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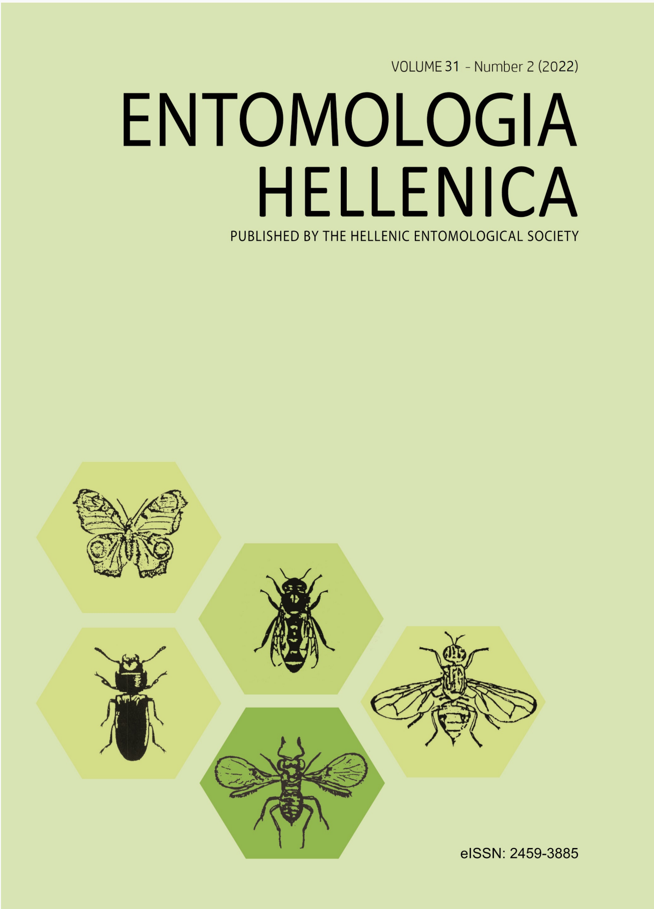

Evaluation and economic analysis of ecofriendly biological approaches for the management of shoot and fruit borer (Earias vittella F.) of okra

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Evaluation and economic analysis of ecofriendly biological approaches for the management of shoot and fruit borer (*Earias vittella* F.) of okra

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ABSTRACT

During the years 2020 and 2021, the effects of various biopesticides and the egg parasitoid *Trichogramma chilonis* on the okra shoot and fruit borer were investigated in open fields. All interventions outperformed over the untreated control. The entomopathogenic bacterium *Bacillus thuringiensis* was the most promising biopesticide tested, with the lowest shoot (5.49% and 6.87% in 2020 and 2021, respectively) and fruit damage (4.95% and 5.65% in 2020 and 2021, respectively), followed by the entomopathogenic fungi *Beauveria bassiana* (7.08%, 8.04% shoot damage and 6.78% and 6.73% fruit damage during 2020 and 2021, respectively). Interestingly, all biopesticides evaluated were shown to be safe for the polyphagous predators occurring in the okra habitat, such as the ladybird beetles *Menochilus sexmaculatus* and *Micraspis discolour* and the spiders *Marpissa* spp. and *Oxyopes lineatipes*. However, emamectin benzoate 5% SG was the most effective treatment in terms of minimizing okra shoot and fruit damage, resulting in a maximum percent reduction over control (PROC) (85.54 and 80.90 against shoot damage and 76.60 and 71.33 against fruit damage during 2020 and 2021, respectively). Each treatment's economics were also analysed. The experimental plots treated with emamectin benzoate had the highest cost:benefit ratio (1:11.16), while *B. thuringiensis* had the highest (1:7.06) among the biopesticide / parasitoid releasing plots.

KEY WORDS: *Earias vittella*; biopesticides; okra; pest management; fruit yield; economic return.

Introduction

Okra shoot and fruit borer (OSFB), *Earias vittella* (Fabricius, 1794) (Nolidae: Lepidoptera), is an oligophagous pest of okra (*Abelmoschus esculentus* (L.) Moench) that causes considerable damage in India. Both the vegetative and reproductive phases of okra are harmed by the OSFB larvae. During the vegetative stage, larvae bore into new shoots and feed on internal tissues, flower buds and fruits, during the reproductive stage. As a result, the infested shoots die early (Rai et al. 2014b). The infested fruits become malformed in shape, which drastically lowers their market value. In India, OSFB alone damages 21-51% of fruits, while in Bangladesh, 52.33-70.75% (Choudhury et al. 2021; Halder & Rai 2021).

Chemical pesticides are still the preferred choice of many Indian farmers due to their ease of use, quick action, and ease of application (Roy et al. 2017). However, the difficulties associated with chemical pesticide applications, such as resistance development of target pests, resurgence of sucking insects, secondary

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pest outbreaks, elimination of non-target organisms, pollution, and consumer health dangers, cannot be ruled out (Ahmad & Arif 2009; Halder et al. 2019; 2021).

Because of their target-specificity, self-perpetuity and safety, biological pest management methods employing macro- and micro-organisms is gaining appeal among growers and consumers. Plant-based insecticides, such as azadirachtin, which is generated mainly from the seeds of the neem tree (*Azadirachta indica* L.), are widely used to control various insect pests of various crops, particularly vegetables (Halder et al. 2010; Kodandaram et al. 2014). The soil bacterium *Bacillus thuringiensis* (Bt) produces delta-endotoxins, which are harmful to a variety of phytophagous insects and can be employed as biopesticides (Rai et al. 2014b). In the same way, *Beauveria bassiana* (Balsamo-Crivelli) and *Metarhizium anisopliae* (Metschnikoff) Sorokin are two significant entomopathogenic fungi (EPF) that are employed as mycoinsecticides against a variety of insect pests around the world (Butt et al. 2001; Zimmerman 2007a,b). In India, little research has been done on the ecofriendly management of OSFB. As a result, the current research was carried out to establish the individual efficacy of these bioagents under field conditions, as well as their cost:benefit ratio, to set up appropriate pest management modules.

Materials and Methods

Experimental site: The field experiments were conducted on a research farm of ICAR-Indian Institute Vegetable Research in Varanasi (82°52' E longitude and 25°12' N latitude), Uttar Pradesh, India. The experimental site is located in the Indo-Gangetic plains' alluvial zone, with silt loam soils that are low in organic carbon (0.43 percent) and available nitrogen (185 Kg/ha).

Seed sowing and crop management: During the rainy season (July - October) of 2020 and 2021, seeds of okra (cv. Kashi Pragati) were sown in a randomized block design with four replications and seven treatments, including an untreated control, in a plot size of 5 x 4 m with plant to plant spacing of 45 cm and row to row distance of 60 cm, during the first week of August. The prescribed fertilizer concentrations (N:P:K = 120:60:60) were utilized as a starting point. Standard agronomic practices were followed, except for plant protection measures. Hand weeding and irrigations were provided as needed.

Experimental design and treatments: The experiment adopted a randomized block design (RBD) with four replications, during the rainy season (July - October) in 2020 and 2021. Three entomopathogenic fungi as talc-based formulation viz., *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae) (1×10^8 cfu/g) NBAIR strain, *Metarhizium anisopliae* (Metchnioff) Sorokin (Hypocreales: Clavicipitaceae) NBAIR strain (1×10^8 cfu/g) and *Bacillus thuringiensis* Berliner (Bacillales: Bacillaceae) NBAIR strain were considered for the experiments. Azadirachtin 300 ppm were procured from the local market. Apart from these microbial insecticides, the egg parasitoid, *Trichogramma chilonis* (NBAIR strain) was also released in a separate plot (75 m^2) 500 m from the main plot. A chemical insecticide approved by the Central Insecticide Board and Registration Committee, Ministry of Agriculture & Farmers Welfare, Govt. of India, emamectin benzoate 5% SG was used as a chemical control treatment. The treatment details along with their concentrations were: T1: *Metarhizium anisopliae* (NBAIR strain) 1×10^8 spores/ g @ 5 g/L; T2: *Beauveria bassiana* (NBAIR strain) 1×10^8 spores/ g @ 5 g/L; T3: *Trichogramma chilonis* (NBAIR strain) @ 50,000 parasitoids/ha, 6 releases at weekly interval; T4: *Bacillus thuringiensis* (NBAIR strain) @ 2 ml/L; T5: Azadirachtin 300 ppm @ 5 ml/L; T6: emamectin benzoate 5% SG @ 0.3 g/L; T7: Untreated control. A high-volume knapsack sprayer equipped with a hollow cone nozzle was used for all applications. The spray volume was 600 L/ha during sunny and warm conditions with no or little wind. The first application was performed when the borer population reached the economic threshold level (5% of the fruit damage), followed by two sprays, fifteen days apart.

Shoot infestation: At weekly intervals, the total number of shoots, as well as the number of infested shoots, were visually inspected and recorded from five plants in each plot. The percentage of shoot infestation was calculated using the following formula:

% Shoot infestation =
100x infested shoots number/total shoots number

Fruit infestation and yield: Fruits were harvested at 4-5 days intervals, and the number of healthy and infested fruits was recorded. Healthy and infested fruits were weighed separately per plot and per treatment. During the fruiting season, harvests were made, and the percentage of fruit infestation was calculated using the following formula:

% Fruit infestation (by number) =
100x infested fruits number/total fruits number

The percentage reduction (PR) of the OSFB over the untreated control for each treatment was calculated using the following formula:

PR = 100x (Count in control–Count in treatment)/Count in control)

Observation on beneficial fauna: In addition, 10 days after each spray, the population of polyphagous ladybird beetles (grub, pupa and adults) i.e., *Menochilus sexmaculatus* (Fab.) (Syn: *Cheilomenes sexmaculata* (Fab.)) and *Micraspis discolor* (Fab.) (Coleoptera: Coccinellidae), jumping spiders (spiderling and adult) (*Marpissa* spp.) (Araneae: Salticidae) and true spiders like lynx (*Oxyopes lineatipes* (Koch)) (Araneae: Oxyopidae) was counted from 5 plants/plot and expressed as number of predators/plant.

Data analysis: All above mentioned data from the field experiment were collected, and the mean data from three replications was analyzed using the SAS program (Johnston 1993). For the parameters of shoot and fruit infestation, analysis of variance (ANOVA) was used to determine significant differences between the treatments. Using data from the mean population after spraying, the critical difference (CD) was estimated at a 5% level of significance. The incremental cost-benefit (ICBR) analysis for each treatment was carried out using the following formula:

Incremental Cost-benefit ratio =
Net return (₹ ha-1)/Cost of treatment (₹ ha-1)

Results

Shoot infestation

Table 1 shows the impact of several treatments on okra shoot and fruit borer (OSFB) infestation of shoots. The proportion of shoot infestation was greatly reduced in all treatments. The untreated control plot had the highest percentage of infested shoots (22.69), whereas the emamectin benzoate treated plots had the lowest (3.28%) during the first year. Among the biopesticides examined, *B. thuringiensis* treated plots had the lowest amount of shoot damage in both years (5.49% and 6.87% during 2020 and 2021, respectively).

During both years, the *B. bassiana* treated plots had the second lowest shoot damage (7.08% and 8.04%). The reduction percentage was calculated with respect to the control treatment, and all treatments showed a markedly decreased percentage of shoot infestation as compared to the control.

The highest reduction over the control (PROC) was detected for treatment 4 (*B. thuringiensis* treated plots), with corresponding values of 75.81 and 71.53 for the years 2020 and 2021, respectively, followed by the plots sprayed with *B. bassiana* (68.80 and 66.68 PROC). The lowest reduction was found in the azadirachtin-treated plots as compared to the control.

Fruit damage

In the case of *Earias* spp. fruit damage in okra, a similar pattern was observed. During the year 2020, plots treated with the entomopathogenic bacterium *B. thuringiensis* suffered only 4.95% fruit damage, resulting in a maximum protection percentage of 70.07 above the untreated control among the biopesticides evaluated. The white muscardine fungus *B. bassiana* was the next best biopesticide in 2020, with 6.78% fruit damage and 59.01 PROC. During the following year, the results were constant, with plots sprayed with *B. thuringiensis* showing the lowest fruit damage (5.65%), followed by *M. anisopliae* (6.73% fruit damage and 55.84 PROC), and *B. bassiana* (7.16% fruit damage and 53.02 PROC). However, emamectin benzoate 5% SG was the most effective treatment in terms of fruit damage, with 3.87% and 4.37% fruit damage in 2020 and 2021, respectively, and PROC values of 76.60 and 71.33.

Table 1. Bio-efficacy of different biocontrol agents against shoot damage (%) in okra

Treatment	2020[a]			2021[a]		
	Before spray	After spray	PROC*	Before spray	After spray	PROC[#]
T1	18.65	7.91[bc]	65.14	22.34	10.59[c]	56.11
T2	17.32	7.08[b]	68.80	21.06	8.04[b]	66.68
T3	20.36	11.97[d]	47.25	21.58	13.96[d]	42.15
T4	15.79	5.49[b]	75.81	20.19	6.87[b]	71.53
T5	18.05	12.64[d]	44.30	19.54	13.97[d]	42.11
T6	19.64	3.28[a]	85.54	20.36	4.61[a]	80.90
T7	19.43	22.69[e]	--	21.14	24.13[e]	--
SEm(±)	--	0.86	--	--	0.74	--
LSD (5%)	--	1.79	--	--	1.87	--

[#]PROC = Percent Reduction Over Control; Means followed by same letters in a column are not significantly different at 0.05P; [a]Mean of 15 observations over three sprays of different treatments at 15 days interval.

(T1: *Metarhizium anisopliae* (NBAIR strain) @ 5 g/L; T2: *Beauveria bassiana* (NBAIR strain) @ 5 g/L; T3: *Trichogramma chilonis* (NBAIR strain) @ 50,000 parasitoids/ha, 6 releases at weekly interval; T4: *Bacillus thuringiensis* (NBAIR strain) @ 2 ml/L; T5: Azadirachtin 300 ppm @ 5 ml/L; T6: emamectin benzoate 5% SG @ 0.3 g/L; T7: Untreated control).

SEM: Standard error of mean, LSD: Least Significant Difference

Table 2. Bio-efficacy of different biocontrol agents against fruit damage (%) in okra

Treatments	2020			2021		
	Before spray	**After spray**	**PROC***	**Before spray**	**After spray**	**PROC#**
T1	12.56	7.43[c]	55.08	13.59	7.16[bc]	53.02
T2	13.47	6.78[c]	59.01	15.67	6.73[c]	55.84
T3	11.69	8.29[cd]	49.88	17.58	7.44[bc]	51.18
T4	12.68	4.95[b]	70.07	15.43	5.65[b]	62.93
T5	13.57	9.81[d]	40.69	14.36	7.89[d]	48.23
T6	11.06	3.87[a]	76.60	13.58	4.37[a]	71.33
T7	12.34	16.54[e]	--	14.56	15.24[e]	--
SEm(±)	--	0.39	--	--	0.37	--
LSD (5%)	--	0.88	--	--	0.84	--

[#]PROC = Percent Reduction Over Control; Means followed by same letters in a column are not significantly different at 0.05P; [a]Mean of 15 observations over three sprays of different treatments at 15 days interval.
(T1: *Metarhizium anisopliae* (NBAIR strain) @ 5 g/L; T2: *Beauveria bassiana* (NBAIR strain) @ 5 g/L; T3: *Trichogramma chilonis* (NBAIR strain) @ 50,000 parasitoids/ha, 6 releases at weekly interval; T4: *Bacillus thuringiensis* (NBAIR strain) @ 2 ml/L; T5: Azadirachtin 300 ppm @ 5 ml/L; T6: emamectin benzoate 5% SG @ 0.3 g/L; T7: Untreated control)
SEM: Standard error of mean, LSD: Least Significant Difference

Economic Analysis

The incremental cost:benefit ratio (ICBR) was estimated on the basis of the crop protection expenses incurred and local market value of healthy fruits obtained against the treatment used in the present study (Table 3). It is important to note that the expenses incurred refer to those only related to pest management. It was revealed that the highest ICBR was 1:7.06 as estimated for the *B. thuringiensis* treated plots, followed by *T. chilonis* plots, whereas the lowest was estimated for azadirachtin 300 ppm (1:1.07).

The common predators observed in okra plants were identified as the predatory polyphagous ladybird beetles i.e., *Menochilus sexmaculatus* (Fab.) (Syn: *Cheilomenes sexmaculata* (Fab,)) and *Micraspis discolor* (Fab.) (Coleoptera: Coccinellidae). Also present were the jumping spiders *Marpissa* spp. (Araneae: Salticidae) and true spiders like lynx (*Oxyopes lineatipes* (Koch)) (Araneae: Oxyopidae).

The effect of these bioagents and botanicals on associated natural enemies (NEs) was investigated (Fig. 1). In all the biopesticide treated plots, *T. chilonis* released and untreated control, all stages of these predators were observed. The highest number of predators was recorded at the untreated control plots (3.94 lady bird beetles and 4.18 spiders per plant) followed by the *T. chilonis* plot (3.97 lady bird beetles and 4.07 spiders plant-1). The plots sprayed with emamectin benzoate 5% SG had the lowest spiders (2.87 per plant) and predatory coccinellid beetles (2.49 per plant). The other biopesticides viz., *B.*

bassiana, *M. anisopliae*, *B. thuringiensis* and azadirachtin treated plots had higher natural enemies' populations and these did not differ significantly to each other.

Table 3. Economic analysis of different treatments against the okra shoot and fruit borer

Treatments	Yield of healthy fruits (q ha^{-1})	Increase in yield over control (q ha^{-1})	Increase in yield over control (%)	Cost of increase yield (₹ ha^{-1})	Cost of treatment (₹ ha^{-1})	Net profit (₹)	Incremental Cost: Benefit ratio
T1	107.4^{c}	19.6	22.32	23520	4500	19020	1:4.23
T2	113.9^{d}	26.1	29.73	31320	4500	26820	1:5.96
T3	99.7^{b}	11.9	13.55	14280	2000	12280	1:6.14
T4	116.8^{d}	29.0	33.03	34800	4320	30480	1:7.06
T5	97.1^{b}	9.3	10.59	11160	5400	5760	1:1.07
T6	131.6^{e}	43.8	49.89	52560	4320	48240	1:11.16
T7	87.8^{a}	--	--	--	4500	--	--
LSD (5%)	3.4	--	--	--	--	--	--

Spray volume = 600 L ha^{-1}; Average cost of okra was ₹ 1200 q^{-1}; Means followed by same letters in a column are not significantly different at 0.05P.

Yield related parameters

The effects of different treatments significantly influenced the individual yields of okra (Table 3). Total yield was calculated by hectare of land, and the maximum yield (131 q ha^{-1}) was found in the emamectin benzoate 5% SG treated plots followed by *B. thuringiensis* (116.8 q ha^{-1}) and *B. bassiana* (113.9 q ha^{-1}) treated plots. The minimum healthy fruit yield (87.8 q ha^{-1}) was found in the untreated control treatment.

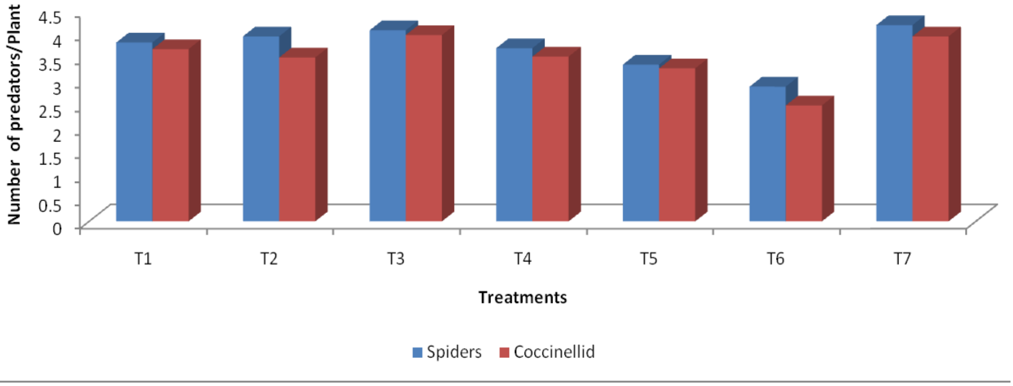

FIG. 1. Effect of different treatments on population of polyphagous spiders and lady bird beetles in okra

Discussion

The entomopathogenic bacterium *Bacillus thuringiensis* treated plots suffered lowest shoot and fruit damages by the okra shoot and fruit borer during both years amongst the biopesticide treated plots. The bacterium after entering the insect gut causes septicemia and eventual death of the insect host. Insects show different kinds of responses to the toxins, depending on the crystal proteins (delta-endotoxins), receptor sites, production of other toxins (exotoxins), and requirements of spores (Halder & Seni 2021). So, *B. thuringiensis* is an effective competitive alternative, compared to conventional insecticides in terms of efficacy and costs of production (Ruiu et al. 2013). Another entomopathogen, *Beauveria bassiana*, is used for the management of a wide range of insect pests. *B. bassiana* 1.15% WP @ 3000 g/ha and 2500 g/ha were highly effective in controlling pod borer populations in chickpea (Bajya et al. 2015). Pest control effectiveness of *B. bassiana* was documented against the maize stem borer, *Chilo partellus* from Kenya (Maniania 1993). Similarly, the black muscardine fungus *Metarhizium anisopliae* is also reported to be effective against a large number of crop pests (Revathi et al. 2011; Halder et al. 2016; Fite et al. 2020). Gaikwad (2013) concluded that *B. thuringiensis* var. *kurstaki* proved to be the toxic to the larvae of *Earias vittella* infesting okra whereas *M. anisopliae* and *B. bassiana* were moderately toxic. In contrast, the chemical insecticide, used in the present study, emamectin benzoate, is a novel group of insecticides, effective against lepidopteran and sucking pests (Rai et al. 2014b; http://ppqs.gov.in/divisions/cib-rc/major-uses-of-pesticides). The mode of action of this new molecule is unique in the panorama of insecticides as it is grouped together with avermectins and milbemycins (IRAC, 2022). It inhibits muscle contraction, causing a continuous flow of chlorine ions in the GABA and H-Glutamate receptor sites (Fanigliulo & Sacchetti 2008; Banik & Halder 2013). This unique mode of action could be the reason for superior activity of this molecule against okra shoot and fruit borer.

It is evident that all the biopesticide treated and *T. chilonis* released okra plots had higher numbers of predatory fauna indicating their target-specificity and safety towards associated non-target organisms. Biosafety of these bioagents and neem-based insecticides have been documented by several workers (Gracy et al. 2011; Rai et al. 2014b; Roy et al. 2017; Zimmerman 2017b).

Among the biopesticide treated plots the highest cost benefit ratio was registered from the *B. thuringiensis* treated okra plots. Relatively higher fruit yield and low cost of plant protection inputs were the reason for its higher ICB ratio. Treatment 3 i.e., inoculative release of *T. chilonis* plots had the second highest ICB ratio (1:6.14). This was due to lowest cost of plant protection inputs amongst the treatments although it had a lower healthy fruit yield (99.7 q per ha). However, the emamectin benzoate treated plots had the maximum ICB ratio (1:11.06) due to its maximum fruit yield (131.6 q per ha) and accompanied with lower input cost.

Conclusion

The present study revealed that among the different biopesticide treatments, *Bacillus thuringhensis* was the best in controlling shoot and fruit borer in okra. Other biopesticides viz., *Beauveria bassiana* and *Metarhizium anisopliae* were also proved effective against OSFB and may be considered as control tools in okra cultivation. However, amongst all examined treatments, emamectin benzoate showed the highest efficacy against OSFB. These individual components should be taken into consideration while formulating ecofriendly pest management module for managing OSFB.

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Αξιολόγηση και οικονομική ανάλυση οικολογικών βιολογικών προσεγγίσεων για την αντιμετώπιση του λεπιδοπτέρου *Earias vittella* F. στη μπάμιας

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ΠΕΡΙΛΗΨΗ

Κατά τη διάρκεια των ετών 2020 και 2021, διερευνήθηκαν οι επιδράσεις διαφόρων βιοπαρασιτοκτόνων και του ωοπαρασιτοειδούς *Trichogramma chilonis* ενάντια στο λεπιδόπτερο *Earias vittella* F. σε ανοιχτή καλλιέργεια. Όλες οι επεμβάσεις είχαν καλύτερη απόδοση σε σχέση με τον μάρτυρα. Το εντομοπαθογόνο βακτήριο *Bacillus thuringiensis* ήταν ο πιο πολλά υποσχόμενος βιολογικός παράγοντας που δοκιμάστηκε, με τη χαμηλότερη προσβολή βλαστών (5,49% και 6,87% το 2020 και το 2021, αντίστοιχα) και καρπών (4,95% και 5,65% το 2020 και 2021 αντίστοιχα), ακολουθούμενο από τον εντομοπαθογόνο μύκητα *Beauveria bassiana* (με 7,08% και 8,04% ζημιά στους βλαστούς και 6,78% και 6,73% ζημιά στους καρπούς κατά το 2020 και το 2021, αντίστοιχα). Είναι ενδιαφέρον ότι όλοι οι βιολογικοί παράγοντες που αξιολογήθηκαν αποδείχθηκαν ασφαλής για τα πολυφάγα αρπακτικά που απαντώνται στον βιότοπο της μπάμιας, όπως οι πασχαλίτσες *Menochilus sexmaculatus* και *Micraspis discolour* και οι αράχνες *Marpissa* spp. και *Oxyopes lineatipes*. Ωστόσο, το εντομοκτόνο emamectin benzoate 5% SG ήταν το πιο αποτελεσματικό όσον αφορά την ελαχιστοποίηση της ζημιάς στους βλαστούς και τους καρπούς της μπάμιας, με αποτέλεσμα τη μέγιστη ποσοστιαία μείωση έναντι του μάρτυρα (PROC) (με 85,54 και 80,90 έναντι της ζημιάς των βλαστών και 76,60 και 71,33 έναντι της ζημιάς των καρπών το 2020 και 2021, αντίστοιχα). Τα οικονομικά στοιχεία κάθε επέμβασης αναλύθηκαν επίσης. Τα πειραματικά αγροτεμάχια στα οποία εφαρμόστηκε το emamectin benzoate είχαν την υψηλότερη αναλογία κόστος/όφελος (1:11,16), ενώ ο *B. thuringiensis* την υψηλότερη (1:7,06) μεταξύ των επεμβάσεων με τους βιολογικους παράγοντες.

