Evaluation of bio-rational insecticides to control *Helicoverpa armigera* (Hübner) and *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) fed on *Vicia faba* L.

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Abstract: Two botanical pesticides, Neem Azal-T/S (NA) and *Quassia amara*, one biopesticide, *Bacillus thuringiensis* subsp. *aizawai* (*Bta*) and one combination of *Bta* + NA were tested against 2nd and 4th instar larvae of the noctuids *Helicoverpa armigera* (Hübner) and *Spodoptera exigua* (Hübner) on field beans under both laboratory and greenhouse conditions. The maximum mortality of 58 and 27 % was obtained in *Bta* + NA treatments in case of 2nd and 4th instar larvae of *H. armigera* under laboratory conditions followed by *Bta* (50 and 14 %) and NA (34 and 7 %) alone treatments. Under greenhouse conditions a mortality of 69 and 26 % was observed in case of *Bta* + NA treatments in 2nd and 4th instar larvae of *H. armigera* followed by *Bta* (67 and 20 %) and NA (56 and 10 %) alone. The mortality of *H. armigera* larvae was higher under greenhouse conditions. There was a significant difference in the mortality between 2nd and 4th instar larvae both under laboratory and greenhouse conditions. The mortality of *H. armigera* was higher in 2nd instar than in the 4th instar larvae in all treatments. Similar results were obtained in case of *S. exigua* both under laboratory and greenhouse conditions. The results indicate that *Bta* and NA have the potential to the control of *H. armigera* and *S. exigua* either independently or in combination, when used at the right stage of the field populations. The *Quassia*-extracts tested did not show a high efficacy against larvae of *H. armigera* and *S. exigua.*

Key words: *Helicoverpa armigera*, *Spodoptera exigua*, control, NeemAzal, *Bacillus thuringiensis aizawai*, Quassins, efficacy

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Helicoverpa armigera (Hübner) and *Spodoptera exigua* (Hübner) (Lep., Noctuidae) are important polyphagous pests of cultivated crops primarily in tropical and subtropical regions (BROWN & DEWHURT 1975). Due to high cost of protecting crops from these pests with chemical pesticides and the increasing resistance and resurgence to many chemical pesticides (ARMES et al. 1992; BREWER & TRUMBLE 1994) there is growing interest in the use of biological products such as bacterial and viral-based insecticides, and parasitoids (NAGARKATTI 1982), predators (KING et al., 1982) and botanical pesticides (RAO et al. 1990). These groups have different mode of action from conventional products (THOMPSON et al. 1999) and their properties? may differ considerably from the conventional chemicals with which growers are familiar. It is therefore important to generate information on the likely differences in the performances of these products to educate growers and facilitate adoption. The objective of this study was to evaluate commercially available biological and botanical pesticides both individually, and partly in combination against *Helicoverpa* and *Spodoptera* species on *Vicia faba* to determine their effects under laboratory and greenhouse conditions. So, this additional information would make existing IPM programmes more effective and sustainable, while decreasing the reliance on synthetic insecticides.

Materials and Methods

Insects

Unhatched eggs of *H. armigera* and *S. exigua* were obtained from the Laboratory for Entomology, Bayer AG, Monheim, Germany. The egg cards were kept at 26 ± 2 °c and 70-90 % relative humidity. When these eggs were hatched, the cultures were maintained on chickpea based artificial diet prepared in the laboratory. The cultures were maintained at 27 ± 2 °c at 16 : 8 L : D photo-period. Generally, second and fourth-stage larvae were used in various experiments and they were starved for 12 h before all experiments.

Bioefficacy evaluation

The various botanical and biological preparations used in laboratory and greenhouse are listed in table 1. *Quassia amara*-extract was delivered by Trifolio-M Company. D-35633 Lahnau. The host plants (*Vicia faba ‘Samba’*) used for the spraying tests in the laboratory and greenhouse were 3 to 5 weeks old and with 7-8 branches. Under laboratory conditions, the tests were carried out in petri dishes (8.5 cm diameter). The tests leaves were collected from the top third of canopy and then dipped in different treatment materials as shown in table 1 for five minutes, and then air dried. The treated leaves were placed into the petri dishes on moistened filter paper (one leave per petri dish) with the adaxial surface uppermost. *Helicoverpa* and *Spodoptera* larvae were then placed onto the leaf disc and then a cover was put onto the dish. Twenty five second and fourth instar larvae of *H. armigera* and *S. exigua* were used in each of the four replications per treatment. For control treatments the leaves were dipped in water only. The experiments were conducted in the laboratory with a temperature of 25 ± 1 °C light regime of 14 h light 10 h dark and relative humidity of 65 ± 5 %. Mortality was assessed every 24 h, 48 h, and 72 h in all the experiments.

Table 1. Summary of the bio-rational insecticides evaluated for efficacy against *Helicoverpa armigera* and *Spodoptera exigua* larvae on *Vicia faba*.

Active ingredient	Product	Rate applied
B. thuringiensis aizawai (*Bta*)	Xentari®	20 ml/5 litre of water
Bta + NA	Xentari® plus NeemAzal®	10+10 ml/5 litre of water
Azadirachtins	NeemAzal® (NA)	20 ml/5 litre of water
Certain oils without azadirachtin	NA formulation ("blank")	20 ml/5 litre of water
Quassin I	*Quassia amara* extract	5 mg a.i./litre of water
Quassin II	*Quassia amara* extract	10 mg a.i./litre of water
Quassin III	*Quassia amara* extract	20 mg a.i./litre of water

For the experiments under greenhouse conditions, the plants of *Vicia faba* were grown 3-5 weeks prior to conducting the experiments in pots having a diameter of 10 cm. When the plants attained about 7-8 branches, the solutions of various treatments were applied with a trigger sprayer, misting to run-off level. Water was used as a control. The spray equipment was drained and triple rinsed after each treatment to avoid any contamination. Second and fourth instars of *H. armigera* and *S. exigua* were placed on each plant and five plants were used in each treatment (25 larvae per treatment). Then the plants were put in cages 50 x 50 x 60 cm, with two meshed and two glass walls and a glass lid. Mortality was measured at 24 h, 48 h and 72 hrs after treatment.

Statistical analysis

For statistical analysis of efficacy of insecticides to *H. armigera* and *S. exigua*, mortality due to the different insecticides was analysed using the programme SPSS 12, ANOVA.

Results

Toxicity of insecticides to H. armigera

Our results show significant differences in the mortality recorded from the different treatments under laboratory and greenhouse conditions. With the exception of quassin (Q) I and II, significantly higher mortality was detected in all the treatments compared with the untreated controls. (table 2 and 3). *B.t. aizawai* (*Bta*) + Neem azal (NA) (58 %) and *Bta* alone (50 %) caused significantly higher mortality than that recorded in the NA, Neem blank and the three quassin treatments in 2nd instar larvae under laboratory conditions (table 2). Whereas in 2nd instar larvae under greenhouse conditions mortality was higher, 67 and 69 % for *Bta* and *Bta* + NA followed by NA (56 %), Q III (23 %), Q II (15 %) and Q I (5 %), respectively. Mortality tended to decline with increasing larval age, and significant differences were recorded between the instars. Fourth-instar larvae suffered significantly lower mortality than that recorded for second-instar larvae (table 2 and 3).

Table 2. Effect of bio-rational insecticides on the 2nd and 4th instar larvae of *H. armigera* under laboratory conditions. Figures followed by different letters are significantly different from each other at p=0.05 (ANOVA, post hoc Tukey)

Treatments	No. of larvae exposed	Mean percentage mortality (2nd instar) after			Mean percentage mortality (4th instar) after		
		24 h	48 h	72 h	24 h	48 h	72 h
Bta	25	37.0	46.0	50.0c	0.0	6.0	14.0b
Bta + NA	25	27.0	34.0	58.0c	1.0	16.0	27.0c
NA	25	18.0	30.0	34.0b	0.0	0.0	7.0a
NA blank	25	0.0	0.0	4.0a	0.0	0.0	1.0a
Quassin I	25	0.0	0.0	2.0a	0.0	0.0	0.0a
Quassin II	25	1.0	1.0	5.0a	0.0	0.0	2.0a
Quassin III	25	0.0	2.0	11.0a	0.0	1.0	5.0a
Tap water	25	0.0	0.0	1.0a	0.0	0.0	0.0a

Toxicity of insecticides to S. exigua

The reductions in total mortality over the post application period were recorded in all the treatments under laboratory and greenhouse conditions, resulting in significant mortality being recorded 3 days after treatment both in 2nd and 4th instar larvae. However, the rate of mortality differed between the treatments, occurring most rapidly in *Bta* + NA (65 and 26 %) and *Bta* alone (58 and 16 %), in the treatments under laboratory conditions in 2nd and 4th instar larvae (table 4). Whereas under greenhouse conditions the highest mortality occurred in *Bta* (68 %) followed by *Bta* + NA (66 %) in case of 2nd instar larvae (table 5). While in 4th instar larvae maximum was in *Bta* + NA (22 %) followed by *Bta* (16 %) and NA (13 %). There were significant differences in percentage mortality in all the treatments in comparison to the control except the treatments with part of the Quassins both under laboratory and greenhouse conditions. Also there was a significant difference in mortality between 2nd and 4th instar larvae of *S. exigua* both under laboratory and greenhouse conditions.

Discussion

The results of our study indicate that the effects of the insecticides we evaluated differed considerably in their age specific mortality. The differences can be attributed to different modes of action of the products and also to the stage of *H. armigera* and *S. exigua* larvae. Our view has been supported by ABBAS & EL-DAKROWRY (1988) and ALI & YOUNG (1996) who observed that the susceptibility of *Helicoverpa* to both conventional and biological insecticides tends to decline with increasing age and size. The rapid decline in the mortality induced by *Bta*, *Bta* + NA and NA to large instar larvae indicates the importance of appropriate spray timings. In *Vicia faba*, crop scouting is infrequent, with sprays often being targeted against populations of late instar larvae. For acceptable levels of control with *Bta* and NA insecticides, the frequency of crop scouting will need to be increased and the timing of application should be accurate, to ensure that populations of early instars are targeted.

In our studies the combination of Bta + NA give the highest mortality compared with other treatments both under laboratory and greenhouse conditions which shows that combination of products can work much better than one ingredient alone. SALMA et al (1984) evaluated several such combinations against *Spodoptera littoralis* (Boisduval) and found that all pyrethroids and most organophophates tested potentiated the activity of *B. thurigiensis*. So the application of neem products with *B. thurigiensis* may be a safe and effective means for controlling *H. armigera* and *S. exigua*. Similarly botanical extracts like *Quassia amara* can also be tried against these pests. But in our studies *Quassia amara*-extracts reduced populations of the pest insects pertly significantly, but in a too low degree. The activity of *Q. amara* shows an antifeedant activity against *H. armigera* and *S. exigua*. FERNANDO et al (2000) and LESKINEN et al (1984) studied the effects of *Q. amara* wood extract against *Hypsipyla grandella* (Zeller) and *Spodoptera eridania* 5th instar larvae, respectively, and observed a clear antifeedant activity against it.

Table 3. Effect of bio-rational insecticides on the 2nd and 4th instar larvae of *H. armigera* under greenhouse conditions. Figures followed by different letters are significantly different from each other at p=0.05 (ANOVA, post hoc Tukey)

Treatments	No. of larvae exposed	Mean percentage mortality (2nd instar) after			Mean percentage mortality (4th instar) after		
		24 h	48 h	72 h	24 h	48 h	72 h
Bta	25	49.0	53.0	69.0d	5.0	5.0	20.0c
Bta + NA	25	35.0	52.0	67.0d	6.0	10.0	26.0c
NA	25	29.0	40.0	56.0d	0.0	2.0	10.0b
NA blank	25	0.0	1.0	2.0a	0.0	1.0	2.0a
Quassin I	25	0.0	0.0	5.0a	0.0	0.0	1.0a
Quassin II	25	8.0	13.0	15.0b	1.0	1.0	6.0ab
Quassin III	25	12.0	16.0	23.0b	2.0	9.0	11.0b
Tap water	25	0.0	0.0	1.0a	0.0	0.0	1.0a

Table 4. Effect of bio-rational insecticides on the 2nd and 4th instar larvae of *S. exigua* under laboratory conditions. Figures followed by different letters are significantly different from each other at p=0.05 (ANOVA, post hoc Tukey)

Treatments	No. of larvae exposed	Mean percentage mortality (2nd instar) after			Mean percentage mortality (4th instar) after		
		24 h	48 h	72 h	24 h	48 h	72 h
Bta	25	28.0	49.0	58.0d	2.0	5.0	16.0e
Bta + NA	25	38.0	54.0	65.0d	4.0	14.0	26.0f
NA	25	30.0	36.0	43.0c	1.0	8.0	12.0de
NA blank	25	1.0	2.0	7.0ab	0.0	1.0	4.0abc
Quassin I	25	0.0	4.0	6.0ab	0.0	1.0	1.0
Quassin II	25	0.0	5.0	10.0bc	0.0	0.0	6.0
Quassin III	25	1.0	7.0	13.0b	0.0	0.0	9.0*
Tap water	25	0.0	0.0	2.0a	0.0	0.0	0.0

Table 5. Effect of bio-rational insecticides on the 2nd and 4th instar larvae of *S. exigua* under greenhouse conditions. Figures followed by different letters are significantly different from each other at p=0.05 (ANOVA, post hoc Tukey)

Treatments	No. of larvae exposed	Mean percentage mortality (2nd instar) after			Mean percent mortality (4th instar) after		
		24 h	48 h	72 h	24 h	48 h	72 h
Bta	25	42.0	56.0	68.0d	3.0	12.0	16.0bc
Bta + NA	25	33.0	46.0	66.0d	10.0	15.0	22.0c
NA	25	23.0	35.0	43.0c	4.0	8.0	13.0b
NA blank	25	0.0	0.0	5.0a	0.0	2.0	3.0a
Quassin I	25	0.0	2.0	6.0a	0.0	3.0	4.0a
Quassin II	25	5.0	8.0	18.0b	2.0	7.0	11.0b
Quassin III	25	7.0	15.0	34.0c	5.0	8.0	15.0bc
Tap water	25	0.0	0.0	0.0a	0.0	0.0	1.0a

PARTHBAN & ANANTHAN (1998) observed that the alternate application of azadirachtin (1500 ppm) and *B.t. kurstaki* (1 litre/ha) significantly reduced the incidence of sucking pests and larval populations of *S. litura* and *H. armigera* in cotton. NA alone treatments gave significantly better results than the control treatments although it was not superior to *B.t. kurstaki* treatments. DAS et al (2000) tested a solvent based neem seed kernel extract (1500 ppm) against *H. armigera* in pigeon pea and observed the best results in the first and second instars, respectively. Similarly PRABHAKAR et al (1986) reported that the use of azadirachtin prolong the development and induce mortality in *S. exigua* at all the stages of larval development. These botanicals and biological products may have high levels of selectivity and the preservation of beneficial populations if tested in the field. MA et al (2000) tested biorational insecticides (neem oil, azadirachtin and *B. thuringiensis kurstaki*) against *H. armigera* in cotton and found that the predators, including lady beetles, lacewings, spiders and predatory bugs, were insensitive to neem seed extract and *B.t.* applications.

All the products except the Quassins in the present study caused levels of mortality of 2nd instar larvae that are near to acceptable levels of control. Consequently, in terms of efficacy, *Bta* and NA could be considered suitable for *H. armigera* and *S. exigua* control in *Vicia faba* if the results can be repeated in the field to fully investigate their field efficacy and side effects to determine their field performance and IPM compatability.

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