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FEEDING DETERRENT AND GROWTH INHIBITORY PROPERTIES OF LARVAL FRASS AGAINST *Spodoptera littoralis* (Boisd.)

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ABSTRACT

The research has been conducted to evaluate the activity of the frass extracts of *Spodoptera littoralis* (Boisd.) against its larvae. Five solvents differ in their polarity namely petroleum ether; chloroform; acetone; ethanol and water were used to extract the frass material. The following points were studied: (a) evaluation the toxicity of different extracts against *S. littoralis* larvae (b) effect of frass extracts on the development of *S. littoralis* larvae (c) evaluation of the antifeedant activity of frass extracts against 4th instar larvae of *S. littoralis*. Results showed that the 10% concentration of frass exhibited strong antifeedant activity. On the other hand ethanol and water extracts (highly polar) caused the highest mortality percentage whilst petroleum ether and chloroform (non-polar) caused the highest percentage of antifeedant activity, respectively. The type and concentration of extracts by water, ethanol, chloroform and acetone affected the sex ratio and development of *S. littoralis*. In general, the biological activity of frass extracts was solvent type-and concentration-dependent.

Key words: ***Spodoptera littoralis*, frass, Antifeedant activity, Deterrent**

INTRODUCTION

A wide range of structural and chemical attributes of plant material has been implicated as a major determinant of food selection in insect. Until recently, most discussions of feeding behaviour have centered on positive aspects of selection that is on the acquisition of required nutrients. The need for primates to select food so as to obtain a diet balanced in essential nutrients is obvious and has been referred of by many authors (Oates *et al*., 1980). Among essential nutrients, emphasis has been placed on such products of primary metabolism in plants as proteins and soluble carbohydrates, and on materials (Nagy and Milton, 1979). With the growing awareness that the vast array of secondary metabolites that plants synthesize may also influence food selection by animals because of their frequently deleterious properties (Oates *et al*., 1980), swain compounds. The suitability of host plant as an oviposition site influenced by the nutritional quality of plant, the presence of absence of toxins, the degree of competition for the food source and exposure to natural enemies (Renwick, 1989).

Chemoreception plays an important role in mediating a diverse range of behaviours, including avoidance (White and Chapman, 1990) while contact chemoreceptors assist with identification of suitable oviposition sites (Ma and Schoonhoven, 1973). The legs, particularly the tarsi that are in contact with the substrate (Gaaboub, 1990 and 2000), also carry many chemoreceptors (Gaaboub and Hustert, 1998). In butterflies *Pieris brassica* stimulation of the tarsi by sugar solutions evokes an automatic extension of the proboscis (Ma and Schoonhoven, 1973). The olfactory stimuli are often the first stimuli encountered by female and starts the long range attraction to the plant, by motivating the female to initiate upwind flight (Renwick and Chew, 1994).

Deterrents affecting oviposition choice in moths have been found in damaged host plants (Rothschild and Schoonhoven 1977, Turlings *et al.* 1991). The deterrent activity of larval frass is dependent on larval density or not is very important. The persistence of the deterring activity of larval frass on the host plant, and its solubility, the minimum amount of frass that is necessary for significant oviposition deterrence and the relation between frass and contact chemoreceptor on tarsus have been studied (Gaaboub and Halawaa, 2003). Field observation by (Campion *et al.*, 1977) revealed that *S. littoralis* emigrated from areas with high population densities. Possibly, *S. littoralis* females respond to oviposition deterrents by emigration, in order to look for a place where the offspring will find suitable developmental conditions. Young larvae can also be more sensitive to food quality and toxins than older larvae, and as a consequence of this are more selective about their diet (Renwick, 1989 and Jones, 1991).

The use of synthetic pesticides for plant protection against agriculture pests during the last 50 years created many problems. Namely, pest resistance, environmental pollution and disturbance in natural balance. Thus we are

trying to find out a new method to control the pest and our work study the relation between frass as antifeedant and toxic material against *S. littoralis* larvae

MATERIAL AND METHODS

Tested Insect:

Laboratory strains of the cotton leaf worm *S. littoralis* (Boisd.) were used in the present work. The culture of *S. littoralis* was originated from eggs obtained from laboratory strain established in the Department of Plant Protection, Faculty of Agriculture Moshtohor, Zagazig University. The eggs were kept in glass jar covered with gauze under laboratory condition of 27 ± 1 C°. and 65 ± 5 % RH. When the egg became dark enough in color fresh castor leaves were introduced daily into the jars as dietary medium for the hatched larvae. This procedure continued till the second larval instar. The 3rd instar larvae were isolated in glass jars capacity 1 kg at the rate of 15 larvae to avoid cannibalism. The jars were cleaned daily. The number of larvae was limited to 10 larvae per jar, when larva reached the last instar then transferred to a clean jars, provided with moistened sawdust 4 cm thick and allowed to pupate. Old pupae of both sexes were transferred to cylindrical cages covered with gauze for oviposition. The adults were provided daily with fresh 10% sugar solution.

Frass extraction:

Extraction of frass was carried out on 100 g of larval frass, were successively extracted with petroleum ether 60:80, acetone, ethanol and water, for 48 hr. at room temperature. Each extract was evaporated separately under vacuum to complete dryness.

Table (1): Percentage of each extract of different solvent based on 100/g larval frass of *S. littoralis* larvae.

Solvents used	% Crude extraction/100g Frass
Petroleum ether	2.44
Chloroform	4.32
Acetone	6.139
Ethanol	1.827
Water	5.71

1. Bioassay for feeding deterrence of frass extracts

Tests for feeding deterrence were carried out using the fourth instar larvae of a laboratory culture of *S. littoralis*. The larvae were starved for 6 hours before treatment and divided into 3 replicates (10 larvae for each replicate). Discs of 39.12-mm^2 area of castor leaves were impregnated with the extract under investigation and allowed to dry. Only one disc was offered to each tested larva. Untreated discs were introduced to larvae as blank control. Discs impregnated with the same solvents allowed drying then offered to test insects as control with solvents. The eaten area was estimated after 24 hours by planimeter.

The percentage of feeding reduction over control was used as factor for determining the presence of feeding deterrent effect. The antifeeding activity of the larval frass extracts was evaluated on the basis of the feeding ratio of the treated and untreated leaf discs. The antifeeding activity was calculated by using the formula of (Saleh *et al.* (1986) as follow

$$\text{Antifeeding activity} = 1 - \frac{\text{\% of. Eaten area in treatment}}{\text{\% of Eaten. Area in control}} \times 100$$

2- Biological effects:

All treated larvae were left for 24 hrs. and supplied with fresh castor leaves and kept under observation until the emergence of adults. Adults were supplied daily with 10% sugar solution. Mortality counts during the larval, pupal and adult stages were recorded. Abnormal pupation and the percentage of malformed adults were recorded for each treatment. Control tests were carried out by the use of solvents only alongside untreated blanks.

3- Toxicity effects:

In order to study the toxicity of the larval frass extracts, preliminary screening tests were carried out to the larval frass extracts of five different solvents each at five concentration levels of 10, 7.5, 5, 2.5 and 1.25 % w/v). Three replicates (10 larvae each) were used for each concentration. Insect larvae were kept under controlled temperature 27 ± 1 C°. Percentage of total mortality was recorded after the end of the larval stage. Percentage mortalities were corrected according to Abbots formula (Abbott, 1925).

Corrected mortality = [(Observed mortality % - control mortality %)/(100 – control mortality %)] x 100

RESULTS AND DISCUSSION

The potential destructiveness of the cotton leaf worm *S. littoralis*, in crops such as cotton, tomato, sweet potato…. etc has been long recognized. This pest is considered by many to be one of the principals limiting factors in the development cotton industry. Laboratory strains of the cotton leaf worm S. *littoralis* (Biosd.) were reared to obtained large amount of larval excretion on our studies. Moths, larvae, pupae and egg masses of the cotton leaf worm were obtained from a standard colony maintained on castor bean leaves (*Ricinus Communis* L.).

The solvents of different polarity, namely, petroleum ether, chloroform acetone, ethyl alcohol and water. The residue obtained from each solvent was weighed and diluted to five concentrations (10, 7.5, 5, 2.5 and 1.25 %) to tested:

1. Feeding deterrence (Antifeedant activity).
2. Biological effects.
3. Toxicity effects.

1. Antifeedant activity of different larval frass extracts on larvae of *S. littoralis*

Data in table (2) and fig. (1A&B) indicated that, *S. littoralis* larvae were deterred from feeding on leaf discs previously treated with different types of larval frass extracts. It was cleared that, the different solvents varied widely in their content of toxic and undesirable materials for development of *S. littoralis* due to the degree of solvent polarity. The percentage antifeedant activity were 66, 65 & 58%, respectively when 10% concentration of chloroform, acetone and petroleum ether extracts of larval frass were tested. These results may be attributed to the non-polar solvents, which are rich in terpenes that create highest protection percentages with less consumed area of the plant leaves. Terpenes component in larval frass of *S. littoralis* was (eugenol, thymol, carvacol, nerolidol and phytol) reported by (Hilker and Kelin, 1989). The physiological activity of terpenoids in feeding activity was bitter-tasting and toxic (Harborne, 1993). The main active constituents which present in non-polar solvent extract was terpens in high concentration, but the highly polar solvent extract were poor in terpenes. On other hand, the lowest effect was recorded with water extract (highly polar solvent) giving 22% antifeedant activity at the same concentration. It was noticed that, with decreasing the concentration extract the antifeedant activity were decreased. The total amount of crude extract is shown in table (1).

Table (2): Effect of larval frass extracts on antifeedant activity and consumed area of treated castor leaves eaten by the 4th instar larvae of *S. littoralis*.

Solvent	Conc. %	Consumed area in $(mm)^2$ treated 24 hr.	% of eaten area	Antifeedant activity
Petroleum ether extract	10.00	15.72 ± 0.39	2.57	58.00
	7.50	29.15 ± 0.67	4.77	22.00
	5.00	31.92 ± 0.75	5.22	15.00
	2.50	33.31 ± 1.02	5.45	11.00
	1.25	36.31 ± 0.84	5.94	3.00
	0.00	37.37 ± 0.75	6.11	0.00
Chloroform extract	10.00	11.94 ± 1.36	1.95	66.00
	7.50	26.46 ± 0.42	4.33	25.00
	5.00	27.45 ± 0.76	4.49	22.00
	2.50	28.77 ± 0.19	4.71	19.00
	1.25	31.06 ± 0.66	5.08	12.00
	0.00	35.37 ± 0.25	5.79	0.00
Aceton extract	10.00	11.45 ± 0.38	1.87	65.00
	7.50	16.34 ± 0.27	2.67	51.00
	5.00	21.64 ± 0.38	3.54	35.00
	2.50	27.58 ± 0.56	4.51	17.00
	1.25	28.28 ± 0.34	4.63	15.00
	0.00	33.05 ± 0.78	5.41	0.00
Ethanol extract	10.00	31.41 ± 0.58	5.14	28.00
	7.50	35.02 ± 0.58	5.73	20.00
	5.00	35.70 ± 0.36	5.84	18.00
	2.50	38.83 ± 0.45	6.35	11.00
	1.25	40.76 ± 0.66	6.67	7.00
	0.00	43.78 ± 0.57	7.16	0.00
Water extract	10.00	40.71 ± 0.18	6.66	22.00
	7.50	44.19 ± 0.74	7.23	15.00
	5.00	47.29 ± 0.80	7.74	10.00
	2.50	50.02 ± 0.44	8.18	4.00
	1.25	51.04 ± 0.52	8.35	2.00
	0.00	52.05 ± 1.27	8.52	0.00

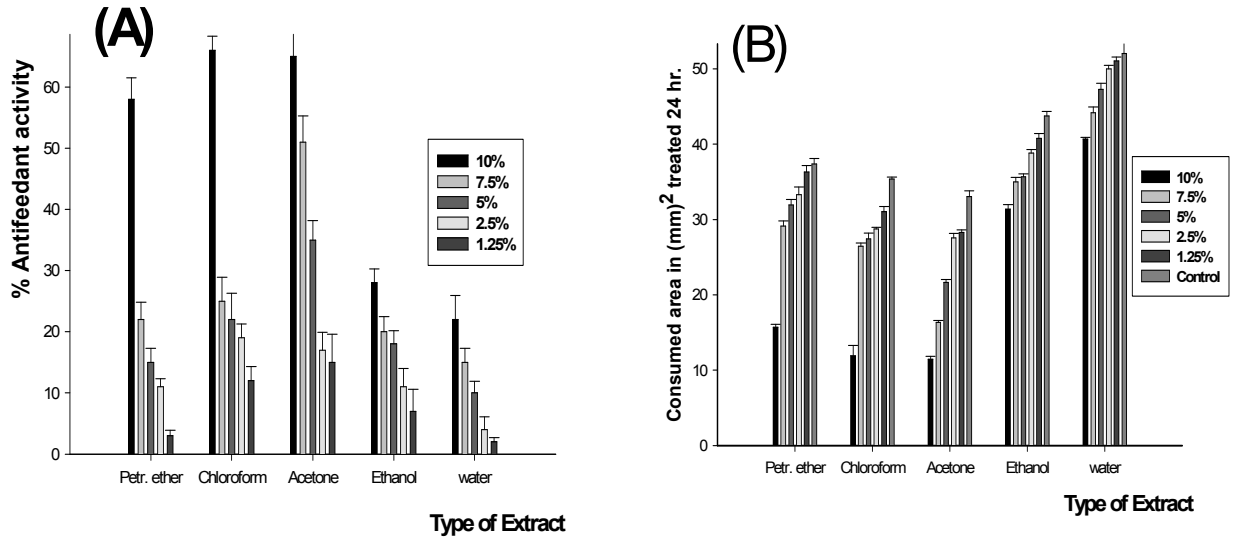

Fig. (1) Effect of larval frass crude extract concentrations on (A) antifeedant activity of 4th instar larvae of *S. littoralis* and (B) consumed area of the treated castor leaves.

2. *Biological effects of different larval frass extracts on larvae of* S. littoralis

Extracts were studied to investigate the biological effects, which may occur after exposure of 4th larval instar to these extracts.

2.1. *Effect of larval frass extract on insect's development*

Data in table (3) show the development of S. *littoralis* larvae after the exposure to frass extract with the different five solvents (petroleum ether, chloroform, acetone, ethyl alcohol and water). The highest percentage of dead larvae was recorded at 10 % concentration of ethyl alcohol and water extract (85.39 & 80.56 %) respectively. Nevertheless, the lowest number of dead larvae was recorded with petroleum ether at 1.25 %, which gave only (8.33%). The study of the development effects showed that larval frass tested extracts adversely affected on developmental aspects of the pest. These results were in agreement with (Ismail *et al*., 1996 and Gaaboub, *et al.*, 2005) that, the non polar solvents extract was recorded lowest mortality percentage.

2.2. *Effect of larval frass extract on insect's sex ratio*

The sex ratio was affected by the different types or concentrations of larval frass extract as shown in table (3). It was clear that; female ratio was greater than male one with non-polar solvents extract (petroleum ether and chloroform). On other hand, all other types of extracts make the opposite. The type of chemical constituents such as terpenes, ketones and aldehes (Hilker and Kelin, 1989), which present in each extract may play one of the important roles in this ratio (Gaaboub and Halawa, 2003 and Gaaboub, *et al*., 2005). The type of food affected on the sex ratio as in *Plutella xylostella* infestation and percentage parasitism in the field in .

Michigan was higher on broccoli than on the other Brassica crops, but the female/male ratio of *Diadegma. insulare* was not significantly different (Idris and Grafius, 1993).

The developmental effects of showed that *Neotorularia aculeolata* (Family *Cruciferae*) tested plant adversely affected all developmental aspects of the pest (Gaaboub, *et al.*, 2005). These results are in agreement with (Mogahed and El-Gengaihi, 1998) who found that both *Diplotaxis acris* and *D. hara* (family *Cruciferae)* had developmental inhibition against *S. littorails.*

In fig (2B) the mean number of egg masses and total eggs on the *Nerium oleander* leaves previously with treated *S. littoralis* frass extracts of S. *littoralis* was significantly lower than the number of egg masses and total eggs of control and the same solvent alone did not give the same result. In several species of Lepidoptera, feeding larvae and larval frass indicate occupancy of the host plant and deter egg deposition (Dittrick *et al*., 1983; Mitchell and Heath, 1985; Renwick and Radke, 1980 and 1981, Rothschild and Schoonhoven, 1977 and Williams et al., 1986). One of the hypotheses is that only larvae at high densities excrete oviposition-deterring substances to which females respond by avoiding egg deposition. Several studies of *S. littoralis*

Table (3): Biological activity of frass extracts *S. littoralis* on larval, pupal and adult stages as affected by frass crude extract concentration.

Solvents	% Conc.	Larval stage		Pupae stage			Adult stage		
		% corrected Mortality	Duration day	% mortal.	% malform.	Durat. day	% malform.	Sex ratio F.	M.
Pet. ether extract	10.00	27.78	6.50	8.00	7.41	7.18	16.67	15	9
	7.50	16.67	6.71	8.00	16.67	7.67	13.04	10	13
	5.00	13.89	6.50	16.00	19.35	7.64	9.52	13	8
	2.50	11.11	6.50	13.79	9.38	7.77	8.00	17	8
	1.25	8.33	6.22	12.50	5.88	7.60	7.14	15	13
	0.00	0.00	10.60	0.00	8.33	7.00	2.94	16	18
Chlorof. extract	10.00	74.29	6.57	37.50	11.11	7.80	0.00	3	2
	7.50	62.86	5.67	25.00	7.69	7.29	0.00	3	5
	5.00	57.14	5.71	14.29	6.67	6.25	15.38	8	5
	2.50	45.71	6.10	11.76	10.53	6.33	13.33	9	6
	1.25	40.00	6.00	0.00	0.00	6.50	5.56	12	6
	0.00	0.00	7.00	0.00	2.68	6.50	2.94	18	16
Acetone extract	10.00	48.57	6.80	10.53	15.00	8.27	6.25	10	6
	7.50	45.71	5.86	41.18	21.05	9.00	40.00	5	5
	5.00	42.86	5.63	11.76	15.00	9.50	20.00	6	9
	2.50	40.00	5.63	10.53	9.52	9.00	17.65	7	10
	1.25	34.29	5.63	6.25	30.43	8.89	5.88	7	9
	0.00	0.00	8.5	0.00	5.71	5.29	11.76	18	16
Ethanol extract	10.00	85.39	11.00	0.00	0.00	22.00	0.00	2	4
	7.50	72.97	10.14	25.00	6.25	23.00	0.00	4	4
	5.00	56.76	9.08	13.33	9.09	22.33	7.14	8	6
	2.50	51.35	9.89	12.59	11.11	21.78	8.33	5	7
	1.25	49.92	10.8	5.26	13.64	22.00	5.56	8	10
	0.00	0.00	21.63	0.00	5.41	8.83	0.00	20	14
Water extract	10.00	80.56	7.33	0.00	12.50	24.00	0.00	3	4
	7.50	77.78	14.00	0.00	16.67	21.00	0.00	3	4
	5.00	72.22	12.00	10.00	9.09	22.33	0.00	4	5
	2.50	66.67	12.11	9.09	8.33	19.57	0.00	6	5
	1.25	61.11	11.14	0.00	0.00	21.42	0.00	5	9
	0.00	0.00	11.50	0.00	2.78	19.47	3.03	21	12

indicate a change of metabolism when larval density increases (Hodjat, 1970; Rivnay and Meisner, 1965; Zaher and Moussa, 1961; Gaaboub, 1990 and Hilker and Klein, 1989). Metabolic changes might cause a change of frass compounds. These changes in frass of larvae, which were reared at high densities, might be a signal to gravid females indicates that the site is unsuitable for oviposition. It is worthy to note that oviposition by females of *Ephestia kuehniella* was strongly deterred by specific amount of secretion from conspecific larvae, while oviposition by females of *Plodia interpunctella* was stimulated by the same amount secretion. Thus, the females of the two species showed an oppsite behavioural response to the same stimulus (Anderson *et al.*, 1995).

2.3. Effect of larval frass extract on insect's malformation

The highest number of abnormal pupae were resulted after feeding of 4th instar larvae on low concentration of acetone and ethanol alcohol extracts (1.25%) 30.43 & 13.64 % respectively, while in high concentration of petroleum ether and chloroform extract give the highest number of malformation. Larval frass extracts differ in with different concentrations were giving between 30.43 to 5.88 % for all used concentration. These may be due to the high concentration extract contain a toxic effect to insect, while low concentration acting as the juvenile hormone (Table 3 and Fig. 2) (Gaaboub and Halawa, 2003 and Gaaboub *et al.*, 2005).

3. Toxicity effects of different larval frass extracts on larvae of *S. littoralis* (insecticidal activity studies)

The insecticidal activity of different larval frass extracts was studied and shown in table (3) and fig (2A). The different extracts of larval frass extract showed a potent effect and an ovicidal activity against *S. littoralis*. The richness of petroleum ether and acetone extract with

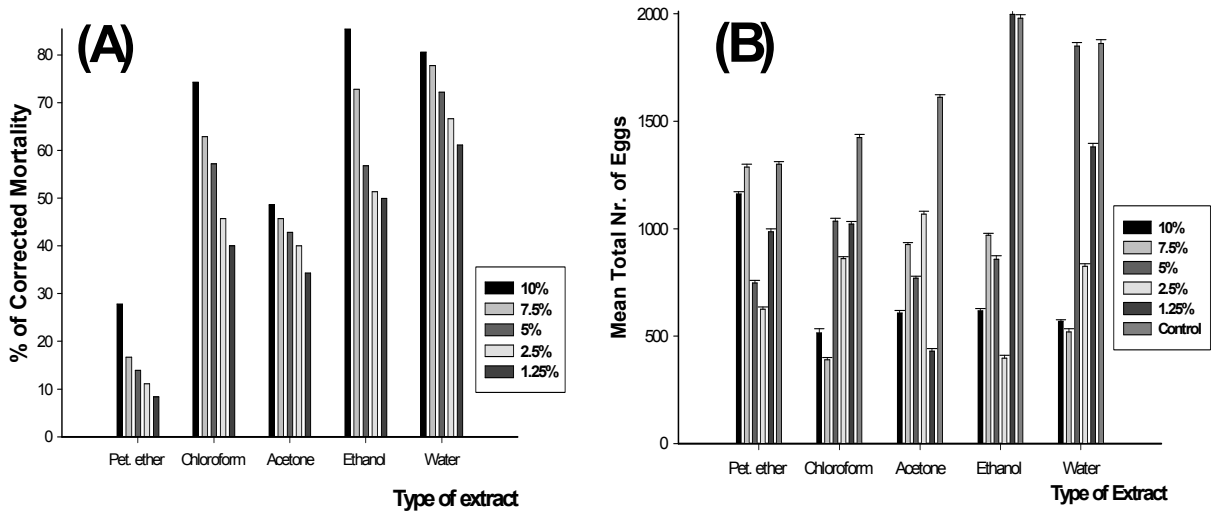

Fig. (2): Effect of larval frass crude extract concentrations on (A) % Mortality of 4th instar larvae of *S. littoralis* and (B) mean number of eggs deposited by insects fed by caster leaves treated with different solvents.

terpenes compounds was the reason of the high protection percentagesas indicated from less consumed area in the plant leaves with less mortality percentages. The alcoholic and aqueous extracts of larval frass of *S. littoralis* were remarkably toxic against *S. littoralis* larvae. These results may be attributed to highly polar solvents was rich in alkaloids, which the physiological activity of alkaloids was many toxic and bitter-tasting against the insect (Harborne, 1993). The alcoholic and aqueous extract of *N. aculeolata* were remarkably toxic against *S. littoralis* larvae and this in agreement with that reported by (Mogahed and El-Gengahi, 1998, Gaaboub *et al*., 2005). On the other hand, the richness of chloroform, ethanol and water extracts with aldeheds, tannins (Hilker, 1985) was the reason of the decreasing the protection percentages and the consumed area of the treated leaves and increasing mortality and malformation percentages. These may be due to the different active materials that encourage the insects to eat the plant, but they increased the mortality and malformation effect (Table 3 and Fig. 2).

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