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Effects of microbial biopesticides against non-target insect pest

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ABSTRACT

Microbial biopesticides are considered as an eco-friendly strategy for pest management in organic and integrated production systems. However, questions have been raised on non-target effect of microbial bioagents on natural enemies. The Present study, hence addresses the gap in our understanding on the effect of bioformulations on natural enemies as parasites and predators. Microbial biopesticides developed at AAU, Jorhat with bioagents viz., Trichoderma viride, T. harzianum, Pseudomonas fluorescens, Beauveria bassiana, Metarhizium anisopliae, Lecanicillium lecanii, Bacillus thuringiensis were tested in both field and pot conditions at the prescribed concentrations against non-target insect pest. In the field experiment of tomato crop, the coccinellid count after 1st and 2nd sprays of different biopesticides showed no statistically significant difference as compared to the control at 3, 7 and 10 days after spraying. Similarly, in the pot experiment of okra crop, it was observed that there was no statistically significant difference in coccinellid beetles between the control pot and the treated pots, both after 1st and 2nd spray of biopesticides at 3, 7 and 10 days after spraying. In both cases, the population of coccinellids, the pests' natural adversaries, was unaffected negatively by the biopesticides.

Keywords: Beneficial microbes, biopesticides, eco-friendly, non-target insects

INTRODUCTION

With the growing awareness about health and environmental conditions, farming by organic means, without using any harmful agrochemicalsis gaining popularity and momentum across the world and growing as a fasteff ective source of income generation in mostly rural areas (Mie *et al.*, 2017). There is a high demand for organic food inboth domestic and international markets which is growing about (20-25) % annually; as a result, the area under organic farming has been increasing consistently (Das, 2020, Bora *et al.*, 2024). Bio pesticides are gaining popularity due to their eco-friendly nature and accuracy in managing pests and diseases of vegetable crops (Riddick *et al.,* 2009; Bora *et al.*, 2015; Nath *et al.*, 2016; Bora and Bora, 2020; Bora and Rahman, 2022).Thesebiopesticides are derived from natural resources and are thus organic means of pestmanagement that can be an important and useful component of integrated pest anddisease management strategy. Most entomopathogenic fungi have wider rangesof hosts (Melnick *et al.,* 2009; Bhattacharyya *et al.*, 2023; Borkakati *et al.*, 2023), and utilization of microorganisms of such type tomanage pests and insects may involve lots of risk factors towards the growth anddevelopment of predatory insects that are of much importance in the biologicalcontrolofpests (Bhattacharyya *et al.*, 2024).Therefore, before their application in the farmer's field, their effecton the natural enemies needs to be known, so research is of utmost importance (Deka *et al.*, 2021). Natural enemies, including predators, are crucial for controlling pest populations in agriculture and natural ecosystems. By preying they help maintain ecological balance, reduce crop damage, and minimize the need for chemical pesticides, thus promoting sustainable pest management practices. Their targeted approach to pest suppression and ability to adapt to changing pest dynamics make them invaluable assets in integrated pest management strategies (Borkakati *et al.*, 2018). Natural enemies, coccinellids play a significant role in controlling pests in both tomato and okra crops. They are voracious predators of aphids, scale insects, and other soft-bodied pests that can infest these plants. By feeding on these pests, coccinellids help to maintain the health and productivity of

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tomato and okra plants, reducing the need for chemical pesticides and promoting sustainable pest management practices (Ghose, 2022; Ambethgar *et al.*, 2024). With this knowledge and back ground, our present study was conducted to evaluate the bioefficacy of certain biopesticides developed by Assam Agricultural University, Jorhat against the non-target, beneficial natural enemies found in a natural ecosystem of tomato and Okra crops. *Beauveriabassiana* was reported safe to natural enemies and found to be harmless against beneficial soilinsects whereas *Metarhizium anisopliae* showed 10% and 4% mortality rates when usedagainst beneficial insects (Thungrabeab, 2006; Bhattacharyya *et al.*, 2022). Amichot *et al.* (2016) reported the biosafety of *Bacillus thuringiensis* bio-formulations on the emerging *Trichogramma chilonis* and confirmed that *Bacillus* issafeagainst*Trichogramma*. Agale *et al.* (2019) conducted a detailed study on the effect of selected biopesticides on natural enemies in the pigeon-pea (*Cajanuscajan*L.) crop and the field experiment revealed that all the selected bio pesticides treatments were found to be safer for the natural enemies which even helped in enhancing the activity of natural enemies' populationin thepigeon-peacropfield.

MATERIALS AND METHODS

Source of biopesticides

Several biopesticides, including Bioveer, Biozium, Biomonas, Biobt, Biosona, Biometa, Biollium, Biotime, and Biogreen-5 developed by the Biocontrol Laboratory, Department of Plant Pathology under DBT- North East Centre for Agricultural Biotechnology (DBT-NECAB), Assam Agricultural University, Jorhat was utilized for the present study.

Effect of bio pesticide formulations on non-target insects under field conditions

Field evaluation was done regarding the effect of the biopesticide formulations against the beneficial insect population. For that, a particular plot in the Experimental Farm of the Department of Horticulture, AAU, Jorhat. The experimentwas laid out in field conditions endorsing randomized block design (RBD), with threereplications. Thenetarea for the experiment was 200 sq.m (20m x 10m). Then etarea was divided into 3 blocks. Each block was further divided into 10 equal plots measuring 4 sq.m (2 m x 2 m) each, respectively. There were altogether 30 plots. Interspacing between blocks and plots were 1m and 0.5m, respectively. For the field experiment, tomato crop (variety: Pusaruby) was selected and cultivated with standard horticultural practices. Spacing was maintained at 75x30cm. First irrigation was provided immediately after transplanting and subsequent irrigations at an interval of (10-15) days depending on the soil and weather conditions. Garden hoe was used in the field at 20 and 40 DAP for weeding. Coccinellid beetles are present in a greater amount and their efficacy wastested against the respective biopesticides.

The biopesticide formulations were sprayed in recommended doses asmentioned in Table 1. The number of natural enemies was recorded after each treatment was given. Out of the sixteen plants present in a single plot, seven plants were selected for counting the beneficial insects,coccinellid beetles, and thedata wasrecorded at 0, 3, 7, and 10 days of treatment (Kumar *et al.,* 2020), and the 2nd spraywas given 15 days after the 1st spray for each of the 30 plots of tomato plants presentinthefield.

Table1: Bio-pesticidesusedtostudy their effects against non-target insect pest

Bio-pesticides	Bio-agents present
Biosona@1%	*Beauveriabassiana*
Biollium@ 1%	*Lecanicilliumlecanii*
Biometa@ 1%	*Metarhiziumanisopliae*
Bioveer@1%	*Trichodermaviride*
Biogreen–5 @ 1%	*Trichodermaviride* and plant growth promoting microorganisms
Biozium@ 1%	*Trichodermaharzianum*
Biobt@ 1%	*Bacillusthuringiensis*
Biomonas@ 1%	*Pseudomonasfluorescens*
Biotime@ 1%	*Metarhizium anisopliae, Pseudomon asfluoresecensand Trichodermaharzianum*
Control (only water)	N/A

Effect of biopesticides on non-target insects under Potcondition

For the pot condition, a total of 30 pots were taken for the research work and the pots were filled with the potting mixture. The okra seeds were planted in the filled pots and enough irrigation was provided so the plants couldflourish well. The pots were seeded in March and the seedlings gave good vegetative growth in April 2022**.** A total of 3 replication sconsisting of 10 treatments perreplication were done. For the pot experiment, okra crop (variety: Bhindi S-51) was planted with standard horticultural practices. First irrigation was done after sowing the seeds in the potsand subsequent irrigations were given based on the requirement of the plant andconsideringthesoiland weather conditions. Only coccinellid beetles were maintained in acontrolled environment inside a net house and net chambers were installed foraphidand beetlerearing. Aphids were reared so that the coccinellids could feed on them and they were captured with in some insect-proof net chambers that were prepared specially for each of the 30 pots, respectively (Fig 1). An individual net chamber having a dimension of (1.5mx1m) was used for each pot and aphid's werer eared there to provide proper food for the beneficial non target insects, coccine llidbeetles. The nine biopesticide formulations inrecommended doses as mentioned in Table 1 were sprayed twice at 15 days intervals to counttheir effect on the beneficial insects, coccinellid beetles, and the data was recorded at 0, 3, 7 and 10 days of treatment.

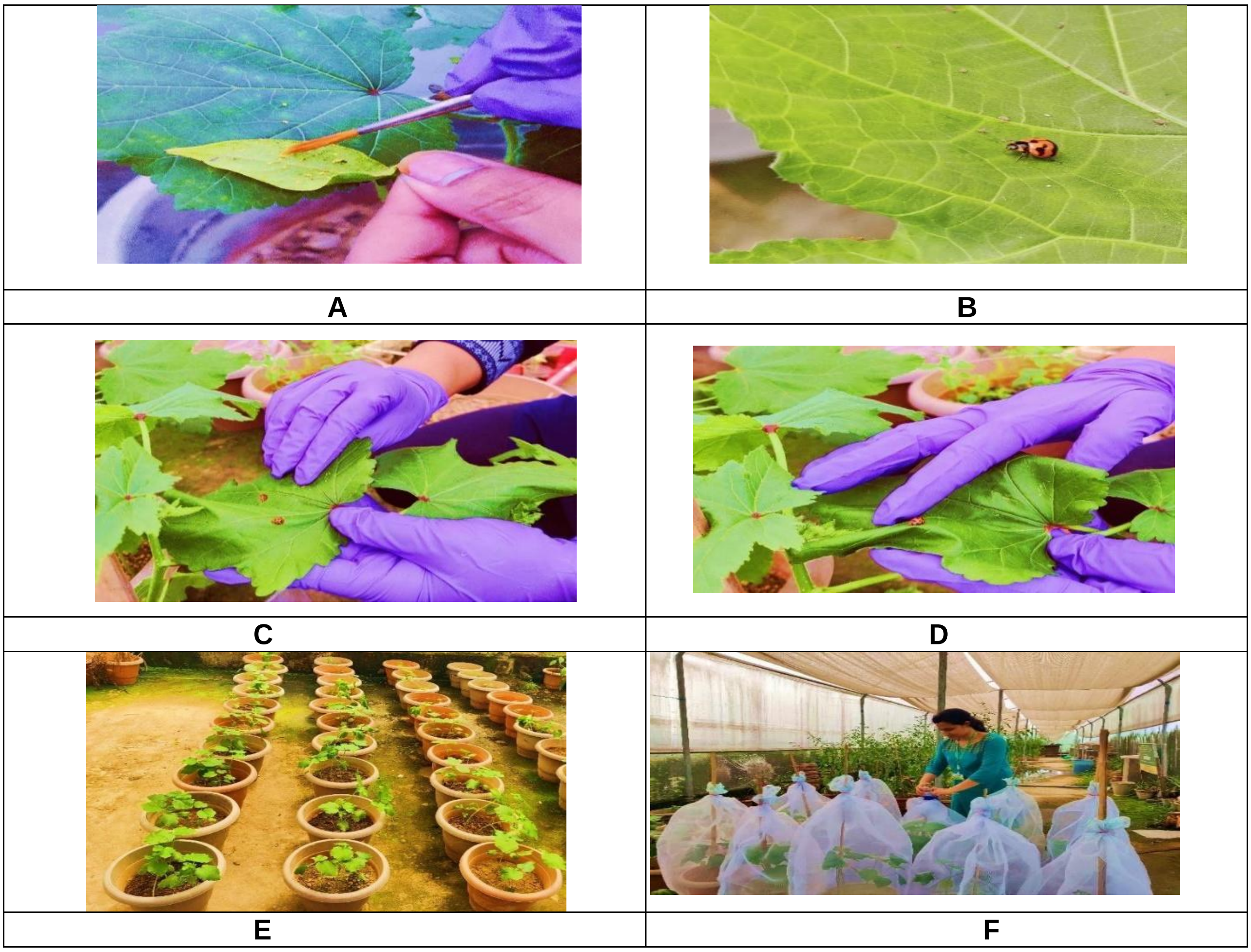

Fig. 1(A-D):Collection and rearing of a phids and coccinellids (*Coccinellatransversalis*)

A) Artificialreleaseofaphidsintheokraleaves
(B) and (C) Coccinellids collected from the external environment were reared
(D) Releasing the coccinellids inside the net chambers
(E) Pots with Ok raseedlings
(F)Tying the bamboo sticks with net chambers for each pot

RESULTS AND DISCUSSION

Effect of bio-pesticide formulations on Non-target Insects

The biopesticide formulations, Bioveer, Biozium, Biomonas, Biobt, Biosona, Biometa, Biollium, Biotime, and Biogreen-5 consisting of microbial biocontrol agents as their key ingredient,developed by the Biocontrol Laboratory, Department of Plant Pathology under DBT- North East Centre for Agricultural Biotechnology (DBT-NECAB), Assam Agricultural University, Jorhat used for studying their effect on beneficial insects present in the vegetable ecosystem. The experiment was conducted in both field and pot conditions and the data was recorded at 0, 3, 7 and 10 days 'intervals. Two sprays of the biopesticide formulations were given, the first spray was immediately after the plant reached vegetative growth and the 2nd spray was after 15 days of the previous spray.

Effectofbiopesticideformulationsonnon-targetinsectsunderfieldconditions

The coccinellid count after 1st and 2nd sprays of different biopesticides showed no statistically significant difference as compared to the control as mentionedin Table 2. Thus, it can be concluded that there is no ill-effect of the bio-pesticides over the natural enemies, coccinellid beetles.

Table 2: Field effect of bio-pesticides against coccinellids (1stand2nd spray-onCoccinellidBeetlecount)

Treatments	Pre- treatment tcount*	1st spray			Pre-treatment count*	2nd spray		
		3 days*	7 days*	10 days*		3 days*	7 days*	10 days*
T_1 (Bioveer@1%)	1.28	1.42	1.47	1.47	1.52	1.35	1.41	1.41
T_2 (Biozium@ 1%)	1.23	1.38	1.42	1.47	1.52	1.33	1.44	1.46
T_3 (Biobt@ 1%)	1.14	1.33	1.38	1.33	1.38	1.31	1.20	1.31
T_4 (Biomonas@ 1%)	1.28	1.42	1.47	1.42	1.42	1.47	1.07	1.17
T_5 (Biosona@1%)	1.18	1.28	1.68	1.38	1.38	1.43	1.27	1.39
T_6 (Biollium@ 1%)	0.95	1.18	1.23	1.28	1.23	1.32	1.21	1.39
T_7 (Biometa@ 1%)	0.99	1.13	1.09	1.14	1.18	1.13	1.35	1.41
T_8 (Biotime@ 1%)	1.09	1.23	1.18	1.14	1.19	1.28	1.40	1.31
T_9 (Biogreen–5@ 1%)	1.18	1.33	1.38	1.43	1.33	1.38	1.10	1.30
T_{10}(CONTROL)	0.89	1.35	1.43	1.40	1.42	1.48	1.30	1.33
SE± (d)	0.45	0.30	0.32	0.25	0.27	0.20	0.16	0.14
C.D.at5 %	NS	NS	NS	NS	NS	NS	NS	NS

Meanofthreereplications

Effectofbiopesticideformulationsonnon-targetinsectsunderpotconditions

In the pot condition, it was observed that there was no statistically significant difference incoccinellid beetles between the control pot and the treated pots, both after 1st and 2nd spray of biopesticides (Table 3). Thus, biopesticides can be applied at recommended doses producing nonnegative effect on the non-targete dinsect, coccinellidbeetles. The interaction between natural enemies in the field is crucial for determining the success of biological control programs. Numerous studies have examined the interactions between entomopathogenic fungi (EPFs) and other biological control agents, such as parasitoids and predators (Bayissa *et al.*, 2016). In our study, results obtained by experimenting with two biopesticide sprays align with findings by Rizwan *et al.* (2021), who investigated the effects of *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin on the life table parameters of the generalist predator, *Coccinella septempunctata* L. Their study concluded that *B. bassiana* and *M. anisopliae* do not significantly impact the performance and biology of *C. septempunctata.* This compatibility suggests that both EPFs can be effectively integrated into pest management programs involving *C. septempunctata*.

Table 3: Pot condition effect of bio-pesticides against coccinellid beetles (1st and 2nd spray)

Treatments	Pre- treatment count*	1st spray			Pre-treatment count*	2nd spray		
		3 days*	7 days*	10 days*		3 days*	7 days*	10 days*
T_1 (Bioveer@1%)	3.00	2.67	2.67	2.67	2.67	2.67	2.67	2.67
T_2 (Biozium@ 1%)	3.00	2.67	3.00	2.67	2.33	2.67	2.67	2.33
T_3 (Biobt@ 1%)	3.00	3.00	2.67	2.67	2.67	2.67	2.67	2.67
T_4 (Biomonas@ 1%)	3.00	2.67	2.67	2.67	2.67	2.67	2.67	2.67
T_5 (Biosona@1%)	3.00	2.67	2.67	2.67	2.67	2.67	2.67	2.67
T_6 (Biollium@ 1%)	3.00	3.00	3.00	3.00	3.00	2.67	2.67	2.67
T_7 (Biometa@ 1%)	3.00	3.00	2.67	2.67	2.67	2.67	2.67	2.67
T_8 (Biotime@ 1%)	3.00	3.00	3.00	3.00	3.00	2.67	2.33	2.33
T_9 (Biogreen–5@ 1%)	3.00	3.00	3.00	3.00	3.00	3.00	2.67	2.67
T_{10} (CONTROL)	3.00	3.00	3.00	2.67	2.67	2.67	2.67	2.33
SE± (d)	0.00	0.29	0.33	0.39	0.39	0.44	0.47	0.47
C.D.at5 %	NS	NS	NS	NS	NS	NS	NS	NS

**Mean of three replications*

Further supporting this, Sayed *et al.* (2021) assessed the pathogenicity of a local *Beauveria bassiana* isolate on all developmental stages of the predators *Coccinella undecimpunctata* and *Hippodamia variegata*. Their findings indicated no significant adverse effects on predator mortality or key biological parameters, including survival, development duration, adult longevity, and fecundity. Similarly, Ramanujam *et al.* (2017) found that *B. bassiana* is safe for *C. septempunctata* and effectively controls *Brevicoryne brassicae* (L.) under field conditions. Additionally, Ullah *et al.* (2019) evaluated the virulence of *Isaria fumosorosea* and *B. bassiana* against the reduviid predator *Rhynocoris marginatus* (Heterop, Reduviidae). They reported no significant impact on the predation and survival rates of this biological control agent. These findings collectively suggest that biopesticides or EPFs like *B. bassiana* and *M. anisopliae* are safe for coccinellid predators in the field, supporting their inclusion in integrated pest management strategies. Further, Waiganjo *et al.* (2007) investigated the use of entomopathogenic fungi and neem-based biopesticides for controlling pests in brassica crops while also aiming to conserve natural enemies within the same field. Their study, employing a randomized block design, demonstrated that biopesticides could be effectively integrated into pest management strategies without adversely affecting the populations of natural enemies such as coccinellids in fields infested with aphids and moths. Similarly, James *et al.* (1995) conducted a thorough field evaluation of the entomopathogenic fungus *Beauveria bassiana*, examining its persistence and its effects on both the pea aphid and non-target coccinellid predators in alfalfa plants. Their findings indicated that *B. bassiana* did not harm the coccinellid population. Further supporting these findings, Agale *et al.* (2019) explored the impact of various biopesticides on natural enemies in pigeon-pea (*Cajanus cajan* L.) crops. Their field experiments revealed that all tested biopesticides were safe for natural enemies and even contributed to enhancing the activity and population of these beneficial insects in the pigeon-pea fields. Additionally, Thungrabeab (2006) examined the effects of *Beauveria bassiana* and *Metarhizium anisopliae* on non-target insects under laboratory conditions, concluding that both biopesticides were non-pathogenic to natural enemies. These studies collectively highlight that biopesticides, including entomopathogenic fungi and plant-based products, can be utilized in pest management programs without detrimental effects on natural enemy populations, thereby supporting the conservation of beneficial predators like coccinellids in agricultural ecosystems.

CONCLUSION

The above research findings showed that the used biopesticides in this study have no detrimental effects on natural enemies and will surely benefit the farmers as they can use biopesticides for the management of pestswithout having any detrimental effect on the natural enemy population, and thus eco-friendly management of pests can beachieved without harming the environment. This work has demonstrated the feasibility of combining or integrating natural enemies with certain pesticides including systemic insecticides, insect growth regulators, selective feeding blockers, microbials, miticides, and fungicides.

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