Bio efficacy of microbial formulations of *Beauveria bassiana* and *Sl*NPV against *Spodoptera litura* F. and *Aphis craccivora* Koch. in vegetable cowpea

Abstract: The study/experiment was conducted in the Department of Agricultural Entomology, College of Agriculture, Vellayani during kharif 2022 with an objective to test the bio efficacy of liquid and bair formulations of *Beauveria bassiana* Bb 6063 and *Sl*NPV against two most damaging pests of vegetable cowpea *Spodoptera litura* F. and *Aphis craccivora* Koch. Emulsifiable suspension (ES) formulation (comprising of Bb 6063 conidia and *Sl*NPV @1.78 x 10^{10} Conidia mL^{-1} and 5.24 x 10^{8} POB mL^{-1} respectively, in 95% sesamum oil and 5% Span- 20 + Triton-X-100 (29:71)) and bait formulation (consisting of *Sl*NPV @4.13 x 10^{7} POB mL^{-1} in, 75% wheat flour + 25% chickpea flour base matrix) developed in the current study, was evaluated under field conditions. NPV bait when applied alone and in combination with ES formulation, recorded low population of *S. litura* (1.33, 1.00 larvae $plot^{-1}$ respectively, after two rounds of application), though inferior to chemical check Flubendiamide 39.35 SC (0 larvae $plot^{-1}$). However, the leaf damage intensity score in treatments with Flubendiamide (1.53), ES formulation applied along with NPV bait (1.73) and NPV bait alone (1.57) at 10 days after two rounds of treatments were statistically on par with each other and significantly lower than rest of the treatments. Treatments with ES formulation alone and in combination with NPV bait application recorded lowest *Aphis craccivora* population also (0.67, 0.33 aphids $plot^{-1}$) as against 9.33 aphids $plot^{-1}$ in untreated control. Thus, ES formulation of Bb 6063 and *Sl*NPV as well as *Sl*NPV bait can be employed in the management of *S. litura*, wherein the former treatment is effective in controlling *A. craccivora* as well.

Keywords: Bioformulation, entomopathogen, *Sl*NPV, *Beauveria bassiana*, *Spodoptera litura*, *Aphis craccivora*, Cowpea

Introduction

Spodoptera litura, is a highly polyphagous pest of ~~I~~international importance. It is known to cause extensive leaf damage on several crops like groundnut, maize, sorghum, banana and is a major pest affecting economic parts of cotton, tobacco and many vegetables (Noma *et* al., 2010; CABI., 2022). Aphids are major sucking pests of cowpea. Apart from sucking sap and debilitating the plant, they act as vectors of devastating viral diseases. To tackle this menace, farmers often resort to multiple spray regimes of different insecticides within a cropping season. The frequency and dose of application often increases due to the development of pesticide resistance (Armes *et al.*, 1999) and accounts to contamination of food and environment through residues, necessitating the need for alternate ecofriendly management strategies.

Microbial control is an excellent option to contain pesticide resistant populations of *S. litura*. Entomopathogenic fungi (EPF) and Nuclear polyhedrosis Virus (NPV), are well documented entomopathogens (Kaur and Padmaja, 2008; Anand and Tiwary, 2009; Ravishankar and Venkatesha, 2010). However, their large-scale adoption is lagging behind, due to slow speed of kill and lack of efficient formulation technology.

Liquid formulations of EPF, either oil or water based help to reduce spray volume and runoff, ensuring efficient target impact besides easiness in handling and storage. Late instar larvae of *S. litura* exhibit a behavioral peculiarity of hiding in soil and crawling back to crop canopy during night. Since bait formulations lure the hiding larvae, pesticide infused food are of often used (Shahanaz, 2018). Attractive food baits loaded with infective propagules of entomopathogens could act as a congenial delivery system. Bait formulations of NPV increase the effectiveness by increasing ingestion of the virus (Bell and Kanavel, 1975).

The present study investigates the effect of bioformulations of EPF and NPV infecting *S. litura* and *A. craccivora* populations and explores possibilities of using both as an alternative to separate chemical application to control them.

Material and methods

Bioformulation of entomopathogenic microbes *viz*., *Beauveria bassiana* Bb 6063 and *Sl*NPV were used in this study. Mother culture of Bb 6063 was procured from Department of Agricultural Entomology, College of Agriculture, Vellayani, Kerala Agricultural University, and was maintained in potato dextrose broth/ agar (PDB/A). Fungal conidia were obtained by liquid fermentation in PDB and subsequent filtration and pelleting by centrifugation. *Sl*NPV cultures were procured from Banana Research Station- Kannara, Kerala Agricultural University and maintained in *S. litura* larvae. The polyhedral occlusion bodies (POB) from diseased cadaver were extracted by differential centrifugation. The dose mortality response of entomopathogens were ascertained in a separate laboratory bio assays and subsequent probit analysis, which is beyond the scope of this paper. The effective doses of pathogens thus obtained were formulated into a liquid and bait formulation and its efficacy was evaluated against *S. litura*.

Field experiment was conducted at the Instructional ~~f~~Farm, College of Agriculture, Vellayani, to evaluate the efficacy of microbial formulations *viz.*, Emulsifiable Suspension (ES) formulation of Bb 6063 + *Sl*NPV and Wheat flour- Chickpea flour (WF- CH) based bait formulation of *Sl*NPV developed. Beds of 90 x 90 cm were raised in three blocks, separated by channels in the rice fallows. Ten-day old seedlings of cowpea variety *Kanakamoni* were transplanted onto these beds at a spacing of 30 x 30 cm. All agronomic practices except plant protection activities were followed as per recommendations in Package of Practices, Kerala Agricultural University.

Design: RBD

No. of treatments: 8

No. of replications: 3

The following treatments were applied:

T1: Aqueous suspension (AS) of NPV

T2: AS of EPF

T3: AS of microbial mixture (LC_{50} of Bb 6063 and *Sl*NPV)

T4: Liquid formulation

T5: Bait formulation

T6: Liquid + bait formulation

T7: Chemical check Flubendiamide 39.35% SC (72 g ai ha^{-1})

T8: Untreated control

- AS of NPV: *Sl*NPV @ 4.13 x 10^7 POB mL^{-1}
- AS EPF: Bb 6063 @ 3.05 x 10^8 Conidia mL^{-1}
- AS of microbial mixture: Bb 6063 @1.78 x 10^8 Conidia mL^{-1} + *Sl*NPV @ 5.24 x 10^6 POB mL^{-1}).
- **Liquid formulation:** 1% emulsion of ES formulation of Bb 6063 and *Sl*NPV (comprising of Bb 6063 conidia and *Sl*NPV @1.78 x 10^{10} Conidia mL^{-1} and 5.24 x 10^8 POB mL^{-1} respectively, in 95% sesamum oil and 5% Span- 20 + Triton-X-100 (29:71))

- **Bait formulation:** Rehydrated bait formulation of *Sl*NPV @4.13 x 10^7 POB mL^{-1} in, 75% wheat flour + 25% Chickpea flour base matrix
- **Liquid + Bait formulation:** 1% ES of Bb6063 and *Sl*NPV + rehydrated bait formulation

Emulsion treatments were applied using a high-volume pneumatic sprayer onto the plants, completely covering the foliage to the point of run-off. Bait formulation was applied after rehydration by placing it in soil @ 10 g $plot^{-1}$ placed in 5 bait stations. Two rounds of application were done at 35 and 65 days after planting (DAP). Observations of pest and natural enemy population, leaf area damage of crop were recorded before treatment as well as at 3, 5, 7 and 10 days after each round of treatment. *S. litura* larvae in each plot was counted by observing the foliage as well as soil and expressed as numbers per plot. Leaf area damage caused by *S. litura* feeding, was scored visually as described below (Plate 1)

List 1 : Leaf area damage percentage scale

Score	Leaf area damage (%)
1	0-5
2	6-15
3	16-25
4	26-50
5	> 50

Leaf area damage of ten leaves selected at random from two random plants per plot was scored one day prior to treatment application and at 3, 5, 7 and 10 DAT and the mean leaf area damage intensity score was worked out using the formula

Damage intensity score (DIS): ∑ (Leaf damage score x No. of leaves in that score)

Total No. of leaves

Population of *A. craccivora* was recorded from two tagged plants per plot one day prior to treatment and at fixed intervals as in 3.5.1 and expressed as numbers per plant. Population of different natural enemies (Plate 2) per plot (*Coccinellid* spp. and spiders) were recorded at different intervals after treatment and expressed as numbers per plot. Biometric evaluation of two random plants per plot were recorded at 15 days after each spraying. Observation on plant height and number of leaves per plant were observed. Pods collected from each plot was weighed at harvest and yield expressed as t ha^{-1}.

Results and Discussion

Lowest population of *S. litura* was recorded from Flubendiamide 39.35 % SC and was superior to all other treatments on all days in both treatment schedules (Table 1). After first treatment, population decreased from 10.67 (pre-treatment) to 1.00 (10 DAT) and after second treatment, it got reduced from 6.67 to 0. Plots that received treatments liquid + bait and bait formulation alone, were identical to each other in terms of their efficacy in controlling *S. litura*. At 10 days after first treatment, Flubendiamide 39.35 % SC recorded significantly low population (1.00 larvae $plot^{-1}$), followed by Liquid + bait formulation (3.00 larvae $plot^{-1}$), bait formulation (3.33 larvae $plot^{-1}$) and were on par with each other. Similar trend was observed 10 days after second treatment also. Flubendiamide 39.35 % SC recorded no larvae and was significantly superior to other treatments, followed by 1.00, 1.33 larvae $plot^{-1}$ in liquid + bait formulation and bait formulation treated plots, respectively. Although they performed less than chemical check, they were superior to other treatments in this study.

S. litura is a nocturnal defoliator with a unique character of hiding below crop stand in the daytime. They find relief among soil clods, stubbles and weeds, away from sight and sunlight. These hiding larvae become active by nightfall and march back to crop and devour plant parts. Baits utilize this behavioral trait. Encounter with food sources more attractive than natural diet urges the marching larvae to have a nibble from it, enroute to the crop. However, these baits are embedded with occlusion bodies of *Sl*NPV that immediately targets the gut membranes, debilitating host within 2-3 days. These enfeebled larvae attract healthy counterparts towards them as the NPV multiplying inside their bodies makes them palatable, inviting cannibalism.

Combined application of liquid formulation and bait although statistically register on par with bait formulation alone, there is a slight decrease in larvae spotted across days in combined treatments than bait alone. Combined application of liquid and bait formulation delivers more POB per unit area, thereby ensuring more ingested POB. However, POB may be inactivated by sunlight and effect of formulation in field persistence of POB's needs to be explored.

The mean leaf damage intensity score (DIS) (Table 2) remained minimum in Flubendiamide treated plots during both treatment schedules. At 10 days after first treatment, significantly low DIS was recorded in Flubendiamide 39.35 % SC (1.40), bait formulation (1.53) and liquid + bait formulation (1.60) treated plots and were statistically on par with each other. Mean leaf damage intensity score recorded in plots treated with Flubendiamide 39.35 % SC at 10 days after second treatment was statistically lowest (1.53) among all treatments and was followed by DIS in bait formulation (1.57) and liquid + bait (1.73). Leaf damage computed in untreated control and AS EPF treatment were the highest on all days of observation.

Heliothis virescens NPV in cottonseed oil bait was equally effective as standard insecticide treatment in controlling tobacco budworm *Helithis virescens* (F.) in cotton (Andrews *et al.*, 1975). Average leaf damage due to *S. litura* feeding on mung bean plots sprayed with *Sl*NPV suspension (1.96x 10^9 POB mL^{-1}) was 55% while it was 45% in plots treated with chlorpyriphos plus monocrotophos (0.3 kg ai ha^{-1} and 0.36 kg ai ha^{-1}) (Krishnaiah *et al*., 1985). Single application of *Sl*NPV at 250 LE ha^{-1} was equally effective as two applications of chlorpyriphos (200 g ai ha^{-1}) in controlling *S. litura* on groundnut (Dhandapani, *et al*., 1993), and pod yield was significantly higher than untreated check and was on par with chemical check in NPV treated replications. Rao and Krishnayya (1996) reported synergistic effect in baits prepared with half recommended dose of *Bt* and diflubenzuron, against *S. litura*. This formulation fared well than its unformulated aqueous suspension of NPV, both when applied at same dose of 50 LE ha^{-1}. NPV of diamond back moth, micro encapsulated with gelatin and sodium alginate caused 70% mortality to target insect after 3 days exposure to sunlight, while the non-formulated NPV could cause less than 40% mortality (Samaneh and Marzban, 2016).

S. litura is notoriously famous for evolving insecticide resistance. Larval conditioning to chemical presence on leaves is an initial indication to resistance development. This conditioning is reflected in the increased leaf damage after second spray. However, this conditioning did not translate to a resistance, which was indicated by the population reduction. This could also be due to escape of a single or few larvae to nearby weeds immediately after spraying and resumed feeding on cowpea after moulting to 4^{th} or 5^{th} instar. Justification apart, Flubendiamide is an ideal candidate for management of *S. litura*, under low population levels of infestation. It being a green label chemical and with a unique mode of action, makes it safer to natural enemies and not target organisms as well. Flubendiamide is well documented for its efficacy against *S.litura* on cowpea (Kumar and Sarada (2015)., Venkataiah *et al.*, (2015)., Reddy, *et al*., (2018)., Reddy and Paul (2019)., Himanshu and Srivastava (2020)).

Both liquid formulation and AS EPF + NPV had the same microbial load in them. However liquid formulation was superior in its effect to *S. litura*. At 10 DAT of first spray, mean population of *S. litura* in liquid formulation applied plots were 4.67 while in the latter it was 6.00. Similarly, after second spray, a population of 3.00 and 4.33 respectively were recorded from both the treatments. Although the field dose of microbes delivered remained same, liquid formulation retained the viability and preserved virulence of microbes far better than their unformulated counterparts.

S. litura population and corresponding mean damage intensity score (DIS) in plots treated with aqueous suspension of EPF were comparable to that observed from untreated control. In the laboratory screening studies, Bb6063, although pathogenic, was not virulent enough to cause complete mortality. Maximum mortality recorded under controlled conditions were below 40 per cent. The low virulence combined with environmental inactivation of conidia under field conditions have attributed to low efficacy in population control and corresponding increase in leaf damage.

Significantly low aphid populations was observed in treatments liquid formulation and liquid + bait formulation (1.67 aphids plant^{-1}, each), followed by Flubendiamide 39.35 % SC (5.00 aphids plant^{-1}) and AS EPF+ NPV (6.67 aphids plant^{-1}), and were on par with each other, at 10 days after first treatment. Similar trend was observed after second treatment as well. *B. bassiana* is a generalist pathogen and highly effective against homopterans. Aerial conidia of *B. bassiana* is easily dispensed in oil as it is hydrophobic. A protective layer of oil around conidia ensures it is evenly spread over treated surface and enhance UV tolerance. During emulsion formation, the larger droplets are broken down to micro/ nano emulsified oil droplets. These oil droplets spread over exposed insect cuticle, exerting a physical poison effect. Simons, *et al.,* (1977) documented detrimental effects of emulsified oils sprayed on leaf surface, on the feeding and virus transmission ability of aphid *Myzus persicae* (Sulzer). The pre- probing time was increased significantly leading to reduced ingestion of sap on oil coated leaves than untreated leaves. Nithya and Rani (2019) developed a chitin enriched groundnut oil based formulation of *Lecanicillium lecanii* Zare and Gams which was equally effective in controlling *A. craccivora* as chlorpyriphos 0.05%. the formulation offered 98.93% and 96.74% reduction at 5 and 50% level of infestation respectively.

All treatments tested in this study had no overall impact on natural enemies *viz.*, spiders and coccinellids (table 4 and 5). At 5 DAT after first spraying, Flubendiamide 39.35 % SC treated plots recorded lowest spider population, followed by liquid and liquid + bait treated plots, however this reduction in population was short- lived, and soon sprung back to non- significant levels among treatments. Wettable powder formulation of *B.bassiana* was found safe to spiders and coccinellids in on comparison to chlorantraniliprole, rice ecosystem (Bajya and Ranjith, 2018). Oil formulations of *B. bassiana* were found to be safe to coccinellid predator, *Cryptolaemus montrouzieri* Mulsant (Prithiva *et al*., 2018). When formulated at 10^8 spores ml^{-1} an adult survival of 92.62 % was ensured, while at lower doses 100% adult survival was observed.

Biometric and yield observations are compiled in table 6. Although maximum yield of 3.74 t ha^{-1} was recorded from Flubendiamide 39.35 % SC treated plots, overall difference among treatments were statistically insignificant. Field populations of pests observed over the duration of the experiment were not damaging enough to cause any detrimental effect on the yield. Pre-treatment count of *S. litura* before first spray averaged around 10 and that before second spray, 6~~six~~. Moreover, their population was affected by different treatments before attaining damaging levels. Severe impact on yield is caused either when complete defoliation occurs or when *S. litura* targets economic part *ie.*, cowpea pods. Such damaging population was not documented during this study. However, the population reduction effect of liquid + bait and bait formulation on *S. litura* can be successfully employed wherein the population is epidemic and highly devastating. In such a scenario definite impact of yield could also be noted. Yield of cowpea obtained from groundnut oil-based formulations of *L. lecanii* was second only to the chemical applied plots (Nithya, 2015). However, the bioformulations were very safe to natural enemies and not target organisms in cowpea ecosystem.

Conclusion

Thus, ES formulation of Bb 6063 and *Sl*NPV (comprising of Bb 6063 conidia and *Sl*NPV @1.78 x 10^{10} Conidia mL^{-1} and 5.24 x 10^{8} POB mL^{-1} respectively, in 95% sesamum oil and 5% Span- 20 + Triton-X-100 (29:71)) as well as *Sl*NPV bait (consisting of *Sl*NPV @4.13 x 10^{7} POB mL^{-1} in, 75% wheat flour + 25% Chickpea flour base matrix) developed in the current study can be employed in the management of *S. litura*, wherein the former treatment is effective in controlling *A. craccivora* as well.

References

Anand R and Tiwary BN. Pathogenicity of entomopathogenic fungi to eggs and larvae of *Sodoptera litura*, the common cut worm. *Biocontrol Sci. Technol.* 2009; 19(9): 919-929.

Andrews GL, Harris FA, Sikorowski PP, and McLaughlin RE. Evaluation of *Heliothis* Nuclear Polyhedrosis Virus in a Cottonseed Oil Bait for Control of *Heliothis virescens* and *H. zea* on Cotton. *J. Econ. Entomol*, 1975; 68(1): 87–90. Available: https://doi.org/10.1093/jee/68.1.87

Armes NJ, Wightman, JA, Jadhav DR and Rao GVR. Status of insecticide resistance in *Spodoptera litura* in Andhra Pradesh, India. *Pesticide Sci*. 1995; 50(3): 240-248.

Bajya D R and Ranjith M. Field efficacy of Beauveria bassiana against rice leaf folder and its safety to spiders and coccinellids. *Indian J. Entomol.* 2018; 80(1): 68-72.

Bell MR and Kanavel RF. Potential of bait formulations to increase effectiveness of nuclear polyhedrosis virus against the pink bollworm. *J. Econ. Entomol.* 1975;68(3): 389-391.

CABI (Centre for Agriculture and Biosciences International). Datasheet report for *Spodoptera litura* F. (Taro Caterpillar). CABI Crop Protection Compendium. 2014 Available online: https://www.cabidigitallibrary.org/doi/10.1079/cabicompendium.44520

Himanshu T and Srivastava RP. Persistent toxicity of spinosyn and diamide against *Spodoptera litura* (F.) on cowpea and soybean. *Indian J. Entomol.* 2020; 82(1): 183-188.

Kaur G and Padmaja V. Evaluation of *Beauveria bassiana* isolates for virulence against *Spodoptera litura* (Fab.) (Lepidopetra: Noctuidae) and their characterization by RAPD- PCR. *Afr.J. Microbiol. Res.* 2008;2(11): 299-307.

Krishnaiah K, Ramakrishnan N, and Reddy PC. Control of *Spodoptera litura* Fabr. on black gram by nuclear polyhedrosis virus. *Indian J. Agrl. Sci.* 1985; 55 (12): 775- 776.

Kumar GS and Sarada O. Field efficacy and economics of some new insecticide molecules against lepidopteran caterpillars in chickpea. *Curr. Biotica*. 2015; 9(2):153-158.

Formatted: Font: (Default) Times New Roman, 12 pt, Not Italic, English (India)

Nithya PR. Improved Formulation of *Lecanicillium lecanii* (Zimmermann) Zare and Gams and its evaluation against sucking pests. M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 2015; 201p.

Nithya PR and Rani OP. Enriched bioformulations of *Lecanicillium lecanii* (Zimmermann) Zare and Gams against sucking pests of yard long bean, *Vigna ungiculata* L. Walp *sesquipedalis*. *J. Entomol. Res*. 2019; 43(4): 445-450.

Noma T, Colunga-Garcia M, Brewer M, Landis J and Gooch A. Oriental leafworm, *Spodoptera litura*. Michigan State University's invasive species fact sheets.2010 Available online: www.canr.msu.edu/ipm/uploads/files/Forecasting_invasion_risks/orientalLeafworm.pdf

Prithiva JN, Ganapathy N, Jeyarani S, and Ramaraju K. Relative safety of *Beauveria bassiana* (Bb 112) oil formulation to *Cryptolaemus montrouzieri* Mulsant. *J Biol Cont*. 2018; 32(3): 212-214.

Rao MB, Krishnayya PV. Effect of diflubenzuron and *Bacillus thuringiensis* var. *kurstaki* baits on the growth and development of *Spodoptera litura* (Fab.) larvae. *Pesticide Res. J.* 1996; 8(1): 80-83.

Formatted: Font: (Default) Times New Roman, 12 pt, Not Italic, English (India)

Ravishankar BS and Venkatesha MG. Effectiveness of SlNPV of *Spodoptera litura* (Fab) (Lepidoptera: Noctuidae) on different host plants. *J. Biopesticides.* 2010*;* 31(1): 168-171.

Formatted: Line spacing: single

Reddy BKK, Hampiah J and Padmanabhan A. Evaluation of Insecticide Mixtures Against Larval Population of Leaf Eating Caterpillar, *Spodoptera Litura* in Cowpea. *Intl. J. Agric.Sci.* 2018*;*10(12): 0975-3710.

Reddy BKK and Paul A. Field efficacy of insecticide mixtures against the pod borer and leaf eating caterpillar in cowpea. *J. Pharmacognosy and Phytochemistry*. 2019; 8(5): 1224-1227.

Samaneh N. and Marzban R. Stability of *Bacillus thuringiensis* and NPV microencapsulated formulation under sunlight . *Intl. J. Adv. Biotechnol. Res*. 2016; 7(3): 2224–2230.

Shahanaz MR. Insecticide based bait formulation against tobacco caterpillar *Spodoptera litura* F. M.Sc. (Ag) thesis, Kerala Agricultural University, Thrissur, 2018; 97p.

Formatted: Line spacing: single

Simons JN, McLean DL and Kinsey MG. Effects of Mineral Oil on Probing Behavior and Transmission of Stylet-borne Viruses by *Myzus persicae*. *J. Econ. Entomol*, 1977; 70(3): 309–315, https://doi.org/10.1093/jee/70.3.309

Venkataiah M, Kumar AB, and Chauhan S. Efficacy of newer insecticides against *Spodoptera litura* in groundnut (*Arachis hypogaea* L). *J. oilseeds Res,* 2015; 32(2): 152-154.

Table 1: Mean population of *Spodoptera litura* in cowpea plots treated with different microbial formulations

*Treatment	**Mean population plot^{-1}									
	First treatment					Second treatment				
	Pre-treatment	3DAT	5 DAT	7 DAT	10 DAT	Pre-treatment	3DAT	5 DAT	7 DAT	10 DAT
AS NPV	9.33 (3.05)	9.67 (3.1)	9.00 (3)^{a}	5.67 (2.38)^{bcd}	5.33 (2.41)^{bc}	6 (2.44)	5.33 (2.31)^{a}	5.33 (2.31)^{ab}	5 (2.35)^{a}	4.33 (2.2)^{ab}
AS EPF	9.67 (3.11)	9.00 (3)	9.33 (3.05)^{a}	7.33 (2.71)^{ab}	7.33 (2.8)^{b}	6 (2.44)	5.67 (2.37)^{a}	5.33 (2.3)^{abc}	5.33 (2.39)^{a}	5.33 (2.39)^{a}
AS EPF + NPV	9.67 (3.1)	9.00 (2.99)	8.00 (2.83)^{a}	6.33 (2.51)^{bc}	6.00 (2.54)^{bc}	7 (2.64)	6 (2.44)^{a}	5.33 (2.31)^{ab}	4.67 (2.27)^{ab}	4.33 (2.19)^{ab}
Liquid formulation	10.00 (3.16)	9.33 (3.05)	7.67 (2.77)^{a}	5.00 (2.23)^{cde}	4.67 (2.27)^{cd}	6.667 (2.58)	5.67 (2.38)^{a}	4.67 (2.16)^{bc}	3.00 (1.87)^{bc}	3.00 (1.86)^{bc}
Bait formulation	10.67 (3.26)	9.67 (3.1)	6.00 (2.44)^{b}	4.33 (2.08)^{de}	3.33 (1.95)^{d}	6.333 (2.51)	5.67 (2.38)^{a}	4.33 (2.08)^{bc}	2.33 (1.68)^{c}	1.33 (1.34)^{cd}
Liquid + bait formulation	10.00 (3.16)	9.00 (2.97)	5.00 (2.23)^{b}	3.67 (1.91)^{e}	3.00 (1.87)^{d}	7 (2.64)	6.33 (2.51)^{a}	4.00 (1.99)^{c}	2.33 (1.64)^{c}	1 (1.17)^{de}
Flubendiamide 39.35 % SC (72 g ai ha^{-1})	10.67 (3.26)	6.33 (2.51)	3.67 (1.9)^{c}	2.33 (1.52)^{f}	1.00 (1.1)^{e}	6.667 (2.58)	3.33 (1.79)^{b}	1.33 (1.14)^{d}	0.33 (0.88)^{d}	0 (0.71)^{e}
Untreated control	10.00 (3.16)	11.00 (3.31)	9.33 (3.05)^{a}	9.33 (3.05)^{a}	10.67 (3.34)^{a}	6.333 (2.52)	6.67 (2.58)^{a}	6.33 (2.52)^{a}	6 (2.55)^{a}	5.33 (2.41)^{a}
SE (m)	0.269	0.387	0.443	0.458	0.601	0.181	0.262	0.318	0.412	0.442
CD 0.05%	NS	NS	0.312	0.353	0.412	NS	0.433	0.315	0.444	0.517

* AS: Aqueous suspension.AS NPV: 4.13 x 10^{7} POB mL^{-1}; AS EPF: 3.05 x 10^{8} Conidia mL^{-1} ; AS EPF+NPV: 1.78 x 10^{8} Conidia mL^{-1} + 5.24 x 10^{6} POB mL^{-1}

Liquid formulation: 1% emulsion of Sesamum oil ES with Bb 6063 (1.78 x 10^{10} Conidia mL^{-1}) + *Sl*NPV (5.24 x 10^{8} POB mL^{-1})

Bait formulation: 4.13 x 10^{7} POB mL^{-1} *Sl*NPV in WF-CH base matrix (10 g plot^{-1})

** Mean of 3 replication (Values in parenthesis are square root transformed) DAT: Days after treatment

Table 2: Mean leaf damage intensity score of *Spodoptera litura* in cowpea plots treated with different microbial formulations

*Treatment	** Mean leaf damage intensity score plot^{-1}									
	First treatment					Second treatment				
	Pre-treatment	3DAT	5 DAT	7 DAT	10 DAT	Pre-treatment	3DAT	5 DAT	7 DAT	10 DAT
AS NPV	1.4 (1.18)	1.83 (1.35)[a]	2.10 (1.45)[bc]	2.10 (1.45)[d]	2.53 (1.59)[c]	1.10 (1.05)	1.37 (1.17)[ab]	1.50 (1.22)[bc]	1.63 (1.28)[b]	1.87 (1.37)[b]
AS EPF	1.43 (1.2)	1.60 (1.26)[b]	2.27 (1.51)[ab]	2.67 (1.63)[b]	2.87 (1.69)[b]	1.20 (1.1)	1.37 (1.17)[ab]	1.57 (1.25)[ab]	2.17 (1.47)[a]	2.37 (1.54)[a]
AS EPF + NPV	1.50 (1.22)	1.70 (1.3)[ab]	2.10 (1.45)[bc]	2.27 (1.51)[c]	1.93 (1.39)[d]	1.03 (1.02)	1.23 (1.11)[bc]	1.40 (1.18)[bcd]	1.73 (1.32)[b]	1.83 (1.35)[b]
Liquid formulation	1.53 (1.24)	1.87 (1.37)[a]	2.00 (1.41)[c]	1.77 (1.33)[e]	1.93 (1.39)[d]	1.17 (1.08)	1.47 (1.21)[a]	1.47 (1.21)[bc]	1.67 (1.29)[b]	1.77 (1.33)[bc]
Bait formulation	1.4 (1.18)	1.57 (1.25)[b]	1.57 (1.25)[d]	1.53 (1.24)[f]	1.53 (1.24)[e]	1.07 (1.03)	1.23 (1.11)[bc]	1.33 (1.16)[cd]	1.53 (1.24)[b]	1.57 (1.25)[cd]
Liquid + bait formulation	1.53 (1.24)	1.83 (1.35)[a]	2.00 (1.41)[c]	1.63 (1.28)[ef]	1.60 (1.26)[e]	1.00 (1)	1.40 (1.18)[a]	1.27 (1.13)[d]	1.53 (1.24)[b]	1.73 (1.32)[bcd]
Flubendiamide 39.35 % SC (72 g ai ha^{-1})	1.47 (1.21)	1.33 (1.15)[c]	1.33 (1.15)[e]	1.40 1.18)[g]	1.40 (1.18)[e]	1.17 (1.08)	1.10 (1.05)[c]	1.37 (1.17)[cd]	1.3 (1.14)[c]	1.53 (1.24)[d]
Untreated control	1.4 (1.18)	1.60 (1.26)[b]	2.37 (1.54)[a]	3.40 (1.84)[a]	3.5 0(1.87)[a]	1.20 (1.1)	1.47 (1.21)[a]	1.77 (1.33)[a]	2.07 (1.44)[a]	2.4 (1.55)[a]
SE (m)	0.025	0.041	0.072	0.132	0.146	0.021	0.029	0.036	0.06	0.068
CD 0.05%	NS	0.08	0.077	0.052	0.09	NS	0.066	0.08	0.09	0.081

* AS: Aqueous suspension.AS NPV: 4.13 x 10^7 POB mL^{-1}; AS EPF: 3.05 x 10^8 Conidia mL^{-1} ; AS EPF+NPV: 1.78 x 10^8 Conidia mL^{-1} + 5.24 x 10^6 POB mL^{-1}

Liquid formulation: 1% emulsion of Sesamum oil ES with Bb 6063 (1.78 x 10^{10} Conidia mL^{-1}) + *Sl*NPV (5.24 x 10^8 POB mL^{-1})

Bait formulation: 4.13 x 10^7 POB mL^{-1} *Sl*NPV in WF-CH base matrix (10 g plot^{-1})

** Mean of 3 replication (Values in parenthesis are square root transformed) DAT: Days after treatment

Table 3: Mean population of *Aphis craccivora* in cowpea plots treated with different microbial formulations

*Treatment	**Mean population plant^{-1}									
	First treatment					Second treatment				
	Pre treatment	3DAT	5 DAT	7 DAT	10 DAT	Pre treatment	3DAT	5 DAT	7 DAT	10 DAT
AS NPV	13.33 (3.67)	10.00 (3.09)	13.33 (3.6)^{abc}	15.00 (3.9)^{ab}	20.00 (4.51)^{a}	5 (2.23)	5.67 (2.38)^{ab}	6.67 (2.58)^{a}	7.67 (2.77)^{a}	8.67 (3.03)^{a}
AS EPF	15.00 (3.9)	11.67 (3.4)	10.00 (3.16)^{bcd}	8.33 (2.94)^{bc}	7.33 (2.78)^{b}	4.33 (2.08)	4.00 (1.99)^{bcd}	4.00 (1.99)^{b}	2.33 (1.52)^{b}	2.33 (1.68)^{b}
AS EPF + NPV	13.33 (3.67)	10.00 (3.09)	8.33 (2.85)^{cd}	6.33 (2.61)^{c}	6.67 (2.64)^{b}	4.33 (2.08)	3.33 (1.82)^{cd}	3.00 (1.73)^{b}	2.33 (1.52)^{b}	2.00 (1.58)^{b}
Liquid formulation	11.67 (3.37)	8.33 (2.85)	6.67 (2.55)^{d}	5.00 (2.35)^{c}	1.67 (1.25)^{c}	4.67 (2.14)	3 (1.72)^{cd}	2.00 (1.41)^{c}	1.00 (1)^{c}	0.67 (1.05)^{c}
Bait formulation	15.00 (3.9)	16.67 (4.04)	15.00 (3.84)^{ab}	16.67 (4.13)^{a}	19.00 (4.41)^{a}	5.33 (2.3)	5.67 (2.37)^{ab}	7.00 (2.63)^{a}	7.67 (2.76)^{a}	9.00 (3.08)^{a}
Liquid + bait formulation	13.33 (3.23)	9.00 (2.95)	7.33 (2.68)^{d}	5.00 (2.35)^{c}	1.67 (1.25)^{c}	5 (2.23)	2.67 (1.63)^{d}	2.00 (1.41)^{c}	1.33 (1.14)^{c}	0.33 (0.88)^{c}
Flubendiamide 39.35 % SC (72 g ai ha^{-1})	11.67 (3.37)	10.00 (3.09)	8.33 (2.85)^{cd}	6.67 (2.4)^{c}	5.00 (2.35)^{b}	5 (2.23)	4.33 (2.08)^{abc}	4.00 (2)^{b}	3.33 (1.82)^{b}	2.67 (1.77)^{b}
Untreated control	11.67 (3.47)	15.00 (3.84)	16.67 (4.07)^{a}	18.33 (4.31)^{a}	23.33 (4.86)^{a}	4.67 (2.16)	6.00 (2.44)^{a}	6.33 (2.52)^{a}	8 (2.83)^{a}	9.33 (3.13)^{a}
SE (m)	1.234	0.962	0.949	1.253	1.806	0.18	0.299	0.425	0.608	0.773
CD 0.05%	NS	NS	0.822	1.017	1.029	NS	0.414	0.31	0.342	0.329

* AS: Aqueous suspension.AS NPV: 4.13 x 10^{7} POB mL^{-1}; AS EPF: 3.05 x 10^{8} Conidia mL^{-1}; AS EPF+NPV: 1.78 x 10^{8} Conidia mL^{-1} + 5.24 x 10^{6} POB mL^{-1}

Liquid formulation: 1% emulsion of Sesamum oil ES with Bb 6063 (1.78 x 10^{10} Conidia mL^{-1}) + *Sl*NPV (5.24 x 10^{8} POB mL^{-1})

Bait formulation: 4.13 x 10^{7} POB mL^{-1} *Sl*NPV in WF-CH base matrix (10 g plot^{-1})

** Mean of 3 replication (Values in parenthesis are square root transformed) DAT: Days after treatment

Table 4: Mean population of *Coccinella* spp. in cowpea plots treated with different microbial formulations

*Treatment	**Mean population plot^{-1}									
	First treatment					Second treatment				
	Pre treatment	3DAT	5 DAT	7 DAT	10 DAT	Pre treatment	3DAT	5 DAT	7 DAT	10 DAT
AS NPV	1.33 (1.34)	1.67 (1.46)	1 (1)	1 (1.17)	1 (1.23)	1.33 (1.14)	1.33 (1.34)	1 (1.23)	0.67 (1.05)	0.67 (1.05)
AS EPF	1 (1.23)	1.33 (1.34)	1.67 (1.28)	1.33 (1.34)	1 (1.23)	1 (1)	1 (1.23)	0.67 (1.05)	1 (1.23)	0.67 (1.05)
AS EPF + NPV	1.33 (1.34)	1.33 (1.34)	1.33 (1.14)	1 (1.23)	1 (1.17)	1.33 (1.14)	1 (1.23)	1.33 (1.34)	1.67 (1.46)	1.33 (1.34)
Liquid formulation	1.33 (1.27)	1.67 (1.46)	1.33 (1.14)	1.33 (1.34)	1.33 (1.34)	1.33 (1.14)	1 (1.23)	1.33 (1.34)	1.33 (1.34)	1 (1.23)
Bait formulation	1.33 (1.34)	1.33 (1.29)	2 (1.38)	1 (1.23)	1 (1.23)	1.33 (1.14)	1.67 (1.46)	1.33 (1.34)	1 (1.23)	1 (1.23)
Liquid + bait formulation	1 (1.23)	1 (1.23)	1.67 (1.28)	1.33 (1.34)	1 (1.23)	1 (1)	1 (1.23)	0.67 (1.05)	1 (1.23)	1 (1.23)
Flubendiamide 39.35 % SC (72 g ai ha^{-1})	1.33 (1.34)	0.33 (0.88)	1.33 (1.14)	1 (1.23)	1.33 (1.34)	1.33 (1.14)	0.67 (1.05)	0.33 (0.88)	1 (1.23)	1 (1.23)
Untreated control	1.33 (1.34)	1.67 (1.46)	1.33 (1.14)	1.67 (1.46)	1.67 (1.46)	1.33 (1.14)	1.67 (1.46)	1.33 (1.34)	1.33 (1.34)	1.67 (1.46)
SE (m)	0.124	0.141	0.12	0.104	0.098	0.09	0.098	0.12	0.092	0.095
CD 0.05%	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

* AS: Aqueous suspension.AS NPV: 4.13 x 10^{7} POB mL^{-1}; AS EPF: 3.05 x 10^{8} Conidia mL^{-1} ; AS EPF+NPV: 1.78 x 10^{8} Conidia mL^{-1} + 5.24 x 10^{6} POB mL^{-1}

Liquid formulation: 1% emulsion of Sesamum oil ES with Bb 6063 (1.78 x 10^{10} Conidia mL^{-1}) + *Sl*NPV (5.24 x 10^{8} POB mL^{-1})

Bait formulation: 4.13 x 10^{7} POB mL^{-1} *Sl*NPV in WF-CH base matrix (10 g plot^{-1})

** Mean of 3 replication (Values in parenthesis are square root transformed) DAT: Days after treatment

Table 5: Mean population of spiders in cowpea plots treated with different microbial formulations

*Treatment	**Mean population plot^{-1}									
	First treatment					Second treatment				
	Pre treatment	3DAT	5 DAT	7 DAT	10 DAT	Pre treatment	3DAT	5 DAT	7 DAT	10 DAT
AS NPV	1.33 (1.34)	1.33 (1.34)	2.00 (1.56)^{ab}	2.00 (1.38)	2.00 (1.41)	1.67 (1.28)	1.67 (1.46)	1.33 (1.34)	2 (1.38)	2 (1.38)
AS EPF	1.00 (1.23)	1.33 (1.34)	1.33 (1.34)^{ab}	1.67 (1.28)	2.33 (1.52)	1.67 (1.24)	2 (1.58)	1.67 (1.46)	1.33 (1.14)	1.67 (1.28)
AS EPF + NPV	1.00 (1.17)	1.33 (1.34)	1.33 (1.34)^{ab}	1.33 (1.14)	1.67 (1.28)	1.33 (1.14)	2 (1.56)	1.67 (1.39)	2 (1.38)	2 (1.41)
Liquid formulation	1.33 (1.34)	0.67 (1.05)	1.00 (1.23)^{bc}	1.33 (1.14)	1.67 (1.28)	1.67 (1.28)	1.67 (1.46)	1.33 (1.34)	1.33 (1.14)	1.67 (1.28)
Bait formulation	1.67 (1.46)	1.67 (1.46)	1.67 (1.46)^{ab}	2.00 (1.41)	2.33 (1.52)	1.67 (1.28)	1.67 (1.46)	1.67 (1.46)	2 (1.41)	2.33 (1.52)
Liquid + bait formulation	1.33 (1.34)	0.67 (1.05)	1.00 (1.23)^{bc}	1.67 (1.28)	1.67 (1.28)	2 (1.38)	2 (1.58)	1 (1.23)	1.67 (1.28)	2 (1.38)
Flubendiamide 39.35 % SC (72 g ai ha^{-1})	1.00 (1.23)	0.33 (0.88)	0.33 (0.88)^{c}	1.33 (1.14)	1.33 (1.14)	1.67 (1.28)	0.67 (1.05)	1.33 (1.34)	1.67 (1.28)	1.67 (1.28)
Untreated control	1.33 (1.34)	1.67 (1.46)	2.00 (1.58)^{a}	2.00 (1.41)	2.33 (1.52)	2 (1.38)	2.33 (1.68)	2 (1.58)	2 (1.41)	2.33 (1.52)
SE (m)	0.109	0.139	0.143	0.115	0.119	0.141	0.138	0.135	0.124	0.127
CD 0.05%	NS	NS	0.349	NS	NS	NS	NS	NS	NS	NS

* AS: Aqueous suspension.AS NPV: 4.13 x 10^7 POB mL^{-1}; AS EPF: 3.05 x 10^8 Conidia mL^{-1} ; AS EPF+NPV: 1.78 x 10^8 Conidia mL^{-1} + 5.24 x 10^6 POB mL^{-1}

Liquid formulation: 1% emulsion of Sesamum oil ES with Bb 6063 (1.78 x 10^{10} Conidia mL^{-1}) + *Sl*NPV (5.24 x 10^8 POB mL^{-1})

Bait formulation: 4.13 x 10^7 POB mL^{-1} *Sl*NPV in WF-CH base matrix (10 g plot^{-1})

** Mean of 3 replication (Values in parenthesis are square root transformed) DAT: Days after treatment

Table 6: Biometric observations of cowpea plants that received treatments with different microbial formulations

*Treatment	50 DAP		80 DAP		Yield (t ha^{-1})
	Plant height (cm)	No. of leaves	Plant height (cm)	No. of leaves	
AS NPV	78.33 (8.84)	11.50 (3.39)	121.33 (11.02)	21 (4.58)	2.61 (1.61)
AS EPF	77.17 (8.77)	10.00 (3.16)	116.33 (10.79)	17 (4.12)	2.52 (1.59)
AS EPF + NPV	76.83 (8.75)	11.00 (3.31)	116.33 (10.79)	18 (4.24)	2.63 (1.61)
Liquid formulation	76.67 (8.74)	13.00 (3.59)	117.17 (10.82)	17.5 (4.18)	2.78 (1.65)
Bait formulation	80.17 (8.94)	13.50 (3.66)	115.5 (10.75)	17 (4.11)	3.15 (1.77)
Liquid + bait formulation	83.83 (9.13)	14.50 (3.76)	120 (10.95)	20 (4.47)	3.15 (1.76)
Flubendiamide 39.35 % SC (72 g ai ha^{-1})	79.83 (8.91)	13.00 (3.58)	112.5 (10.61)	17.5 (4.17)	3.74 (1.92)
Untreated control	83.83 (9.13)	16.00 (3.98)	116 (10.77)	19.5 (4.41)	2.41 (1.52)
SE (m)	1.649	0.526	0.729	0.455	0.17
CD 0.05%	NS	NS	NS	NS	NS

* AS: Aqueous suspension.AS NPV: 4.13 x 10^7 POB mL^{-1}; AS EPF: 3.05 x 10^8 Conidia mL^{-1} ; AS EPF+NPV: 1.78 x 10^8 Conidia mL^{-1} + 5.24 x 10^6 POB mL^{-1}

Liquid formulation: 1% emulsion of Sesamum oil ES with Bb 6063 (1.78 x 10^{10} Conidia mL^{-1}) + *Sl*NPV (5.24 x 10^8 POB mL^{-1})

Bait formulation: 4.13 x 10^7 POB mL^{-1} *Sl*NPV in WF-CH base matrix (10 g plot^{-1})

** Mean of 3 replication (Values in parenthesis are square root transformed) DAT: Days after treatment

Plate 1: Leaf damage scoring

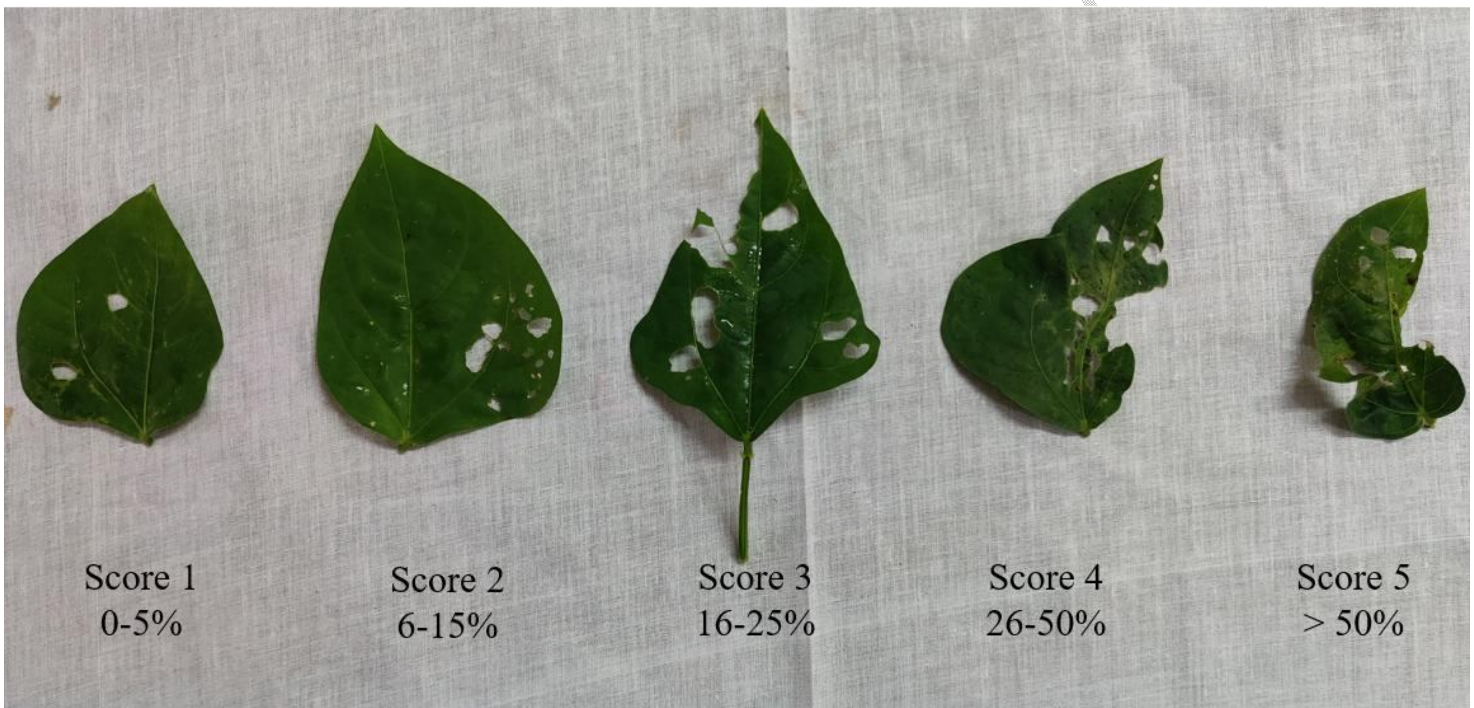

Plate 2: Pests and natural enemies observed

1. Leaf damage due to *S. litura*

2. Bait feeding

3. Cadaver exhibiting *Sl*NPV infection

1. Grubs and adults of *Coccinella* spp.

2. Spider