

Evaluation of Brigade-BL (*Beauveria bassiana*-Entomopathogenic Fungi) Against Leaf Folder/Roller (*Hedylepta indicata*) in Soybean (*Glycine max* (L.) Merrill)

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ABSTRACT

Soybean (*Glycine max* (L.) Merrill) is one of the most important oilseed legumes in the world as well as in India, which is attacked by several insects, particularly the leaf folder (*Hedylepta indicata*) causes significant yield losses. Hence, a field bio-efficacy of Brigade-BL (*Beauveria bassiana*) an entomopathogenic fungi was evaluated against leaf folder of soybean at ARI (MACS) Pune during kharif-2015 season. Results obtained indicated that Treatment No. T-3 (Two sprayings of Brigade BL @ 2.0 ml/L) treatment resulted in reduction of leaf folder/roller larvae/m upto 45-39% individually and 95-56% in combination with chemical insecticides. Similarly, spraying of Brigade-BL @ 2.0 ml/L resulted in increase of plant height (7.44%), leaf area (7.68%), Pods/plant (21.40%) harvest index (17.90%) and yield by 19.79% over control and 4.07% over chemical insecticides. Similarly, spraying of Brigade-BL did not show any phytotoxic effect on soybean plants.

Keywords- Soybean, *Glycine max*, liquid bio-insecticide, Brigade-BL, leaf roller/folder, *Hedylepta indicata*, *Beauveria bassiana*, phytotoxicity.

I. INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) now has been established as one of the most important oilseed crop in the world accounting for more than 50% of oilseeds produced and 30% of the total supply of oil vegetable oils. It is unique crop having both high quality protein (40%) and oil (20%) contents. It is also helps in improving soil fertility through nitrogen fixation. The protein from soybean is equivalent to that of meat, milk products and eggs in quality. Soybean is used in formation of low cost nutritionally balanced protein foods and drinks most essential for protein deficient countries. In the ancient times, soybean was used as a medicinal plant in China as a specific remedy for proper functioning of heart, liver, kidneys, and stomach (Kale, 1936). In USA, soybean was used in earlier days as a forage crop in

combination with corn. It is one of the most popular protein ingredients in the world in manufacturing of livestock feeds like soybean flakes, soybean beetles for feeding fish, soaps, varnishes, printing inks, paints, enamels, insecticides, leather, greases, wallboards etc. Soybean oil is used in the manufacture of food preparations like cooking oils, salad dressings, sandwiches, vegetable oils, pharmaceuticals, cosmetics etc. Bhatnagar (1986) has reviewed the potentials of soybean for industry and food uses and reported a comparison with other food sources for protein and nutritive value.

Soybean was once considered as a crop that had negligible losses due to insect infestation. But over a period of its development in the country, it has become one of the preferred hosts for most of the insect-pests and suffers from severe damage and yield losses. This is one of the major reasons for not realizing full yield potential. Chemical insect control methods have been used by most of the farmers till now. But now, the chemical insecticides are proving inadequate for successful management of major and minor insect pests like pod borer, tobacco caterpillar, leaf folder, Bihar hairy caterpillar and girdle beetle. Hence, it has to be borne in mind that no single approach would provide adequate insect control. Therefore, the Integrated Pest Management (IPM) strategy that provides a sustainable management of most of the damaging insect pest needs to be adopted. Chemical control is commonly practiced by farmers for getting more profits; but continuous utilization of chemical insecticides have created many problems. Only use of chemical control leads to problems of pest resistance, pesticide residue, destruction of beneficial fauna and environmental pollution. Under such circumstances, the use of biological insecticide/entomopathogenic fungi in pest management is found to be ecologically viable for solving above problems.

II. MATERIALS AND METHODS

A field experiment was conducted to study the effect of endophytic Brigade-BL (*Beauveria bassiana* 1.15% A.L.) along with chemical insecticides against prevailing insect pests (Leaf folder/leaf roller) of soybean. Hence, a study was conducted during 2015-16 Kharif season at Agharkar Research Institute's Research Farm, Hol, Taluka - Baramati, District Pune, Maharashtra State. The site of the experimental farm is situated at 18°14'N latitude, 74°21'E longitude and altitude of 548.6 m from mean sea level. Average rainfall is about 500 mm. However, during this season only 272.40 mm. rainfall was received during June to October. Soybean seeds were treated with Bavistin @ 2.5 g/kg seed and later on inoculated with *Bradyrhizobium japonicum* culture @ 5.0 g/kg seed. For general weed control pre-emergence spray of Pendimethalin @ 1.0 kg a.i. (active

Treatments Details:

Tr. No.	Treatments(Product)	Dose	Remarks
T ₁	PoP (2 sprays)	Quinalphos 3 ml/L	1st preventive at 42 DAS & 2nd Need base spray at 56 DAS
T ₂	Brigade-B (2 sprays)	1 ml/L	1st preventive at 42 DAS & 2nd Need base spray at 56 DAS
T ₃	Brigade-B (2 sprays)	2 ml/L	1st preventive at 42 DAS & 2nd Need base spray at 56 DAS
T ₄	PoP (1st) + Brigade-B (2nd)	Quinalphos 3 ml/L + 1 ml/L	1st preventive at 42 DAS & 2nd Need base spray at 56 DAS
T ₅	PoP (1st) + Brigade-B (2nd)	Quinalphos 3 ml/L + 2 ml/L	1st preventive at 42 DAS & 2nd Need base spray at 56 DAS
T ₆	Brigade-B (1st) + Chemical (2nd)	1 ml/L + Rynaxypyr 0.08 ml/L	1st preventive at 42 DAS & 2nd Need base spray at 56 DAS
T ₇	Brigade-B (1st) + Chemical (2nd)	2 ml/L + Rynaxypyr 0.08 ml/L	1st preventive at 42 DAS & 2nd Need base spray at 56 DAS
T ₈	Chemical (1st) + Brigade-B(2nd)	Rynaxypyr 0.08 ml/L + 1 ml ml/L	1st preventive at 42 DAS & 2nd Need base spray at 56 DAS
T ₉	Chemical (1st) + Brigade-B(2nd)	Rynaxypyr 0.08 ml/L + 2 ml/ L	1st preventive at 42 DAS & 2nd Need base spray at 56 DAS
T ₁₀	Control (No spray)	-	-

PoP : Package of Practice

Foliar two sprayings of insecticides/Brigade-BL were carried out as per the treatments given in Table-1. About 500 Litre/ha volume of water was used with Knapsack Sprayer using NMD nozzle.

Data were recorded on plant height leaf area, pods/plant were recorded on random 10 plants/plot from each replication. Data on number of leaf roller (folder) larvae per meter row length were recorded at 1 DBT, 5 DAT and 10 DAT at three places per plot. Visual Defoliation Score (VDS) was recorded at peak period of infestation in 1-9 scale. Biological yield was recorded on net plot basis and used for calculating the harvest index. Harvest index (%) was calculated as (seed yield/biological yield) x 100. Seed yield was recorded on net plot basis and converted into kg/ha. Data collected was statistically analysed by adopting standard procedure

(ingredient)/ha was used. The soil is medium black with 7.5 pH and all the plots were well drained. Meteorological data showed that minimum and maximum temperature ranged between 20.50°C to 22.80°C and 29.68°C to 32.96°C respectively, during the crop season. Rainfall received during the crop season was (272.40 mm) very less and unevenly distributed. Crop was irrigated with well/canal irrigation during water stress conditions due to which the crop growth was satisfactory. Similarly, incidence of insect pests was very low.

The experiment was laid out in a Randomised Block Design (RBD) with three replications. A promising soybean variety 'MACS-1188' was sown at 45 cm row spacing and 5-7 cm. plant to plant distance in 5m X 3.6m (eight rows) gross plot size. A basal dose of 20:80:20:30 NPKS kg/ha was applied at the time of sowing with soil application. The Treatment details are as below:-

(ANOVA) as suggested by Panse and Sukhatme (1985).

Brigade-BL formulation contains spores of *Beauveria bassiana* in a liquid formulation. It is a bio-insecticide for management of soil and foliar insect pest infestation of crops. The active principles in Brigade-BL (spores of *Beauveria bassiana*) germinate or stick to the insect. These spores germinate on the insect body, penetrate and destroy the tissues. The fungus proliferates in the body of the insect causing mycosis, so that the insect stop feeding resulting in insects death. Under moist conditions fluffs of fungus forms spores on the dead insect causing spread of the fungus and affect other insects. Similarly, it is residue free, non-toxic, no pre-harvest internal, organic certified and environmentally free and safe for foliar application.

III. RESULTS AND DISCUSSION

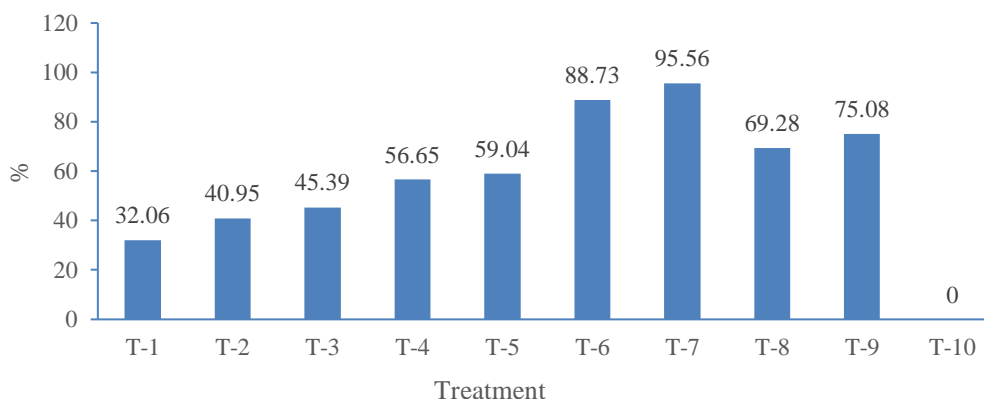
Data obtained on number of leaf folder/roller larvae/meter, visual defoliation score; plant height, leaf area, pods/plant, 100 seed weight, harvest index and seed yield are presented in Table-1 and figures 1. Significant findings are presented and discussed below:-

a) Leaf roller/folder/meter (*Hedylepta indicata*)

Data presented in Table-1 indicated that result obtained one days before treatment were non-significant in all the treatments. However, all the treatments showed significantly reduction in number of leaf roller/folder larvae/m over control (3.27). Similarly, T-7 treatment (Brigade-BL + Chemical) recorded minimum larvae/m (0.23 and 0.13) on 5 DAT and 10 DAT respectively followed by T-6 (Brigade BL @ 1.0 ml/L + Chemical @ 0.08 ml/L) with 0.60 and 0.33 larvae/m; T-9 (chemical @ 0.08 ml/L + Brigade-BL @ 2.0 ml/L) with 1.33 and 0.73

larvae/m; T-8 (Chemical @ 0.08 ml/L + Brigade-BL @ 1.0 ml/L) with 1.67 and 0.90 larvae/m, as compare to control (3.27 and 2.93 larvae/m). Likewise, Brigade-BL @ 2.0 ml/L (T-3) recorded 2.30 and 1.60 larvae/m at 5 DAT and 10 DAT respectively followed by Brigade-BL @ 1.0 ml/L (T-2) with 2.57 and 1.73 respectively as compared to control (3.57 and 2.93). Likewise, T-7, T-6, T-9 and T-8 recorded significantly reduction in number of larvae/m than PoP (T-1, Quinolphos @ 3.0 ml/L) treatment also. Similarly, T-7 treatment recorded 93.56% and 95.56% reduction in number of larvae/m on 5 DAT and 10 DAT respectively followed by T-6 (83.19% and 88.73%), T-9 (2.74% and 75.08%), T-8 (53.22% and 69.25%), T-5 (54.94% and 59.04%), T-4 (47.62% and 58.65%), T-3 (35.57% and 45.39%), T-2 (28.01% and 40.95%) and T-1 (40.34% and 32.06%) over control treatment.

% reduction of leaf folder 10-DAT

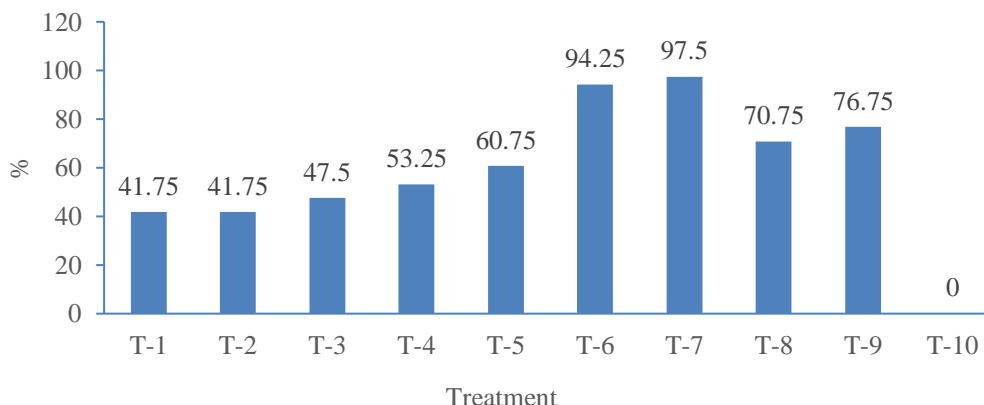


b) Visual Defoliation Score (VDS)

Visual defoliation score (VDS) was recorded at peak period of infestation in 1-9 scale. VDS ranged from 0.10 (T-7) to 4.0 (T-10, control plot). All the treatments recorded significantly less VDS than control treatment. Treatment T-7 (Brigade-BL @ 2.0 ml/L + Chemical) recorded least VDS (0.10) followed by T-6 (Brigade-BL

@ 1.0 ml/L + Chemical with 0.23, T-9 (0.93), T-8 (1.17), T-5 (1.57), T-4 (1.87), T-3 (2.10) and T-2 / T-1 with 2.33 as compared to control treatment (4.0). Similarly, T-7 recorded maximum reduction in defoliation score (97.50%), followed by T-6 (94.25%), T-9 (76.75%), T-8 (70.75%), T-5 (60.75%), T-4 (53.25%), T-3 (47.75%) and T-2 and T-1 (41.75%) over control treatment.

% reduction VDS



c) Plant height (cm)

Foliar sprays of chemical insecticides as well as bio-insecticide do not differ plant height significantly. Plant height ranged from 40.70 cm (T-10, control) to 46.97 cm (T-5 treatment). Among all the treatments T-5 (PoP + Brigade-BL @ 2.0 ml/L) treatment recorded maximum plant height (46.97 cm) followed by T-7 (46.73 cm), T-6 (46.67 cm), T-4 (46.57 cm) and T-1 (45.87 cm) treatment.

d) Leaf area (sq. cm.)

The differences for leaf area were non-significant for different doses of chemical insecticides as well as bio-insecticides (Brigade-BL) individually or in combination with each other. However, T-7 (Brigade-BL @ 2.0 ml/L + Chemical) gave maximum leaf area (37.07 cm) followed by T-1 (Quinolphos @ 3.0 ml/L) with 36.93 cm; T-5 (PoP + Brigade-BL @ 2.0 ml/L) with 36.23 cm.

e) Pods/Plant

No. of pods/plant influenced significantly due to application of Bio-insecticides individually or in combination with chemical insecticides. Significantly, maximum pods/plant (36.52) was recorded in T-5 (PoP + Brigade-BL @ 2.0 ml/L treatment followed by T-7 (36.13), T-9 (35.15), T-3 (34.77); T-8 (34.64) and T-4 (34.36) treatments which are on par with each other. T-5 treatment gave 27.51 more pods/plant over control treatment followed by T-7 (26.15%), T-9 (22.73%), T-3 (21.40%), T-8 (20.95%), and T-4 (19.97%) treatment.

f) 100 seed wt.

The differences for 100 seed weight were non-significant for chemical insecticides and Brigade-BL

individually or in combination with each other. However, T-3 (Brigade-BL @ 2.0 ml/L) gave higher 100 seed weight (14.70 g) followed by T-5 (14.6 g); T-2, T-6, T-7 and T-9 with 14.5 g and minimum with control treatment (14.2 g).

g) Harvest index (%)

The differences for harvest index (%) were significant due to application of different doses of chemical insecticides and bio-insecticides (Brigade-BL) and their combination as compare to control. However, T-9 (chemical + Brigade-BL @ 2.0 ml/L) gave significantly more harvest index % (61.06%) followed by T-5 (PoP + Brigade-BL @ 2.0 ml/L) with 61.02% and Brigade-BL @ 2.0 ml/L (T-3) with 60.39% over control treatment. Likewise, T-9 treatment gave 19.21% more harvest index than control treatment followed by T-5 (19.13%) and T-3 (17.90%) treatment which are on par with each other.

h) Seed yield (kg/ha)

All the treatments recorded significantly more seed yield than control treatment. Seed yield ranges from 2435 kg/ha (control) to 3048 kg/ha (T-7). The maximum seed yield (3048 kg/ha) was recorded from T-7 (Brigade-BL @ 2.0 ml/L + Chemical) treatment followed by T-9 (2961 kg/ha), T-5 (2952 kg/ha); T-3 (2917 kg/ha), T-4 (2874 kg/ha) and T-8 (2855 kg/ha) treatment. All the above treatments gave more yield than PoP (T-1) treatment. Similarly, T-7 treatment gave 25.17% more yield than control treatment followed by T-9 (21.60%), T-5 (21.23%), T-3 (19.79%), T-4 (18.02%), T-8 (17.25%) and T-6 (16.59%) treatment.

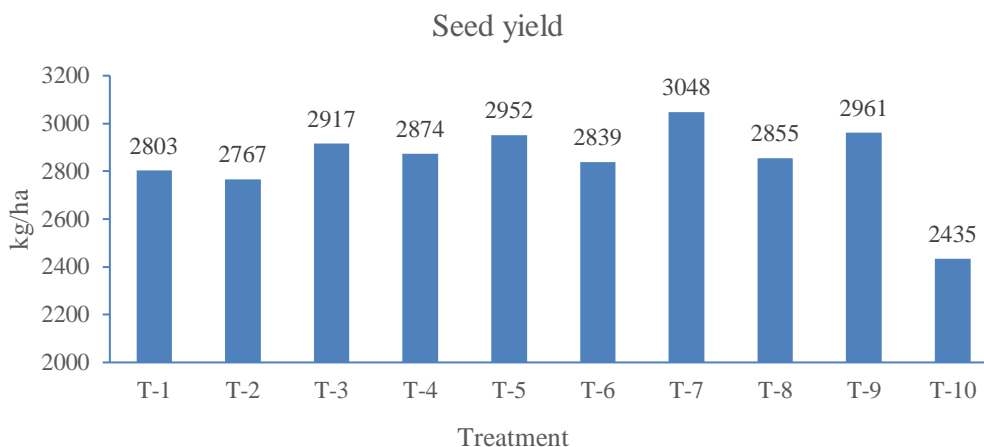


Table 1: Bio-efficacy of Brigade-BL against leaf roller larvae of soybean

Tr. No.	Treatments	Plant height (cm)	No. of leaf folder/roller larvae/meter			VDS (1-9 scale)	Leaf area sq.fm.	pods/plant	100 seed wt (g)	Harvest index (%)	Seed yield kg/ha
			1-DBT	5-DAT	10-DAT						
T-1	PoP (Two sprays of Quinalphos 3 ml/L)	45.97 12.70	3.73	2.13* 40.34	1.67* 32.06	2.33* 41.75	36.93 10.90	32.43 13.23	14.3 0.70	56.92 11.13	2803* 15.11
T-2	Brigade-BL @ 1.0 ml/L (2 sprays)	42.80 5.16	4.03	2.57* 28.01	1.73* 40.95	2.33* 41.75	35.45 6.46	33.57 17.21	14.5 2.11	57.54 12.34	2767* 13.63
T-3	Brigade-BL @ 2.0 ml/L (2-sprays)	43.73 7.44	3.63	2.30* 35.57	1.60* 45.39	2.10* 47.50	35.86 7.68	34.77* 21.40	14.7 3.52	60.39* 17.90	2917* 19.79

T-4	PoP (1st spray) + Brigade-BL 2nd @ 1.0 ml/L	46.57 14.42	3.47	1.87* 47.62	1.27* 56.65	1.87* 53.25	35.19 5.68	34.36* 19.97	14.4 1.41	58.41 14.03	2874* 18.02
T-5	PoP (1st spray) + Brigade-BL @ 2.0 ml/L(2nd)	46.97 15.41	4.00	1.63* 54.34	1.20* 59.04	1.57* 60.75	36.23 8.79	36.52* 27.51	14.6 2.82	61.02* 19.13	2952* 21.23
T-6	Brigade-BL @ 1.0 ml/L (1st) + Chemical (2nd)	46.67 14.67	4.23	0.60** 83.19	0.33** 88.73	0.23** 94.25	35.49 6.58	33.26 16.13	14.5 2.11	57.03 11.34	2839* 16.59
T-7	Brigade-BL @ 2.0 ml/L (1st) + Chemical (2nd)	46.73 14.82	3.67	0.23** 93.56	0.13** 95.56	0.10** 97.50	37.07 11.32	36.13* 26.15	14.5 2.11	57.80 12.85	3048* 25.17
T-8	Chemical (1st) + Brigade-BL @ 1.0 ml/L (2nd)	41.47 1.89	3.53	1.67* 53.22	0.90** 69.28	1.17** 70.75	33.59 0.87	34.64* 20.95	14.3 0.70	57.60 12.46	2855* 17.25
T-9	Chemical (1st) + Brigade-BL @ 2.0 ml/L (2nd)	41.67 2.29	4.00	1.33* 62.74	0.73** 75.08	0.93** 76.75	33.99 2.07	35.15* 22.73	14.5 2.11	61.06* 19.21	2961* 21.60
T-10	Control (No spray)	40.70	3.77	3.57	2.93	4.00	33.30	28.64	14.2	51.22	2435
	C.D.at 5%	NS	NS	0.86	0.72	1.09	NS	5.26	NS	8.64	251

(%) increase/decrease over control, * Significantly superior over control

i) Phytotoxicity

Data presented below indicated that among the tested treatments of Brigade-BL, none of the treatments

showed phytotoxicity when the observations were recorded at 1, 3, 5, 7 and 10 days after treatment.

Treatments	Chlorosis	Necrosis	Wilting	Scorching	Hyponasty	Epinasty
T-1	0	0	0	0	0	0
T-2	0	0	0	0	0	0
T-3	0	0	0	0	0	0
T-4	0	0	0	0	0	0
T-5	0	0	0	0	0	0
T-6	0	0	0	0	0	0
T-7	0	0	0	0	0	0
T-8	0	0	0	0	0	0
T-9	0	0	0	0	0	0
T-10	0	0	0	0	0	0

In the present study, Brigade-BL formulation containing *Beauveria bassiana* resulted in reduction of leaf folder/roller larvae/m upto 45.39% individually and 95.56% in combination with chemical insecticides. Similarly, two sprayings of Quinolphos resulted only 32.06% reduction of leaf roller larvae/m. as compared to control treatment. Similarly, spraying of Brigade-BL @ 2.0 ml/L resulted in increase of plant height (7.44%), leaf area, (7.68%), pods/plant (21.40%), harvest index (17.90%) and yield by 19.79% over control and 4.07% over Quinolphos (chemical insecticides)

For sustainability in agriculture has resulted in numerous government initiatives to develop environmentally friendly agricultural practices. During

2003, the Canadian Government initiated the Pesticide Risk Reduction Programme to provide infrastructure for the development and implementation of reduced risk approaches for managing pests in crops (Anonymous, 2003). Similar programs were also initiated in USA (Jones, 2004) and UK (Departmental Report 2005) sought to reduce environmental risk with older chemical insecticides by replacing them with low risk alternatives. These programs make the assumption that natural insecticides present less risk to the environment than synthetic insecticides (James, 1990) and influential scientific papers purporting greater sustainability of organic practice (Regonold et. al. 2001). Many studies have compared organic, conventional and integrated pest

management (IPM) production systems as a whole, but the conclusions reached in these studies are widely divergent. However, Suckling et. al. (1999) in New Zealand apple production suggested an integrated approach was more sustainable. But a study made during 2001 by Reganold et.al (2001) of the same system in Washington favored an organic management approach.

The caterpillars of leaf folder (*Hedylepta indicata*) damage the crop either by folding the leaves or webbing together many leaves skeletonise them completely. Leaf folders can cause up to 9.3% yield loss in soybean crop in the Meghalaya state of northeast India (Azad Thakur, 1985). Recently, eco-friendly approaches based on bio-pesticides are gaining popularity for pest management programs. Moreover, exploitation of systemic pesticides for seed treatment is believed to be a less hazardous to non-target fauna than foliar spraying and soil application. Entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* are reported as potential bio-agent against a variety of insects, especially under humid climate (Thakur and Sandhu 2010; Sandhu et. al. 2012). Neem based pesticides are also reported to be effective against several insects, including soybean defoliators (Biswas and Islam, 2012). Similarly, Firke et. al. (2018) indicated that the soybean seeds may be soon during mid July and the soybean crop could be sprayed alternatively with neem seed kernel extracts and *Beauveria bassiana* during flowering to suppress the damage caused by major pests of soybean. Foliar spray of Imidacloprid may be given only if the stem fly damage exceeds economic threshold level (Firke et. al., 2018).

Recently, Kanitkar et. al. (2019) studied the efficacy of Brigade-BL (*Beauveria bassiana*) and other insecticides against thrips, fruit borer and mites on chilli crops. Their results indicated that three spraying of Brigade-BL @ 5.0 ml/L significantly control thrips (57.96%), fruit borer (65.09%) and mites (30.47%) and recorded 22.38% more green chilli yield over control treatment. Similarly, Kanitkar et. al. (2020) studied the field bio-efficacy of Brigade-BL for the management of mealy bugs on Thomson Seedless grapes. Their results indicated that application of Brigade-BL @ 5.0 ml/L with two to five sprayings resulted in reduction of mealy-bug colonies up to 67.82% in foundation pruning and 75.68% in fruiting pruning and increase in grape yield of 92.44% over control. The present findings are in confirmative with the earlier reports of Brigade-BL.

Mirhaghparast et. al. (2013) studied the effect of *Beauveria bassiana* on cellular immunity intermediary metabolism of fruit borer (*Spodoptera littoralis*). Their results clearly revealed different effects of *B.bassiana* on cellular immunity and phenoloxidase activity in the fifth larval instars of *S.littoralis*. These differences were due to surface properties of spores and their capability in production of secondary metabolites, which are toxic on hemolytic and other tissues. Moreover, the changes of enzymatic activity involved in intermediary metabolism of fungal spores and secondary metabolites on chemical composition of insect hemolymph (Sujak et. al., 1978;

Shitosuku and Kato, 1996). Hence, it could be concluded that pathogenicity of an entomopathogenic fungi may occur via overcoming on immune responses and discrepancies of intermediary metabolism. These findings show significant role of these agents against agricultural pests and might be used to improve their quality and efficiency in future.

Entomopathogenic fungi, particularly *Beauveria bassiana* is an attractive bio-pesticide for use in integrated pest management, as they have host specificity with proven safety (Bateman et. al. 1993). The entomopathogenic fungi are the promising agents that are used against pests for several decades. These organisms include taxa of several fungal groups like *Beauveria bassiana* and *Metarhizium anisopliae* are the two most recognized species (Vincent et. al. 2007). *B.bassiana* and *M.anisopliae* grow naturally through the world and acts as parasites of many arthropod species causing white and green muscardine diseases due to the color of their spores (Vincent, 2007). Besides entomopathogenic fungi caused natural mortality on insects, these agents are environmentally safe, so there is a worldwide interest of their using and improvement for biological control of insects.

IV. CONCLUSION

The present investigation of Bio-efficacy of Brigade-BL and other chemical insecticides against leaf folder/roller (*Hedylepta indicata*) on soybean was carried out during Kharif 2015 season. The results obtained indicated that T-3 (Brigade-BL @ 2.0 ml/L) treatment resulted in reduction of leaf folder larvae/m up to 45.39% individually and 95.56% in combination with chemical insecticides. Similarly spraying of Brigade-BL @ 2.0 ml/L resulted in increase of plant height, leaf area, pods/plant, harvest index and yield by 19.79% over control and 4.07% over chemical insecticide.

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