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Research Article

Conventional *Versus* Biorational Insecticides for Controlling Mustard Aphid and Their Effects on Beneficial Insects

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ABSTRACT

Mustard is one of the most important oilseed crops in Bangladesh. However, its production is severely constrained by aphid infestation. Farmers usually spray conventional insecticides to control aphids that negatively affect beneficial insects and environment. Thus, alternatives to conventional insecticides are required. We tested three biorational insecticides (Nimbicidine @ 1ml/L, Spinosad: Libsen 45SC @ 0.5 ml/L and Lufenuron: Hayron 5EC @ 0.5ml/L of water) against aphid infestation and assessed their effect on ladybird beetle and honey bee and compared these effects with two conventional insecticides (Diazinon: Sabion 60EC @ 2ml/L and Cypermethrin: Cypraplus 10EC @ 1ml/L of water) and with an untreated control. The study was conducted in a randomized complete block design with each treatment replicated thrice in the field of Department of Entomology, Bangladesh Agricultural University. Number of infested plants per plot, aphid per plant and per pod, number of ladybird beetle and honey bee per plot was counted and yield per plot was calculated. All tested insecticides reduced aphid infestation compared to untreated control indicating all of them were effective against aphid. However, Lufenuron and Nimbicidine was most effective to reduce plant infestation and number of aphids per plant and pod. Spinosad and Sabion were found fairly effective whereas Cypraplus was found to be least effective. Lufenuron and Nimbicidine did not reduce the number of ladybird beetle and honey bee visit per plot, however, Spinosad and conventional insecticides reduced their number. Lufenuron treated plots provided highest yield (1.35 t/ha) followed by Nimbicidine (1.15 t/ha), Spinosad (1.10 t/ha) and Sabion (1.00 t/ha) whereas Cypraplus treated plots provided lowest yield (0.9 t/ha). In terms of reducing aphid population thus plant infestation and increasing yield, all biorational insecticides performed better than conventional insecticides, however, concerning effect on ladybirds and honey bees, only Lufenuron and Nimbicidine can be recommended to farmers.

Introduction

Mustard is one of the leading oilseed crops in the world. About 72 million tons of mustard seed produced from an area of 36.59 million hectares (ha) of land worldwide and it supplies about 12% of the world's edible oil (FAO, 2022). It is cultivated in all countries of Indian sub-continent including Bangladesh. In Bangladesh, mustard is commonly known as "Sarisha" and is cultivated during Rabi season. It occupies 69% (330.76 thousand ha) of the total acreage of oilseed cultivation and 40% (410 thousand metric tons) of the total oilseed production in 2021-22 (BBS, 2022). However, the production quantity is not yet enough. In 2020, annual edible oil demand of the country was 3 million tons out of which 2.73 million tons was imported (Hassan, 2022).

Mustard yield is severely constrained each year by infestation of various insect pests (Kumar and Chauhan, 2005). Among those insect pests, mustard aphid (*Lipaphis erysimi*) is the most destructive one (Das, 2002; Dhillon *et al.*, 2022). Both the nymph and adult aphid suck cell sap from leaves, twigs, flower buds, inflorescences and pods of the plants. Siliqua is the most suitable part for development of this pest, and maximum damage is caused at silique formation stage (Koirala, 2020). As a consequence, the plant shows stunted growth, curled leaves, flowers wither and pod formation are hindered leading the lower oil content in seeds (Yadav and Rathee, 2020). Aphids also secret honey dews that influence the growth of sooty mould fungus which reduces the photosynthesis of the plant and thus compromises the yield (Singh and Singh, 2016). Yield loss in mustard due to aphid infestation has

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been reported to 87-98% in India and Bangladesh (Patel *et al.*, 2004; Hossain *et al.*, 2015).

To combat with mustard aphids, farmers of Bangladesh mostly rely on variety of broad-spectrum chemical insecticides spraying as those products are readily available and cheap to buy (Hoque et al., 2002; Douglas, 2016). It has been reported that farmers usually apply different types of insecticides in every week (Dasgupta, 2005). Application of conventional insecticides might provide some sort of control. However, they are highly toxic and persistent to the environment (Gill and Garg, 2014). Indiscriminate use of insecticides leads to the development of resistance to target pests, kills natural enemies and other beneficial insects that enhances pest resurgence, secondary pest outbreak and hampers crop pollination (Dutcher, 2007; Goulson *et al.*, 2018). Moreover, regular spraying causes farmers' health hazards, environmental pollution and accumulation of toxic chemicals in the food (Matthews, 2015; Rani *et al.*, 2021).

To avoid all these insecticidal hazards, alternative to chemical insecticides is required. In this regard, Integrated Pest Management (IPM) might be a good option for farmers. However, IPM is very difficult to implement by smallholder farmers of Bangladesh as this is very knowledge and labour intensive procedure (Nahar *et al.*, 2020). Moreover, Bangladeshi farmers are habituated with pesticide use; withholding pesticide and application of purely strategic IPM (no pesticide) might not work for these farmers (Nahar *et al.*, 2020). In this regard, recommending farmers to spray insecticides that are least harmful to beneficial insects, farmers health and environment seems most logical. In this context, biorational insecticides might be a good choice for farmers because these insecticides are claimed to be relatively safer or have little impact on beneficial insects and environments compared to other conventional insecticides used by farmers (Rosell *et al.*, 2008). Among the biorational insecticides, Spinosad, Nimbicidine and Lufenuron are the most commonly used to control various insect pests (Horowitz *et al.*, 2009). Spinosad, obtained from soil bacteria *Saccharopolyspora spinosa*, reported to control various insect pests and claimed to be relatively safer for natural enemies (Biondi *et al.*, 2012). The mode of action of Spinosad insecticides is a neuronal mechanism which is highly active, by both contact and ingestion (Salgado, 1998). Similarly, Nimbicidine, extracted from neem trees, *Azadirachta indica* acts as an antifeedant and stomach poison. It affects the vital physiological activities like moulting, cell division, reproduction of insects and finally causing death (Mordue, 2004). Lufenuron, a chitin synthesis inhibitor, also recommended in many pests control programme in recent days due to is excellent inhibition capacity (Adel, 2012).

In mustard ecosystem, ladybird beetles and honey bees are considered as two very important beneficials. Ladybirds has been reported as the most efficient predator of aphid (Singh and Singh, 2013) and honey bees as primary pollinators of mustard crop to increase production (Hayter and Cresswell, 2006). Usually conventional insecticides kill these beneficial insects but spraying of biorational insecticides might not affect them (Abrol and Shankar, 2017). Effect of various insecticides against aphids has already been well-researched (e.g. Akter *et al.*, 2021; Saha *et al.*, 2021), however, field studies on the side effects of biorational and conventional insecticides on predators and pollinators of oilseed crops are few (e.g. Dutta *et al.*, 2016, 2017). Therefore, the main aim of the present study is to compare the effect of biorationals and frequently used conventional insecticides by farmers against mustard aphid and assess their impact on abundance of a predator, ladybird beetle and a pollinator, honey bee.

Materials and Methods

Experimental site and layout

The field experiment was conducted during the Rabi season with a mustard variety BARI Sarisha-14 at the field laboratory of Department of Entomology (24.75˚N latitude and 90.5˚E longitude), Bangladesh Agricultural University. The experiment was laid out in a Randomized Complete Block Design with six treatments each replicated thrice. Area of each plot was 1 m × 1 m and distance between plots was kept 0.5 m to avoid treatment influence.

Land preparation and plant cultivation procedure

Fresh and disease-free seeds of BARI Sarisha-14 were collected from the Department of Agronomy, Bangladesh Agricultural University. The land was prepared thoroughly by ploughing and cross-ploughing followed by laddering and proper leveling to have a good tilth, and all kinds of weeds, stubbles, crop residues were removed from the field. Seeds are sown at the rate of 7 kg/ha. The full amount of triple super phosphate (170 kg /ha), muriate of potash (125 kg/ha), cowdung (1000 kg/ha) were applied during final land preparation as a basal dose and urea (240 kg/ha) was applied in two splits. One half of urea was added during final land preparation and the remaining was added before flowering. Irrigation, weeding and other intercultural operations were provided whenever needed.

Application of treatments (insecticides)

For this experiment, three biorational insecticides (Nimbicidine, Spinosad and Lufenuron) were tested and compared their effects with two conventional insecticides (Diazinon and Cypermethrin) and an untreated control. Thereby, treatments were implemented as follows: (i) Nimbicidine @ 1 ml/L of water, (ii) Spinosad (Libsen 45SC) @ 0.5 ml/L of water, (iii) Lufenuron (Hayron 5EC @ 0.5 ml/L of water, (iv) Diazinon (Sabion 60 EC) @ 2ml/L of water, (v) Cypermethrin (Cypraplus 10 EC) @ 1ml/ L of water, and (vi) Untreated control. All plots were monitored regularly for onset of aphid infestation, and spraying was done for first time when all plots were infested. All experimental plots except the control plots were sprayed with a hand sprayer at morning time at 15 days interval.

Monitoring of plant infestation and abundance of insects

At the beginning of the study, total number of mustard plants per plot was counted. Afterwards, number of healthy and infested plants per plot was counted. A plant was considered infested with typical symptom of aphid infestation and presence of aphid on the plant. The percentage of aphid infestation was calculated by dividing the infested plants by total number of plants and multiplied by hundred.

Three random plants per plot were selected and tagged to record abundance of aphids. Number of aphids from these plants were counted using hand lens. At the reproductive stage of the plant, number of aphids per pod was counted visually from three random plants per plot. Later, mean number of aphids was calculated over replications of each treatment.

Harvesting of pod was done at maturity of the crop at 90 days after sowing. Harvested plant of each plot was bundled separately, properly tagged and threshed. The seeds were collected, cleaned and weighed. Finally, yield per plot was calculated and converted to ton/ha.

All plants per plot was visually inspected with least disturbance and ladybird beetle per plot was counted. Plots were monitored from morning till afternoon for consecutive three days during full bloom to observe the peak foraging time of honey bees. Afterwards, number of honey bees per plot was counted with hand counter during peak foraging time (11.00 a.m.-12.00 p.m.).

Statistical analysis

Data were analyzed using statistical software R version 3.5.3. Before analysis, normality and homogeneity of the data were tested by Shapiro-Wilkinson and Levene's test. Data were analyzed using a standard analysis of variance (ANOVA) with post-hoc mean separation according to Tukey's HSD test.

Results and Discussion

In the present study, few biorational insecticides (Nimbicidine, Spinosad and Lufenuron) and conventional insecticides (Diazinon: Sabion 60 EC and Cypermethrin: Cypraplus 10 EC) were tested to manage a major pest of mustard, mustard aphid (*Lipaphis erysimi*), and the effect of those insecticides on ladybird beetle, one of the most important predator of mustard aphid and on honey bees, most important pollinators of mustard.

Effect of selected insecticides on number of aphids per plant

Both biorational and conventional insecticides significantly ($P<0.05$) reduced the number of aphids per plant compared to untreated control plots (Table 1). Among the insecticides tested, the highest reduction of aphid number occurred in Lufenuron treated plots whereas lowest reduction occurred in of Cypraplus treated plots (Table 1). Average number of aphids per plant in Lufenuron treated plots was 18.35 whereas aphid number was nearly thrice (47.17) in Cypraplus treated plots. It signifies that conventional insecticide Cypraplus was not as much as effective as biorational insecticide Lufenuron. Nimbicidine also substantially reduced the number of aphids compared to both conventional insecticides namely Cypraplus and Sabion. Unfortunately, biorational Spinosad could not reduce aphid number as much as the conventional Sabion did. Less efficacy of the conventionals and Spinosad might be linked to the resistance development. It has been reported that aphid developed resistance against conventional insecticides after repeated spraying (Nauen *et al.*, 2003). On the other hand, biorational insecticides, particularly Lufenuron and Nimbicidine has been rarely reported for resistance development and they have comparatively less negative effect on environment (Ayilara *et al.*, 2023). Therefore, Lufenuron and Nimbicidine could be reported to manage mustard aphids.

Effect of selected insecticides on number of aphids per pod

The highest number of aphids (28.65) on pod was observed at untreated control plots as aphid multiplication was high when no insecticide was used. All the biorational treated plots showed significantly ($P<0.05$) less number of aphids per pod compared to conventional insecticide treated plot (Table 1). Among the biorational insecticides, the best result was observed when plot was treated with Lufenuron. The average number of aphids per pod was 9.2 which was the lowest among all treatments (Table 1). Nimbicidine and Spinosad had a moderate effect on the reduction of aphid number per pod. In contrast, Cypraplus and Sabion were least effective on aphid reduction. The mean number of aphids per pod was 20.97 and 17.37 for Cypraplus and Sabion, respectively. Relatively lower efficacy of conventional insecticides against aphid has been reported earlier (Amer *et al*., 2010).

Table 1. Effect of selected insecticides on aphid abundance and plant infestation

Treatments	Number of aphids per plant	Number of aphids per pod	Average plant infestation (%)
Nimbicidine	25.5 ± 12.66 e	11.55 ± 3.97 e	20.99 ± 10.23 e
Spinosad	38.5 ± 12.66 c	14.51 ± 4.76 d	29.55 ± 9.92 c
Lufenuron	18.35 ± 12.60 f	9.20 ± 3.73 f	16.11 ± 10.51 f
Sabion	31.5 ± 12.66 d	17.37 ± 4.38 c	26.22 ± 10.14 d
Cypraplus	47.17 ± 12.53 b	20.97 ± 4.65 b	34.11 ± 9.82 b
Untreated control	93.88 ± 3.30 a	28.65 ± 6.08 a	62. 00 ± 0.00 a

Values within a column followed by the same letter did not differ significantly ($P>0.05$) according to Tukey's HSD test. Values presented here are the average of the replications (n=3) ± standard deviation.

Effect of selected insecticides on plant infestation

Both conventional and biorational insecticides significantly ($P<0.05$) reduced the plant infestation compared to untreated control (Table 1). The untreated control plot showed the highest infestation by mustard aphid. The average infestation of this plot was 62% whereas infestation in treated plot ranged from 16-34% only (Table 1). Among the insecticides tested, biorationals particularly Lufenuron and Nimbicidine performed better than conventional insecticides. Lufenuron was found to be the most effective treatment against mustard aphid as plant infestation was only 16.11% in Lufenuron treated plots. Among biorationals, Spinposad did not reduce the infestation as much as Lufenuron and Nimbicidine treated plots. Although the plant infestation in Spinosad was significantly lower than untreated control plots, however, infestation remained still higher (29.55%). In contrast of biorational insecticides, conventional Cypraplus treated plots showed the highest percentage of aphid infested plants (34.11%).

Based on the effects of all tested insecticides, it is concluded that Lufenuron reduced plant infestation most. The results are in line with Dutta *et al.* (2016). Although Spinosad has been reported to control other insects effectively, however, in the present study, Spinosad is found less effective against aphid compared to other biorationals. Less efficacy of Spinosad against sucking insect pests has also been reported by other studies (Vimala et al., 2010; Kumar et al., 2017) which might be related to the resistance development by sucking pests against Spinosad.

Effect of selected insecticides on yield

Biorational and conventional insecticides treated plots both produced better yield than untreated control plots ($P<0.05$). The lowest yield was produced in untreated control plot which was 0.30 t/ha (Fig. 1). However, biorational insecticides treated plots produced better yield than conventional insecticides treated plots. Among the biorationals, Lufenuron treated plot produced the highest yield (1.35 t/ha). The second highest yield (1.15 t/ha) was observed from Nimbicidine treated plots followed by Spinosad treated plots. Conventional insecticides Sabion and Cypraplus treated plots showed lower yield compared to biorational treated plots.

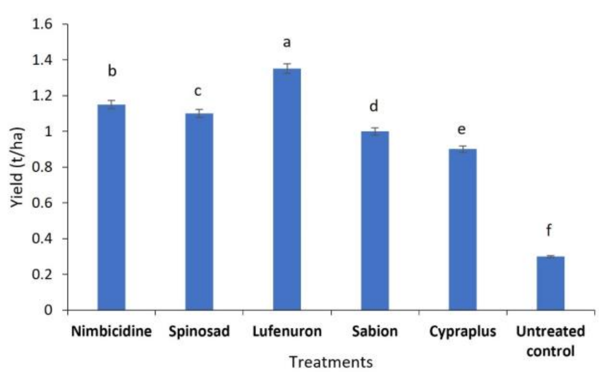

Figure 1. Effect of selected insecticides on the yield of mustard. Bars with different letters differ significantly ($p<0.05$) according to Tukey's HSD test. Effor bar represents standard errors of the mean

Effect of selected insecticides on the abundance of predatory ladybird beetle and pollinator honey bees

Each of the treatments significantly reduced the number of ladybird beetle per plots compared to untreated control, however, least reduction occurred in the plots treated with Nimbicidine (Table 2). The number of ladybird beetle in Nimbicidine treated plot (7.16) and untreated control plot (7.89) was comparable. Next to Nimbicidine, Lufenuron was the second safest insecticide among all treatments. Average number of ladybird beetle was 6.10 for Lufenuron sprayed plots. In contrast to all those, conventional insecticides Cypraplus and Sabion greatly affected the number of ladybird beetle compared to untreated control. Average number of ladybird beetle per plot in control plot was 7.89 whereas it was 3.36 and 4.56, respectively for Cypraplus and Sabion treated plots. Due to its harmful effects on ladybirds (major predator of aphid), Sabion and Cypraplus should be discouraged to spray in mustard fields. Although Spinosad has been considered as biorational, however, in the present study, it is found toxic to ladybird beetle as like as conventional insecticide Cypraplus and Sabion. There are many studies reporting the toxicity of conventional broad spectrum insecticides on predatory insects (Sanchez-Bayo, 2012) which reflected in this study as well. In contrast, biorational insecticides have been found safer to natural enemies (Smith and Krischik, 2000). However, like this study, toxicity of a biorational Spinosad on ladybirds also reported by Galvan *et al.* (2005). Thereby, despite being biorational, Spinosad also be discouraged to spray.

Table 2. Effect of selected insecticides on abundance of ladybird beetles and honey bees

Treatments	Number of ladybird beetles per plot	Number of honey bees per plot
Nimbicidine	7.16 ± 2.22 b	6.30 ± 2.23 b
Spinosad	3.86 ± 2.22 e	3.90 ± 2.22 e
Lufenuron	6.10 ± 2.22 c	5.29 ± 2.23 c
Sabion	4.56 ± 2.22 d	4.30 ± 2.23 d
Cypraplus	3.36 ± 2.22 e	3.40 ± 2.23 e
Untreated control	7.89 ± 1.74 a	8.32 ± 2.24 a

Values within a column followed by the same letter did not differ significantly (P>0.05) according to Tukey's HSD test. Values presented here are the average of the replications (n=3) ± standard deviation.

Number of honey bees visiting per plot was reduced in all treatment plots compared to untreated control plots (Table 2). However, among the insecticide tested, biorationals were found safer compared to conventional insecticide. Among biorationals, Nimbicidine was found most safest as honey bee number was comparable in Nimbicidine (6.30 bees per plot) and untreated control plots (8.32 bees per plot). Next to Nimbicidine, Lufenuron was also found safer to honey bees but Spinosad was found toxic as it reduced number of bees (3.90 bees per plot) close to the Cypraplus (3.40 bees per plot) and Sabion (4.30) treated plots. Number of honey bees in Spinosad, Cypraplus and Sabion treated plots became half of the untreated control plots. Similar effect was found in the study reported by Miles et al., 2003. As honey bees play a vital role in mustard pollination, therefore, these three insecticides should be considered highly toxic to honey bees and thus discouraged to spray in mustard field.

Conclusion

Major aim of the present study was to find out best insecticide(s) that can manage mustard aphid without compromising its predator ladybirds and pollinator honey bees. Both the biorational and conventional insecticides reduced number of aphids from plants and pods and thus plant infestation compared to untreated control. However, conventional insecticides drastically reduced number of ladybird beetles and honey bees. Biorational Spinosad was also found toxic to these beneficial insects but Lufenuron and Nimbicidine found safer. These two biorationals was also found most effective in reducing aphid numbers and thus plant infestation. Thereby, both Lufenuron and Nimbicidine can be safely recommended to farmers. However, a large field study with proper economic analysis should be conducted in future.

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