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Effect of Several Biopesticides on Alfalfa Weevil Larvae, *Hypera brunneipennis* (Boheman)

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Abstract: Commercial and locally extracted biorational pesticides were tested by leaf dipping against the Egyptian alfalfa weevil (EAW), *Hypera brunneipennis* (Boheman). They included BioNeem, horticultural spray oil, K^{+}Neem, garlic barrier, hot pepper wax and cypermethrin 10% EC, at the recommended rates. Water extracts of *Calotropis procera* latex, *Azadirachta indica* seeds and *Citrullus colocynthis*, in addition to methanol extracts of *Solenostemma argel* and *Rhazya stricta*, at rates 0.1-2.0%. Spray oil, *C. procera* and cypermethrin were the only pesticides that significantly caused mortality consistently. The smallest leaf area consumed by EAW larvae was in leaves treated with neem seeds, *C. procera* latex, hot pepper wax, *S. argel*, spray oil and *R. stricta*. No mortality was observed, in all experiments, in neem seeds, *S. argel, R. stricta* and hot pepper wax. Bioneem, *C. procera*, Spray Oil and Cypermethrin were very effective in both inhibition of feeding and causing mortality of larvae. Garlic barrier and *C. colocynthis* stimulated feeding more than the control.

Key words: Biopesticides, alfalfa weevil larvae, hot peper wax, cypermethrin

Introduction

The Egyptian alfalfa weevil (EAW), *Hypera brunneipennis* (Boheman), is a bivoltine, monophagous species which is considered to be the most serious and destructive pest of alfalfa in the central parts of Saudi Arabia. In these regions the newly emerged adults go into estivation in April-May and resume activity around October-November, where they mate and lay eggs in mid-December (Aldryhim and Elshafei, 1991). One annual generation is recorded in Egypt for the EAW (Hammad *et al.*, 1967), whereas 2 generations were present in central Saudi Arabia (Aldryhim and Elshafei, 1991), occurring specifically throughout the winter months. Larval populations reach their peak densities in February, where extensive damage to alfalfa crop is usually witnessed, reducing both yield and quality of the crop.

Current control procedures of EAW rely primarily on application of classical synthetic insecticides such as malathion, Gardona, methoxychlor or pyrethroids. Relatively new compounds have been recommended in the US as being more effective against larvae and adults, such as Furadan, Lorsban and the pyrethroids Warrior, Danitol and Baythroid (Shaw *et al.*, 1998; Shamiyeh *et al.*, 1998). However, still humans face the hazards associated with their use on the crop when they consume dairy and poultry products from animals exposed to these chemicals. Moreover, due to increase in cases of insecticide resistance and adverse effects on beneficial organisms, particularly the environmentally safe forms used in biological control of pests, thoughts were directed towards finding better alternative means of control.

An alternative to conventional insecticides is the use of biological control agents and biorational pesticides, being selective and have no ecological and human health risks and cheap (Stiener and Elliot, 1987; Stauffer and Rose, 1997; Miller and Uetz, 1998). Botanical insecticides, compounds extracted from plants, are the principal constituents of biorational pesticides (Sukumar *et al.,* 1991; Arnason *et al.,* 1989, Isman, 1994). They have been shown to be effective against a wide array of insect species. Among these are seeds and leaves of the neem tree, *Azadirachta indica* A. Juss (Ascher, 1993; Schumutterer, 1990); Harmal, *Rhazya stricta* Decaisne; (El Hag *et al.*, 1996); *Thymus* spp., (Mansour *et al.,* 2000); cloves, *Syzygeum aromaticum* (L.) (Hassanali *et al.,* 1990); Argel, *Solenostemma argel* (Del.) Hayne (Hamed, 2001; Kamel *et al.*, 2000; El-Kamali, 2001). Biorational pesticides also include horticultural oils, insecticidal soaps, fungi such as BotaniGard (a commercial formulation of the entomopathogenic fungus *Beauveria bassiana*), Garlic barrier, bacteria (such *Bacillus thuringiensis* var. *isrealensis*, *B. popilliae*) and insect growth regulators (such as methoprene).

This investigation utilized plant extracts, horticultural oils, insecticidal soaps, garlic barrier, hot pepper wax and cypermethrin (for comparison), as biorational insecticide for control of EAW larvae.

Materials and Methods

Test insects: Alfalfa weevil larvae were collected, early in the morning, with a sweep-net in the alfalfa field at the College of Agriculture Research Center, Meleida. Insects were sorted out in the laboratory, maintained on fresh alfalfa plants kept in water and second instar weevil larvae were selected later for the tests.

Pesticides tested: Commercial biorational pesticides were mixed at the labeled rate and applied. They included: BioNeem, (0.09% azadirachtin, Safer Inc. Mn., USA), all Seasons Horticulture and Dormant Spray Oil® (98.0% petroleum oil, Bonide products Inc., NY, USA), K^+Neem: insecticidal soap concentrate (25.0% potassium salts of fatty acids, derived from neem seed oil, organic Inc. PA, USA), garlic barrier (100% garlic water, Garlic Research Labs., Glendale, Canada), Hot pepper wax (0.00018% capsaicin and other capsaicinoids, Hot Pepper Wax Inc., PA, USA); cypermethrin 10% EC (Cypermethrin 10%, Hubi Sanonda, China). Water extracts of *Calotropis procera* (Ait.) R. Br. (Ascelpiadaceae) latex, *Azadirachta indica* A. Juss seeds and *Citrullus colocynthis* (L.) Schrader (Cuccurbitaceae); methanol extracts of *Solenostemma argel* (Del.) Hayne (Asclepiadaceae) and *Rhazya stricta* Decaisne (Apocyanaceae), as botanical products were also included. The rates tested were: BioNeem, 25 ml l^{-1}; All seasons spray oil, 12.5 ml l^{-1}; K^+Neem, 20 ml l^{-1}; garlic barrier, 100 ml l^{-1}; hot pepper wax, 30 ml l^{-1}; cypermethrin, 0.5 ml l^{-1}; *C. procera* Latex, 1.0%; *A. indica* 2%; *C. colocynthis*, 0.1%; *S. argel*, 0.1% and *R. stricta* 0.1%. Tween 80, 0.2% was added as a surfactant to each concentration and to the water control as well. For comparison to the biorationals, we included the cypermethrin as a conventional commonly used pesticide.

Bioassay: Fresh alfalfa stems were collected from a 2-year old stand and brought into the laboratory where 4 groups of 3 intact leaflets of comparable size, were detached and their area was determined (Li-Cor 3000 Portable Area Meter). Leaflets were then dipped into the desired concentration; air dried in the laboratory and each was placed in a petri-dish and kept moist by attaching the petiole holding them into a wet sponge. Three EAW larvae were introduced into each petri-dish which represented one replicate. The leaflets in controls were dipped into water and surfactant. Each treatment was replicated four times in a completely randomized design. Dishes were kept in the laboratory during the bioassay period under 25±2°C, RH 50-60 and 12:12 L: D. Twenty-four hours later mortality was assessed and leaf area consumed was determined by re-measuring their area in the area meter. The experiment was repeated three times performed simultaneously. Data were analyzed by ANOVA and the means were separated using the Duncan's (Duncan, 1955) multiple range test.

Results

All the materials tested were of plant origin, except the cypermethrin, which was used for comparison. Survival of the larvae varied among the replicate bioassays except in the control, which was 100%. Spray oil, *C. procera* and cypermethrin were the only pesticides that significantly ($P<0.05$) caused mortality consistently, in all 3 experiments (Table 1), however, 24-hr time was not enough to affect death by starvation due to anti-feeding caused by the material. Among the biorationals, the smallest leaf area consumed by EAW larvae was in leaves treated with neem seeds, *C. procera* latex (Table 1); hot pepper wax and *S. argel* (Table 2) and *S. argel, R. stricta*, spray oil and neem seeds (Table 3). No mortality was observed, in all 3 experiments, in neem seeds, *S. argel, R. stricta* and hot pepper wax (Tables 1, 2 and 3). Materials such as Bioneem, *C. procera*, spray oil and cypermethrin were very effective in both inhibition of feeding and causing mortality of larvae that attempt to feed. A third category of these materials is represented by garlic barrier and *C. colocynthis*, which stimulate feeding, sometimes more than the control, (Tables 1, 2 and 3) but caused none or least mortality.

Discussion

Phytochemical investigations demonstrated that alkaloids, flavonoids, sterols, tannins and volatile oils are perhaps the main constituents in most of the materials used. *R. stricta*, locally known as Harmal, is a herb widely distributed in Saudi Arabia and is being used in traditional medicine (Migahid, 1978; Al-Yahia *et al.*, 1990). Rich alkaloidal contents in *R. stricta* of different classes have been reported (Rahman and Fatima, 1982; Ahmad *et al.*, 1983). El Hag *et al.* (1996, 1999) reported on its mosquitocidal activity. *A. indica* seeds contain the neem extract azadiracthin and other active ingredients, which are toxic and have ovicidal, antifeedant and growth inhibitory effects on over 200 species of arthropods (Su and Mulla, 1998; Koul, 1992; Schumutterer, 1990). These compounds were reported to cause diverse behavioral and physiological effects such as repellency, feeding and oviposition deterrence besides reproduction and growth inhibition. El Hag *et al.* (1999) found that *R. stricta* and neem seeds have toxic and growth retarding influence on larvae of *A. ipsilon* and *H. brunneipennis* when fed on leaves dipped into 3000 ppm methanolic extracts. *A. ipsilon* live larval weights and pupation and production of offspring in *H. brunneipennis* were significantly reduced. The results obtained in this study agree with this.

C. procera (Ushar), grows abundantly in Saudi Arabia and other tropical countries. Its latex contains cardiac glycosides, triterpenoides, anthocyanins, alkaloids, resins and proteolytic enzymes (Khan *et al.*, 1981; Al-Yahya *et al.*, 1990) and has been shown to have an anthelmintic, antidysentric, antifungal and molluscicidal activity (Al-

Table 1: Effect of biorational pesticides on alfalfa weevil Larvae, 1st experiment: 11 Feb. 2002

Treatments	Rate	Area consumed(cm^2)	% Mortality
C. colosynthis (aqu. extr.)	0.1%	4.44a	0.00b
BioNeem	25 ml l^{-1}	2.83ab	1.50a
Spray oil	1205 ml l^{-1}	1.79ab	1.25a
Control	-	3.39ab	0.00b
Hot wax	30 ml l^{-1}	3.08ab	0.00b
K$^+$ Neem	20 ml l^{-1}	3.04ab	0.00b
R. stricta (meth. extr.)	0.1%	1.58ab	0.00b
Garlic barrier	100 ml l^{-1}	1.29ab	0.00b
Cypermethrin	0.5 ml l^{-1}	0.48b	0.50b
S. argel (meth. extr.)	0.1%	1.20ab	0.00b
C. procera (latex)	1.0%	0.96b	0.25b
Neem seeds (aqu. ext.)	2.0%	0.25b	0.00b

Aqu. = aqueous, meth. = methanolic, extr. = extract.
*Means in the same column followed by the same letter are not significantly different at the 5% level, Duncan's multiple range test.

Table 2: Effect of biorational pesticides on alfalfa weevil Larvae, 2rd experiment: 4 March 2002

Treatments	Rate	Area consumed(cm^2)	% Mortality
C. colosynthis (aqu. extr.)	0.1%	5.41a	0.25a
Control	-	3.98ab	0.00a
Garlic barrier	100 ml l^{-1}	4.37ab	0.00a
C. procera (latex)	1.0%	3.80ab	0.25a
Spray oil	12.5 ml l^{-1}	3.67ab	0.75a
Cypermethrin	0.5 ml l^{-1}	3.23ab	0.75a
Neem seeds (aqu. ext.)	2.0%	2.54ab	0.00a
BioNeem	25 ml l^{-1}	1.83ab	0.00a
R. stricta (meth. extr.)	0.1%	1.81ab	0.00a
K$^+$ Neem	20 ml l^{-1}	1.72ab	0.00a
S. argel (meth. extr.)	0.1%	1.42b	0.00a
Hot pepper wax	30 ml l^{-1}	1.14b	0.00a

Aqu. = aqueous, meth. = methanolic, extr. = extract.
*Means in the same column followed by the same letter are not significantly different at the 5% level, Duncan's multiple range test.

Table 3: Effect of biorational pesticides on alfalfa weevil Larvae, 3rd experiment: 11 March 2002

Treatments	Rate	Area consumed(cm^2)	% Mortality
Garlic barrier	100 ml l^{-1}	3.18a	1.25a
Control	-	2.37ab	0.00b
C. procera (latex)	1.0%	2.22ab	0.25b
BioNeem	25 ml l^{-1}	1.36ab	0.00b
K$^+$ Neem	20 ml l^{-1}	1.75ab	0.50b
Hot pepper wax	30 ml l^{-1}	1.44ab	0.00b
Spray oil	12.5 ml l^{-1}	0.74b	1.25a
Cypermethrin	0.5 ml l^{-1}	0.27b	0.25b
Neem Seeds (aq. ext.)	2.0%	1.07b	0.00b
R. stricta (meth.extr.)	0.1%	0.94b	0.00b
S. argel (meth.extr.)	0.1%	0.59b	0.00b

Aqu. = aqueous, meth. = methanolic, extr. = extract.
* Means in the same column followed by the same letter are not significantly different at the 5% level, Duncan's multiple range test.

Qarawi *et al*., 2001; Larhsini *et al.,* 1997; Ageel *et al.,* 1987). Its insecticidal activity has been reported by Al-Doghairi and Elhag (2002) on mosquitoes. Its effect on EAW, as an agricultural pest, encourages and justifies investing more research on its outdoor use.

S. argel is a medical African herb, that has long been used by Africans for its antispasmodic, purgative, loss of appetite and carminative effects. Monoterpene glucosides, pregnane glucosides, flavonoids and tannins as well as some other steroids and alkaloids were isolated and identified from different parts of the herb (Hamed, 2001; Kamel *et al*., 2000). It has also been known for its anti-inflammatory, anti-ulcerous, immunostimulatory as well as antimicrobial and fungicidal properties (Hahn *et al.,* 1998). El-Kamali (2001) and Al-Doghairi *et al.* (2002) have reported its mosquitocidal effect. This is the first record of *S. argel* use in control of agricultural pests. Its performance is considered excellent, the smallest leaf area consumed by EAW larvae was observed in leaves treated with neem seeds, *C. procera* latex; hot pepper wax and *S. argel*; and *S. argel, R. stricta*, spray oil and neem seeds (Tables 1, 2 and 3). Nevertheless, no mortality was observed, in all 3 experiments, in neem seeds, *S. argel, R. stricta* and hot pepper wax. Perhaps these materials exert their effect by their anti-feeding action, thus, although larvae did not feed, they do not die in 24 h time. Materials such as BioNeem, *C. procera*, spray oil and cypermethrin were very effective in both inhibition of feeding and causing mortality of larvae that attempt to feed. BioNeem, neem seeds and spray oil have oil in them, perhaps causing the EAW larvae to suffocate, or causing treated leaves less attractive to the larvae. Al-Doghairi (2000) found that horticultural oil SunSpray an effective flea beetle, *Phyllotreta pusilla,* repellent which lowered their infestation on broccoli; however, he found garlic extract the least satisfactory, among the materials he tested. Garlic barrier and *C. colocynthis* represent a third category among these materials which stimulate feeding, sometimes more than the control, but caused none or least mortality.

Thus *R. stricta*, *S. argel, C. procera* and *A. indica* seeds are cheap local materials that are potentially rich in bioactive substances of varied chemical nature that may be made use of in the suppression of pest populations. These materials are effective, safe and easy to use, however their use in outdoor field situations needs further investigations. Spray oil and hot pepper wax are equally effective and environmentally safe, however, they are relatively costly. BioNeem, K$^+$Neem and other formulations of neem are not far better than the locally extracted products if we consider the cost. These materials could be useful tools to be considered among effective biopesticides to be incorporated in pest management programmes.

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