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Increase in defense response of biocontrol agents for reduction of *Cercospora* leaf spot of greengram (*Vigna radiata* L.)

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Abstract

In addition to the prevalent practice of seed inoculation with nitrogen fixing bacteria, several other biological control agents are also applied to green gram in the form of seed treatment particularly for organic cultivation of green gram. To study the effect of such seed inoculation treatments with biological agents on reduction of cercospora leaf spot disease as well as its component characters, a field experiment was conducted during summer season, 2015 at the organic plot of the Instructional Cum Pratap (SG-1) Farm of Assam Agricultural University (AAU), Jorhat. Seeds of green gram variety were treated with microbial formulations of *Rhizobium*, *Bacillus megaterium*, *Trichoderma harzianum*, *Trichoderma viride* and their combinations. The seven treatments consisted of- *Rhizobium* (T₁), *Trichoderma harzianum* (T₂), *Trichoderma viride* (T₃), *Bacillus megaterium* (T₄), *Trichoderma harzianum* + *Trichoderma viride* + *Bacillus megaterium* (T₅), *Rhizobium* + *Trichoderma harzianum* + *Trichoderma viride* + *Bacillus megaterium* (T₆) and Control (T₇). The experiment was laid out in Randomised Block Design with four replications. The unit plot size was 1.5 m x 3 m with a row to row spacing of 30 cm and 10 cm between plants. 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 (T₆) recorded significantly higher response in terms of reduction of percentage of disease occurrence. These results, thus, indicated that the combined inoculation treatment of *Rhizobium* with other biological agents improves vigour characteristics, seed yield as well as increasing the efficiency of defense response mechanism thereby reducing the occurrence of cercospora leaf spot in green gram.

Keywords: greengram, rhizobium species, *Trichoderma harzianum*, *Trichoderma viride*, *Bacillus megaterium*, cercospora leaf spot

Introduction

The term “pulses” is limited to crops harvested solely for dry grain, thereby excluding crops harvested green for food, which are classified as vegetable crops. Green gram [*Vigna radiata* (L.) Wilczek] 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) [8] 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) [13]. In addition, it contains iso-flavonoids having estrogens and antioxidant activities that can be used in prevention of diseases such as Cancer (Brouns, 2002) [11]. The cultivation of green gram during summer season gains wider acceptance with the availability of new varieties for additional income, improvement in soil fertility and efficient land utilization (Dodwadia and Sharma, 2012) [3]. In recent years, organic farming is considered as an important component of environmentally sound sustainable agriculture. Therefore there is an urgent need to reduce the usage of chemical fertilizers and in turn increase the usage of organic manures which are known to improve the physico-chemical properties of the soil and supply the nutrient to the plants. Although, this crop is capable of fixing atmospheric nitrogen through *Rhizobium* species living in root nodules, however, under our 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) [16, 6].

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The biological seed treatment involves the use of biological organisms to enhance nutrient fixation and availability for utilization by the crop plants and to control the pathogen located in the seed. Bacteria like *Rhizobium spp.*, invades the root hairs of mungbean and result in the formation of nodules, where free air nitrogen is fixed. To ensure an optimum *Rhizobia* population in the rhizosphere, seed inoculation of legumes with an efficient *Rhizobia* strain is necessary. This helps in improving nodulation, N₂ fixation, crop growth and yield of leguminous crops (Henzell, 1988) [5]. *Bacillus megaterium* aids in transformation of soil phosphorous into an assimilable form and is therefore an integral part of the soil phosphorous cycle as they help in releasing Phosphorous through solubilization. Biological seed treatment is usually very specialized and uses specific microorganisms that attack or interfere with specific pathogens or types of pathogens. With regards to plant diseases, suppression can be accomplished in many ways. Biological control is one of the viable, eco-friendly propositions, which can substantially minimize the disease (Cook, 1985) [2]. In addition to seed inoculation with nitrogen fixing bacteria several other biological control agents are also applied to green gram for management of diseases affecting the crop in form of seed treatment. *Trichoderma spp.*, are biocontrol agents widely used in management of fungal diseases of crop plants exhibiting mycoparasitism against a wide range of plant pathogens (Sharma *et al.*, 2014) [15]. Their bio-control potential has also been explored against several foliar diseases by seed or soil application indicating their effectiveness in inducing disease resistance in the crop plants. Therefore, the present study was undertaken to investigate certain seed production aspects in summer green gram grown under organic conditions in summer season. The specific objective was to study the effect of seed treatment with microbial formulations on disease incidence, and its component characters and seed quality of green gram.

Materials and Methods

Site and season

The experiment was conducted during summer season, 2015-2016 at the organic plot of the Instructional Cum Research

(ICR) Farm of Assam Agricultural University (AAU), Jorhat. The farm is situated at 26 degree 46' N latitude and 94 degree 16' E longitude. The altitude is 86.6 m above mean sea level. The soils of the experimental site belonged to the order inceptisol with sandy loam texture with a pH 4.8. The status of organic matter was high.

Experimental material

The experimental materials used in the study and their sources are given below:

Greengram seeds of the variety Pratap (SG-1) collected from AICRP on National Seed Project (Crops), of the Department of Plant Breeding & Genetics, AAU, Jorhat was used as a source material for conduction of the experiment. On the other hand, the biofertilizers or the bioagents such as *Rhizobium*, species of *Trichoderma* such as *Trichoderma viride* and *Trichoderma harzianum* and bacterial biocontrol agent such as *Bacillus megaterium* were collected from the Department of Soil Science and Plant Pathology of Assam Agricultural University, Jorhat Assam, India.

Treatments

Seeds of the variety Pratap (SG-1) were treated with bioagents as follows to constitute the following treatments:

Four g of *Rhizobium* along with 0.025% of carboxyl methyl cellulose (CMC) was dissolved in 1000 ml of water and used as T1 simultaneously prepared as a solution for soaking of seeds before sowing the seeds (Fig 1). After one and a half hour of soaking, the seeds were shade dried overnight. *Trichoderma harzianum* which was used as T2 was prepared by dissolving 5 ml liquid based fungal inoculum along with 0.025% of carboxyl methyl cellulose (CMC) in 1000 ml of water as a solution for soaking of the seeds before sowing the seeds. The seeds were dried overnight after being treated with the biocontrol agent. Similar procedure was followed for the treatment T3, (*Trichoderma viride*), T4 (*Bacillus megaterium*), T5 (*Trichoderma harzianum* + *Trichoderma viride* + *Bacillus megaterium*), T6 (*Rhizobium* @ 4 g + *Trichoderma harzianum* + *Trichoderma viride* + *Bacillus megaterium*), T7 Control (un-inoculated).



Fig 1: (A) *Bacillus megaterium* (B) *Rhizobium* species (C) *Trichoderma harzianum*

Layout of the experiment

The experiment involving the seven seed treatments of green gram variety Pratap and un-treated control was sown in a field experiment laid out in Randomized Block Design with four replications. Five rows of 3 m length represented each treatment in a replication, with row to row spacing of 30 cm.

A plant to plant spacing of 10 cm was maintained through thinning after 25 days of sowing.

Application of fertilizer

FYM @ 2t ha⁻¹ was applied to the experimental area. No inorganic fertilizer was applied.

Cultural operations

Manual weeding, thinning and other intercultural operations were done after 25 days of sowing as recommended.

Meteorological Record

Meteorological data on temperature, relative humidity, rainfall and bright sunshine hours during the crop period were obtained from the Department of Agrometeorology, AAU, Jorhat.

Observational Procedure

Disease incidence (%)

The disease incidence was recorded after 50 days on each treatment of four replications. The disease incidence percentage (DI) was calculated as follows:

$$DI = \frac{\text{Diseased plants in a plot}}{\text{Total plants in a plot}} \times 100$$

Statistical analysis of data

The mean data were analysed following standard statistical procedures (Panse and Sukhatme, 1985., Gomez and Gomez, 1984).

(a) Analysis of variance

The statistical model used in the present analysis of variance was as follows:

$$Y_{ij} = m + g_i + r_j + e_{ij}$$

Where, Y_{ij} = the observation in the i^{th} treatment/entry in the j^{th} replication.

m = general mean.

g_i = effect of j^{th} replication.

e_{ij} = error component associated with i^{th} entry in the j^{th} replication

The mean data of each character were subjected to analysis of variance following the randomised block design (RBD). The differences of means of individual treatments were tested by working out the critical (CD) both at 5% and 1% level of probability as follows:

CD = SED x t for error d.f. at 5% or 1% level of probability

SED = $\sqrt{2}$ Error MS/r

Results and Discussions

Evaluation on effect of seed treatment with microbial formulations on disease incidence, seed yield and its component characters and seed quality of green gram

The data on incidence of Cercospora leaf spot disease (Table1) revealed that the lowest percentage of incidence on disease 3.95% was recorded in the treatment *Rhizobium*+ *Trichoderma harzianum*+ *Trichoderma viride* + *Bacillus*

megaterium @ 5 ml/1000 ml of water each (T_6) followed by *Trichoderma harzianum*+*Trichoderma viride*+*Bacillus megaterium* @ 5 ml/1000 ml of water each (T_5) with mean values of 4.45% and *Rhizobium* @ 4g/1000 ml of water (T_1) with mean values of 4.47%, whereas, the highest disease incidence 8.14% was recorded in the un-treated control (T_7). All the treatments performed better than the un-treated control with significantly lower level of disease incidence. The treatments with *Trichoderma harzianum*+*Trichoderma viride*+*Bacillus megaterium* @ 5 ml/1000 ml of water each (T_5) and *Rhizobium* @ 4g/1000 ml of water (T_1) performed equally well and were statistically *at par*. Application of bio-fertilizers like *Rhizobium* in the form of seed treatment is a recommended practice and has been effectively used for reducing the application of inorganic fertilizers or totally avoiding the use of such fertilizers particularly in organic farming situations. Several other biological agents are also applied as seed inoculants to crops including mungbean for control of diseases. Application of such biological agent alone or in combination of biological agents is likely to affect the emergence, growth characteristics and seed yield. Implications of the findings of the present study on effect of some seed inoculation treatments on seed yield and quality of green gram are discussed in this manuscript. Paulitz and Linderman, (1991) [11] reported that the mycorrhizal fungi, *Trichoderma* and *Pseudomonas* strains are well known combinations to control most of the soil, air, root borne pathogens. The results corroborated with the findings of Natarajan and Rao (1996) [10] who studied potential use of *Trichoderma* as an effective bio-control agent against *R. bataticola* in most of the crop plants. Antibiotic production by *Trichoderma spp.* is a well-documented phenomenon and the reason for its inclusion in bio-control agents (Howell, 1998; Hazarika *et al.*, 2000; Mar Vazquez *et al.*, 2000; Tsimilli-Michael *et al.*, 2000; Rudresh *et al.*, 2005) [7, 4, 9, 17, 14]. It was also observed that the highest reduction on incidence of disease was recorded in the treatment *Rhizobium*+ *Trichoderma harzianum* + *Trichoderma viride* + *Bacillus megaterium* @ 5 ml/1000 ml of water each (T_6) followed by *Trichoderma harzianum* + *Trichoderma viride* + *Bacillus megaterium* @ 5 ml/1000 ml of water each (T_5) and *Rhizobium* @ 4 g /1000 ml of water (T_1). The seed inoculants applied could have helped the plants indirectly to escape from the diseases caused by pathogens due to the production of anti-microbial metabolites in the host. Present findings are in confirmation with earlier workers observed by Rahman *et al.* (2002) who concluded that when mungbean seeds were coated with the conidial suspension of *T. viride* effectively control (93.00%) seed-borne *M. phaseolina* and also reduction of seed borne fungi of black gram with treatment of *T. viride* (88.00%) followed by *B. subtilis* (56.00%) and *P. fluorescens* (48.00%).

Table 1: Mean values for disease incidence from studies on effect of seed inoculation treatments on seed yield and quality of green gram

Treatments	Disease incidence (%)
T_1 : <i>Rhizobium</i> @ 4g/1000 ml of water	4.47
T_2 : <i>Trichoderma harzianum</i> @ 5 ml/1000 ml of water along with 0.025% CMC	4.65
T_3 : <i>Trichoderma viride</i> @ 5 ml/1000 ml of water along with 0.025% CMC	5.40
T_4 : <i>Bacillus megaterium</i> @ 5 ml/1000 ml of water along with 0.025% CMC	4.61
T_5 : <i>Trichoderma harzianum</i> + <i>Trichoderma viride</i> + <i>Bacillus megaterium</i> @ 5 ml/1000 ml of water each	4.45
T_6 : <i>Rhizobium</i> + <i>Trichoderma harzianum</i> + <i>Trichoderma viride</i> + <i>Bacillus megaterium</i> @ 5 ml/1000 ml of water each	3.95
T_7 :Control	8.14
SE.d (\pm)	0.19
C.D (5%)	0.40

Summary and Conclusion

The present investigation was conducted to assess the effect of seed treatment with microbial inoculation of *Rhizobium spp.*, *Trichoderma spp.*, and *Bacillus megaterium* and two of its combination treatments on seed yield and quality of seeds of a green gram variety Pratap (SG-1), grown in a field experiment in RBD with four replications in the summer season. Treatment of the seeds with the four microbial formulations namely viz., *Rhizobium*, *Trichoderma harzianum*, *Trichoderma viride*, *Bacillus megaterium* and consortial bio-formulation treatments were found to be effective in reducing the incidence of disease over that of untreated control. However, *Rhizobium* + *Trichoderma harzianum* + *Trichoderma viride* + *Bacillus megaterium* (T₆) was recorded to be the most effective treatment followed by *Trichoderma harzianum* + *Trichoderma viride* + *Bacillus megaterium* (T₅) and *Rhizobium* (T₁). Lower disease incidence and resultant increase in seed yield by seed treatment with microbial agents might be attributed to rapid multiplication of antagonists in the soil and its colonization in the root of seedlings, thereby preventing the establishment of pathogens in the rhizosphere. Seed treatment with the bio-control agents has been offered as a solution to this problem that will maximize the probability of obtaining a good stand of healthy and vigorous plants. Based on the results of the experimental evidences, it 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 apart from enhancing the growth and productivity, it also helped in reducing the occurrence of diseases in green gram. The bio-control agents apart from increasing the defense responses of the plants have also been shown to increase the seed germination, emergence and seedling growth characteristics.

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