CATRINA (2010), 5 (1): 73 -75
© 2010 BY THE EGYPTIAN SOCIETY FOR ENVIRONMENTAL SCIENCES

Antifeedant activity of spindle tree *Euonymus europaeus* (Celastraceae) seed extract against diamond back moth *Pluttella xylostella* (L.) (Lepidoptera: Plutellidae)

Shereen M. Elbanna

Zoology Dept., Faculty of Science, Suez Canal University, Ismailia, Egypt

ABSTRACT

The diamond back moth; *Plutella xylostella* (L.) was long considered a relatively significant pest. Its impact was overshadowed by such serious defoliators as *Pieris rapae* (Linnaeus). It attacks plants in the family Cruciferae causing serious damages. In this research for new insect naturally occurring antifeedant substances, spindle tree *Euonymus europaeus* (Celastraceae) seeds was assayed for antifeedant and/or insecticidal activities. The alkaloid fraction, isolated from the seeds of the spindle tree was found to be responsible for the significant antifeedant activity of this tree using crude extract against 4th instar larvae of the diamond back moth.

Key words: Antifeedant, *Euonymus europaeus, Plutella xylostella,* alkaloid

INTRODUCTION

The diamond back moth has many hosts, including cabbage, broccoli, cauliflower, collards, kale, Brussels sprouts, kohlrabi, turnip, radish, mustard, and watercress. The larvae initially feed as leaf miners but soon emerge to feed on undersides of leaves. Investigations of plant defence mechanisms against herbivores, and more especially the role of plant allelochemicals, have been extensively investigated over the last decades. These studies are of major interest because of their potential application in integrated pest management (IPM) programs, and in providing new leads for environmentally safe pesticides (Balandrin *et al*., 1985). Although so far, a few compounds only have reached the level of application in the field (Klocke & Kubo, 1991), the search for more potent insect control agents of natural origin continues. Given the growing problem of insecticide resistance and the hazards of insecticide use, antifeedants are among the most promising alternative pest-control methods (Powell *et al*., 1992). Plants of the family Celastraceae are distributed in all parts of China; some of these plants were traditionally used in China to protect other plants from insect damage (Jacobson, 1958). Some of these plants contain sesquiterpene polyol esters which exhibit insect antifeedant and/or insecticidal effects (Wakabayashi *et al*., 1988). The Chinese bittersweet, *Celastrus angulatus* Max. (Celastraceae), is a traditional insecticidal plant widely distributed and used in China. Plants of the family Celastraceae produce alkaloids, of which some exhibit insect antifeedant and insecticidal activity. *Euonymus* is an unusual genus since some of its species contain cardiac glycosides and alkaloids (Bishay *et al*., 1973). The presence of alkaloids in the plants belonging to *Euonymus* genus was reported. *Euonymus europaeus* L., the sole representative of this genus in central and Western Europe and is known as spindle tree. In this paper, the possible antifeedant effect of the crude extract of *Euonymus europea* (Celastraceae) against *Plutella xylostella* (Lepidoptera: Plutellidae) was investigated

MATERIALS AND METHODS

Extraction of the plant material:

The *Euonymus europaeus* L. seeds were collected, freeze-dried, crushed and grounded. Dried and powdered seeds (20gm) were subjected to Soxhlet extraction at room temperature for 72 h. The extract was concentrated by evaporation under reduced pressure at 30 °C, to yield an orange gum. Further purification methods were followed, and pure alkaloid fraction was isolated from the seed crude extract.

The insects:

Early fourth instar larvae of *Plutella xylostella* (Linnaeus) were bioassayed in this study. The plant which was used for the leaf-disc test and rearing insects is Chinese cabbage.

Rearing the insects:

Plants; Chinese cabbage leaves were collected and used for rearing the test insects. Dry leaves were removed from the adult cages, which is covered in eggs. To synchronise the eggs place in a Stewart box for a day or two, so any neonates that have crawled onto the leaf die off. The leaves with eggs were placed onto a fresh plant just before they hatch. Fresh leaves were placed into the adult moth cages for oviposition. The leaves were placed top side down as the moths prefer to lay their eggs on the underneath of the leaves. A fresh plant was added to any cage with larvae that need more food. The 4th instar larvae generally gravitate upwards when about to pupate. The pots were left in the cage as they are often covered in pupae. The life cycle took place in 14 days at 28°C and (16/8; L/D).

* Corresponding Author: shelbana@gmail.com

Bioassay of *Pluttela xylostella* strain:

Leaf-disc test: In control tests, five dishes were treated only with pure acetone 1%. After complete evaporation of the solvent by air drying, early fourth instar larvae were placed in each dish (5 dishes, 10 larvae per each). The dishes were maintained under controlled conditions (28 °C in 16/8; L/D). The experiment was repeated three times. Young larvae were preferred since they are more sensitive to the antifeedant effect of allelochemicals (Lewis & Van Emden, 1986). During this bioassay, maintaining constant moisture in the test arena was necessary, and it was equally important for the discs to remain in place, so that measurement of individual disc surfaces can be done accurately. This is done usually by placing a wet filter paper disc in the bottom of the dish and pinning down the disks on the bottom of the dish (Kogan & Goeden, 1969).

Leaf discs of known area were treated with known amounts of the plant extract dissolved in acetone. The early fourth instar larvae were allowed to feed on the discs for 24 h. The areas consumed were measured under a binocular microscope by counting 1 mm squares exposed when the partially eaten disc was placed on a circle the exact size of the disc drawn on mm-ruled paper. After 24 h, the number of knocked down larvae or dead larvae was recorded. Each treatment consisted of 5 plastic Petri dishes with 10 insects each. The consumed areas of the discs were measured, when the consumed areas of the sample discs were less than 20% of those of the control discs, the samples were considered to have strong feeding inhibitory activity.

Bioassay experiment:

The crude extract was dissolved in 1% acetone in distilled water, one ml of each solution applied on the surface of the Chinese cabbage leaf. The leaf was placed on the top of a filter paper and placed in a Petri dish and the solution left for half an hour to dry. 10 early fourth instar larvae of *Plutella xylostella* were placed on the leaf and the dishes were left at 28 °C. The control was represented by 5 dishes, 10 larvae each. The extract was represented by 5 dishes, 10 larvae each. The treated larvae left for 24 hours before recording mortality. The larvae were observed for repellence (drive away without feeding). The dead larvae were counted for insecticide action and the percentage of consumed cabbage leaf was calculated for antifeedant and deterrent effect of the seed extract.

Statistical analysis:

For each set of experiments, the percentage of area consumed was later calculated by reference to the control surface. A value of less than 20 indicates feeding deterrent activity. A value of zero indicates that the treated discs were not consumed. A value exceeding 80 would indicate a strong feeding stimulation effect.

RESULTS AND DISCUSSION

It is generally accepted that most of the secondary products of the plants are important for its fitness. They may either serve to attract pollinating animals or to inhibit the growth of microorganisms and of other plants or to repel the feeding of herbivorous animals (Harborne, 1987). Some of these compounds such as the limonoid, azadirachtin not only act as deterrent but also have insecticidal activity if absorbed or eaten by insects (Simmonds & Blaney, 1996). The diversity of these defence compounds is high; and many are very effective at protecting plants from being eaten. However, only a few have been developed into commercial products such as insecticides. Some plant species of the Celastraceae, especially the powdered root bark of Chinese bittersweet, *Celastrus angulatus* Max., were traditionally used in China to protect plants from insect damage (Wakabayashi *et al*., 1988). In this experiment, there was no larval mortality on the leaves treated with acetone. About 60 % of the leaf was eaten when the leaves treated only with acetone, indicating no antifeedant effect for acetone (Fig. 1). Almost all larvae on the leaves treated with acetone didn't moult to a pupal stage, indicating that acetone doesn't have any deterrent, insecticidal, growth suppresant or antifeedant effect. In the experiment, where the crude seed extract was applied to the leaf disc, all the larvae moulted rapidly to become pupae before the control treatment, indicating growth rate effect of the crude extract as (table 1). In the experiment where the leaf was treated with crude seed extract, only 1% of the leaf disc was eaten, indicated antifeedant activity of the l crude seed extract. The antifeedant activity was significant. The current results showed that the *Euonymus europaeus* crude seed extract has exhibited significant antifeedant activity against fourth larval instar of *Plutella sp* moth. This is in agreement with Wu (1991) who found that *Celastrus angulatus*, another plant from the same family, exhibited antifeedant activity against several insect species. Meanwhile, the above results showed that most of the larvae when placed on the leaf treated with crude seed extract, most larvae transformed to pupae and shorten larval stage, indicating growth rate enhancement effect of the crude seed extract. This can be explained as most of the larvae tried to avoid the plant seed extract by going through the immobile pupae stage. The previous findings suggest that bad or abnormal environmental conditions can enhance the larvae to go through pupal stage. Using purification of the plant crude seed extract, pure alkaloid mixture was obtained. Application of alkaloid mixture on the leaf disc, showed significant larval quick transformation to pupae and 1% of the leaf disc was eaten. Early fourth larval instars didn't feed or it goes to pupate very quickly rather than the larvae treated with acetone. This indicated that the alkaloid mixture has antifeedant and growth enhancement activity. The results showed that alkaloid mixture and crude seed extract didn't have repellence effect as there are no larvae driven away from the leaves. On the other hand, neither the crude seed extract nor the alkaloid has any lethal effect, as no dead larva was recorded in these experiments. This finding is in disagreement with the findings that the pyridine alkaloid nicotine is of one of the few plant-derived alkaloids that has been developed as an insecticide. The concentration of nicotine in foliage can be induced and it has been shown to increase by 28% in leaves exposed to *Lepidopteran* larvae, compared to control leaves (Baldwin, 1998 & 1999). Further purification by preparative thin layer chromatography (tlc) on silica gel narrowed the antifeedant to a band sensitive to Dragendorff's reagent, and thus was probably an alkaloid compound. Comparing the pure alkaloid compound and the mass spectrum showed that this compound is evonine. The effect of the evonine was the same for both 1mg/ml and 0.1 mg/ml but the effect was demolished at lower concentration; 0.01mg/ml. The results revealed that the tested plant seed extract can be used as antifeedant or growth rate enhancement against *Plutella xylostella* L. management program as the spindle tree contains toxic compounds especially for the immature stage.

Table (1): The effect of the *Euonymus europaeus* seeds extract on insect *Pluttella xylostella* (L.) feeding, growth rate and larval death

Treatment	%Plant eaten	No. larvae	No of alive larvae	No. of pupae
Control	60	10	9	1
	80	10	10	0
	90	10	10	0
Acetone	5	10	10	0
	70	10	10	0
	80	10	10	0
Alkaloid	1	10	1	9
(evonine)(	2	10	0	10
1000 ppm)	2	10	1	9
Treatment	**%Plant eaten**	**No. larvae**	**No of alive larvae**	**No. of pupae**
Control	100	10	10	0
	75	10	10	0
	90	10	10	0
Acetone	70	10	10	0
	60	10	10	0
	80	10	10	0
Alkaloid	1	10	7	3
(evonine)	2	10	4	6
(100 ppm)	0	10	6	4
Treatment	**%Plant eaten**	**No. larvae**	**No of alive larvae**	**No. Of pupae**
Control	100	10	10	0
		10	10	0
		10	6	4
Acetone	70	10	10	0
	90	10	7	3
	100	10	8	2
Alkaloid	50	10	3	7
(evonine)	40	10	3	7
(10 ppm)	70	10	2	8

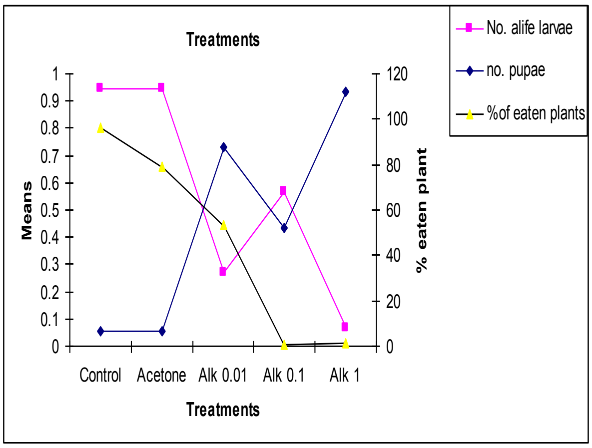

Fig (1): The effect of the *Euonymus europaeus* seeds extract on insect *Pluttella xylostella* (L.) feeding, growth rate and larval death.

References

BALANDRIN M.F., KLOCKE J.A., WURTELE E.S., AND BOLLINGER W.H. (1985). Natural plant chemicals: sources of industrial and medicinal materials. American Association for the Advancement of Science **228; (4704):** 1154-1160

BALDWIN, I.T. (1999). Inducible nicotine production in native *Nicotiana* as an example of adaptive phenotypic plasticity. *J. Chem. Ecol.* **25**: 3–30.

BALDWIN, I.T. (1998). Jasmonate-induced responses are costly but benefit Plants under attack in native populations. *Proc. Natl. Acad. Sci.* **95:** 8113–8118.

BISHAY D W., KOWALEWSKI Z., AND PHILLIPSON J. D. (1973). The identification of peptide alkaloids from Euonymus europaeus L. (Celastraceae). The Journal of Pharmacy and pharmacology **24:** 169-1972

HARBORNE, J. B. (1987). Chemical signals in the ecosystem. Annals of Botany, **60:** 39-57

KLOCKE J A. & KUBO I. (1991). Defense of Plants through Regulation of Insect Feeding behavior. *The Florida Entomologist*, **74; 1:** 18-23.

JACOBSON, M. (1958). Insecticides from plants, a review of the literature, 1941–1953. Agricultural Handbook No. 154, USDA, US Government Printing Office. Washington, DC, pp. 44.

LEWIS A. C. AND VAN EMDEN. H. F. (1986). Assays for insect feeding, pp. 95-119. *In* J. R. Miller and T. A. Miller (eds.), Insect-Plant Interactions. Springer-Verlag, New York.

POWELL G., HARDIE J. AND PICKETT J. A.(1992). Laboratory evaluation of antifeedant compounds for inhibiting settling by cereal aphids. Entomologia Experimentalis et applicata **84:** 189-193

SIMMONDS M. S. & BLANEY W. M. (1996). Azadiractin: advances in understanding its activity as an antifeedant. Entomologia Experimentalis et applicata. **80:** 23-26

WAKABAYASHI, WU, W. J., WATERS, R. M. REDFERN, R. E. MILLS, G.D. DEMILO, JR, A.B. LUSBY W.R. AND ANDRZEJEWSKI, D. (1988) Celangulin: a non-alkaloid insect antifeedant from *Celastrus angulatus*. J. Nat. Prod. **51:** 537–542

WU, W. (1991). A review of the studies on insecticidal action of Chinese bittersweet, *Celastrus angulatus* Max. Plant Protection **17:** 34–38.

Received May 17, 2010
Accepted August 15, 2010