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RESEARCH ARTICLE

Biological control of tephritid fruit flies *Bactrocera* spp. in Himachal Pradesh, India

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Abstract

Over the years, chemical-based insecticides have been used to enhance crop yield in the agricultural industry. However, the hazards associated with these insecticides have highlighted the need for an alternative method that is economical, eco-friendly, and safe. In this investigation, the efficacy of various biological materials, such as Neem kavach, *Beauveria bassiana* (Balsamo), and clay, was evaluated against fruit flies under open field conditions. The results revealed that the highest infestation was recorded in the control (71.67 %), while the lowest infestation (25.67 %) was observed in the plot treated with *B. bassiana* (1.5 %). This was followed by Neem kavach-treated plot (4 %) with an infestation rate of 26.67 %, and the clay-treated plot (15 g/L) with an infestation rate of 38.67 %, after the third spray. The impact of these biological agents on cucumber yield was also evaluated. The highest yield, 9.36 kg/plot, was obtained from the *B. bassiana*-treated plot, followed by Neem kavach-treated plots with yields of 8.94 and 7.28 kg/plot, respectively. The lowest yield of 4.58 kg/plot was recovered in the untreated plots. These findings suggest that the application of these biological agents is highly effective, as they repel egg-laying (oviposition deterrence), thereby minimizing fruit infestation and maximizing yield profit.

Keywords

biopesticides; biological control; *B. bassiana*; *C. sativus*; cucumber

Introduction

Tephritid flies are a highly destructive group of insect pests that cause significant economic losses in agriculture, particularly affecting a variety of vegetables, fruits, and flowers (1, 2). Commonly known as "fruit flies" due to their close association with fruits and vegetables (3), these pests inflict extensive damage on many economically important crops. Among the most affected species are cucumber, sweet melon, sweet pepper, pumpkin, sponge cucumber, wax apple, and tomato (4, 5).

India is a major producer of fruits and vegetables, which are crucial sources of nutrition for its large population. With increasing globalization, the country faces the dual challenge of feeding its own population and exporting fruits and vegetables to other countries. Cucumber (*Cucumis sativus* Linnaeus) is one of the most important and popular vegetable crops grown worldwide. In India, cucumbers and gherkins cover an area of 26,088 ha, producing a total of 123,846 metric tonnes valued at 114 million USD. Globally, the estimated total cucumber production is 91,258,272 metric tonnes (6, 7).

About 50 % of damage to cucurbits is attributed to fruit fly infestations (8). *Bactrocera cucurbitae* (Coquillett) and *Bactrocera tau* (Walker) cause significant losses, including direct damage to fruits that leads to decreased quality, yield, and marketability, as blemished fruits are rejected by customers (9-13). These fruit flies not only affect cucurbitaceous vegetables and fruits but also solanaceous fruits (14). The total global damage caused by tephritid fruit flies is estimated to exceed US$2 billion annually, encompassing harvesting, packing, and marketing losses (12). Due to their highly invasive nature, fruit flies are considered federal quarantine pests in India and many other countries, as they cause extensive damage to a wide range of fruits and vegetables, particularly cucurbitaceous vegetables (13). Fruit flies puncture the fruit skin and oviposit beneath it. The eggs hatch, and larvae at all instar stages cause damage to the fruit, typically leading to fruit falling to the ground just before or during the maggot pupation stage. This results in a reduction in both the yield and quality of harvestable fruit (15). Managing fruit flies is particularly challenging due to their high reproductive potential, wide host range, adaptability to various climates, and overlapping generations (5).

Fruit flies lay their eggs at both the tender and ripening stages of fruits. Once hatched, the maggots feed on the fruit pulp, causing premature ripening. This leads to fruits falling off the plants and rotting, with most infested fruits decaying on the ground (16). Various management techniques, including chemical insecticides, have been employed against fruit flies. However, chemical insecticides have several drawbacks, such as environmental harm, pest resistance, and adverse effects on natural enemies and non-target species (17). Due to increased public awareness about environmental issues and the growing demand for organic products, the use of chemical insecticides has decreased. Consequently, the reliance on chemical insecticides and pesticides for pest control is becoming less socially, environmentally, and economically viable (18).

Biological control has proven effective for managing fruit flies, given the low tolerance threshold in commercial fruit production (19, 20). Consequently, it is imperative to identify management strategies that are safe for humans, non-target biological systems, and the environment. In this context, the present investigation focuses on the effective management of tephritid flies using eco-friendly alternatives, including the application of biopesticides and clay.

Materials and Methods

General cultures of *B. cucurbitae* were established from infested cucumber fruits collected from the field. These fruits were placed in rearing cages measuring 90 × 45 × 45 cm, which were equipped with a removable tray at the base filled with sieved and sterilized sand up to a height of 20-25 cm. Adult fruit flies were provided with cucumber fruit for oviposition. Additionally, a mixture of dry glucose and protein hydrolysate (Protinex®, Pfizer Ltd.) in a 1:1 ratio was supplied in a petri dish as food for the adult fruit flies. The host fruits and the diet were replenished as needed. To facilitate egg laying, the fruits were slightly peeled on one side. The bases of the rearing cages were immersed in plastic cups or plates filled with water to prevent predatory ants from entering. The rearing was done at 26±2°C temperature and 70% relative humidity.

The study was conducted on an experimental farm located at 30° 75' 60'' north latitude, 77° 29' 90'' east longitude, at an elevation of approximately 1900 m (6233 ft) above mean sea level. Cucumber seeds (var. Amrit F_1 hybrid) were purchased from a nearby market and sown in coco pits with a soil and FYM (farmyard manure) mixture in a 1:1 ratio. The average temperature during the study ranged from 17 to 20 °C, with an average relative humidity of 75-81 %, under a light regime of 12 hrs light and 12 hrs dark. The experiment was conducted under open field conditions. An area of 60 sq m was prepared and ploughed. From this ploughed area, 28 plots were established, each measuring 2 sq m. The cucumber plants were transplanted into these plots at a density of 5 plants per plot, with a planting distance of 50 cm, following a randomized block design (RBD). Regular watering was carried out, and Agrobium® (NPK-8:25:25) was applied at a rate of 2.5 g per plant as fertilizer.

To initiate fruit fly infestation, cultured fruit flies were released into the field at a rate of 30 pairs of adults and 20 pupae per plot. Biological materials (Neem kawach and *Beauveria bassiana* Balsamo), along with clay powder, were procured from the market and applied against *Bactrocera* spp. to evaluate their effectiveness in managing fruit fly infestation in cucumber. The first foliar application was administered prior to the fruit's ripening stage, followed by two additional applications at 10- days intervals, total three sprays were applied. The required concentrations (Table 1) were prepared by mixing the test materials with water and applied using a knapsack sprayer on the cucumber crop. The experiment was conducted twice, with each treatment having four replicates. Data were collected before and after each spray at 10-day

Table 1. List of different treatments along with their concentrations.

Treatment	Concentration (%)	Application time	Observations
Neem kavach (1500 ppm)	2.0	5:30-6:30 PM	Fruit infestation and yield
	4.0	5:30-6:30 PM	Fruit infestation and yield
***Beauveria bassiana* (1.0 % W.P)**	1.0	5:30-6:30 PM	Fruit infestation and yield
	1.5	5:30-6:30 PM	Fruit infestation and yield
Clay powder	10	5:30-6:30 PM	Fruit infestation and yield
	15	5:30-6:30 PM	Fruit infestation and yield
Control	Water only	5:30-6:30 PM	Fruit infestation and yield

intervals to assess the efficacy of the treatments. The impact on oviposition deterrence was also evaluated. Statistical analysis was performed using OPSTAT software, applying a two-factor analysis of variance. The critical difference was determined by comparing treatments at $p < 0.05$.

Results

Developmental biology of B. cucurbitae

The developmental biology of *B. cucurbitae* was studied under laboratory conditions (Table 2). Results from the investigation show that the incubation period ranged from 1 to 3 days, with an average of 2 days, and hatchability was 93 %. The total larval period varied from 4 to 7 days, averaging 5.5 days, while the pupal period ranged from 9 to 12 days, with an average duration of 10.5 days. Thus, the entire developmental period of *B. cucurbitae* was 19.5 days. Regarding reproductive biology, the oviposition period for females ranged from 30 to 38 days, with an average of 34 days, while for males it ranged from 28 to 36 days, averaging 32.25 days. The oviposition and post-oviposition periods ranged from 28 to 32 days and 2 to 5 days, respectively, with average durations of 30 days and 4.5 days. During its lifespan, a female *B. cucurbitae* laid between 180 and 250 eggs, averaging 233.5 eggs per female. The sex ratio of *B. cucurbitae* was 1:2 (male to female).

Effect on fruit infestation

Three biological agents—Neem kavach, *B. bassiana*, and clay—were applied at various concentrations, alongside a control treatment, as foliar sprays on cucumber plants to manage and mitigate infestations caused by fruit flies, specifically *Bactrocera* spp. (Fig. 1). The results indicated that the lowest infestation rate of 39 % occurred after the initial application of *B. bassiana* at a concentration of 1.5 %. This was followed by Neem kavach at 4 % concentration, which resulted in a 40.33 % infestation rate, and clay powder at 15 % concentration with an infestation rate of 48.33 %. Significant differences in infestation rates were observed among the various treatments targeting fruit flies, with the highest infestation rate recorded in the control group at 63.67 %.

Table 2. Developmental biology and reproductive phases of *Bactrocera cucurbitae.*

Biological parameter		Duration (days)		Survival (%)
		Mean ± SE	Range (days)	
Incubation period		2±0.645	1-3	93
Total larval period		5.5±0.645	4-7	93
Pupal period		10.5±0.645	9-12	53
Total developmental period		19.5±0.645	18-21	-
Pre-oviposition period		18.5±0.645	15-20	-
Oviposition period		30±0.816	28-32	-
Post-oviposition period		4.5±1.323	2-5	-
Adult longevity	**Male**	32.25±1.65	28-36	-
	Female	34±1.41	30-38	-
Fecundity (eggs/female)		233.5±27.21	180-250	-

The infestation rates (oviposition) decreased to 36 %, 37.33 %, 45 %, and 65.67 % after the second spray, and further to 25.67 %, 26.67 %, 38.67 %, and 71.67 % after the third spray of *B. bassiana* (1.5 %), Neem kavach (4 %), clay (15 %), and the control, respectively (Table 3). Cucumber plants treated with these different solutions exhibited significantly lower fruit infestation rates (oviposition deterrence) compared to untreated plants ($F = 4.58$, $DF = 6$, $p < 0.05$). All treatments were notably more effective than the control. Fruit infestation continued to decrease across all treatments after the third spray. When comparing the

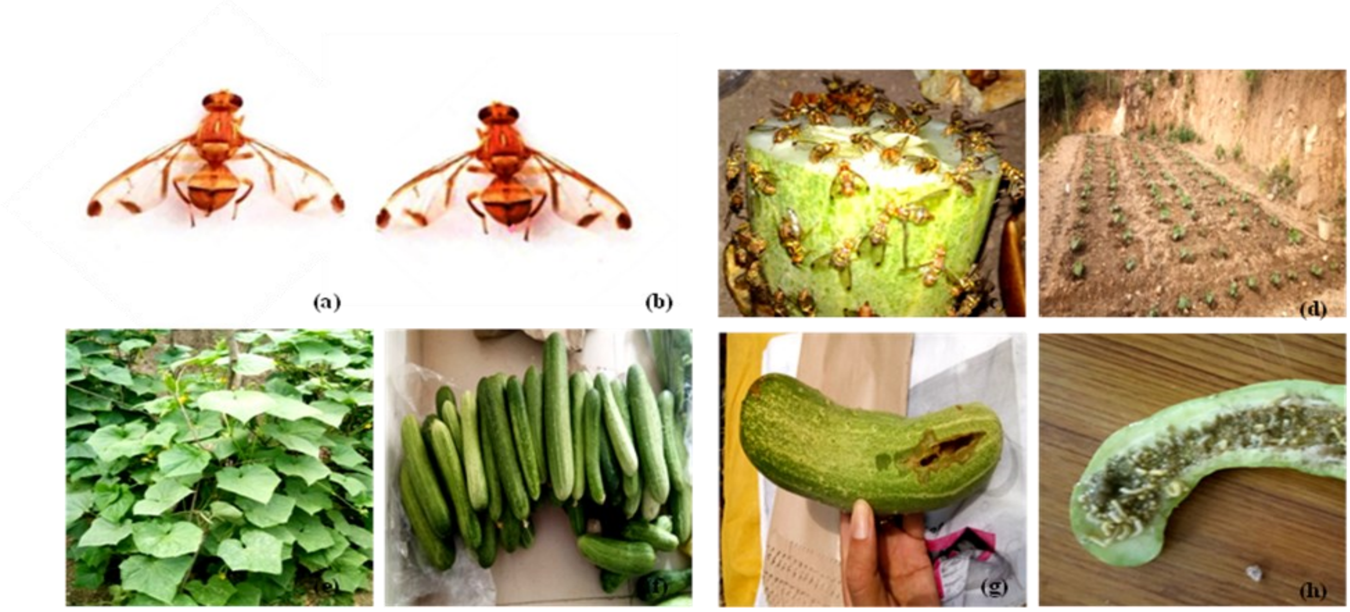

Fig. 1. Effect of biopesticides on *Bactrocera cucurbitae* on cucumber plants (a) *B. cucurbitae* (female); (b) *B. cucurbitae* (Male); (c) Adults on cucumber; (d) Cucumber crop grown in open field condition; (e) Applications of bio-agents; (f) Harvested cucumber fruits; (g-h) Infested fruits.

Table 3. Field efficacy of biopesticides and clay on fruit fly infestation in cucumber.

Treatment	Concentration (%)	Fruit infestation (%) at 10-day interval			Mean fruit infestation (%)
		After spray I	After spray II	After spray III	
Neem kavach (1500 ppm)	2.0 %	45.67 (42.49)	40.67 (39.58)	30.00 (33.15)	38.78 (38.41)
	4.0 %	40.33 (39.39)	37.33 (37.59)	25.67 (30.34)	34.44 (35.78)
Beauveria bassiana (1.0 % W.P)	1.0 %	42.00 (40.37)	39.33 (38.80)	31.33 (34.01)	37.55 (37.73)
	1.5 %	39.00 (38.80)	36.00 (36.82)	26.67 (30.93)	33.89 (35.45)
Clay	10 %	51.67 (45.94)	49.67 (44.79)	45.00 (42.10)	46.34 (44.28)
	15 %	48.33 (44.02)	45.00 (42.10)	38.67 (38.42)	44.00 (41.52)
Control		63.67 (52.94)	65.67 (54.14)	71.67 (57.85)	67.00 (54.98)

$CD_{0.05}$; Treatment (T) = 2.76; Spray interval (I) =1.808; Interaction (T×I)= 4.78

means of the various treatments, the lowest infestation rate (33.89 %) was observed in cucumber plots treated with *B. bassiana*, followed by Neem kavach (34.44 %) and clay (41.52 %). In contrast, the control group showed a 67 % infestation rate. The critical difference was 7.50, and the Fisherman's Least Significant Difference (LSD) was 12.72. Pearson's correlation coefficients for yield were calculated as r = 0.52 after the first spray, r = 0.57 after the second spray, and r = 0.62 after the third spray, indicating the impact of the different treatments on fruit yield.

Effect on fruit yield

The fruit weight was also measured to assess the impact of various treatments on cucumber yield. The data concerning yield showed that the highest cucumber yield, 9.36 kg per plot, was achieved in plots treated with *B. bassiana* (1.5 %), followed by Neem kavach (4.0 %) and clay (15 %), which yielded 8.94 kg and 7.28 kg per plot, respectively (Fig. 2). In contrast, the lowest yield of 4.58 kg per plot was observed in the untreated plots. Significant variability in yield was noted after three consecutive sprays. The critical difference was 0.94, and Pearson's correlation coefficients for yield were calculated as r = 0.556 after the first spray, r = 0.46 after the second spray, and r = 0.55 after the third spray, highlighting the impact of the different treatments on fruit yield. The Fisherman's Least Significant Difference (LSD) was 8.77. Cucumber plants treated with the various solutions exhibited a significant increase in fruit yield compared to untreated plants ($F = 11.40$, $DF = 6$, $p < 0.05$).

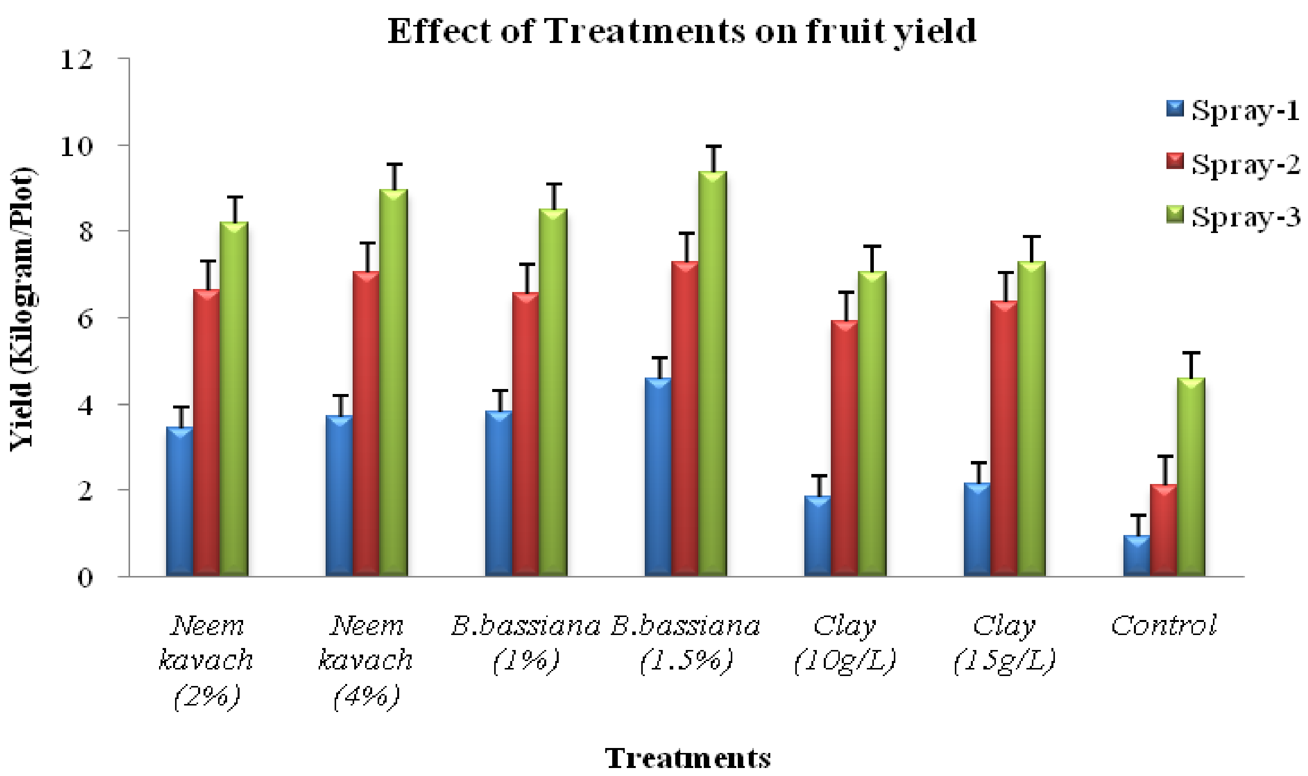

Fig. 2. Effect of different treatments on cucumber yield.

Discussion

During this study, it was observed that applications of *B. bassiana* were highly effective in reducing fruit fly infestations, followed by neem kavach and clay. These findings align with previous research indicating that non-volatile components of neem play a role in reducing egg laying (21). Oviposition deterrence has been documented for azadirachtin extracts against pests like the melon fly *B. cucurbitae* and the oriental fruit fly *Bactrocera dorsalis* (Hendel) (13, 22). Earlier studies have also reported reduced oviposition rates with aqueous neem extracts at concentrations of 3.0 % and 5.0 %, as well as neem oil (Neemix) at 4.5 % concentration against the Mexican fruit fly *Anastrepha ludens* (Anstlu) (23). Additionally, formulations such as neem seed kernel extract (NSKE) at 5 % concentration, bait spray (Malathion 50 g + molasses 500 g + 50 L water), and cypermethrin have been evaluated against fruit flies, resulting in minimal damage when applied consecutively, while untreated plots showed maximum damage to bottle gourd fruits, consistent with the current findings (24).

Reports indicated the lowest fruit damage percentage (7.33 %) occurred with commercial neem products (Nimbex 0.15 %), while the highest damage (54.33 %) was observed in untreated checks, consistent with the current study findings (25). Previously, Thakur explored the oviposition deterrence of fruit flies using neem, garlic, and Melia extracts at 3.0 % concentration each. Results showed substantial deterrence rates of 93.2 %, 92.3 %, and 91.2 % on cucumber fruits in laboratory conditions, supporting the present findings (26). Sharma and Kumar applied cypermethrin 25 EC and neem oil for managing fruit flies in cucumber, noting that cypermethrin 25 EC yielded 191.48 and 183.33 q/ha, respectively, followed by neem oil at 189.01 and 181.48 q/ha (27). These results align with Perri *et al.,* who reported significant reductions in fruit infestation levels in kaolin-treated trees compared to untreated ones (28), bolstering the outcomes of the current study. Additionally, research on the effects of kaolin and copper on olive fruit flies showed lower infestation levels and fewer oviposition stings per olive (29).

The current findings corroborate earlier studies by Thakur and Gupta, who investigated various concentrations of azadirachtin (0.005 %, 0.01 %, and 0.015 %), neem oil (1 %, 2 %, and 3 %), pongamia oil (1 %, 2 %, and 3 %), *B. bassiana* (0.1 %, 0.5 %, and 1 %), and clay (6 %, 8 %, and 10 %) for their effectiveness in deterring oviposition by the fruit fly *B. tau* on cucumber under laboratory conditions. They reported that azadirachtin (0.015 %), neem oil (3 %), pongamia oil (3 %), and clay (10 %) exhibited significant deterrence rates of 89.09 %, 80.24 %, 83.40 %, and 89.29 %, respectively, compared to the control (30). Similarly, research on the application of kaolin particles and cinnamon essential oil on sour cherry pests and fruit quality demonstrated reduced fruit injury in fruits treated with kaolin particle film, aligning with the findings of the present study (31).

Conclusion

In the present investigation, three biological agents were applied as foliar sprays on cucumber plants to manage and reduce fruit fly infestation. The results showed that applications of *B. bassiana* were highly effective in decreasing fruit infestation (oviposition deterrence) by fruit flies, followed by Neem kavach and clay. Maximum fruit damage was observed in the untreated plots. Additionally, it was found that higher concentrations of the bio agents, along with increased application and exposure times, resulted in greater oviposition deterrence. Therefore, it can be concluded that the application of these biological agents effectively repels egg-laying, thereby minimizing fruit infestation and maximizing yield profit.

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Authors' contributions

PT gave the concept. PT performed the experiment, wrote the manuscript. SS, SS, RS, PKR, AY and ANY contributed in the reviewing and drafting of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare that there is no conflict of interest.

Ethical issues: None.

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