ENTOMOLOGY

Azadirachtin in combination with emamectin benzoate and abamectin increases efficacy against brinjal shoot and fruit borer, *Leucinodes orbonalis* Guenee

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Abstract

Brinjal shoot and fruit borer (BSFB) is a serious insect pest of brinjal, causing significant damage and yield loss in Bangladesh. Chemical insecticides having toxic effects are frequently applied to control this pest. The present study was conducted with two insecticides, emamectin benzoate and abamectin individually or in combination with plant-origin azadirachtin to combat BSFB. The insecticides were applied at 7-day intervals using a knapsack sprayer. Though all the insecticides control BSFB, emamectin benzoate 1% + azadirachtin and abamectin 1.8% + azadirachtin were found to be most effective, providing 89.34% and 81.55% shoot infestation reduction, 93.34% and 85.11% fruit infestation reduction (number basis), and 94.91% and 85.27% fruit infestation reduction (weight basis), respectively. The highest marketable yield was obtained from abamectin 1.2% + azadirachtin (2.32-ton ha^{-1} $harvest^{-1}$). The same treatment confirmed the highest gross yield (2.72-ton ha^{-1} $harvest^{-1}$). However, the highest individual weight of marketable fruit was obtained from abamectin 1.2% + azadirachtin (47.30 g). Therefore, emamectin benzoate 1% + azadirachtin and abamectin 1.8% + azadirachtin can be recommended for BSFB control, while abamectin 1.2% + azadirachtin is recommended for increasing fruit yield. However, further investigation is required.

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Introduction

Brinjal (*Solanum melongena* Lin.) is a nutritious vegetable of the Solanaceae family. It contains bioactive phytochemicals like vitamins, minerals, polyphenols, isoflavones, antioxidants, *etc.* (Sharma & Koushik, 2021), like the high food value crop soybean (Khatun *et al.*, 2023) and cereal grains (Khatun & Mollah, 2024). These bioactive compounds have anti-obesity, anti-inflammatory, angiotensin-converting enzyme inhibitory, and many other properties (Khatun *et al.*, 2022; Khatun *et al.*, 2023). So, brinjal is used as a good appetizer, cardiotonic, aphrodisiac, laxative, and reliever of inflammation (Kalawate & Dethe, 2012). More than 100 varieties of brinjal are cultivated throughout the world, having variations in fruit size, shape, color, and taste (Shelton *et al.*, 2020).

Production of this high-value crop is affected by different insect pests, including brinjal shoot and fruit borer (BSFB), *Leucinodes orbonalis* G. (Mollah *et al.*, 2023), cotton aphid, *Aphis gossypii* G. (Mollah & Khatun, 2024), green leafhopper, *Amrasca biguttula biguttula* I. (Mollah, 2024a), whitefly, *Bemisia tabaci* G., and many others. Among these, the BSFB is abundant in the summer season while aphid, whitefly, and green leafhopper are in both the winter and summer seasons in brinjal or many other vegetables (Mollah, 2022; Mollah & Khatun, 2024). Insect pests infest brinjal plants at any stage of the plant, from seedling to fruiting (Latif *et al.*, 2010). At the vegetative stage, BSFB larvae enter the tender shoots, causing withering, drying, and drooping of the damaged shoots. In the fruiting stage, the larvae bore into developing fruit, feed inside, and cause ~50 % fruit damage (Mollah *et al*., 2022a). Sometimes, infestation may cause 30-60% yield loss, although the crop is frequently sprayed with chemical insecticides (Mondal & Akter, 2018). The frequent application of insecticides causes mortality to predators (Mollah *et al*., 2012a), parasitoids (Mollah & Khatun, 2023), nontarget insects (Mollah *et al*., 2022b), secondary pest outbreaks along with residual effects on the produces (Hussain *et al.*, 2020).

Nowadays, emphasis is put on the use of integrated pest management techniques (Singh *et al.*, 2007) that include plant extracts (Gokce, 2010; Mollah *et al*., 2017), microbial origin insecticides or microbes (Mollah *et al.*, 2022c; Ahsan *et al.*, 2024), biological control agents, pheromone traps, *etc*., being non-toxic and safe biodegradable alternatives to the conventional chemical control (Dolui & Debnath, 2010). These have also been reported to control different insect pests of brinjal (Sood *et al.*, 2023; Mollah & Khatun, 2024), country bean (Mollah *et al.*, 2012b; Mollah *et al.*, 2013a) or yard long bean (Amin *et al.*, 2017). The above-mentioned bioinsecticides are also reported to increase crop yield (Mollah *et al*., 2013b; Singh *et al*., 2018). We know the individual effect of plant-based azadirachtin or microbial-origin emamectin benzoate or abamectin on BSFB and the yield of brinjal, but we do not know their combined effect. Therefore, this study aims to observe the effect of azadirachtin in combination with emamectin benzoate or abamectin on the yield of brinjal by combating BSFB.

Materials and Methods

Study location and soil characters

The present study was conducted in the research field during the Kharif season at Gazipur, Bangladesh, with the local variety ‘Khotkhotia’ for evaluation of the combined effect of plant-based azadirachtin and microbial emamectin benzoate, or abamectin to combat BSFB, as well as their effect on fruit production. This area belongs to the Agro-Ecological Zone of Madhupur tract (AEZ-28) where p^H is around 5.8-6.5, and cation exchange capacity is 25.58 with silty clay loam textured soil (Mollah & Hassan, 2023). This area is positioned at 24.09ºN latitude, 90.26ºE longitude and 8.4 m above sea level.

Design of experiment and treatments

A randomized complete block design with four replications was adopted for this study. The plot was 2.8 m long, 1.4 m wide, and 0.7 m apart from rows and plants. Every plot consists of 8 plants in 2 rows. Watering, weeding, and fertilization were applied whenever necessary. The adopted treatments were: emamectin benzoate 1% (T1), emamectin benzoate 1% + azadirachtin 1% (T2), abamectin 1.2% (T3), abamectin 1.2% + azadirachtin 1% (T4), abamectin 1.8% (T5), abamectin 1.8% + azadirachtin 1% (T6) and untreated control (T7). Details of the treatments are provided in Table 1. The insecticides were provided by Russell IPM Ltd. (Deeside, UK). To prepare the spray mixture with insecticides, a certain amount of water was directly added to the sprayer tank to get the desired concentration. Treatments were applied weekly on the whole plant canopy with a knapsack sprayer, 500-750 L ha^{-1} depending on the plant canopy size. A combination of treatments was applied sequentially at alternative week. Each treatment was replicated in four plots. Insecticide mixtures were applied through spraying from the vegetative stage to the last harvest.

Collection and processing of data

Treated plants were observed regularly to record infested and healthy shoots as well as fruits. The number of infested and healthy shoots was recorded separately from all plants in a plot before the spraying date (6^{th} day) to determine the percentage of shoot infestation. Fruit harvesting from all the plants of each plot was also performed before the day of spraying (6^{th} day). Healthy fruits were separated from the infested fruits, and their weight and number were recorded separately for the percent fruit infestation calculation. For calculating gross yield, the yield of infested fruits was combined with the healthy fruits. The yield was expressed as ton/hectare. Dividing the total fruit weight by the total fruit number, the weight of each fruit was calculated. The percentage of shoot damage, fruit damage, and their change over control was calculated following Mollah and Hassan (2023) [Eqs. 1-3]:

$$\text{Percent (\%) shoot infestation} = \frac{\text{number of infested shoots}}{\text{total number of shoots}} \times 100 \quad \text{[Eq. 1]}$$

$$\text{Percent (\%) fruit infestation} = \frac{\text{number/weight of infested fruit}}{\text{total number/weight of fruit}} \times 100 \quad \text{[Eq. 2]}$$

$$\text{Percent (\%) change over control} = \frac{\text{mean of treatment} - \text{mean of control}}{\text{mean of control}} \times 100 \quad \text{[Eq. 3]}$$

Table 1. Information on the bioinsecticides used in this study.

Treatments	Active ingredients	Manufacturer	Dose
T1	Emamectin benzoate 1%	Russell IPM Ltd., UK	2 mL L^{-1} water
T2	Emamectin benzoate 1% Azadirachtin 1%		2 mL L^{-1} water 1 mL L^{-1} water
T3	Abamectin 1.2%		2 mL L^{-1} water
T4	Abamectin 1.2%		2 mL L^{-1} water
	Azadirachtin 1%		1 mL L^{-1} water
T5	Abamectin 1.8%		2 mL L^{-1} water
T6	Abamectin 1.8% Azadirachtin 1%		2 mL L^{-1} water 1 mL L^{-1} water
T7	Control	N/A	N/A

T1, emamectin benzoate 1%; T2, emamectin benzoate 1% + azadirachtin 1%; T3, abamectin 1.2%; T4, abamectin 1.2% + azadirachtin 1%; T5, abamectin 1.8%; T6, abamectin 1.8% + azadirachtin 1%; T7, untreated control; N/A, not available.

Statistical analysis and data presentation

Microsoft Excel was used for calculation and arranging the collected raw data. PROG GLM in the SAS program (SAS, 2002) was used for one-way analysis of variance of all the continuous variable data. Means were compared with the least significant difference test at the 0.05 level of type I error. Values presented in the graphs are the mean of 4 replications from 7 observations.

Results

Effect of insecticides on shoot infestation

The combination of insecticides played a significant ($p<0.05$) role in reducing the infestation on shoots (Figure 1). From the treatments, T2 and T6 were most effective in decreasing the shoot infestation to 1.49±0.21% and 2.58±0.19%, respectively (Figure 1A). However, in control, shoot infestation was recorded at 13.98±1.02%. For reducing shoot infestation over control, T2 (89.34%) was found to be the most potent, which was followed by T6 (81.55%) (Figure 1B). However, the other treatments also reduced shoot infestation by more than 60% compared to control.

Insecticides successfully control fruit infestation

All the insecticides were found to be significant ($p<0.05$) in reducing the infestation in fruits by count (Figure 2). Among the insecticides, the lowest fruit infestation was observed for T2 (3.70±0.62%) and T6 (8.28±0.96%) (Figure 2A). The others were also found effective in controlling fruit infestation as T5 (11.05±1.29%), T1 (15.40±1.52%), T4 (15.71±1.41%), and T3 (18.93±1.57%) (Figure 2A).

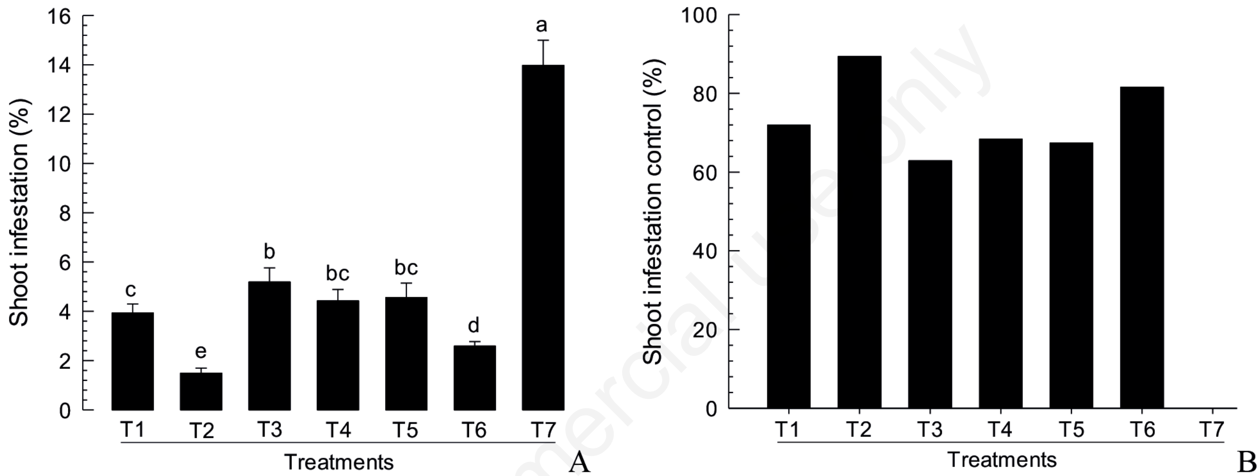

Figure 1. Effect of insecticides to reduce shoot infestation in brinjal. A) Percentage of shoot infestation; B) reduction of shoot infestation over control. Error bars represent standard deviation. Different letters above the error bar denote significant differences ($p>0.05$, least significant difference test) among the treatments. T1, emamectin benzoate 1%; T2, emamectin benzoate 1% + azadirachtin 1%; T3, abamectin 1.2%; T4, abamectin 1.2% + azadirachtin 1%; T5, abamectin 1.8%; T6, abamectin 1.8% + azadirachtin 1%; T7, untreated control.

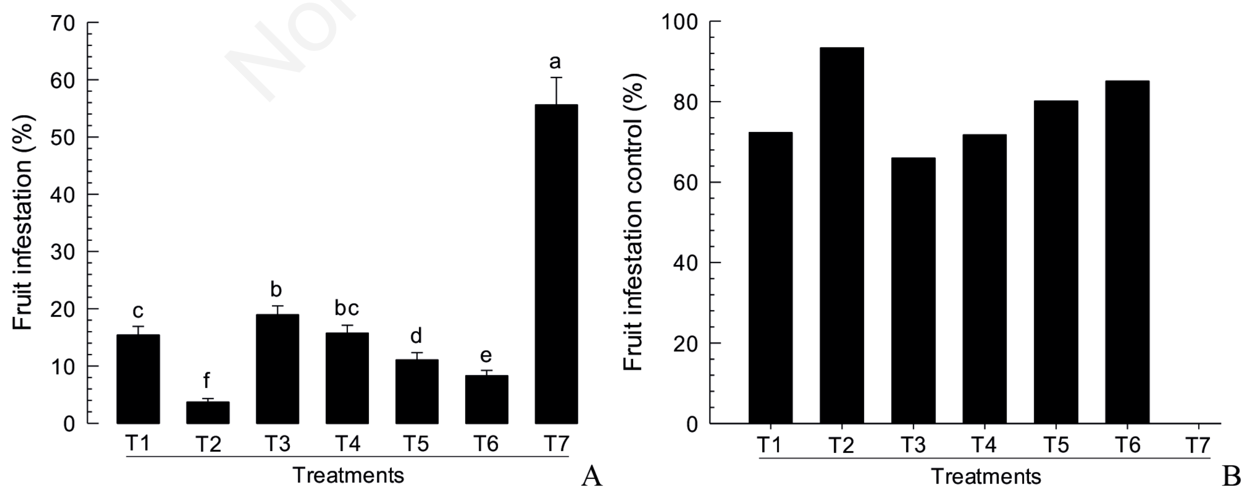

Figure 2. Role of insecticide to reduce fruit infestation (by count) in brinjal. A) Percentage of fruit infestation; B) fruit infestation reduction over control. Error bars represent standard deviation. Different letters above the error bar denote significant differences ($p>0.05$, least significant difference test) among the treatments. T1, emamectin benzoate 1%; T2, emamectin benzoate 1% + azadirachtin 1%; T3, abamectin 1.2%; T4, abamectin 1.2% + azadirachtin 1%; T5, abamectin 1.8%; T6, abamectin 1.8% + azadirachtin 1%; T7, untreated control.

However, the maximum infestation was observed for untreated control (55.59±4.80%). For fruit infestation reduction over control, T2 was found to be the most potential, providing 93.34% control, which was followed by T6, T5, T1, T4, and T3, providing 85.11%, 80.12%, 72.30%, 71.74%, and 65.95% reduction of fruit infestation, respectively (Figure 2B).

Similarly, insecticides have a significant ($p<0.05$) role in reducing fruit infestation based on weight (Figure 3). The minimum fruit infestation was observed for T2 (2.78±0.69%) which was followed by T6 (8.05±0.52%), T5 (10.71±0.67%), T1 (14.01±1.65%), T4 (14.80±1.17%), and T3 (16.66±1.22%). However, the maximum infestation was recorded from untreated control (54.66±4.85%) (Figure 3A). In the context of fruit infestation reduction over control, T2 (94.91% control) was found to be the most effective, followed by T6 (85.27% control), T5 (80.41% control), T1 (74.37% control), T4 (72.92% control) and T3 (69.54% control) (Figure 3B).

Insecticide for decreasing infested fruit yield

The applied insecticides significantly ($p<0.05$) decreased infested fruit yield (Figure 4). Infested fruit yield (ton ha^{-1} $harvest^{-1}$) was recorded at its lowest in T2 (0.04±0.02), followed by T3 (0.13±0.03) and T6 (0.14±0.02). The maximum yield was recorded from control (0.96±0.11), followed by T4 (0.40±0.07) (Figure 4A). In this context, T2 yielded the most effective reduction of infested fruits (93.83%), followed by T3 (86.67%) and T6 (85.73%) (Figure 4B).

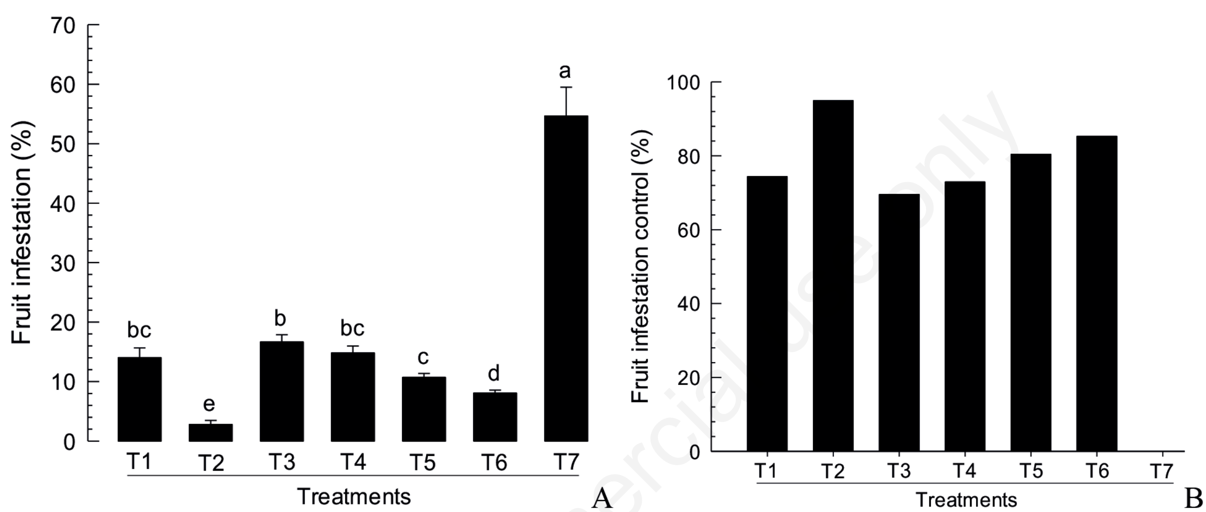

Figure 3. Role of insecticides for fruit infestation (weight basis) reduction in brinjal. A) Percentage of fruit infestation; B) Fruit infestation reduction by over control. Error bars represent standard deviation. Different letters above the error bar denote significant difference ($p>0.05$, least significant difference test) among the treatments. T1, emamectin benzoate 1%; T2, emamectin benzoate 1% + azadirachtin 1%; T3, abamectin 1.2%; T4, abamectin 1.2% + azadirachtin 1%; T5, abamectin 1.8%; T6, abamectin 1.8% + azadirachtin 1%; T7, untreated control.

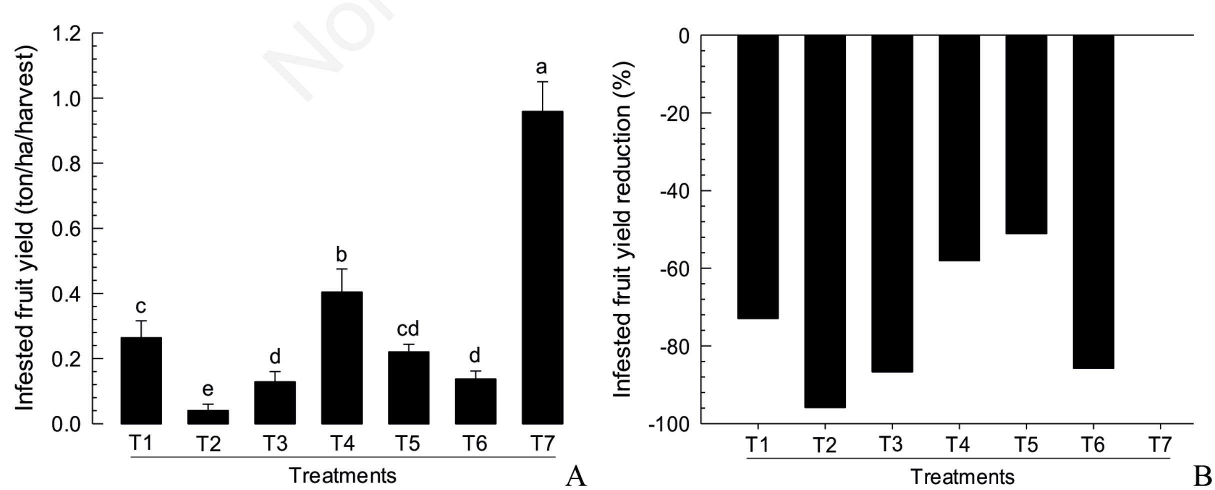

Figure 4. Role of insecticide to decrease infested fruit yield. A) Infested fruit yield; B) Percent reduction of infested fruit yield over control. Error bars represent standard deviation. Different letters above the error bar denote significant differences ($p>0.05$, least significant difference test) among the treatments. T1, emamectin benzoate 1%; T2, emamectin benzoate 1% + azadirachtin 1%; T3, abamectin 1.2%; T4, abamectin 1.2% + azadirachtin 1%; T5, abamectin 1.8%; T6, abamectin 1.8% + azadirachtin 1%; T7, untreated control.

Insecticides increase the marketable yield

The yield of healthy or uninfected or marketable fruit was also increased significantly ($p<0.05$) by treated insecticides (Figure 5).

Among the treatments, the highest marketable yield (ton ha^{-1} harvest^{-1}) was obtained from T4 (2.32±0.30), followed by T5 (1.84±0.21) and T1 (1.61±0.09); conversely, the lowest marketable yield was harvested from T3 (0.64±0.13), followed by T2 (1.35±0.39) and T6 (1.56±0.19).

However, for untreated control, it was 0.79±0.08-ton ha^{-1} harvest^{-1} (Figure 5A). The maximum increase of marketable fruit over control was found in T4 (193.67%), which was followed by T5 (132.91%), T1 (103.80%), T6 (97.47%), T2 (70.89%), T4 (59.62%) and it was decreased for T3 (18.99%) (Figure 5B).

Insecticide for gross yield

Insecticides had a significant ($p<0.05$) effect on the gross yield of brinjal (Figure 6). Maximum gross yield (ton ha^{-1} harvest^{-1}) was recorded from T4 (2.72), which was statistically different and followed by T5 (2.06) and T1 (1.87), T6 (1.70), T2 (1.39) and T3 (0.77) (Figure 6A) Control produced 1.75-ton ha^{-1} harvest^{-1}. Maximum increase of gross yield over control was recorded from T4 (55.43%) which was followed by T5 (17.71%), and T1 (6.86%) (Figure 6B). However, gross is decreased for T3 (56.00%), T2 (20.57%), and T6 (2.86%) (Figure 6B).

Insecticide affects individual fruit weight

Insecticides had a significant ($p<0.05$) effect on preventing weight loss. The healthy fruits gained higher individual weight compared to infested fruits in every case (Figure 7), but a significant difference was found in the case of T1 and T2. The highest individual weight of infested fruit was recorded in T4 (44.12±2.64 g) which was followed by T5 (43.68±3.93 g); whereas, in control, it was 42.47±2.13g and others were less than the control. On the contrary, for healthy fruits, the maximum was recorded from T4 (47.30±2.57

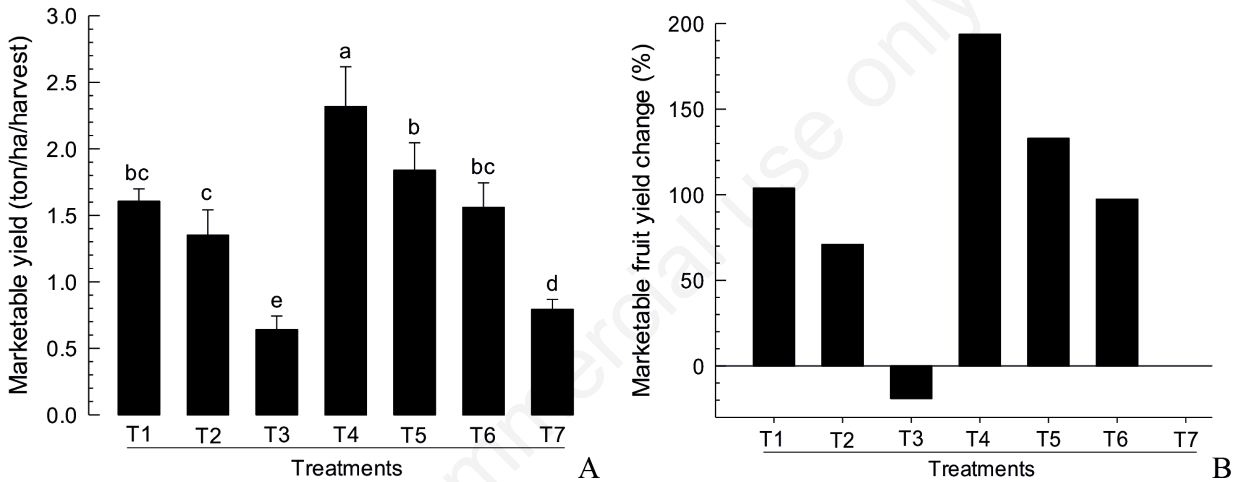

Figure 5. Role of insecticide on healthy fruit yield. A) Infested fruit yield; B) percent change of infested fruit yield over control. Error bars represent standard deviation. Different letters above the error bar denote significant differences ($p>0.05$, least significant difference test) among the treatments. T1, emamectin benzoate 1%; T2, emamectin benzoate 1% + azadirachtin 1%; T3, abamectin 1.2%; T4, abamectin 1.2% + azadirachtin 1%; T5, abamectin 1.8%; T6, abamectin 1.8% + azadirachtin 1%; T7, untreated control.

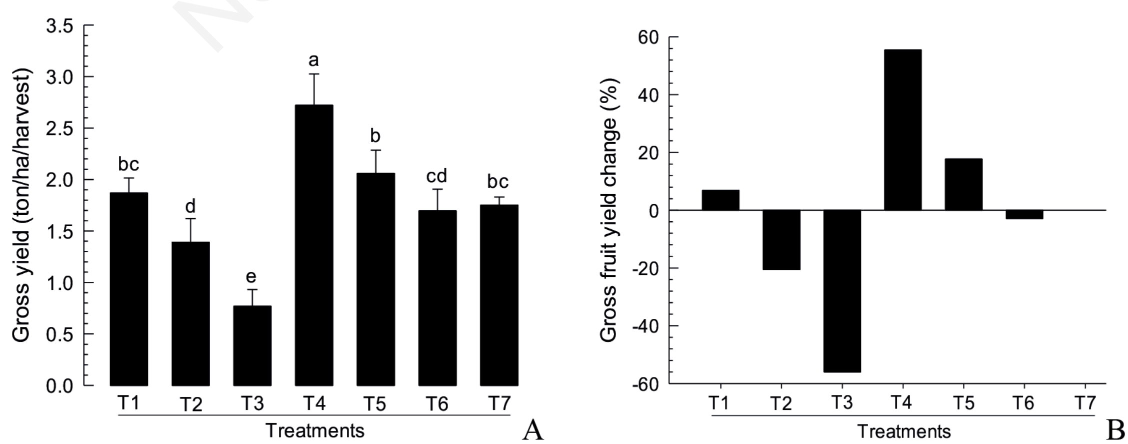

Figure 6. Role of insecticide on gross yield of brinjal. A) Gross fruit yield; B) percent change in gross fruit yield over control. Error bars represent standard deviation. Different letters above the error bar denote significant differences ($p>0.05$, least significant difference test) among the treatments. T1, emamectin benzoate 1%; T2, emamectin benzoate 1% + azadirachtin 1%; T3, abamectin 1.2%; T4, abamectin 1.2% + azadirachtin 1%; T5, abamectin 1.8%; T6, abamectin 1.8% + azadirachtin 1%; T7, untreated control.

g) followed by T1 (46.49±2.15 g) and T5 (44.99±1.26 g), while in control it was 44.10±2.24 g. Others produce less than control.

Discussion

The present study showed that both emamectin benzoate and abamectin in combination with azadirachtin were effective in combating BSFB compared to their individual application. Emamectin benzoate + azadirachtin and abamectin + azadirachtin provided maximum control of shoot infestation (89.34 and 81.55%, respectively), number basis fruit infestation (93.34 and 85.11%, respectively), weight basis fruit infestation (94.91 and 85.27%, respectively) (Figure 8). Mollah *et al.* (2022a) reported that a combination of abamectin, emamectin benzoate, and azadirachtin control more than 75% of shoot, as well as more than 50% of fruit infestation in brinjal. Another study by Mollah *et al.* (2022c) reported that abamectin + emamectin benzoate reduced 75.76% of shoot infestation and 70.88% of fruit infestation. Anand *et al.* (2014) reported that emamectin benzoate, in combination with azadirachtin-based Neem Baan, provided the lowest fruit infestation by both number and weight basis. Abamectin + azadirachtin also provided a maximum increase of marketable (193.67%) and gross (55.43%) yield over control. Mollah *et al.* (2022a) also reported that azadirachtin, in combination with abamectin and emamectin benzoate, increases 87.50% of fruit yield. Another study by Anand *et al.* (2014) reported that emamectin benzoate, in combination with azadirachtin-based Neem Baan, produced maximum fruit yield (16.89 ton/ha). The maximum individual fruit weight was also recorded from abamectin + azadirachtin-treated plants.

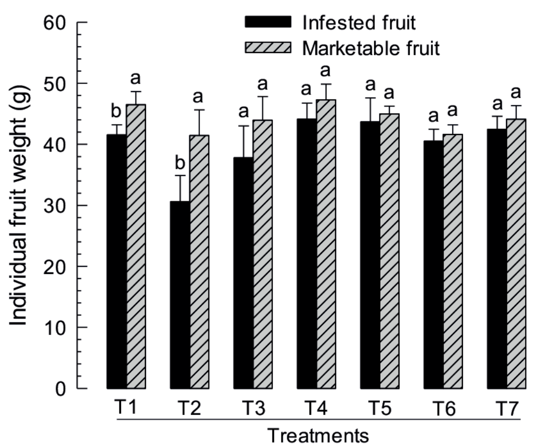

Figure 7. Impact of insecticides on individual fruit weight of brinjal. Comparative individual fruit weight of healthy and infested fruit (a) and percent change in individual fruit weight over deviation (b). Different letters above the error bar denote significant differences (p>0.05, least significant difference test) among the treatments. T1, emamectin benzoate 1%; T2, emamectin benzoate 1% + azadirachtin 1%; T3, abamectin 1.2%; T4, abamectin 1.2% + azadirachtin 1%; T5, abamectin 1.8%; T6, abamectin 1.8% + azadirachtin 1%; T7, untreated control.

Both emamectin benzoate and abamectin are bacterial secondary metabolites. Bacteria or other pathogens release different types of metabolites upon invasion into the host body (Kim, 2018). Bacterial metabolites have host immunity suppression activity (Mollah & Kim, 2020), apoptotic activity to degrade DNA (Mollah *et al.*, 2020a), and toxic or insecticidal activity (Horikoshi *et al.*, 2017; Mollah *et al.*, 2020b). For immune suppression, these metabolites bind with the immune proteins, such as dorsal switch protein 1 (DSP1) (Mollah, 2024b), phospholipase A_2, or any other. The binding of these metabolites with DSP1 inactivates the DSP1-specific immune pathway like the Toll-spätzle immune pathway (Mollah, 2023). The immune-suppressed insects are easily killed by the apoptotic or toxic activity of the bacterial metabolites.

The potency of emamectin benzoate or abamectin might come from their above-mentioned attributes. However, azadirachtin is well known for its insecticidal, feeding deterrent, and ovicidal activity. The combined effect of azadirachtin and bacterial metabolites emamectin benzoate or abamectin might provide an additive effect on BSFB control. However, the reason for increased yield and increased fruit weight is yet to be understood.

Conclusions

Farmers face a serious challenge as brinjal is vulnerable to attack from BSFB as a major pest, causing significant losses in yield and market value. This study found that emamectin benzoate 1% + azadirachtin and abamectin 1.8% + Azadirachtin effectively reduce

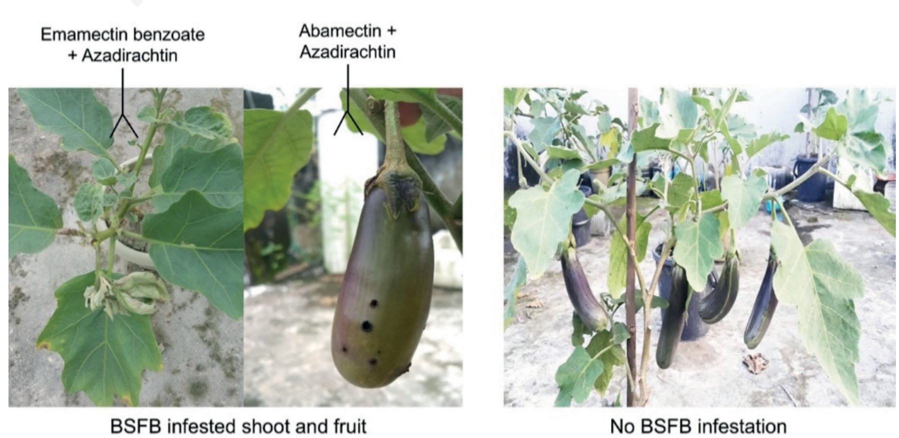

Figure 8. Schematic representation of the study. Among the treatments, emamectin benzoate + azadirachtin and abamectin + azadirachtin control shoot and fruit infestation, respectively. BSFB, brinjal shoot and fruit borer.

the BSFB infestation in the field. Additionally, abamectin 1.2% + azadirachtin increased the fruit yield as well as individual fruit weight. However, further study is required to analyze the residual effect of these insecticides as well as their effect on plant growth.

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