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Efficacy of *Bacillus thuringiensis* var. aizawai and NeemAzal-T/S against the old world bollworm *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae)

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Abstract

The efficacy of XenTari® containing *Bacillus thuringiensis var. aizawai* (Bta), NeemAzal -T/S® (Azadirachtin A) and their combination were tested against the second and fourth larval instars of the old world bollworm (OWB), *Helicoverpa armigera* (Hübner). The Bta was applied in three concentrations of 0.5, 1, and 1.5 kg/ha while NeemAzal concentrations were 1, 2, and 3L/ha. The results showed a significant difference in the mortality percentage between the different concentrations. The Bta was more effective in the fourth larval instars. Seven days, after treatment, the mortality percentage in both second and fourth instar larvae reached 97% at concentration of 1 ml/l water. At the concentration of 2.7 g/l water, the mortality percentage caused by Bta in both second and fourth instar larvae increased gradually until the sixth day, where it reached 90% and 93%, respectively. On the other hand, the efficacy of NeemAzal-T/S® on the second and fourth instar larvae reached 97% seven days after treatment when applied at concentration of 1 ml/l water. The combination of XenTari® and NeemAzal-T/S® resulted in 100% mortality in the second instar larvae compared with respective mortality of 70% and 83%, when either of the two biopesticides was applied alone.

Keywords: *Helicoverpa Armigera;* Biopesticides*;* Azadirachtin*; Bacillus thuringiensis*

1. Introduction

The old world bollworm (OWB), *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) is one of the major pests of cotton, legumes, and more than 180 other plant species. It is a cosmopolitan and highly polyphagous insect pest (Tay et al., 2013). OWB has a vast worldwide distribution ,probably in the whole of Africa, near and Middle East, Southern Europe ,Central and South-East Asia, the eastern part of Australia, New Zealand and a number of pacific Islands (Kranz, et al., 1978). In east Africa, it attacks various crops including cotton, legumes, maize, sorghum, sunflower, tobacco, and tomato .In South Africa, crops attacked include peas, beans, wheat, cotton, maize ,grain sorghum, oats, barley, sunflower, tobacco, citrus, cucurbits, potato ,tomato, Lucerne, chickpea and groundnuts (Schmutterer, 1969). The annual crop losses, in the old world, due to OWB is estimated to be about 2 billion US$ annually (Tay et al., 2013).

The importance and success of *H. armigera* is largely due to its well-developed survival strategies, which include diapause and high dispersal capacity (Fitt, 1989). Adults of OWB can migrate over long distances, borne by wind, for example from southern Europe to the UK (Pedgley, 1985) and through movement in international trade mainly on ornamental plants, on cut flowers, in cotton bolls, and in tomato fruits.

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The control of this notorious pest, especially in vegetables, with chemical pesticides has become more expensive and most of the time ineffective. Additionally, the moth has developed resistance against many widely used insecticides (Forrester, 1993; Martin, et al., 2002; Yang, et al., 2013). The bacterium, *B. thuringiensis* (*Bt*) has been widely used for pest management because of its specificity to insect pests, biodegradability, lack of toxicity to human, and safety to most beneficial insects and other animals. (Van Frankenhuyzen, 1993). Likewise, neem insecticides have proven to be effective in controlling agricultural pests, in addition to their rapid degradation in the environment, safety to consumer, and natural enemies of pests (Schmutterer, 1990; El-Shafie and Basedow, 2003; El-Shafie and Almahy, 2012). Therefore, the present study was carried out with the objective of investigating, through laboratory screening, the potential of using *Bacillus thuringiensis var. aizawai*, and NeemAzal-T/S (A commercial neem preparation) as insect control agents against the old world bollworm *H. armigera*.

2. Material and methods

2.1. Insects

Larvae of the OWB were collected from tomato grown in Shambat Research Station Experimental Field of the Department of Plant Protection, College of Agricultural Studies, Sudan University of Science and Technology (SUST). The collected larvae were brought to the laboratory and reared at room temperature of 27 - 30℃ and were individually kept in glass Petri dishes (9 cm in diameter) to avoid cannibalism. The larvae were provided daily with small pieces of okra fruits as source of food until pupation and adult moths' emergence. The adults were fed on a piece of sponge placed in small plastic cups (4 × 3 cm in diameter) and impregnated with 10% sugar solution. Fine cloths were provided for adults as egg laying substrate. The cloths containing eggs were daily placed in glass bottles until egg hatching. The established colony was reared for at least two generations to ensure good quality of larvae that are free from any residue of insecticides. Neonate larvae were immediately transferred into Petri dishes, and provided with small pieces of okra fruit. The developing larvae were carefully followed until they reached the second and then the fourth instars, which were used in the bioassays trials. The implications of the results for sustainable management of vegetable insect pests are discussed.

2.2. Biopesticides

Commercial formulations of XenTari® containing a balanced blend of four potent toxin proteins (Cry 1D, Cry 1C, Cry 1Ab and Cry 1Aa) and spores of *Bacillus thuringiensis var. aizawai* (Bta) and NeemAzal-T/S® (Azadirachtin A) were used in the trials. The trade names, the active ingredients, and the application rates of the two biopesticides in are given in Table 1.

2.3. Bioassays

Small pieces of okra fruit were placed in fine clothes and dipped for 30-40 sec. in each prepared concentration. The treated okra, were then left to dry for 10-20 min. under room conditions. Ten larvae of the second instar were placed in a Petri dish, and provided with 10 pieces of treated okra. The ten larvae were considered as an experimental unit and replicated three times for each treatment. The treated okra was discarded and replaced with untreated fresh okra. The larvae were then given ad libitum access to untreated fresh okra until they all died or otherwise reached pupal stage.

For the fourth instar larvae, ten small plastic cups, each contained two to three pieces of treated okra were prepared. One larva was placed in each cup to avoid cannibalism. The treated pieces of okra were replaced with fresh ones after 48 h. The larvae were daily provided with fresh untreated okra fruit until the end of the experiment (pupation or death of all larvae). Each treatment was replicated three times.

2.4. Statistical analysis

Treatments were arranged in a completely randomized design and the data was statistically analyzed using analysis of variance (ANOVA).Treatment means were separated using Fisher's Least Significance Difference (LSD) test.

3. Results and discussion

From the third day of treatment until the end of the experiment, a dose-dependent mortality effect on the larvae due to treatment with XenTari® (Bta) was observed. At the concentration of 2.7 g/l water, the mortality percentage in both second and fourth instar larvae increased gradually until the sixth day, where it reached 90% and 93%, respectively (Table 2). Bta at a concentration of 0.9 g/L water caused mortality percentage of 67% and 77% in both second and

fourth instar larvae, respectively, while the concentration of 1.8 g/L water resulted in mortality percentages of 70% and 87%) against the second and fourth instar larvae, respectively. The dead larvae appeared to be dark brown to black in color, swelling shriveled and excrete dark liquids. The high mortality percentage of Bta in the fourth larval instars, as compared with the second instar, may be due to high food consumption rate exhibited by the larvae. The second instar larvae stopped feeding shortly after treatment and most of them died thereafter. This finding was similar to that reported by Marrone and Macintosh (1993). In this respect, Vaidya, et al. (1995) reported that *Bacillus thuringiensis* var. *aizawai* (Certain) gave a mortality range of 40-100% in different concentrations when tested against the larvae of cabbage caterpillar *Pieris brassica* Linn. Under laboratory conditions. And also the effects of Bt.a., on the survival and development of the larvae of greater wax moth *Galleria mellonella* L. were investigated in the laboratory against first, second and third instar. The second instar being most susceptible, complete mortality was caused within 4 days after treatment (Eldinahdul, 1988). Basedow et al. (2012) reported a mortality percentage of 77% on larvae of the greater wax moth caused by *Bacillus thuringiensis* var. *aizawai* (XenTari).

Table 1 The biopesticides, their active ingredients and application rates used in the trials

Trade name	Active ingredient	Application rate	Producing company
XenTari®	Protein strain (ABTS-1857) of *Bacillus thuringiensis var. aizawai* 10.3% (w/w)	0.9, 1.8, and 2.7 g/ litre water	Valent Bioscience Corporation, USA
NeemAzal-T/S®	Azadirachtin A 1% (w/w)	0.5, 1.0, and 1.5 ml/litre water	Trifolio-M GmbH Company Lahnau, Germany

Table 2 Mean mortality percentage, caused by XenTari® (Bacillus thuringiensis var. aizawai), against the 2nd and 4th instar larvae of Helicoverpa armigera (Hüb.) under laboratory conditions

Treatments	Days after treatment											
	1st day		2nd day		3rd day		4th day		5th day		6th day	
	2nd instar	4th instar	2nd instar	4th instar	2nd instar	4th instar	2nd instar	4th instar	2nd instar	4th instar	2nd instar	4th instar
0.9 g/L H_2O	33 c	7 a	40 bc	27 ab	43 b	47 b	50 b	53 b	53 b	63 b	67 b	77 b
1.8 g/L H_2O	20 bc	3 a	53 c	20 ab	63 c	50 b	70 c	60 b	70 bc	77 bc	70 b	87 b
2.7 g/L H_2O	10 ab	0 a	27 b	40 b	47 c	77 c	70 c	83 c	80 c	90 c	90 c	93 b
Control (H_2O)	0 a	0 a	0 a	0 a	0 a	0 a	0 a	7 a	0 a	10 a	10 a	10 a
CV. (%)	12.4	4.2	11.7	19.5	18.2	21.7	19.5	20.3	21.2	27	21.1	36.7
SE ±	1.04	0.41	0.82	1.53	1.12	1.19	1.00	1.00	1.04	1.8	0.91	1.22

Means followed by the same lower case letter (s) are not significantly different at 5% level of significance

The efficacy of NeemAzal-T/S® on the second and fourth instar larvae, when tested in different concentrations, is shown in Table 3. In the first day after treatment, in both second and fourth instar larvae, the mortality percentage ranged between (3%- 10%) in different concentrations compared to untreated control. Seven days, after treatment, the mortality percentage in both second and fourth instar larvae reached 97% at concentration of 1 ml/l water. There was no significant difference among the three-tested concentration. Larvae were observed to move far away from the treated food especially in case of second instar larvae. Moreover, the larvae did not molt until death has occurred six to seven days after treatment. The treated larvae also consumed less food compared with the untreated control. These results are also similar to those reported by Gupta, (2002) who observed that Neem formulations containing azadirachtin were more effective against the bollworms. In general, neem derivatives often modify the development of insects by their influence on the hormonal system, especially on ecdysteriods (Schmutterer, 1990), leading to growth regulatory effects, exhibited by growth inhibition, malformation and mortality (Mordue (Luntz) and Blackwell, 1993; Shannag et al. 2015). Spraying of azadirachtin at low concentration of 2 ppm on seedlings of barely provided complete protection against the old world bollworm *Helicoverpa armigera* (Hüb.). It is well known that the order Lepidoptera are very sensitive to

azadirachtin with antifeedancies ranging from < 1-50 ppm, depending on the species. The effective dose (ED_{50}) which causes 50% inhibition of feeding in Lepidoptera is < 0.001-50 ppm (Mordue (Luntz) and Nisbet, 2000).

Table 3 Mean mortality percentage, caused by NeemAzal-T/S® against the 2nd and 4th instar larvae of *Helicoverpa armigera* (Hüb.) under laboratory conditions

Treatments	Days after treatment													
	1st day		2nd day		3rd day		4th day		5th day		6th day		7th day	
	2nd instar	4th instar	2nd instar	4th instar	2nd instar	4th instar	2nd instar	4th instar	2nd instar	4th instar	2nd instar	4th instar	2nd instar	4th instar
0.5 ml/0.3 L H_2O	3 a	0 a	7 a	43 b	10 ab	50 b	57 b	63 b	73 b	73 b	83 b	77 b	90 b	87 b
1 ml/0.3 L H_2O	0 a	10 a	3 a	23 b	10 ab	47 b	53 b	53 b	73 b	70 b	83 b	93 b	97 b	97 b
1.5 ml/0.3 L H_2O	0 a	3 a	0 a	20 b	20 b	40 b	67 b	63 b	73 b	63 b	87 b	80 b	87 b	90 b
Control (H_2O)	0 a	0 a	0 a	0 a	0 a	0 a	0 a	7 a	0 a	10 a	10 a	10 a	10 a	10 a
C.V (%)	2.91	9.44	4.20	14.43	7.71	17.54	15.50	34.20	24.85	41.80	14.63	39.60	22.13	28.00
SE ±	0.29	0.91	0.41	1,12	0.71	1.15	0.90	1.83	1.12	1.91	0.50	1.40	0.65	0.82

Means followed by the same lower case letter (s) are not significantly different at 5% level of significance

Table 4 Mean mortality percentage, caused by combination of XenTari® (*Bacillus thuringiensis var. aizawai*) and NeemAzal-T/S® aga/inst the 2nd and 4th instar larvae of *Helicoverpa armigera* (Hüb.) under laboratory condition.

Treatments	Days after treatment													
	1st day		2nd day		3rd day		4th day		5th day		6th day		7th day	
	2nd instar	4th instar	2nd instar	4th instar	2nd instar	4th instar	2nd instar	4th instar	2nd instar	4th instar	2nd instar	4th instar	2nd instar	4th instar
XN(0.9g/H_2O) +NA(0.5ml/ 0.3 L H_2O)	10 b	17 b	13 b	30 b	33 b	50 b	77 c	67 c	80 b	87 b	100 d	90 b	-	93 b
XN (1.8 g/L H_2O)	20 bc	3 a	53 c	20 ab	63 c	50 b	70 c	60 b	70 bc	77 bc	70 b	87 b	-	87 b
NA (1ml/0.3 L H_2O)	0 a	10 a	3 a	23 b	10 ab	47 b	53 b	53 b	73 b	70 b	83 b	93 b	97 b	97 b
Control (H_2O)	0 a	0 a	0 a	0 a	0 a	0 a	0 a	7 a	0 a	10 a	10 a	10 a	10 a	10 a
C.V (%)	5.40	4.80	6.96	21.50	8.80	14.40	12.90	13.30	20.70	27.60	16.90	31.90	-	30.6
SE ±	0.50	1.90	0.50	1.80	0.65	0.19	0.65	0.71	0.91	1.08	0.58	0.96	-	0.87

XN = XenTari®, NA = NeemAzal-T/S®, combination (50% XN + 50% NA) n Means followed by the same lower case letter (s) are not significantly different at 5% level of significance.

The combination of XenTari® (Bta) and NeemAzal-T/S® caused 100% mortality on the second instar larvae six days after the treatment (Table 4). This efficacy was significantly different from that caused by either biopesticide alone. Cornale, et al. (2001) reported that NeemAzal-T/S® demonstrated an efficacy of 90% and above when it was tank-mixed with other biopesticides such as natural pyrethrum and/or a commercial product based on *Beauveria bassiana* (Naturalis). It is worth to mention here that the combination of NeemAzal-T/S® and *Bacillus thuringiensis* against the larvae of gypsy moth on oak trees reduced the average defoliation by 93.8%. When Bt. and neem were applied separately, the reduction in defoliation was 22% and 7.1%, respectively, (Turcani, 2001). Abedi et al. (2014) evaluated the lethal and sub lethal effects of azadirachtin and *Bacillus thuringiensis var. kurstaki* (Btk) on the third instar larvae of

H. armigera under laboratory conditions. They found that the lethal time (LT_{50}) values of azadirachtin and Bt were 4.8 and 3.6 days, respectively. These results are in line with the findings of the present investigation, where complete mortality for both biopesticides occurred in around 6-7 days after treatment. Neem oil and entomo-pathogenic fungi have been reported to be compatible (Halder et al. 2017), and hence can be used as effective components in integrated pest management programs (IPM) of major vegetable pests. Neem and Bt. biopesticides represent a benign source of killing agents against insect pests and could be safely used in the management of vegetable pests as well as in organic farming agriculture. Traditional neem formulations and available and affordable for the poor farmers and can be made at the village level and simply applied to manage insect and mite pests.

4. Conclusion

The larvae of the old world bollworm *H. armigera* showed high susceptibility to *Bacillus thuringiensis* var. *aizawai* (Bta) and azadirachtin. The combination of the two products resulted in 100% mortality in second larval instar six days after treatment, which clearly demonstrates a synergistic interaction. For azadirachtin to be synergistic with Bta, it should be applied in a concentration that causes growth regulating effect rather than anti-feeding repellency. The two biopesticides could be used in organic farming agriculture for integrated pest management of the old world bollworm and similar lepidopteran pests.

Compliance with ethical standards

Disclosure of conflict of interest

There is no conflict of interest.

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