

Original Research Article

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Combination of Plant Growth Promoting Rhizobacteria and Biopesticides: An Effective Means of Suppressing Pod Borer Complex in Mungbean

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ABSTRACT

An experiment was conducted to evaluate the effect on seed biopriming in integration of bio-pesticides on the infestation of pod borer complex of mungbean in the Institutional farm of Institute of Agriculture, Visva Bharati University during 2014-2015. Seed bio-priming was done by *Pseudomonas fluorescence* and other bio-agents, where the bio-pesticides used were *Beauveria bassiana*, *Verticillium lecanii* and *Metarhizium anisopliae*. Studies on different biochemical parameters of the plants were made to highlight on the impact on pod borer infestation. The results of the experiment suggested that seed bio-priming with *Pseudomonas fluorescence* along with consecutive spray of *Beauveria bassiana* and *Verticillium lecanii* were found effective to reduce the infestation of pod borer complex upto 75.02% as compared to untreated check. It also reduced the percentage of damaged pods upto 11.86%. However, *Pseudomonas fluorescence* had a clear impact towards enhancement of phenol and ortho-dihydroxy phenol content along with peroxidase activities in mungbean leaf thus imparting resistance to pod borer complex.

Keywords

Bio-control agents,
Helicoverpa armigera,
Maruca testulalis,
Vigna radiata

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Introduction

Mungbean *Vigna radiata* (L.) Wilczek, an important legume crop is gaining importance due to its wide adaptability in different climatic conditions, tolerance to water stress and salinity, capacity of fixing atmospheric nitrogen and checking the soil nutrient depletion (Pataczek *et al.*, 2018). It is a rich

source of protein, carbohydrate and other micronutrients in daily diet and used as iron-rich whole food source for baby food (Nair *et al.*, 2013). India is one of the largest producers of mungbean in the world with a production of 1.90 million tonnes of mungbean grains having acreage of 4.07 million hectares (Anonymous, 2018). However, the country imports mungbean every year due to huge gap

between demand and supply. The crop suffers from low productivity due to lack of good varieties, poor agronomic management practices and improper crop protection measures (Singh *et al.*, 2015).

A wide array of insect-pest species infests the crop on regular intervals, which results into huge yield loss upto 38.06% (Lal 1985; Duraimurugan and Tyagi, 2014). Pod borer complex such as gram pod borer *Helicoverpa armigera* and spotted pod borer *Maruca tetulalis* are two most noxious pests of the crop infesting in both vegetative and reproductive stage.

They cause a yield loss upto 36.14% in case of severe infestation (Umbarkar *et al.*, 2011). Growers are mostly relying upon chemical insecticidal measures, which in turn result into resistance and resurgence development as well as environmental hazards (Mahamood *et al.*, 2016).

Microbial bio-pesticides, as an alternative to chemical insecticides are very safe to environment and natural enemies of crop pest. However, it has a disadvantage due to its limited persistency in adverse environmental conditions (Maina *et al.*, 2017).

Now a days, plant growth promoting rhizobacteria and other beneficial soil microbes are used to induce systemic resistance against insect herbivores by enhancing biosynthesis of secondary metabolites and activities of plant defense enzymes (Murugan and Dhandapani, 2007; Pangesti *et al.*, 2017; Rashid and Chung, 2017).

An attempt was made to evaluate the efficacy of the bio-pesticides in integration with seed priming of mungbean by *Pseudomonas fluorescence* and other growth promoting bio-agents in order to achieve sufficient control

against pod borer complex without using chemical insecticides.

Materials and Methods

The field experiment was conducted during Post Kharif season of 2015 and 2016 in Agricultural Farm of Institute of Agriculture, Visva-Bharati located at Birbhum district of West Bengal having latitude and longitude 23°40.182'N and 87°39.555'E respectively. The experimental site is situated in the sub-humid sub-tropical lateritic belt of West Bengal.

The experiment was carried out in Randomized Block Design (RBD) having seven treatments with four replications. The variety of mungbean, PDM 139 was sown on 05th of September in both the year with a plot size of 4 m x 3 m. Row to row distance was maintained in 30 cm. The treatments are mentioned in Table 1.

The bio-inoculum for trials were prepared and supplied by the Department of Plant Pathology of Uttar Banga Krishi Viswavidyalaya with required population in formulation (10^8 for each *Pseudomonas*, Phosphate Solubilizing Bacteria, *Azotobacter* and *Rhizobium*) following standard protocol (Park *et al.*, 2005). Seeds of mungbean were primed with all these bio-inoculant in 1:1 ratio @ 5g/kg of seed for 30 minutes. Bio-primed seeds of mungbean (variety: PDM 139) were sown for the experimental purpose. Recommended agronomic practices were followed throughout the crop growth period (Bhowmick *et al.*, 2008). However, nitrogen fixer in the name of 'Symbion-N', which is a biofertilizer with selective strains of nitrogen fixing bacteria such as *Rhizobium*, *Azospirillum*, *Acetobacter* and *Azotobacter* is made available from 'T.Stanes and Company Limited', Tamil Nadu, India. Mungbean seeds were bio-primed with 'Symbion-N' @ 10 ml/kg of

seeds. Similarly, biopesticides like *Beauveria bassiana*, *Verticillium lecanii* and *Metarhizium anisopliae* were also obtained from 'T.Stanes and Company Limited', Tamil Nadu, India in the name of 'Bio Power', 'Bio Catch' and 'Bio Magic' respectively.

Two sprays were carried out at 40 days after sowing (DAS) and 55 DAS. The data on infestation of pod borer complex was recorded from randomly chosen 2 rows of one metre length from each plot on the basis of number of larvae. The data were taken before spraying as a pre count data and subsequently on 3rd, 7th, 10th and 14th days after the spray schedules. Percentage of pod damage by pod borer complex was recorded during harvest at 65th and 75th day after sowing.

Moreover, different biochemical parameters of mungbean leaf were studied to assess the impact of bio-inoculation of seed and its relationship with pod borer infestation. Fresh leaves were randomly collected from each plot at 40th day after sowing before spray of any biopesticides. Estimation of total phenol and ortho-dihydroxyphenol (OD phenol) was done following the procedure described by Mahadevan and Sridhar (1982). However, protein content of leaf was estimated following Lowry's method (1951). Polyphenol oxidase and peroxidase activity was measured following the procedure described by Mahadevan and Sridhar (1982) and Addy and Goodman (1972) respectively. Correlation studies were also conducted to study the major contributing biochemical parameter(s) along with its impact on pod borer infestation.

Data on number of pod borers in one meter of row length thus obtained were square root transformed ($\sqrt{x + 0.5}$, where x is the number of insects) and tested for analysis of variance. Efficiency of each treatment over untreated control was measured through the formula derived by Henderson and Tilton (1955). In

case of the percentage pod damage during harvest, the data was angular transformed and tested for analysis for variance. The data on biochemical parameters thus obtained were also tested for analysis of variance. All the analysis was carried over in the statistical software SAS 9.2. The data was computed in Micro-soft Excel.

Results and Discussion

The efficacy of bio-pesticides on pod borer is depicted in Table 2 which represents the pooled data of two years (2015-2016).

It is clearly revealed that all the treatments were found superior over the check. Initial observation at 40 days after sowing (pre-spray count) showed comparatively less infestation of the pod borers in treatment T₁ and T₃ recording 1.78 and 1.88 number of pod borer/one metre of row length respectively, which was at par with other five treatments except treatment T₂ and untreated check (T₇). In both the treatments T₁ and T₃, *Pseudomonas fluorescence* was used for priming of seeds. There was a decreasing trend in number of pod borers in four treatments out of six, which were treated with biopesticides (T₁ to T₄) upto 10 days after spraying. However, the lowest number of pod borer was found in the treatment T₁ recording 0.62/one metre of row length at 14 days after spraying of *Beauveria bassiana* and *Verticillium lecanii*. It was at par with the treatment T₃ (0.64/one metre row length) involving the same biopesticides. These two treatments were found superior than all other treatments. Highest number of pod borer except untreated check were observed in treatment T₂ (1.16/one metre row length) at 14 days after spraying of *Metarhizium anisopliae* and *Verticillium lecanii*. The percentage control was found highest in treatment T₁ and T₃ with *Beauveria bassiana* and *Verticillium lecanii*, which ranges from 74.4 to 75.02%.

Moreover, all the treatments significantly reduced the damage of pods by pod borer complex as compared to untreated control, which ranged from 7.03% to 11.86%. The percentage pod damage was found lowest in treatment T₃ (7.03%), which was at par with treatment T₁ (7.82%). The results obtained from the experiment are in clear conformity of earlier works, where good field level control of *Helicoverpa armigera* and *Maruca testulalis* was achieved through *Beauveria bassiana*, *Verticillium lecanii* and *Metarhizium anisopliae* (Bayu and Prayogo, 2018; Choudhary *et al.*, 2017; Knox *et al.*, 2016; Revathi *et al.*, 2011). Moreover, seed priming with *Pseudomonas fluorescense* along with foliar application of *Beauveria bassiana* is also very effective to control pod borer complex (Soundararajan and Chitra, 2011).

Bio-chemical parameters of plants are related with the defence mechanisms of the plant against its herbivores. Thus, an attempt has been made to study the effect of bio inoculated mungbean on incidence of pod borer and its relation with biochemical parameters of plants. Phenol, OD phenol, peroxidase, polyphenol oxidase and protein of mungbean leaf was studied and presented in Table 3.

The results of the experiment revealed that there is a marked difference in the phenol content in the different treatments. The highest phenol content was found in the treatment T₁ (2.472 mg/g of fresh leaf), which was followed by treatment T₃ (2.218 mg/g of fresh leaf). The treatments T₂ and T₄ were statistically at par with each other recording phenol content 2.027 and 1.941 mg/g of fresh leaves respectively. The lowest phenol content was found in the untreated check suggesting that bio-inoculations have induced enhancement of phenol content within the plant system. The studies in the leaf phenol

content of different treatments thus elucidates that the best enhancement of phenol content was found in the bio-inoculation with *Pseudomonas fluorescense*. There is also a significant difference in the OD Phenol content of leaves in different treatments. Similar trend was also observed in case of OD phenol. The higher OD Phenol content was observed in the treatments T₁ and T₃ having bio-inoculation with *Pseudomonas fluorescense* resulting 1.012 and 1.008 mg/g fresh leaves respectively. The lowest enhancement of OD phenol in leaves among the treatments was in T₆ (0.779 mg/g fresh leaves), where only nitrogen fixers were used as seed dresser. However, the highest polyphenol oxidase activity (0.769 δ -495/mint./ μ g protein) was observed in the treatment with T₄ having bio-inoculation with nitrogen fixer and azotobacter. This was followed by T₁ (PSB, rhizobium and *Pseudomonas fluorescense*) recording 0.726 δ -495/mint./ μ g protein, which was at par with treatment T₄. Similarly, peroxidase level in all treatments was found significantly higher as compared to check (0.452 δ 420/mint./ μ g protein). Highest level of peroxidase was observed in T₁ (1.258 δ 420/mint./ μ g protein), which was at par with treatment T₃ (1.224 δ 420/mint./ μ g protein). In both the treatments, *Pseudomonas fluorescense* was used as seed dresser. Therefore, *Pseudomonas fluorescense* had a clear impact in enhancement of plant secondary metabolites like phenol, OD phenol, peroxidase and polyphenol oxidase level in mungbean plant. However, there was not much variation in the protein content among the different treatments. The protein content varied from 17.378 to 17.749 mg/g of fresh leaves. Treatments T₁, T₂ and T₃ were at par with each other. The protein content of the untreated check (17.400 mg/g of fresh leaves) was statistically at par with treatments T₄, T₅ and T₆.

Table.1 Details of the treatments

Treatment Number	Details of Treatment
T1	Seed dressing with Phosphate solubilising bacteria (PSB), <i>Rhizobium</i> (<i>Rh</i>) and <i>Pseudomonas fluorescense</i> (<i>Pf</i>) @ 5.0 g/kg of seed in 1: 1 ratio and one spray of <i>Beauveria bassiana</i> (<i>Bb</i>) followed by <i>Verticillium lecanii</i> (<i>VI</i>) @ 5.0 g/l of water
T2	Seed dressing with Phosphate solubilising bacteria (PSB), <i>Rhizobium</i> and <i>Azotobacter</i> (<i>Az</i>) @ 5.0 g/kg of seed in 1: 1 ratio and one spray of <i>Metarhizium anisopliae</i> (<i>Ma</i>) followed by <i>Verticillium lecanii</i> @ 5.0 g/l of water
T3	Seed dressing with Nitrogen fixer (<i>Nf</i>) @ 10ml/kg of seed and <i>Pseudomonas fluorescense</i> @ 5.0 g/kg of seed and one spray of <i>Beauveria bassiana</i> followed by <i>Verticillium lecanii</i> @ 5.0 g/l of water
T4	Seed dressing with Nitrogen fixer @ 10ml/kg of seed and <i>Azotobacter</i> @ 5.0 g/kg of seed and one spray of <i>Metarhizium anisopliae</i> followed by <i>Verticillium lecanii</i> @ 5.0 g/l of water
T5	Seed dressing with Phosphate solubilising bacteria (PSB) and <i>Rhizobium</i> @ 5.0 g/kg of seed in 1: 1 ratio and one spray of <i>Beauveria bassiana</i> followed by <i>Metarhizium anisopliae</i> @ 5.0 g/l of water
T6	Seed dressing with Nitrogen fixer @ 10ml/kg of seed and one spray of <i>Beauveria bassiana</i> followed by <i>Metarhizium anisopliae</i> @ 5.0 g/l of water
T7	Control Plot as Check where no seed dressing with bioinoculant was done and water was sprayed only

Table.2 Efficacy of bio-inoculum and bio-pesticides against pod borer complex

Treatment	No. of larvae/one metre row					% Control at 14 DAS	% Pod damage (average of two harvest)
	Pre-Spray at 40 th Day	3DAS	7 DAS	10 DAS	14 DAS		
T₁ (PSB + Rh + Pf + Bb + VI)	1.78 (1.51)*	1.08 (1.26)	0.73 (1.11)	0.56 (1.03)	0.62 (1.06)	74.44	7.82 (16.24)**
T₂ (PSB + Rh + Az + Ma + VI)	2.43 (1.71)	1.84 (1.53)	1.19 (1.30)	1.08 (1.26)	1.16 (1.29)	64.97	11.86 (20.14)
T₃ (Nf + Pf + Bb + VI)	1.88 (1.54)	1.20 (1.30)	0.66 (1.08)	0.63 (1.06)	0.64 (1.07)	75.02	7.03 (15.38)
T₄ (Nf+ Az + Ma + VI)	1.91 (1.55)	1.24 (1.32)	0.74 (1.11)	0.63 (1.06)	1.13 (1.28)	56.59	9.94 (18.38)
T₅ (PSB + Rh + Bb + Ma)	1.95 (1.57)	0.99 (1.22)	0.48 (0.99)	0.75 (1.12)	1.13 (1.28)	57.48	8.62 (17.07)
T₆ (Nf + Bb + Ma)	2.00 (1.58)	1.06 (1.25)	0.59 (1.04)	0.78 (1.13)	1.18 (1.30)	56.71	9.47 (17.92)
T₇ (Untreated Control)	3.50 (2.00)	3.65 (2.04)	3.92 (2.10)	4.24 (2.18)	4.77 (2.30)	-	21.58 (27.68)
CD at 5%	0.16	0.14	0.15	0.18	0.20		2.31
SEm (±)	0.07	0.06	0.06	0.08	0.09		0.74

*Figure in parentheses are square root transformed value, ** Figure in parentheses are angular transformed value, DAS : Days after spraying

Table.3 Biochemical parameters of mungbean leaf

Treatment	Total phenol (mg/gm. of fresh leaves)	OD phenol (mg/gm. of fresh leaves)	PPO (δ -495/mint./ μ g protein)	Peroxidase (δ 420/mint./ μ g protein)	Protein mg/gm. of fresh leaves
T ₁ (PSB + Rh + Pf + Bb + Vl)	2.472	1.012	0.726	1.258	17.697
T ₂ (PSB + Rh + Az + Ma + Vl)	2.027	0.933	0.574	1.076	17.672
T ₃ (Nf + Pf + Bb + Vl)	2.218	1.008	0.672	1.224	17.749
T ₄ (Nf + Az + Ma + Vl)	1.941	0.832	0.769	1.056	17.410
T ₅ (PSB + Rh + Bb + Ma)	1.526	0.812	0.436	0.593	17.378
T ₆ (Nf + Bb + Ma)	1.504	0.779	0.463	0.678	17.433
T ₇ (Untreated Control)	1.208	0.629	0.431	0.452	17.413
CD at 5%	0.125	0.021	0.067	0.055	0.076
SEm(\pm)	0.041	0.070	0.029	0.017	0.026

The correlation studies between infestation level of pod borers with biochemical parameters of mungbean leaf like total phenol, OD phenol, peroxidase, polyphenol oxidase and protein showed a significant negative relationship between the OD Phenol content with pod borer infestation having correlation coefficient 0.76 at 5% level of significance.

Therefore, higher OD phenol content in leaf of mungbean plant through seed bio inoculation had adversely affected the population build up of pod borers. The corresponding regression equation showed as $4.99 - 4.12x$, where x represents the number of pod borers in one meter row length. The coefficient of regression (R^2) was found as 0.67 which simply means that OD phenol contributed 67% variation in population build up of pod borers in mungbean crop. Similar findings were observed by many research workers. *Pseudomonas fluorescence* is capable of enhancing the level of phenolics and defense related enzyme activities in plants, thus

imparting induced resistance against *Helicoverpa armigera* (Jeyarani *et al.*, 2005; Rajendran *et al.*, 2007; Muthukumaran, 2016). Therefore, it can be concluded that *Pseudomonas fluorescence*, the most effective bio-inoculant may be used in pre-sowing seed priming of mungbean to enhance the secondary plant metabolites in the plant system for imparting resistance to some extent against the pod borer complex. Moreover, biopesticides like *Beauveria bassiana* and *Verticillium lecanii* may be applied at 15 days interval starting from 40 days of crop age to get effective control of pod borer complex in mungbean crop.

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