)	1.67	25.48	9.22	15.00
T_7: Control	(85.50)	38.66	(12.00)	17.75	(41.00)	1.13	21.03	8.00	7.50
SE.d (±)	2.85	0.92	0.76	3.21	4.34	0.12	1.33	0.87	0.74
C.D (5%)	5.98	3.12	1.60	6.95	9.12	0.25	2.79	1.83	1.55

85.75% in *Trichoderma harzianum* along with *Trichoderma viride* and *Bacillus megaterium*@ 5 ml/1000 ml of water each (T_5) which were found to be statistically *at par.*

Speed of emergence

The results on speed of emergence exposed that the speed of emergence in days ranged from 17.75 to 42.14 and has been clearly shown in Table 2 Significantly higher speed of emergence 42.14 was recorded in the treatment *Rhizobium* combined with *Trichoderma harzianum, Trichoderma viride* and *Bacillus megaterium* (T_6) compared to the treatment with *Trichoderma harzianum* along with *Trichoderma viride* and *Bacillus megaterium* (T_5) with 36.20 which did not show any significant results and were found to be statistically *at par.*

Seedling dry weight (mg)

Similarly the results on seedling dry weight revealed that highest dry weight of seedling 1.67mg was recorded in the treatment with *Rhizobium* along with *Trichoderma harzianum, Trichoderma viride* and *Bacillus megatarium* (T_6) closely followed by 1.62 mg in *Trichoderma harzianum* along with *Trichoderma viride, Bacillus megaterium* (T_5). Similar results were also recorded in cotton (Vanangamudi *et al.,* 1987), (Kumudha and Gomathinayagam, 2007) and paddy (Rajasekaran *et al.,* 2015) and had come to the conclusion that the reason might be due to the interference of bio-fertilizer with the seedlings metabolism which ultimately increased the biomass of green gram seedlings.

Shoot length (cm)

The data on shoot length revealed that highest shoot length with 25.48cm was recorded in the treatment with *Rhizobium* in combination with *Trichoderma harzianum, Trichoderma viride* and *Bacillus megaterium* @ 5 ml/1000 ml of water each (T_6) followed by 23.35cm in *Trichoderma harzianum* combined with *Trichoderma viride* and *Bacillus megaterium* @ 5 ml/1000 ml of water each (T_5). These results are in accordance with the findings of Patra and Bhattacharyya (1998), who reported that seed inoculated plants, exhibited significantly greater root and shoot length as compared to un-inoculated control plants.

Root length (cm)

The records on root length has been shown in which clearly revealed that the highest root length with 9.22 cm was recorded in the treatment consisting of *Rhizobium* combined with *Trichoderma harzianum, Trichoderma viride* and *Bacillus megaterium* @ 5 ml/1000 ml of water each (T_6) followed by mean root root length of 9 cm in *Trichoderma harzianum* along with *Trichoderma viride* and *Bacillus megaterium* @ 5 ml/1000 ml of water each (T_5).The present findings are in line with the findings of Anandaraj and Leema Rose Delapierre (2010) who also concluded that the combined inoculation of *Rhizobium spp., P. fluorescens* and *B. megaterium* enhanced shoot and root growth of *Vigna radiate*.

Table 2: Mean values for pods per plant, seeds per pod, seed yield, Stover yield and 100 seed weight from studies on effect of seed inoculation treatments on seed yield and quality of green gram.

Treatments	Pods plant^{-1}	Seeds pod^{-1}	Seed yield (kg ha^{-1})	Stover yield (kg ha^{-1})	100 seed weight (g)
T_1: *Rhizobium* @ 4g/1000 ml of water	34	12.50	878	1748	3.54
T_2: *Trichoderma harzianum* @ 5 ml/1000 ml of water along with 0.025% CMC	29	12.00	730	1690	3.42
T_3: *Trichoderma viride* @ 5 ml/1000 ml of water along with 0.025% CMC	28	11.75	673	1682	3.40
T_4: *Bacillus megaterium* @ 5 ml/1000 ml of water along with 0.025% CMC	30	12.25	840	1710	3.49
T_5: *Trichoderma harzianum+Trichoderma viride+Bacillus megaterium*@ 5 ml/1000 ml of water each	35	13.00	880	1760	3.57
T_6: *Rhizobium+ Trichoderma harzianum+ Trichoderma viride+ Bacillus megaterium* @ 5 ml/1000 ml of water each	37	13.25	992	1870	3.63
T_7: Control	25	11.25	663	1536	3.25
SE.d (±)	1.93	0.71	91.80	40.56	0.22
C.D (5%)	4.07	1.50	192.87	85.21	0.11



Fig 1: Results showing the effect of microbial formulations on nodulation of green gram with the following treatments: T_1: *Rhizobium* T_2: *Trichoderma harzianum* T_3: *Trichoderma viride* T_4: *Bacillus megaterium* T_5:*Trichoderma harzianum+ T. viride+ B.megaterium* T_6:*Rhizobium + Trichoderma harzianum+ T. viride+ B.megaterium* T_7: Control.

Nodules plant^{-1}

In case of nodules plant,$^{-1}$ the records revealed that the highest number of effective nodules, 15 was recorded in the treatment with *Rhizobium* along with *Trichoderma harzianum, Trichoderma viride* and *Bacillus megaterium* @ 5 ml/1000 ml of water each (T_6) followed by 13 nodules plant^{-1} in *Trichoderma harzianum* in combination with *Trichoderma viride and Bacillus megaterium* @ 5 ml/1000 ml of water. Similar findings were also reported by Rashid *et al.* (1999), Ramaswami and Oblisami (1986) that the increase in nodules plant^{-1} is due to the application of inoculation in combination with *Rhizobium* (Fig 1).

Seeds pod^{-1}

In case of seeds pod^{-1} the records revealed that highest number of seeds pod^{-1} 13.25 was recorded in the treatment combined with *Rhizobium, Trichoderma harzianum, Trichoderma viride* and *Bacillus megaterium* @ 5 ml/l of water each (T_6) followed by 13.00 in *Trichoderma harzianum* combined with *Trichoderma viride* and *Bacillus megaterium*@ 5 ml/1000 ml of water each (T_5). These results were also in agreement with Priya and Reddy (2008) for number of pods plant^{-1}; RoopaLavanya and Bini Toms (2009) and Priya and Reddy (2008) for number of seeds pod^{-1}; Roopa Lavanya and Bini Toms (2009) and Reddy *et al*. (2011) for 100-seed weight.

Seed yield (kg ha^{-1})

The combined treatment with *Rhizobium* along with *Trichoderma harzianum, Trichoderma viride* and *Bacillus megaterium* @ 5 ml/1000 ml of water each (T_6) recorded the highest seed yield 992 kg ha^{-1} as compared to *Trichoderma harzianum* combined with *Trichoderma viride* and *Bacillus megaterium* @ 5 ml/1000 ml of water each (T_5) with a seed yield of 878 kg ha^{-1}. The similar kind of results were also obtained by Rani and Kodandaramaiah, (1997)

who reported that the increase in yield in inoculated treatment might be attributed to increased nodules plant^{-1} and nodule dry weight, resulting in higher dry matter accumulation during the growth period and translocation of more photosynthate to the seed. These results are also in accordance with Shukla and Dixit (1996) who reported that *Rhizobium* inoculation increased mungbean seed yield over un-inoculated plots.

Stover yield (kg ha^{-1})

The results on stover yield revealed that the highest stover yield 1870 kg ha^{-1} was recorded in the treatment in combination with *Rhizobium* along with *Trichoderma harzianum, Trichoderma viride* and *Bacillus megaterium* @ 5 ml/1000 ml of water each (T_6) followed by *Trichoderma harzianum* combined with *Trichoderma viride* and *Bacillus megaterium*@ 5 ml/1000 ml of water each (T_5) with a mean value of 1748 kg ha^{-1}. The highest stovcr yield recorded by (T_6) was attributed to influence higher branches plant^{-1} and increased plant height.

100 Seed weight

The data on seed weight revealed that highest weight of 100 seeds 3.63g was recorded in the treatment with *Rhizobium* in combination with *Trichoderma harzianum, Trichoderma viride* and *Bacillus megaterium* (T_6) followed by *Trichoderma harzianum* along with *Trichoderma viride* and *Bacillus megaterium* (T_5) as well as *Rhizobium* @ 4g (T_1) with mean values of 3.57g and 3.54g. *Rhizobium* inoculation significantly increased the 100-seed weight of chickpea cultivars (El Hadi and Elsheikh, 1999), faba bean (Babiker *et at.,* 1995) and groundnut (Elsheikh and Mohamedzein, 1998) compared to the un-inoculated control.

CONCLUSION

Seed treatment with the bio-control agents has been offered as a resolution to this problem that will maximize the probability of obtaining a good stand of healthy and vigorous plants. The present investigation deals with the effect of microbial formulation treatments on growth and seed yield of green gram (*Vigna radiata*).The experimental evidences indicated that the seed treatment with microbial inoculants of bio-fertilizers/bio-control agents namely *Rhizobium, Bacillus megaterium, Trichoderma harzianum* and *Trichoderma viride* along with the consortial formulations enhance growth, productivity and seed yield of green gram. Based on the results from the present study obtained, it can be concluded that seed treatment with the microbial agents at effective concentration is efficient for enhancing the crop growth attributing characters and seed yield. The seed treatment, under field conditions, improved the seed quality together with an enhancement in the seed yield and 100 seed weight as assessed along with other yield attributing characters of the crop. The bio-control agents have also been exposed to increase the seed germination, emergence and seedling growth characteristics.

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