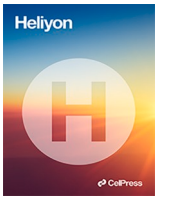
Heliyon 10 (2024) e37175

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Insecticidal potential of *Bacillus thuringiensis*, *Beauveria bassiana* and *Metarhizium anisopliae* individually and their synergistic effect with barazide against *Spodoptera litura*

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ARTICLE INFO

Keywords:
Chemical insecticides
Entomopathogenic bacteria
Entomopathogenic fungi
Pest management
Sustainability

ABSTRACT

Excessive use of insecticides are responsible to contaminate the environment, soil health, developing resistance in the insect pests, introduces new species, toxic to human and eliminates non-target organisms and affects the eco-balance and biodiversity adversely. Application of microbial bio-agents with the chemical insecticides is an assertive way to manage the population of pests, in an addition to dropping down the chemical residues risk to the eco-system. Larval stages of *Spodoptera litura* are prolific eater, caused huge losses globally. Individual and combined effect of chemical insecticides Barazide (Novaluron 5.25 %+Emamectin benzoate 0.9 % SC), entomopathogenic bacterial (*Bacillus thuringiensis* var. *kurstaki*), and entomopathogenic fungus (*Beauveria bassiana* and *Metarhizium anisopliae*) is assessed against the larvae of *S. litura* in bio-assay experiment. The decreasing trend in the observed mortality among insecticides alone is Barazide (95.80 ± 1.16, 85.30 ± 1.85 and 82.00 ± 1.72) > *B. thuringiensis* var. *kurstaki* (88.70 ± 1.01, 79.90 ± 2.01 and 78.00 ± 2.91) > *B. bassiana* (82.60 ± 2.46, 73.90 ± 2.46 and 73.00 ± 4.16) > *M. anisopliae* (78.60 ± 1.46, 68.90 ± 2.96 and 69.00 ± 3.46) after 96 h at its highest inoculation level against 3rd, 4th and 5th instar larvae. The combined application of Barazide @0.1 % with *B. thuringiensis* @1.5%induced mortality cent percent after 72 and 96 h against 3rd and 4th instar. Chi-squared test indicated a significant level of mortality at $p < 0.05$ level at highest dose and the probit analysis showed lowest LC_{50} value at dose 5.15 and 7.63 % with 95 % FL:1.38–19.22 and 2.85–20.39 after 72 and 96 h of exposure against 3rd and 4th instar. The increasing trend in the observed mortality among insecticides used in combination is Barazide + *B. thuringiensis* < Barazide + *B. bassiana* < Barazide + *M. anisopliae*. Insecticides used in combination induced synergism that providing valuable practice to manage insect pests. These results suggested that the combined treatments could be a successful method for controlling the population of *S. litura* and at the same time farmers will decrease the inappropriate misuse and overuse of harmful chemical insecticides.

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https://doi.org/10.1016/j.heliyon.2024.e37175
Received 30 May 2024; Received in revised form 28 August 2024; Accepted 28 August 2024
Available online 29 August 2024

1. Introduction

Tobacco cutworm, *Spodoptera litura* (Fabricius) of order lepidoptera and family noctuidae, is a disreputable polyphagous insect pest of different field crops, including corn, cotton, soybean, groundnut, tobacco, and different vegetables [1]. This insect pest is reported throughout the temperate as well as tropical Australasia, Asia, and Pacific Islands [2]. Early instars larvae of this insect pests are gregarious feeders whereas the later instars scatter and feed greedily, causing the complete defoliation of the plants when number in abundance [3]. Although insecticides are known as the most trustworthy tool in the field of insect pest management, however, the development of pesticide resistance is one of the primary problems with chemical control. A large number of reports are available showing development of resistance in *S. litura* and accumulation of residues in horticultural fruits and agricultural crops [4,5]. Earlier, studies examine the effectiveness of synthetic insecticides against Fall armyworm (FAW) in maize revealed monomehypo as the most effective after 10 days, compared to other insecticides [6,7]. It is clear from the previous investigation that synthetic pesticides are a critical management tool against the FAW [8,9]. The investigation was carried out to check the effectiveness of different insecticides to control FAW in maize crop and reported Fipronil + Emamectin Benzoate 0.35 % G and Chlorantraniliprole 20 % SC as a best insecticides in which the larvae population was observed lowest after 14 days under agro-ecological conditions in Lahore, Pakistan [10]. Impulsive spraying and frequent use of the insecticides comprising chlorinated hydrocarbons, organophosphates, carbamates, and pyrethroids also impersonated a threat to the beneficial creatures [11,12]. To overcome this problem and to reduce the insecticide applications in the fields it becomes essential to go with the alternative techniques to control insect pests.

Microbial control agents such as bacteria, fungi, nematodes and viruses are the alternatives and getting more attention as they are safe to the environment and show pest selectivity [13–16]. The bacteria *Bacillus thuringiensis* dominating the market and taking two percent of insecticidal market share [17]. Besides *B. thuringiensis* other bacteria like *Xenorhabdus* spp., *Photorhabdus luminescens*, *Serratia* spp., *Pseudomonas cedrina*, *Paenibacillus* spp., *Chromobacterium substugae* and *Lysinibacillus sphaericus* have been reported for their insecticidal activity against the lepidopteran, dipteran, and coleopteran pests under the field conditions [18–24]. Entomopathogenic nematodes *Heterorhabditis bacteriophora* and *Steinernema carpocapsae*, are the sustainable, eco-friendly biocontrol measure against the insect pest which contributing in improving food security [15,25]. Biocontrol agents are more beneficial and target specific, abridged potential to develop resistance among the target pests, are safe to other living organisms. In broad, the main barriers in widespread acceptance of microbial control include a long time duration to cause adequate mortality comparable to the insecticides, susceptibility against environmental degradation, narrow host range, and cost of production.

Entomopathogenic micro-organisms have been observed to be effective when combined with the low concentrations of chemical insecticides [26]. When the two different control agents work independently on the similar target host, not causing toxicity to another one, their mutual effects may be synergistic [27,28]. Combining different strategies could enhance the efficiency of the IPM techniques which is a cost-effective as well as time saving substitute in controlling the insect pest population in the agricultural sector. Synthetic insecticides cannot be eliminated but its use could be reduced by using them in combination with the entomopathogenic microorganisms [29]. The new chemical insecticides which showed novel modes of action, such as Spinosad, Emamectin Benzoate, Abamectin, Indoxacarb, Lufenuron, Fipronil, and Chlorantraniliprole have now been familiarized for management of insect pests as these compounds are species-specific and are less harmful [30–32]. To get agricultural outputs which are free from pollution and that have virtuous compatibility with the environment, we have to use noble insecticides at lower doses together with the different strains of entomopathogenic microbes. Combined actions of the insecticide and biological control agents could be more effective than the individual constituents due to their different action modes, which may also delay development of resistance in pests [33]. Entomopathogenic bacteria and fungi appear to be compatible or well matched with wide spectrum of the chemical insecticides that may result in the synergism when they applied in the combination even at the low doses [34–36]. The integration of microbial biocontrol agents and chemical insecticides in pest management enhances efficacy and sustainability, but also presents challenges like compatibility, complexity, cost, and regulatory factors. Considering the importance of combined approaches, this study is planned to appraise bacterial and fungal compatibility with insecticide. Bacterial isolate *B. thuringiensis*, and two isolates of fungus *Beauveria bassiana* and *Metarhizium anisopliae* revealing pathogenicity against the tobacco cutworm when applied in combination treatments with chemical insecticides Barazide (Novaluron + Emamectin benzoate).

2. Materials and methods

To establish culture of *S. litura*, larvae along with the egg masses were collected from cauliflower, capsicum, and tomato and cabbage fields around Baru Sahib (Himachal Pradesh), India. Mass culturing or rearing was carried out in the laboratory of Zoology Department, Eternal University at altitude 1067m as per protocol [37,38]. Castor (*Ricinus communis* L.) leaves were provided as feed for different instars and were kept at 26±2°C temperatures and 65 ± 4 % humidity conditions respectively for healthier development [39]. Emerging adults were transferred to the glass chimneys for oviposition containing feed 15 % sucrose solution soaked on a cotton swab that refreshes daily and are responsible for better development and fecundity [40,41]. Adult females laid eggs in the cluster within 4–5 days of mating. Newly hatched larvae were shifted to plastic jars where castor leaves were provided to these voraciously feeder. Up to third instars, the larvae were kept in groups in plastic vials. After that, they were shifted to the individual vials as they show cannibalistic nature. After larval forms attain the full physiological maturity they are shifted over the moist sterilized sand for pupation in pupation jars (15 × 15 cm). Breeding stock of target insects was retained properly under controlled conditions in the laboratory. The culture of target insect pests was raised for the 3–4 generations under the laboratory before engaging for the experiments. Life cycle of this insect pest lasted around 32–40 days under controlled conditions.

Commercial formulations of biological insecticide *Bacillus thuringiensis* var. *kurstaki* (Mahastra 0.5 % W.P., DOR BT-1, International

Panaacea Limited (IPL), New Delhi, India), *Beauveria bassiana* (Daman 1.0 % W.P. IPL/BB/MI/01, New Delhi, India) and *Metarhizium anisopliae* (Kalichakra 1.0 % W.P., IPL, New Delhi, India) were used. One insect growth regulators (IGR) & Avermectin chemical insecticide "Barazide" formulated with the Novaluron emamectin benzoate (Adama 5.25 + 0.9 % S.C.) with dual action approach which interfere with neuromuscular process and act as a powerful weapon against insects were purchased from local market and are used in this bioassay experiment.

2.1. Insecticides compatibility test

A compatibility test was done by using bacteria *B. thuringiensis* var. *kurstaki* with chemical pesticide Barazide to assess whether they can be used in combination or not. Shake flask and plate assay experiments were conducted to evaluate the compatibility between bacterial culture and insecticide. In the shake flask assay, a single dose of insecticide Barzide (0.1 %) was transferred to the 100 mL Luria Bertani (LB) broth in 250 mL capacity flask. An insecticide-free culture medium was served as the control. A single colony of *B. thuringiensis* was incubated at 180 rpm for 48 h at 30 °C. *B. thuringiensis* culture was centrifuged at 10,000 rpm (revolutions per minutes) for 10 min at 4 °C temperature to notice the bacterial proliferation. A spreader was used to overlay the 100 μL culture suspension over the LB plates. In the four plate quadrants, eight different wells were created. Phosphate buffered saline (PBS) was introduced to a single control well and 0.1 % Barazide was again added to another wells and left to dry for overnight and these plates were incubated at 30 °C for 48 h to check the growth of bacterial cell. The compatibility of chemical insecticide against bacterial culture was demonstrated by the diameter of halo zones around wells in comparison to control well [42].

In-vitro investigation was also conducted to evaluate the compatibility between synthetic chemical Barazide and entomopathogenic fungus *B. bassiana* and *M. anisopliae*. The dose 0.1 % of insecticide was added to the potato dextrose agar (PDA) in a conical flask before solidification. After through mixing, the obtained media was transferred with gentle shaking on the Petri dishes and let it to solidify. By using a micropipette, *B. bassiana* and *M. anisopliae* formulations 0.5, 1.0 and 1.5 % were inoculated in different petri plate on the media prepared. Petri plates were then sealed and incubated in an incubator and were maintained there at 25 ± 1 °C temperature and 80 ± 5 % relative humidity. The media on the Petri plate without insecticide was used as a control. After three days of inoculation, the diameters of colonies were calculated using Vernier calipers. To assess the possible influence of the insecticide Barazide on colony development, treatment groups' growth was compared to that of the control [43].

2.2. Effect of insecticides individually and in-combination

Bio-efficacy of treatments of EPB (*B. thuringiensis* var. *kurstaki*), EPF (*B. bassiana* and *M. anisopliae*) and insecticide Barazide alone was evaluated in the laboratory against 10 larvae of *S. litura* belonging to different instar. Bioassay was implemented with the above mentioned four insecticides with three different concentrations and the insect larval mortality against target insect pest was assessed up to 4 days after application. Initially bioassays with Barazide formulated with the Novaluron emamectin benzoate (5.25 + 0.9 % S.C., Adama) was performed at 0.008 %, 0.001 %, and 0.01 % concentrations against the 3rd, 4th and 5th instar larvae of the insect pest *S. litura*. The stock solution (1000 %) of insecticides was made in 100 mL distilled water and serial dilution was done to prepare the three different concentrations. The commercial formulations of *B. thuringiensis* var. *kurstaki*, *B. bassiana* and *M. anisopliae* were used after serial dilution and bacterial and fungal concentration was adjusted to 0.5, 1.0 and 1.5 %. Leaf dip method was implemented to conduct this experiment as the protocol given different scientist [44–47]. The castor leaves only treated with the distilled water were act as a control. Single leaf disc of nearly 10 ± 11 cm^2 dipped in the different concentrations of insecticides and were used.

To evaluate the efficacy of the combined doses of entomopathogens and insecticide a bioassay experiment was also conducted. Three different bio-agents *B. thuringiensis* var. *kurstaki*, *B. bassiana* and *M. anisopliae* at three doses 0.5, 0.1 and 0.5 % singly with the chemical insecticide Barazide 0.1 % are used under polystyrene tray having 48 wells per tray along with absolute control. The leaves treated with the distilled water were act as control. In this experiment single leaf disc (nearly 10 cm^2) dipped in 0.1 % dose of chemical insecticide along with three different concentrations of biopesticides was used. Fresh leaves were chopped and that pieces were placed in a rearing tube with *S. litura* larvae. These experiments were repeated annually during the two consecutive years. The chopped fresh leaves were given as a feed every 48 h. These experiments were conducted at constant temperature (25 ± 2 °C) and humidity conditions (65 ± 5 %) respectively. The larval mortality was recorded in combined assay after 24, 48 72, 96 h. Larvae are measured dead when no movement of the appendages is seen upon touching them with a brush.

2.3. Statistical analysis

In this experiment, the biannual insect mortality data collected from the bioassay experiment during the two consecutive years was used for statistical analysis. A statistical method, probit analysis was used to estimate the lower as well as median lethal values. The formulae ME = MC + MB/(1-MC) or ME = MC + MF/(1-MC) were used to determine the expected mortality value. A chi square test was employed to ascertain whether the insecticide and biopesticides had a synergistic effect [48] $\chi 2$=(MBI-ME)2/ME or $\chi 2$ = (MFI-ME)2/ME, where MBI was the observed mortality caused by bacteria + insecticide and where MFI was the observed mortality caused by fungus + insecticide. *$p < 0.05$ statistically significant (df 9). If the predicted value of $\chi 2$ is greater than the value in the table, a synergistic action between the two agents was found while if the tabular value is more than the $\chi 2$ value, an additive interaction was noticed.

3. Results

S. litura is a polyphagous insect pest and its larval stages are voracious feeders that frequently obliterate the leaves entirely. Younger larvae have lighter green colour while older larvae change to a brown colour or dark green. The larvae were without any hairs and mainly feed at night. Six larval instars were recognized in the present investigation and 10 larvae were used in each treatment per petri plate to observe the mortality.

3.1. Insecticides compatibility with bacteria and fungi

The study found that *B. thuringiensis* var. *kurstaki* and chemical insecticide Barazide were compatible and promoting bacterial growth. However, significant differences were observed in conidia generation between entomopathogenic fungi when cultured alongside Barazide. The insecticide had a less effect on conidia formation.

3.2. Effect of insecticides individually and in-combination

3.2.1. Effect against 3rd instar larvae

The study explores the impact of insecticides, both individually and in combination. The study found that different concentrations of Barazide alone significantly increased larval mortality in 3rd, 4th, and 5th instar larvae of *S. litura* compared to the control. The mortality rate significantly increased with higher dose concentrations and time exposure, ranging from 24, 48, 72, and 96 h. The study revealed that Barazide, when used at a 0.1 % inoculation level, was the most effective biopesticide, with a maximum mortality rate of 95.80 ± 1.16. This bioassay study showed a minimum mortality rate of 45.30 ± 1.01 at a 0.008 % inoculation level after 24 h. The probit analysis of Barazide revealed the lowest LC_{50} value at a dose of 1.16 %, with 95 % FL ranging from 0.24 to 5.53 after 96 h of exposure. The study revealed that *B. thuringiensis* var. *kurstaki* resulted in a maximum mortality rate of 88.70 ± 1.01 at a 1.5 % dose after 96 h. The probit analysis showed *B. thuringiensis* var. *kurstaki* had the lowest LC_{50} value at 0.19 % dose. Entomopathogenic fungus *B. bassiana* and *M. anisopliae* showed higher mortality rates at 1.5 % inoculation level whereas the lowest mortality was recorded at inoculation level 0.5 % after 24 h (Table 1). The probit analysis in *B. bassiana* and *M. anisopliae* revealed the lowest LC_{50} value at dose 0.56 and 0.40 % with 95 % FL: 0.23–1.36 and FL: 0.23–0.71 respectively (Table 4).

Furthermore, compared to solo treatments in bioassay studies, the current study demonstrated a considerable impact of combining treatments of chemical insecticide with biopesticides. The combination of Barazide (0.1 %) and bacteria *B. thuringiensis* var. *kurstaki* (1.5 %) led to a higher mortality cent percent after 72 h of exposure. Pearson's chi-squared test indicated a significant level of mortality at $p < 0.05$ level at highest inoculation dose Barazide@ 0.1 %+*B. thuringiensis* var. *kurstaki*@1.5 % after 72 h. Additionally, after 72 h of exposure, the probit analysis in the combination of Barazide + *B. thuringiensis* var. *kurstaki* revealed the lowest LC_{50} value at dose 5.15 % with 95 % FL: 1.38–19.22 (Table 5). The combined treatment of Barazide with *B. bassiana* and *M. anisopliae* resulted in higher mortality rates after 96 h of exposure. After 96 h, the combination of different insecticides was statistically significant at the $p < 0.05$ level (Table 1). After 96 exposure hours, the probit analysis revealed that the lowest LC_{50} value for Barazide + *B. bassiana* was 1.75 % with a 95 % FL of 0.34–8.88, and the lowest LC_{50} value for Barazide + *M. anisopliae* was 7.88 % with a 95 % FL of 1.92–32.25 (Table 5).

According to our research, the insecticide barazide increased larval mortality in an additive way when combined with larger concentrations of *B. thuringiensis* var. *kurstaki, B. bassiana, and M. anisoplae*. When *B. thuringiensis* var. *kurstaki, B. bassiana,* and *M. anisoplae* were combined with barazide at 0.1 %, the mortality was increased and the interactions were found to be synergistic against larvae in their third instar.

3.2.2. Effect against 4th instar larvae

After 96 h of exposure, the maximum mortality 85.30 ± 1.85 was seen with Barazide alone at a 0.1 % inoculation level against 4th instar larvae. The study revealed a minimum mortality rate of 38.90 ± 2.78 at a 0.008 % inoculation level of Barazide after 24 h.The probit analysis of Barazide revealed an LC_{50} value of 2.80 % after 96 h of exposure. *B. thuringiensis* var. *kurstaki* caused the highest mortality at a dose of 1.5 %, while the lowest at a dose of 0.5 %. The probit analysis in *B. thuringiensis* var. *kurstaki* revealed the lowest LC_{50} value at a dose of 0.16 %, with 95 % FL ranging from 0.07 to 0.38. *B. bassiana* and *M. anisopliae* showed high mortality rates at 1.5 % inoculation levels, with the lowest at 0.5 % after 24 h (Table 2), and the lowest LC_{50} value at 0.33 % and 0.47 % doses (Table 4).

The study found that the combination of Barazide (0.1 %) with bacteria *B. thuringiensis* var. *kurstaki* (1.5 %) resulted in higher mortality after 96 h of exposure. After 96 h, the highest inoculation dose showed a significant amount of death at a $p < 0.05$ level, according to Pearson's chi-square test. The LC_{50} value at dose 7.63 % with 95 % FL: 2.85–20.39 at the same time interval was revealed by the probit analysis (Table 5). Barazide (0.1 %) combined with *B. bassiana* and *M. anisopliae*@1.5 %, caused greater mortality rates of 85.30 ± 2.56 and 79.90 ± 4.39, respectively. Combining pesticide applications demonstrated significance at the $p < 0.05$ level (Table 2). Barazide + *B. bassiana* (95 % FL: 0.98–25.26) and Barazide + *M. anisopliae* (5.12 %, 95 % FL: 0.74–35.23) had the lowest LC_{50} values, respectively (Table 5).

As per our research, the pesticide Barazide exhibited an additive effect on larval mortality when combined with elevated concentrations of *B. thuringiensis* var. *kurstaki, B. bassiana,* and *M. anisoplae*. While *B. thuringiensis* var. *kurstaki, B. bassiana,* and *M. anisoplae*@0.5 % increased the mortality and the interactions proved to be synergistic against larvae in their fourth instar, barazide@0.1 % combined with these agents improved the mortality.

Table 1
Effect of the combined treatments of chemical insecticides Barazide and bio-insecticides *B. thuringiensis* var. *kurstaki*, *B. bassiana* and *M. anisopliae* against 3rd instar larvae of *S. litura*.

Treatment/Concentration	Observed mortality (%)				Expected Mortality (%)				χ^2value				Calculated value	Interaction
	24h	48h	72h	96 h	24h	48h	72h	96h	24h	48h	72h	96h		
Barazide @0.008 %	45.30 ± 1.01	55.30 ± 2.73	65.70 ± 3.04	83.70 ± 1.71	–	–	–	–	–	–	–	–	–	–
Barazide @0.01 %	56.60 ± 1.91	64.20 ± 2.65	71.90 ± 1.88	90.50 ± 1.19	–	–	–	–	–	–	–	–	–	–
Barazide @0.1 %	63.20 ± 2.08	74.30 ± 1.63	81.10 ± 2.46	95.80 ± 1.16	–	–	–	–	–	–	–	–	–	–
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–	–	–	–	–	–	–	–	–	–
B. thuringiensis var. *kurstaki* @0.5 %	40.90 ± 1.58	49.30 ± 1.43	55.50 ± 1.31	70.90 ± 1.58	–	–	–	–	–	–	–	–	–	–
B. thuringiensis var. *kurstaki* @1.00 %	47.60 ± 1.26	56.20 ± 1.36	63.80 ± 1.27	80.40 ± 1.43	–	–	–	–	–	–	–	–	–	–
B. thuringiensis var. *kurstaki* @1.5 %	56.50 ± 1.19	68.00 ± 1.30	76.20 ± 1.16	88.70 ± 1.01	–	–	–	–	–	–	–	–	–	–
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–	–	–	–	–	–	–	–	–	–
Barazide @0.1 % + *B. thuringiensis* var. *kurstaki* @0.5 %	57.80 ± 2.20	66.70 ± 2.47	77.90 ± 1.67	89.50 ± 2.08	39.45	47.92	59.83	72.67	8.53	5.28	4.19	3.89	21.89*	Synergistic
Barazide @0.1 % + *B. thuringiensis* var. *kurstaki* @ 1.0 %	63.20 ± 1.32	72.30 ± 2.23	81.80 ± 2.47	93.80 ± 2.82	46.24	54.89	65.56	79.22	6.22	5.52	4.02	2.68	18.44*	Synergistic
Barazide @0.1 % + *B. thuringiensis* var. *kurstaki* @1.5 %	72.10 ± 2.08	83.50 ± 1.45	100 ± 0.00	–	55.20	68.79	75.03	–	5.17	3.14	6.23	–	14.54	Additive
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	–
B. bassiana @0.5 %	27.30 ± 2.52	34.40 ± 4.39	49.50 ± 3.39	64.30 ± 2.69	–	–	–	–	–	–	–	–	–	–
B. bassiana @1.00 %	32.70 ± 3.43	43.00 ± 2.26	54.30 ± 2.27	71.30 ± 3.65	–	–	–	–	–	–	–	–	–	–
B. bassiana @1.5 %	39.00 ± 1.31	54.20 ± 2.19	64.70 ± 1.19	82.60 ± 2.46	–	–	–	–	–	–	–	–	–	–
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–	–	–	–	–	–	–	–	–	–
Barazide@0.1 % + *B. bassiana* @0.5 %	39.70 ± 2.18	46.70 ± 3.69	62.70 ± 2.78	78.20 ± 2.55	25.79	33.30	48.25	63.06	7.50	5.24	4.32	3.63	20.69*	Synergistic
Barazide@0.1 % + *B. bassiana* @1.0 %	45.60 ± 2.03	55.60 ± 1.15	67.20 ± 1.53	83.30 ± 2.36	31.26	41.68	53.04	70.12	6.57	4.64	3.78	2.47	17.46*	Synergistic
Barazide @0.1 % + *B. bassiana* @1.5 %	51.40 ± 1.08	66.40 ± 2.08	76.70 ± 1.32	91.70 ± 1.16	37.65	52.98	63.50	81.48	5.02	3.39	2.74	1.28	12.43	Additive
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	–
M. anisopliae @0.5 %	22.20 ± 2.46	28.00 ± 3.51	36.50 ± 2.36	56.00 ± 2.93	–	–	–	–	–	–	–	–	–	–
M. anisopliae @1.00 %	30.10 ± 2.41	35.00 ± 2.34	41.30 ± 1.22	64.20 ± 1.84	–	–	–	–	–	–	–	–	–	–
M. anisopliae @1.5 %	37.00 ± 1.22	42.10 ± 2.16	51.30 ± 1.15	78.60 ± 1.46	–	–	–	–	–	–	–	–	–	–
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00	0.00	0.00	0.00	–	–	–	–	–	–
Barazide@0.1 % + *M. anisopliae* @0.5 %	32.20 ± 2.45	38 ± 2.68	47.20 ± 2.97	68.40 ± 1.88	20.68	26.59	35.17	54.76	6.41	4.89	4.11	3.39	18.80*	Synergistic
Barazide@0.1 % + *M. anisopliae* @1.0 %	40.50 ± 2.41	45.10 ± 2.48	52.10 ± 2.21	75.30 ± 1.36	28.64	33.67	40.01	63.01	4.91	3.88	3.65	2.39	15.06	Additive
Barazide@0.1 % + *M. anisopliae* @1.5 %	46.90 ± 1.98	52.60 ± 2.23	61.40 ± 1.26	89.60 ± 1.31	35.65	40.82	50.49	77.45	3.55	3.39	2.91	1.90	11.75	Additive
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00	0.00	0.00	0.00	–	–	–	–	–	–

Mean ± Standard Error (Mean ± SE) for the mortality observed; Expected mortality ME = MC + MB/(1-MC) or ME = MC + MF/(1-MC), where MC, MB and MF are the percentage mortality observed caused by combined application of insecticide with biopesticides, bacteria and fungus. Test for interaction based on χ^2 with 9 df, using the formula $\chi^2 = (\text{MBI-ME})^2/\text{ME}$ or $\chi^2 = (\text{MFI-ME})^2/\text{ME}$, where MBI is the mortality observed caused by bacteria + insecticide and where MFI is the observed mortality caused by fungus + insecticide. *$p < 0.05$ statistically significant.

Table 2
Effect of the combined treatments of chemical insecticides Barazide and bio-insecticides *B. thuringiensis* var. *kurstaki*, *B. bassiana* and *M. anisopliae* against 4th instar larvae of *S. litura*.

Treatment/Concentration	Observed mortality (%)				Expected Mortality (%)				χ^2value				Calculated values	Interaction
	24h	48h	72h	96 h	24h	48h	72h	96 h	24h	48h	72h	96 h		
Barazide @0.008 %	38.90 ± 2.78	48.10 ± 1.67	59.40 ± 1.73	69.6 ± 1.46	–	–	–	–	–	–	–	–	–	–
Barazide @0.01 %	45.60 ± 1.69	55.70 ± 1.36	65.20 ± 1.36	77.5 ± 1.38	–	–	–	–	–	–	–	–	–	–
Barazide @0.1 %	52.90 ± 1.79	60.90 ± 1.52	73.50 ± 1.19	85.30 ± 1.85	–	–	–	–	–	–	–	–	–	–
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–	–	–	–	–	–	–	–	–	–
B. thuringiensis var. *kurstaki* @0.5 %	32.80 ± 4.48	44.00 ± 3.23	48.90 ± 3.61	66.30 ± 2.48	–	–	–	–	–	–	–	–	–	–
B. thuringiensis var. *kurstaki* @1.00 %	41.60 ± 3.26	48.10 ± 4.38	56.20 ± 2.34	73.50 ± 2.47	–	–	–	–	–	–	–	–	–	–
B. thuringiensis var. *kurstaki* @1.5 %	49.00 ± 4.19	55.00 ± 3.34	65.50 ± 2.36	79.90 ± 2.01	–	–	–	–	–	–	–	–	–	–
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–	–	–	–	–	–	–	–	–	–
Barazide @0.1 % + *B. thuringiensis* var. *kurstaki* @0.5 %	47.50 ± 0.25	59.50 ± 3.17	63.90 ± 2.87	81.20 ± 2.48	31.31	42.62	47.57	65.06	8.37	6.68	5.60	4.00	24.65*	Synergistic
Barazide @0.1 % + *B. thuringiensis* var. *kurstaki* @ 1.0 %	56.90 ± 4.35	62.80 ± 2.27	70.60 ± 3.49	87.40 ± 2.92	40.20	46.77	54.92	72.29	6.93	5.49	4.47	3.15	19.79*	Synergistic
Barazide @0.1 % + *B. thuringiensis* var. *kurstaki* @1.5 %	60.90 ± 4.68	66.90 ± 4.25	78.50 ± 3.23	100 ± 0.00	47.73	53.76	64.28	78.72	3.63	3.21	3.14	4.52	14.50	Additive
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–	–	–	–	–	–	–	–	–	–
B. bassiana @0.5 %	26.80 ± 3.82	36.60 ± 4.49	44.40 ± 2.59	57.30 ± 1.79	–	–	–	–	–	–	–	–	–	–
B. bassiana @1.00 %	34.20 ± 4.48	42.10 ± 3.36	52.20 ± 4.17	63.50 ± 2.35	–	–	–	–	–	–	–	–	–	–
B. bassiana @1.5 %	42.20 ± 4.38	50.00 ± 3.69	60.00 ± 3.59	73.90 ± 2.46	–	–	–	–	–	–	–	–	–	–
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–	–	–	–	–	–	–	–	–	–
Barazide@0.1 % + *B. bassiana* @0.5 %	37.70 ± 3.58	48.20 ± 2.64	56.70 ± 1.58	69.40 ± 2.35	25.09	34.93	42.93	55.86	6.33	5.04	4.41	3.28	19.06*	Synergistic
Barazide@0.1 % + *B. bassiana* @1.0 %	45.30 ± 3.23	53.70 ± 2.65	64.50 ± 3.13	74.30 ± 3.32	32.49	40.57	50.82	62.10	5.05	4.27	3.68	2.39	15.39	Additive
Barazide@0.1 % + *B. bassiana* @1.5 %	54.40 ± 2.98	60.80 ± 3.28	72.30 ± 2.52	85.30 ± 2.56	40.72	48.63	58.67	72.62	4.59	3.04	3.16	2.21	13.00	Additive
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–	–	–	–	–	–	–	–	–	–
M. anisopliae @0.5 %	19.80 ± 3.47	22.60 ± 2.59	29.00 ± 2.37	51.30 ± 3.92	–	–	–	–	–	–	–	–	–	–
M. anisopliae @1.00 %	24.20 ± 2.49	29.00 ± 3.36	34.00 ± 2.29	61.00 ± 4.82	–	–	–	–	–	–	–	–	–	–
M. anisopliae @1.5 %	32.20 ± 3.29	38.00 ± 4.49	47.00 ± 3.45	68.90 ± 2.96	–	–	–	–	–	–	–	–	–	–
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–	–	–	–	–	–	–	–	–	–
Barazide@0.1 % + *M. anisopliae* @0.5 %	28.80 ± 4.25	32.00 ±3.38	39.40 ± 4.93	62.80 ± 5.28	18.28	21.12	27.59	50.05	6.05	5.64	5.05	3.24	19.98*	Synergistic
Barazide@0.1 % + *M. anisopliae* @1.0 %	33.40 ± 3.71	38.20 ± 4.48	44.60 ± 2.29	73.30 ± 3.33	22.76	27.64	32.65	59.83	4.97	4.34	4.37	3.03	16.71	Additive
Barazide@0.1 % + *M. anisopliae* @1.5 %	42.10 ± 2.38	47.80 ± 3.13	58.20 ± 2.66	79.90 ± 4.39	30.98	36.71	45.73	67.74	3.99	3.41	3.44	2.18	13.02	Additive
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–	–	–	–	–	–	–	–	–	–

Mean ± SE for the mortality observed; Expected mortality ME = MC + MB/(1-MC) or ME = MC + MF/(1-MC), where MC, MB and MF are the observed percentage mortality caused by combined application of insecticide with biopesticides, bacteria and fungus. Test for interaction based on χ^2 with 9 df, using the formula $\chi^2 = (\text{MBI-ME})^2/\text{ME}$ or $\chi^2 = (\text{MFI-ME})^2/\text{ME}$, where MBI is the mortality observed caused by bacteria + insecticide and where MFI is the observed mortality caused by fungus + insecticide. *$p < 0.05$ statistically significant.

Table 3
Effect of the combined treatments of chemical insecticides Barazide and bioinsecticides *B. thuringiensis* var. *kurstaki*, *B. bassiana* and *M. anisopliae* against 5th instar larvae of *S. litura*.

Treatment/Concentration	Observed mortality (%)				Expected Mortality (%)				χ^2value				Calculated values	Interaction
	24h	48h	72h	96 h	24h	48h	72h	96 h	24h	48h	72h	96 h		
Barazide @0.008 %	30.40 ± 2.34	38.10 ± 2.61	53.90 ± 2.04	67.30 ± 2.15	–	–	–	–	–	–	–	–	–	–
Barazide @0.01 %	39.10 ± 2.13	44.30 ± 2.41	60.70 ± 1.88	74.40 ± 2.00	–	–	–	–	–	–	–	–	–	–
Barazide @0.1 %	43.60 ± 1.52	51.90 ± 1.99	70.80 ± 1.22	82.00 ± 1.72	–	–	–	–	–	–	–	–	–	–
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–	–	–	–	–	–	–	–	–	–
B. thuringiensis var. *kurstaki* @0.5 %	25.40 ± 3.24	33.60 ± 2.93	45.50 ± 3.21	63.90 ± 1.82	–	–	–	–	–	–	–	–	–	–
B. thuringiensis var. *kurstaki* @1.00 %	31.10 ± 4.71	40.30 ± 2.43	51.00 ± 3.74	70.00 ± 2.89	–	–	–	–	–	–	–	–	–	–
B. thuringiensis var. *kurstaki* @1.5 %	38.20 ± 2.89	47.90 ± 3.14	63.00 ± 2.73	78.00 ± 2.91	–	–	–	–	–	–	–	–	–	–
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–	–	–	–	–	–	–	–	–	–
Barazide @0.1 % + *B. thuringiensis* var. *kurstaki* @0.5 %	35.70 ± 3.83	44.60 ± 2.58	57.00 ± 2.37	75.40 ± 3.35	36.88	46.65	61.80	76.84	4.73	5.22	5.59	4.82	20.36*	Synergistic
Barazide @0.1 % + *B. thuringiensis* var. *kurstaki* @ 1.0 %	40.90 ± 4.12	51.10 ± 2.36	62.00 ± 2.63	82.70 ± 2.23	23.94	32.23	44.22	62.70	5.77	4.74	3.69	2.57	16.77	Additive
Barazide @0.1 % + *B. thuringiensis* var. *kurstaki* @1.5 %	50.10 ± 4.32	62.70 ± 4.09	80.40 ± 3.69	96.10 ± 3.54	29.74	39.00	49.76	68.80	4.18	3.75	3.01	2.80	13.74	Additive
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–	–	–	–	–	–	–	–		–
B. bassiana @0.5 %	19.90 ± 2.52	26.90 ± 3.29	35.40 ± 1.89	55.00 ± 4.59	–	–	–	–	–	–	–	–	–	–
B. bassiana @1.00 %	25.80 ± 3.58	35.70 ± 2.42	43.30 ± 4.27	63.70 ± 5.85	–	–	–	–	–	–	–	–	–	–
B. bassiana @1.5 %	31.90 ± 2.58	43.48 ± 3.99	54.90 ± 3.58	73.00 ± 4.16	–	–	–	–	–	–	–	–	–	–
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–	–	–	–	–	–	–	–	–	–
Barazide@0.1 % + *B. bassiana* @0.5 %	28.10 ± 2.53	35.40 ± 4.24	44.80 ± 3.38	65.10 ± 2.65	18.41	25.53	34.10	53.79	5.10	3.81	3.35	2.37	14.63*	Additive
Barazide@0.1 % + *B. bassiana* @1.0 %	33.20 ± 2.73	44.90 ± 4.25	52.90 ± 2.52	72.10 ± 2.73	24.46	34.41	42.05	62.55	3.12	3.19	2.79	1.45	10.55*	Additive
Barazide@0.1 % + *B. bassiana* @1.5 %	40.00 ± 3.58	52.20 ± 2.38	62.40 ± 4.32	81.40 ± 3.56	30.61	42.25	53.74	71.87	2.88	2.34	1.39	1.26	7.87*	Additive
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–	–	–	–	–	–	–	–		–
M. anisopliae @0.5 %	17.90 ± 3.59	24.90 ± 3.69	33.40 ± 3.65	53.00 ± 2.83	–	–	–	–	–	–	–	–	–	–
M. anisopliae @1.00 %	23.00 ± 3.38	32.90 ± 2.89	43.00 ± 3.19	62.30 ± 1.72	–	–	–	–	–	–	–	–	–	–
M. anisopliae @1.5 %	28.70 ± 3.40	41.48 ± 2.39	51.90 ± 1.82	69.00 ± 3.46	–	–	–	–	–	–	–	–	–	–
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–	–	–	–	–	–	–	–	–	–
Barazide@0.1 % + *M. anisopliae* @0.5 %	25.20 ± 3.42	32.60 ± 3.54	41.50 ± 2.43	62.10 ± 3.68	16.24	23.42	32.02	51.75	4.94	3.59	2.80	2.07	13.04*	Additive
Barazide@0.1 % + *M. anisopliae* @1.0 %	30.40 ± 2.23	40.40 ± 4.04	50.80 ± 3.99	70.10 ± 4.23	21.49	31.49	41.74	61.12	3.69	2.52	1.96	1.31	09.48*	Additive
Barazide@0.1 % + *M. anisopliae* @1.5 %	36.80 ± 5.23	49.80 ± 3.75	59.40 ± 2.76	76.40 ± 3.22	27.26	40.19	50.67	67.80	3.33	2.29	1.50	1.09	08.21*	Additive
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–	–	–	–	–	–	–	–	–	–

Mean ± SE for the mortality observed; Expected mortality ME = MC + MB/(1-MC) or ME = MC + MF/(1-MC), where MC, MB and MF are the observed percentage mortality caused by combined application of insecticide with biopesticides, bacteria and fungus. Test for interaction based on χ^2 with 9 df, using the formula $\chi^2 = (\text{MBI-ME})^2/\text{ME}$ or $\chi^2 = (\text{MFI-ME})^2/\text{ME}$, where MBI is the mortality observed caused by bacteria + insecticide and where MFI is the observed mortality caused by fungus + insecticide. *$p < 0.05$ statistically significant.

Table 4
Pooled mortality data of chemical and bio-insecticides individually against 3rd, 4th and 5th instars larvae of *S. litura* under laboratory conditions.

Percent Mortality in 3rd Instars larvae						
Pesticide Name	Susceptibility (h)	Mean + SE	LC_{50} (μg/mL)	95 % fiducial limit		Person's X^2
				Lower limit	Upper limit	
Barazide	24	195 + 0.047	78.45	9.28	662.83	0.000
	48	185 + 0.049	22.18	3.94	124.72	0.001
	72	179 + 0.052	4.79	0.73	31.52	0.018
	96	143 + 0.076	1.16	0.24	5.53	0.000
B. thuringiensis var. *kurstaki*	24	0.91 + 0.117	1.02	0.44	2.38	0.111
	48	0.89 + 0.118	0.56	0.27	1.13	0.008
	72	0.88 + 0.120	0.40	0.21	0.74	0.004
	96	0.84 + 0.133	0.19	0.10	0.35	0.056
B. bassiana	24	0.92 + 0.122	4.21	1.48	11.99	0.333
	48	0.92 + 0.118	1.29	0.67	2.49	0.055
	72	0.90 + 0.117	0.56	0.23	1.36	0.010
	96	0.87 + 0.125	0.25	0.13	0.48	0.002
M. anisopliae	24	0.93 + 0.125	3.60	1.64	7.92	0.565
	48	0.92 + 0.121	2.82	1.17	6.75	0.378
	72	0.91 + 0.118	1.56	0.63	3.82	0.022
	96	0.88 + 0.121	0.40	0.23	0.71	0.000
Percent Mortality in 4th Instars larvae						
Barazide	24	201 + 0.046	502.23	39.81	6335.86	0.010
	48	196 + 0.047	57.69	3.08	1078.24	0.003
	72	188 + 0.049	8.81	0.93	83.42	0.031
	96	173 + 0.055	2.80	0.46	17.04	0.001
B. thuringiensis var. *kurstaki*	24	0.92 + 0.119	1.65	0.76	3.61	0.51
	48	0.91 + 0.117	1.00	0.30	3.37	0.147
	72	0.90 + 0.117	0.56	0.25	1.24	0.085
	96	0.87 + 0.124	0.16	0.07	0.38	0.294
B. bassiana	24	0.93 + 0.122	2.67	1.20	5.93	0.281
	48	0.91 + 0.118	1.65	0.62	4.38	0.140
	72	0.90 + 0.117	0.77	0.33	1.78	0.285
	96	0.88 + 0.120	0.33	0.15	0.72	0.012
M. anisopliae	24	0.94 + 0.129	6.43	2.55	16.22	0.075
	48	0.94 + 0.125	3.50	1.61	7.60	0.108
	72	0.93 + 0.121	2.09	1.02	4.30	0.001
	96	0.89 + 0.118	0.47	0.23	0.97	0.382
Percent Mortality in 5th Instars larvae						
Barazide	24	205 + 0.047	4448.07	240.14	82,390.60	0.000
	48	201 + 0.046	628.57	50.10	7885.55	0.018
	72	189 + 0.048	27.80	4.07	189.68	0.014
	96	179 + 0.052	2.79	0.37	20.96	0.004
B. thuringiensis var. *kurstaki*	24	0.93 + 0.123	4.09	1.59	10.53	0.247
	48	0.92 + 0.119	1.88	0.77	4.61	0.258
	72	0.90 + 0.117	0.73	0.34	1.58	0.004
	96	0.880.123	0.19	0.08	0.46	0.072
B. bassiana	24	0.94 + 0.128	6.35	2.49	16.21	0.441
	48	0.93 + 0.121	2.33	1.11	4.90	0.495
	72	0.91 + 0.118	1.25	0.64	2.45	0.029
	96	0.88 + 0.119	0.38	0.19	0.78	0.103
M. anisopliae	24	0.94 + 0.131	9.14	3.34	25.01	0.406
	48	0.93 + 0.123	2.67	1.28	5.58	0.275
	72	0.92 + 0.119	1.40	0.70	2.79	0.330
	96	0.89 + 0.118	0.41	0.19	0.91	0.588

3.2.3. Effect against 5th instar larvae

After 96 h of exposure, the treatment with Barazide alone at an inoculation level of 0.1 % resulted in the highest observed mortality, measuring 82.00 ± 1.72. After a 24-h period, the lowest death rate was seen at a 0.008 % inoculation dose. After 96 h of exposure, the probit analysis test revealed the LC50 value at dose 2.79 % with 95 % FL: 0.37–20.96. After 96 h, *B. thuringiensis* caused 78.00 ± 2.91 percent mortality at a dose of 1.5 %. In contrast, after 24 h, the minimum mortality at the 0.5 % inoculation dose was 25.40 ± 3.24. With a 95 % confidence interval of 0.08–0.46, *B. thuringiensis* var. *kurstaki* exhibited the lowest LC_{50} value of 0.19 %. After 96 h of exposure, the percent mortality of *B. bassiana* and *M. anisopliae* at inoculation level 1.5 % was reported to be 73.00 ± 4.16 and 69.00 ± 3.46, respectively. After a 24 h period, the lowest inoculation level of 0.5 % yielded the lowest fatality rates (Table 3). In *B. bassiana* and *M. anisopliae*, the probit analysis test revealed LC_{50} values at doses of 0.38 % and 0.41 % with 95 % FL: 0.19–0.78 and FL: 0.19–0.91, respectively (Table 4).

After 96 h of exposure, the combination treatment of *B. thuringiensis* var. *kurstaki* (1.5 %) and Barazide (0.1 %) resulted in a greater mortality rate of 96.10 ± 3.54. After 96 h, the highest inoculation dose showed a substantial amount of death at a $p < 0.05$ level,

Table 5
Pooled mortality data of chemical and bio-insecticides in combination against 3rd, 4th and 5th instars larvae of *S. litura* under laboratory conditions.

Percent mortality against 3rd Instar larvae						
Pesticide Name	Susceptibility (h)	Mean + SE	LC_{50} (μg/mL)	95 % fiducial limit		Person's X^2
				Lower limit	Upper limit	
Barazide + *B. thuringiensis* var. *kurstaki*	24	174 + 0.052	27.00	8.08	117.08	0.004
	48	160 + 0.049	12.97	1.43	90.26	0.003
	72	155 + 0.062	5.15	1.38	19.22	0.001
	96	–	–	–	–	–
Barazide + *B. bassiana*	24	202 + 0.046	650.70	30.39	13,931.89	0.022
	48	194 + 0.047	78.04	13.77	442.04	0.001
	72	186 + 0.050	6.88	0.86	54.67	0.104
	96	160 + 0.063	1.75	0.34	8.88	0.033
Barazide + *M. anisopliae*	24	206 + 0.047	1794.48	147.96	21,763.78	0.001
	48	202 + 0.046	548.52	48.18	6243.65	0.006
	72	197 + 0.047	98.33	9.81	985.36	0.092
	96	168 + 0.055	7.88	1.92	32.25	0.041
Percent mortality against 4th Instar larvae						
Barazide + *B. thuringiensis* var. *kurstaki*	24	198 + 0.047	54.15	2.77	1056.06	0.000
	48	196 + 0.048	1.27	0.01	113.89	0.210
	72	184 + 0.051	4.42	0.54	36.20	0.009
	96	123 + 0.088	7.63	2.85	20.39	0.014
Barazide + *B. bassiana*	24	202 + 0.046	430.45	53.11	3488.62	0.004
	48	198 + 0.047	74.04	4.87	1125.42	0.042
	72	190 + 0.049	13.47	1.57	115.54	0.003
	96	173 + 0.054	4.98	0.98	25.26	0.092
Barazide + *M. anisopliae*	24	209 + 0.047	4767.75	431.01	52,739.83	0.081
	48	207 + 0.047	1422.63	171.45	11,804.25	0.018
	72	201 + 0.047	294.26	53.90	1606.50	0.095
	96	182 + 0.051	5.12	0.74	35.23	0.000
Percent mortality against 5th Instar larvae						
Barazide + *B. thuringiensis* var. *kurstaki*	24	204 + 0.047	945.13	94.72	9430.06	0.059
	48	197 + 0.048	128.48	20.79	793.91	0.023
	72	176 + 0.051	35.72	10.94	116.66	0.212
	96	130 + 0.076	12.36	4.98	30.70	0.010
Barazide + *B. bassiana*	24	209 + 0.047	9893	613.57	159,520.20	0.039
	48	203 + 0.046	613.41	67.51	5572.85	0.000
	72	197 + 0.047	112.51	15.64	809.03	0.002
	96	179 + 0.052	5.74	0.95	34.59	0.007
Barazide + *M. anisopliae*	24	211 + 0.048	21,435	1275	360,325	0.028
	48	206 + 0.047	982.14	130.73	7378.34	0.002
	72	199 + 0.047	181.57	24.18	1363.17	0.000
	96	187 + 0.050	4.03	0.39	40.87	0.001

according to Pearson's chi-square test. After 96 h of exposure, the probit analysis revealed the LC_{50} value at dose 12.36 % with 95 % FL: 4.98–30.70. Barazide (0.1 %) caused greater mortality rates of 81.40 ± 3.56 and 76.40 ± 3.22, respectively, with *B. bassiana* and *M. anisopliae*@1.5 %. The application of pesticides in combination showed its significance at $p < 0.05$ level (Table 3). The lowest LC_{50} value were recorded at dose 5.74 % in Barazide + *B. bassiana* with 95 % FL: 0.95–34.59 and in Barazide + *M. anisopliae* at dose 4.03 % with 95 % FL: 0.39–40.87 respectively (Table 5). According to our research, Barazide@0.1 % combination with higher concentrations of *B. thuringiensis* var. *kurstaki*, *B. bassiana* and *M. anisoplae* @1.5 % increased the larval mortality in an additive manner. Only Barazide @0.1 % +*B. thuringiensis* var. *kurstaki* @0.5 % enhanced the mortality and the interaction turned out to be synergistic against 5th instar larvae. The increased mortality due to the additive effect as confirmed by Chi-square test, indicated that mortality observed in the combination treatments was caused by independent action of both microbial isolates and insecticide whereas synergistic interaction demonstrated a significant interaction between two treatments.

The decreasing trend in the observed mortality among insecticides alone is Barazide (95.80 ± 1.16, 85.30 ± 1.85 and 82.00 ± 1.72) > *B. thuringiensis* var. *kurstaki* (88.70 ± 1.01, 79.90 ± 2.01 and 78.00 ± 2.91) > *B. bassiana* (82.60 ± 2.46, 73.90 ± 2.46 and 73.00 ± 4.16) > *M. anisopliae* (78.60 ± 1.46, 68.90 ± 2.96 and 69.00 ± 3.46) after 96 h at its highest inoculation level against 3rd, 4th and 5th instar larvae. Similarly, the increasing trend in the observed mortality among insecticides used in combination is Barazide + *B. thuringiensis* < Barazide + *B. bassiana* < Barazide + *M. anisopliae*.

4. Discussion

B. thuringiensis var. *kurstaki*, *B. bassiana* and *M. anisopliae* (commercial formulations) were evaluated in the laboratory and found to show highest pathogenicity against 3rd followed by 4th and 5th instars larvae of *S. litura*. Among at theses biopesticides entomopathogenic bacteria was more effective as compared to entomopathogenic fungus. Similar observations were obtained by Liu et al. [22], Thakur et al. [28] and Sharma et al. [49]. It has been reported the efficacy of commercial formulations of *B. thuringiensis*, *B. bassiana*

and *M. anisopliae* against the 3rd and 4th instars larvae of *S. litura* and observed that it caused more mortality in the 3rd followed by 4th instar larvae in laboratory. They also noticed highest mortality caused by *B. thuringiensis* followed by *B. bassiana*. As per report of Vega-Aquino et al. [50], entomopathogenic fungi provide significant potential for managing lepidopterous insect pests. This is corroborated by the current investigation, whereby entomopathogenic fungi shown acceptable outcomes against various larval stages. The above findings were further confirmed by laboratory bioassays showing *M. anisopliae* efficacy against the different larval instars of *H. armigera* [51]. Kumar et al. [52] conducted an experiment on genetically modified *M. anisopliae*, revealing enhanced virulence and good growth against model insects on dead cadavers. Wang et al. [53] stated that *B. thuringiensis* and *B. bassiana* are the most studied biopesticides that invade and kill insect hosts by producing a secondary metabolites (toxins) such as bassianolide, beauvericin, beauverolides, bassianin, tenellin, oxalic acid and oosporein. Thakur et al. [28] also analysed the virulence of indigenous bio-agent, entomopathogenic nematode and found them effective against *H. armigera*, *S. litura* and *Agrotis segetum*. Sajid et al. [54] also evaluated the toxicity potential of *B. thuringiensis* against 2nd instar larvae of *S. litura* and reported maximum mortality after 72 h of exposure and also stated that microbial control is the best eco-friendly tactic to manage insect pests under laboratory conditions. Earlier there are many reports on the bacterium, *B. thuringiensis* produces Bt-toxin (Cry), which works against the lepidoptera, diptera and coleoptera through binding to their midgut epithelium cell surface that making pores in the apical microvilli membrane and causing death [55–58]. During pathogenesis phase, spore of *B. bassiana* and *M. anisopliae* settle on the cuticle of insect pests where hyphae development takes place which discharge their extracellular hydrolytic enzymes [59–62]. These enzymes assisting its penetration to insect cuticle leads to spores germination and enter the integument by forming appressorium. As a result fungal hyphae develop on hypodermis and continue to multiply in the body and blood cells that disables and crumbles the host immune system leading to the death of insects.

In the present investigation, chemical insecticide Barazide formulated with the Novaluron emamectin benzoate (5.25 % + 0.9 % S. C., Adama) are found most effective among insecticides used against different instar larvae of *S. litura* after 96 h of time exposure. Sreedhar [63] evaluated novaluron + emamectin benzoate against *S. litura* and observed that >90 % mortality up to 8 days after spray in field experiment. Earlier, efficiency of emamectin benzoate against different instar stages of *S. litura* is well recognized [64–66]. Similarly efficacy of novaluron 5.25 %+emamectin benzoate against this insect pest has been reported [66].

This experiment also revealed that the biopesticides used in this bioassay experiment *B. thuringiensis* var. *kurstaki*, *B. bassiana* and *M. anisopliae* are compatible with chemical insecticide Barazide. Our findings are in conformation with Amizadeh et al. [67] who investigated the interaction between various chemical insecticides and *B. thuringiensis* var. *kurstaki* (Bt) but against *Tuta absoluta*. Similar results were also reported by de Souza et al. [68] conducted *in vitro* studies to evaluate the compatibility of chemical insecticides with *B. thuringiensis* in Petri dishes and measured the colony growth and observed that the chemical products that allowed growth more than the control were more compatible. The insecticides carbosulfan, tiametoxan, diafentiuron, acetate, imidacloprid, cyproconazol + thiametoxan and thiametoxan at different concentration were compatible and not affecting the growth of *B. thuringiensis* [69,70]. In the current study, compared to the control, the chemical pesticide Barazide did not reduced Bt colonization. Agostini et al. [71] also performed a compatibility test with lambda-cyhalothrin and thiametoxan and observed no inhibition in the growth of *B. thuringiensis* colonies. Earlier, Khun et al. [72] detected the compatibility of *M. anisopliae* and *B. bassiana* with chemical insecticides and fungicides. They reported indoxacarb, trichlorfon and acephate compatible with *M. anisopliae*, while *B. bassiana* displayed compatibility with acephate, trichlorfon, indoxacarb, spinetoram and sulfoxaflor. Earliar, Moorhouse et al. [73] also recorded no effect of insecticides and fungicides on the *M. anisopliae* on spore germination. Niassy et al. [74] also observed the compatibility of *M. anisopliae* isolate ICIPE 69 with 12 different agrochemicals and reported abamectin among them highly compatible. The compatibility of 27 insecticides, 10 fungicides and 8 herbicides with *M. anisopliae* in laboratory conditions [75].

Different combination treatments carried out in the present studies induced higher larval mortality in *S. litura* than individual bacterial or fungal treatments. Our findings indicated that as compared to the individual application of insecticide, the combined treatment at high dose increased the larval mortality in an additive manner. However, low doses of biopesticides along with insecticide Barazide 0.1 % enhanced the mortality and the interactions of microbes and insecticide turned out to be synergistic. The increased mortality due to the additive effect as confirmed by Chi-square test, indicated that mortality observed in the combination treatments was caused by independent action of both microbial isolates and insecticide whereas synergistic interaction demonstrated a significant interaction between two treatments. The interaction among the insecticides may be additive, synergistic and antagonistic therefore many scientists suggested to assess the compatibility of microbial agents with chemical products [76,77]. Improper insecticide use can lead to pest resistance, environmental contamination, and human intoxication [78,79]. An alternative strategy involves combining insecticides with microbial pathogens, causing higher larval mortality and DNA damage. Our results indicated that combination treatments caused more stress which further enhances pathogenicity and mortality in *S. litura* larvae. The studies are in line with the previous reports indicating genotoxicity due to insecticide exposure to various insects [31,80–84].

Combined application of biological (bacteria, fungi, viruses and nematodes) and chemical products are among the efficient IPM strategy throughout the world [79,85]. The intensity impacts of chemicals on the species and the lineage of pathogens, nature of pesticides, its concentration, time of exposure, and inert materials in the formulation of product used [76,86]. In the present bioassay experiment, the younger instar larvae were recorded more susceptible in which mortality were cent percent after 72 exposure of time at highest dose of Barazide + *B. thuringiensis* var. *kurstaki*. The results also revealed that with the increase in larval instars mortality rate decreased. The studies are in conformity with Liu et al. [87] who noticed more susceptible nature of younger larvae than the other larval stages.

In the present investigation, all four different treatments separately and in combination with chemical Barazide reduced the pest density in *in-vitro* assay. The hierarchy of combined treatments used in the present study reported is Barzide + *B. thuringiensis* var. *kurstaki*, Barazide + *B. bassiana*, Barazide + *M. anisopliae* in the descending order. This laboratory studies showed that integration of

two different methods by using biopesticides in combination with chemical insecticide, are less toxic to the environment and minimize ecological impact. The growing awareness of the negative consequences of chemical control highlights the need for sustainable practices in agriculture. Some researchers also suggested that among the different insect management tactics, chemical control demonstrated as more effective method, but at the same time are highly expensive, more toxic to soil micro-flora, livestocks, plants and fauna [14,88,89]. Keeping in view the negative impact of synthetic chemicals, governments demand environmentally safe alternatives against chemicals, which are less toxic with less mobility to avoid the ground water contamination and inadequate affects on the non-target organisms.

5. Conclusions

Barazide in combination with *B. thuringiensis* var. *kurstaki* is the most effective treatment after 72 h and causes cent percent followed by 91.70 percent mortality in combination of Barazide with *B. bassiana*. Used treatments are reported to be best and showed synergistic potential in combination. Integration of synthetic chemical Barazide and biological insecticides *B. thuringiensis* var. *kurstaki* are concluded to be the most effective and less environmental polluting method in controlling the population of the *S. litura* in *in-vitro* experiment and could be recommended in integrated manner for the management of population of this lepidopteron insect pest. In future field trials should be conducted to validate the long-term effectiveness of combined treatments under diverse environmental conditions. The impact on non-target organisms may be studied to ensure safety and ecological balance. The optimal application rates and timing will be determined to maximize efficacy and minimize costs. New formulations have to be developed to enhance the stability and compatibility of microbial agents with chemical insecticides. Ecotoxicological studies should be conducted to understand potential risks and establish safe usage guidelines.

Data availability statement

Not applicable.

Institutional review board statement

Not applicable.

Informed consent statement

Not applicable.

Funding

The authors would like to extend their sincere appreciation to the Researchers Supporting Project Number (RSP2024R134), King Saud University, Riyadh, Saudi Arabia.

CRediT authorship contribution statement

Anuja Sharma: Writing – original draft, Investigation, Data curation. **Neelam Thakur:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Conceptualization. **Abeer Hashem:** Writing – review & editing. **Turki M. Dawoud:** Writing – review & editing. **Elsayed Fathi Abd_Allah:** Writing – original draft, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors are thankful to the Vice Chancellor and Pro Vice Chancellor Eternal University, Baru Sahib for providing necessary laboratory facilities. The authors would like to extend their sincere appreciation to the Researchers Supporting Project Number (RSP2024R134), King Saud University, Riyadh, Saudi Arabia.

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