*Sci. J. Fac. Sci. Menoufia Univ. Vol. X (1996). 21 - 36*

# REPELLENT, FEEDING DETERRENT, AND GROWTH-INHIBITING EFFECTS OF *CLERODENDRON INERME* AND *PITURANTHOS TORTUOSUS* EXTRACTS AGAINST THE CONFUSED FLOUR BEETLE, *TRIBOLIUM CONFUSUM* (COLEOPTERA : TENEBRIONIDAE)

Emara, T.E. and Assar, A.A.

Zoology Department, Faculty of Science, Menoufia Univ.,

Shebin El-Kom, Egypt

**Key words :** ***Clerodendron inerme*** (Verbenaceae), ***Pituranthos tortuosus*** (Umbeliferae), ***Tribolium confusum***, Repellency, Deterrency, Reprduction.

*ABSTRACT*

*Laboratory studies were carried out to investigate the efficacy of ethanolic leaves extract from* ***Clerodendron inerme*** *Gaerth. (Verbenaceae) and aerial parts extract from* ***pituranthos tortuosus*** *Desf. (Umbeliferae), against the confused flour beetle,* ***Tribolium confusum*** *Duval. The two plant extracts elicited moderate repellency effects that increased with increasing the concentration of the test materials. Feeding deterrent efficiency of both ethanolic crude extracts was evaluated using wheat wafer disk bioassay. The two plant extracts showed moderate food deterrency. Development of larvae (16 ± 2 day - old) fed on wheat flour treated with above crude extracts was affected as the pupation and adult emergence percentages were decreased as compared with control. In addition, the progeny of adult beetles obtained from treated larvae was affected. Reproduction of adult beetles (two weeks old) treated with different doses of both plant extracts and the development of their progeny were affected. The above mentioned effects were dependent on the dose and kind of plant extract applied.* ***Clerodendron inerme*** *extract was highly effective, that it prevented the development of F1 progeny (larvae) into pupae at the doses of, 0.5, 1.0, and 1.5%.*

## INTRODUCTION

Insect infestation of stored grains and their products is considered a serious problem througout the world. In recent years

much effort has been devoted to search for alternatives to the current agents of pest-control using insecticides, activity of plant extracts against stored-products insects has been studied by many authors (Jilani and Malik, 1973; Jilani and Saxena, 1990; and Talukder and Howse, 1993). Control methods using extracts of plant origin were safe and economic as compared with the chemical control application, because of certain problems due to the chemical insecticides use, such as undesirable chemical residues and environmental pollution were detected. The efficiency of plant extracts as food deterrent and repellent for insects was studied by many investigators Jilani and Malik (1973) against stored grain insects; Jilani and Saxena (1990) against lesser grain borer; and Talukder and Howse (1993) against *Tribolium castaneum* in storage. Growth inhibiting and toxic effects of certain plant extracts were evaluated by Abdallah and Kandil (1986) on *Sitophilus granarius* L. and *Tribolium castaneum* Hbst.; El-Nahal *et al.* (1989) and Risha *et al.* (1990) on different stages of some stored product Coleoptera and Jilani *et al.* (1988) on the red flour beetle. Reproduction and development of some coleopteran insects were affected due to the using of different plant extracts : Smet *et al.* (1986) on *Tribolium confusum* and Schmidt *et al.* (1991) on some stored-product Coleoptera. The present study was undertaken using ethanolic botanical extracts of *Clerodendron inerme* and *pituranthos tortuosus* to reveal the repellent, feeding deterrent and growth-inhibiting effects of these plants on the confused flour beetle, *Tribolium confusum.*

## MATERIALS AND METHODS

Leaves of *Clerodendron inerme* Gaerth. (Verbenaceae) and aerial parts of *pituranthos tortuosus* Desf. (Umbeliferae) were collected, air - dried and then pulverized into fine powder with electric mill. Plant

material powder was extracted with absolute ethanol by soaking for 24 hours and then shaking for 48 hours and filtered using vacuum pump. Plant extract was concentrated using rotary evaporator and finally dried using nitrogen. Crude extract of *Clerodendron inerme* and *pituranthos tortuosus*, hereafter were referred to as crude plant extract A and B, respectively. Different concentrations were prepared using ethanol as a solvent. The confused flour beetles, *Tribolium confusum* Duval. were obtained from a laboratory culture maintained at 28 ± 2°C and 60 ± 5% R.H. The beetles were reared in glass jars on diet of whole wheat flour mixed with dried baking yeast with a ratio 19 : 1, respectively.

Plant extracts were evaluated for their repellency effects by using the standard technique described by McDonald *et al.* (1970). Whatman filter paper disks (No. 40), 8.5 cm diameter were cut into two halves and dipped separately for 1 min. in different concentrations of plant extracts. Control disk halves were treated only with the solvent. The filter paper halves were air-dried overnight at room temperature. Each treated disk half was then attached lengthwise, edge to edge to the control half with cellulose tape on the reverse side and placed in a petri-dish. Ten adult beetles (15 ± 2- days - old) were released in the middle of the petri dish. The number of beetles on each half was counted at different intervals : from filter paper treatment (1 - day, 8-day, and 15-day); and from beetles release (15 min, 30 min, 1 hour, 2 hour, 3 hour, 4 hour, and 5 hour). All tests were carried out in dark and the counts were made under red light. Four replicates were applied for each concentration. The mean repulsion percentage (R) was calculated as follows : R = 2 (C - 50), where C = percentage of beetles on the control half. Positive values express repellency and negative values indicate attractancy (McDonald *et al.*, 1970).

Feeding deterrent activity of tested plant extracts was evaluated, using the wheat wafer disk technique (Nawrot *et al.*, 1986). Wheat wafer disks were treated by dipping (1 min) in different concentrations of plant extracts (giving treated disks, E) or in solvent only (giving control disks, K). Treated disks were evaporated for 24 hours at room temperature, weighed and presented to 20 beetles (15 ± 2-days-old). After 5-days, during which, wafer disks were the sole food source. Food consumption of the beetles was recorded under three conditions : a) on pure untreated food, two control disks presented (KK disks); b) on food with choice, one treated disk (E) and one control disk (K); and c) on food with no choice, two treated disks presented (EE disks). At the end of 5 - days period, the disks were reweighed and according to the amount of food consumed in control (KK), choice (EK), and no choice (EE) test, three coefficients for feeding deterrent activity were calculated : the absolute coefficient of deterrency, A = (KK - EE / KK + EE) x 100; the relative coefficient of deterrency, R = (K - E/K + E) x 100; and the total coefficient of deterrency, T = A + R. The total coefficient was the index of activity. Plants with strong antifeedant activity have an index of 150 - 200, while those neutral have an index of 0 - 50.

To assess the effect of botanical extracts on the development of the immature stages, three concentrations of both plant extracts were presented through 20 g of the diet to 25 larvae (16 ± 2 - day - old). Percentage of pupation and adult emergence were determined. The adults emerged from treated larvae were kept in the treated media and mean number of progeny (larvae and pupae)/female was determined at 25th day and 35th day after adult emergence.

In order to determine the effectiveness of plant extracts on reproduction and on progeny development of adult beetles, the extracted dose material was mixed with 20 g of diet and left for evaporation (48 hours). 25 beetles (15 ± 2 - days - old, randomly

selected) were released to the treated flour in glass jars (100 ml capacity) covered with muslin. Three replicates were made for each concentration and the control. After 15-days, the adults were removed and the flour was examined at different periods for the following progeny (larvae, pupae and adults). Larvae and pupae were removed from the media by sieving (0.5 mm mesh), counted and returned back to the media until adult emergence.

## RESULTS AND DISCUSSION

Repellent efficiency of both plant extracts A and B against the beetles of *Tribolium confusum* was illustrated (Table 1) by using standard method of whatman filter paper. The mean repellency percentage of plant extract A and B was : 60.71 and 58.57 at 0.5%; 62.14 and 60.00 at 1.0%; and 63.57 and 59.28 at 2.0% doses, respectively, at one-day after treatment. So, plant extract A and B elicited moderate repellency, that slightly increased with increasing the dose of treatment. The mean repellency elicited by plant extract A was slightly more than that of plant extract B. The repellent potential of both plant extracts A and B slightly decreased with increasing the period between filter paper treatment and the release of beetles. This slight decrease might be due to the evaporation of certain volatile components of plant extract.

On the contrary, ethanol extracts of the same plants elicited strong repellency against *Tribolium castaneum*, as the mean percentages of repellency were : 92.85 and 88.57 at 0.5%; 96.42 and 94.28 at 1.0%; and 90.00 and 94.28 at 2.0% doses, for *Clerodendron inerme* and *pituranthos tortuosus*, respectively (Emara and Ryan, 1996). This means that *Tribolium confusum* is more resistant than *Tribolium castaneum* for these plant extracts. Similar study was carried out using Pithraj seeds, *Aphanamixis polystachya* extract against *Tribolium castaneum* adults (Talukder and Howse, 1993).

Table (1) : Mean repellency percentage of plant extracts to *Tribolium confusum* adults at given time intervals using treated filter paper test.

| Plant extract | Dose % | Mean repellency percentage at the following intervals | | | | | | | Mean | Repellency class** |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 15 min | 30 min | 1 hour | 2 hour | 3 hour | 4 hour | 5 hour | | |
| A | | One-day after treatment | | | | | | | | |
| | 0.5 | 40 | 40 | 50 | 55 | 70 | 80 | 90 | 60.71 | IV |
| | 1.0 | 40 | 45 | 55 | 60 | 75 | 75 | 85 | 62.14 | IV |
| | 2.0 | 45 | 50 | 55 | 60 | 70 | 80 | 85 | 63.57 | IV |
| | | Eight-day after treatment | | | | | | | | |
| | 0.5 | 40 | 40 | 55 | 65 | 70 | 85 | 85 | 62.85 | IV |
| | 1.0 | 40 | 45 | 55 | 60 | 70 | 80 | 90 | 62.85 | IV |
| | 2.0 | 50 | 45 | 60 | 60 | 70 | 80 | 85 | 64.28 | IV |
| | | Fifteen-day after treatment | | | | | | | | |
| | 0.5 | 40 | 40 | 50 | 60 | 60 | 75 | 80 | 57.85 | III |
| | 1.0 | 40 | 40 | 50 | 55 | 55 | 70 | 80 | 55.71 | III |
| | 2.0 | 40 | 40 | 60 | 65 | 60 | 70 | 80 | 59.28 | III |
| B | | One-day after treatment | | | | | | | | |
| | 0.5 | 40 | 60 | 55 | 60 | 60 | 65 | 70 | 58.57 | III |
| | 1.0 | 45 | 55 | 55 | 65 | 60 | 65 | 75 | 60.00 | III |
| | 2.0 | 40 | 55 | 55 | 60 | 60 | 70 | 75 | 59.28 | III |
| | | Eight-day after treatment | | | | | | | | |
| | 0.5 | 35 | 50 | 55 | 55 | 65 | 70 | 70 | 57.14 | III |
| | 1.0 | 40 | 40 | 55 | 65 | 65 | 75 | 75 | 59.28 | III |
| | 2.0 | 40 | 50 | 55 | 60 | 60 | 70 | 75 | 58.57 | III |
| | | Fifteen-day after treatment | | | | | | | | |
| | 0.5 | 40 | 40 | 50 | 55 | 60 | 75 | 70 | 55.71 | III |
| | 1.0 | 40 | 40 | 55 | 60 | 65 | 75 | 75 | 58.57 | III |
| | 2.0 | 40 | 50 | 60 | 55 | 60 | 65 | 70 | 57.14 | III |

A = Extract of *Clerodendron inerme*, leaves; Fam : Verbenaceae

B = Extract of *Pituranthos tortuosus*, leaves and stems; Fam : Umbeliferae

** Various classes for repulsion-percentage are according to the following scale (Talukder and Howse, 1993).

| Class | Repulsion percentage |
|---|---|
| 0 | > 0.01 to < 0.1 |
| I | 0.1 - 20 |
| II | 20.1 - 40 |
| III | 40.1 - 60 |
| IV | 60.1 - 80 |
| V | 80.1 - 100 |

Ethanolic extracts of pithraj seeds elicited strong repellency in terms of mean repellency percentages after 5 hours of beetles release, which were : 86.0 and 85.0 at 0.5 and 1.0%, respectively. Repellent effects of Turmeric oil, Sweetflag oil, and Neem oil have been reported against lesser grain borer (Jilani and Saxena, 1990); and red flour beetle (Jilani *et al.*, 1988). Also, their repellency was decreased with increasing period between filter paper treatment and release of beetles. The latter authors attributed this decrease in repellency due to the evaporation of active components with time.

The plant extracts A and B evinced marked feeding deterrence effects against *Tribolium confusum* beetles, as their total coefficients of deterrency were : 68.64 and 62.08 at 0.5%; 80.05 and 72.64 at 1.0%; and 93.84 and 82.94 at 2.0%, respectively for plant extract A and B (Table 2). The feeding deterrent effect was dose-dependent and *Clerodendron inerme* extract was more effective. Crude extracts of *Clerodendron inerme* and *pituranthos tortuosus* were examined previously against *Tribolium castaneum* for deterrency, their total coefficients of deterrency were : 84.44 and 63.31 at 0.5%; 94.54 and 54.53 at 1.0%; and 93.30 and 71.92 at 2.0% respectively (Emara and Ryan, 1996). Ethanolic extracts of *Aphanamixis polystachya* seeds elicited moderate deterrency against *Tribolium castaneum* adults, as the total coefficient of deterrency was 48.82 at 1.0% dose using the same wafer disc technique (Talukder and Howse, 1993).

*Tribolium confusum* larvae (16 ± 2-days-old) showed sensitivity towards the crude plant extracts A and B, as the pupation and adult emergence percentages have decreased (Table 3). This decrease was dose-dependent as the pupation percentage was, 90.66 and 93.33 at 0.5%, 81.33 and 90.66 at 1.0%, and 64.0 and 86.66 at 1.5%; and the adult emergence percentage was, 92.30 and 97.05 at 0.5%, 86.56 and 93.84 at 1.0%, and 79.54 and 92.85 at 1.5%, for plant extract A and

Table (2) : Feeding deterrent activity coefficients of plant extracts against the confused flour beetle, *Tribolium confusum* adults.

| Plant extract | Dose % | Coefficients of deterrency (Mean) | | | Efficacy of extract |
|---|---|---|---|---|---|
| | | Absolute | Relative | Total | |
| A | 0.5 | 10.39 | 58.25 | 68.64 | ++ |
| | 1.0 | 13.49 | 66.56 | 80.05 | ++ |
| | 2.0 | 26.12 | 67.72 | 93.84 | ++ |
| B | 0.5 | -9.32 | 71.40 | 62.08 | ++ |
| | 1.0 | 0.84 | 71.79 | 72.64 | ++ |
| | 2.0 | 5.41 | 77.53 | 82.94 | ++ |

A = Extract of *Clerodendron inerme*
B = Extract of *Pituranthos tortuosus*

B, respectively. Reproduction potential of adult beetles emerged from above treated larvae was affected as the mean number of larvae/female observed on the 25th day of adult emergence was : 16.37 and 24.84 at 0.5%, 13.65 and 24.63 at 1.0%, and 9.22 and 17.99 at 1.5%, respectively, for plant extract A and B as compared with control (25.93). It is clear that plant extract A was more effective against the development of larvae and the reproduction potential of emerged adults than plant extract B (Table 3).

Adult beetles (15 ± 2 - days - old) reared on whole wheat flour media treated with 0.5, 1.0, and 1.5% of plant extract A and B for 15 days evinced decrease in reproduction (number of larvae) and delay in the progeny development. This decrease in number of larvae observed on the 20th day after treatment was dose-dependent and was greater in plant extract A than B, as the mean number of larvae was : 131.6 and 159.3 at 1.5%; 124.0 and 170.0 at 1.0%; and 156.6 and 196.6 at 0.5% respectively for plant extract A and B in comparison with 257.3 in the

Table (3) : Botanical extract effects on the development of *Tribolium confusum* larvae (16±2 day-old) and progeny of emerged adults.

| Plant extract | Dose % | Pupation % | Adult emergence % | Mean number of progeny (larvae and pupae) observed / female on the following periods after adult emergence. | | | |
|---|---|---|---|---|---|---|---|
| | | | | 25th day | | 35th day | |
| | | | | larvae | pupae | larvae | pupae |
| | 0.5 | 90.66 | 92.30 | 16.37±0.31 | 0.0 | 0.69±0.00 | 0.0 |
| A | 1.0 | 81.33 | 86.56 | 13.65±0.20 | 0.0 | 0.0 | 0.0 |
| | 1.5 | 64.00 | 79.54 | 9.22±0.09 | 0.0 | 0.0 | 0.0 |
| F value | | | | 1722.9** | -- | -- | -- |
| F at 1 % level | | | | 7.59 | -- | -- | -- |
| | 0.5 | 93.33 | 97.05 | 24.84±0.27 | 0.0 | 21.66±0.20 | 5.42±0.08 |
| B | 1.0 | 90.66 | 93.84 | 24.63±0.11 | 0.0 | 21.11±0.24 | 3.67±0.06 |
| | 1.5 | 86.66 | 92.85 | 17.99±0.27 | 0.0 | 16.10±0.10 | 2.92±0.05 |
| F value | | | | 490.0** | -- | 632.0** | 184.4** |
| F at 1 % level | | | | 7.59 | -- | 7.59 | 7.59 |
| C | 0.0 | 97.33 | 98.63 | 25.93±0.06 | 0.0 | 23.76±0.17 | 4.27±0.09 |

A = *Clerodendron inerme* extract; B = *Pituranthos tortuosus* extract; C = control.
** Highly significant at 0.01 level of probability.

case of control (Fig. 1). All the larvae observed in treatments of plant extract A died on 41st day after treatment. A delay in the larval development in treatments of plant extract B was observed. This delay was dose-dependent, as the maxmium mean number of larvae was : 170.3 on 27th day; 205.6 on 34th day; 229.6 on 27th day; and 257.3 on 20th day respectively at 1.5%; 1.0%; 0.5% of plant extract B and control treatments (Fig. 1). The larval development durates up to 69 days due to treatment with 1.5% plant B extract, in comparison with 48 days in control.

Plant extract A highly affected the development of treated adults progeny, as all larvae died at 0.5, 1.0, and 1.5% doses. However, plant extract B caused delaying effects on the development of larvae as the begining mean number of pupae observed was : 1.0, 3.6, 5.6, and 16.3 on 27th day, respectively, at 1.5, 1.0, 0.5% doses and control treatments (Fig. 2). This delay in development of larvae to pupae was also indicated as the maximum mean number of pupae observed was : 55.3 and 82.3 on 48th day; 87.0 on 41st day; and 100.3 on 41st day, respectively, at 1.5% and 1.0%; 0.5%; and control treatments (Fig. 2).

Adult emergence of Fl progeny was affected and delayed due to the treatment of parent adult beetles with plant extract B, as the begining mean number of adults emerged was 13.0 on 41st; 3.6 and 10.0 on 34th day; and 2.0 on 27th day, respectively, at 1.5%; 1.0% and 0.5%; and control treatments. This delay was indicated also as the maximum mean number of adults emerged was : 60.6 on 55th day; 93.0 and 104.0 on 48th day; and 100.0 on 41st day, respectively at 1.5%; 1.0% and 0.5%; and control treatments (Fig. 3).

Oil vapors of Indian, *Acorus calamus* rhizomes did not evince any effects with respect to the toxicity, development and reproduction of *Tribolium confusum*, meanwhile this oil induced a great effect on

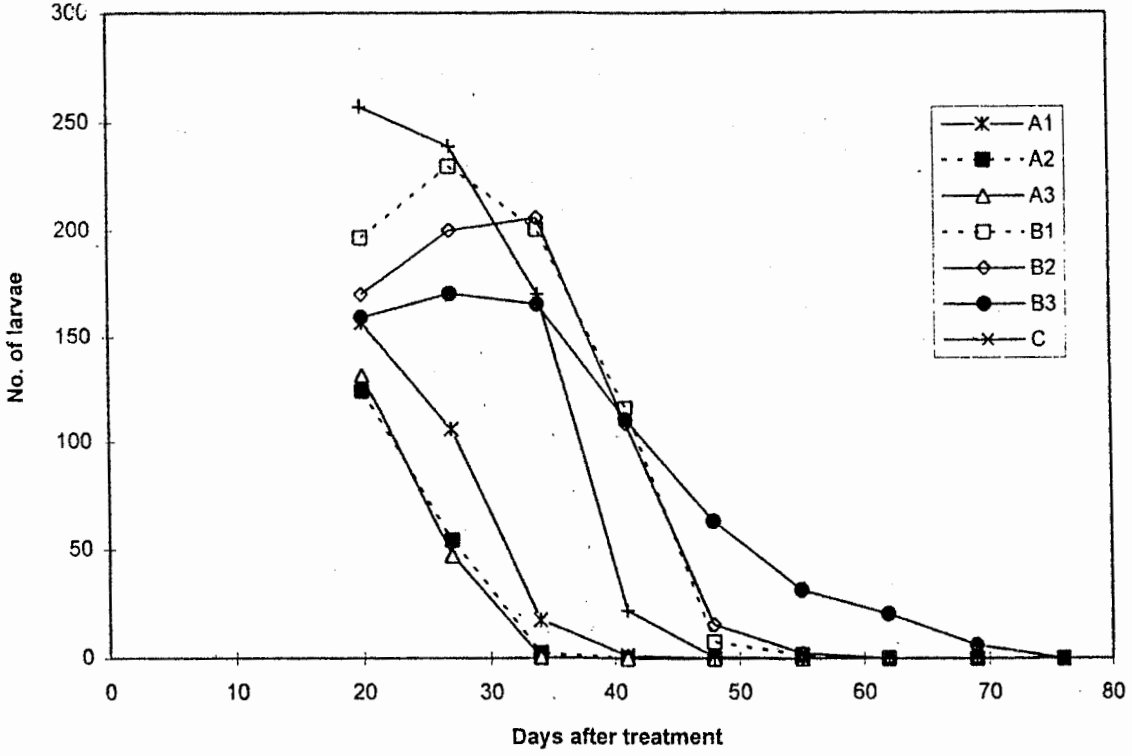


Fig. 1 : Botanical extract effects on the mean number of progeny (larvae) of F1 generation of *Tribolium confusum* Duval. treated as adults.
A1, A2, A3 = *Clerodendron inerme* with concentrations of 0.5, 1.0 and 1.5%, respectively.
B1, B2, B3 = *Pituranthos tortuosus* with concentrations of 0.5, 1.0 and 1.5%, respectively. C = Control.

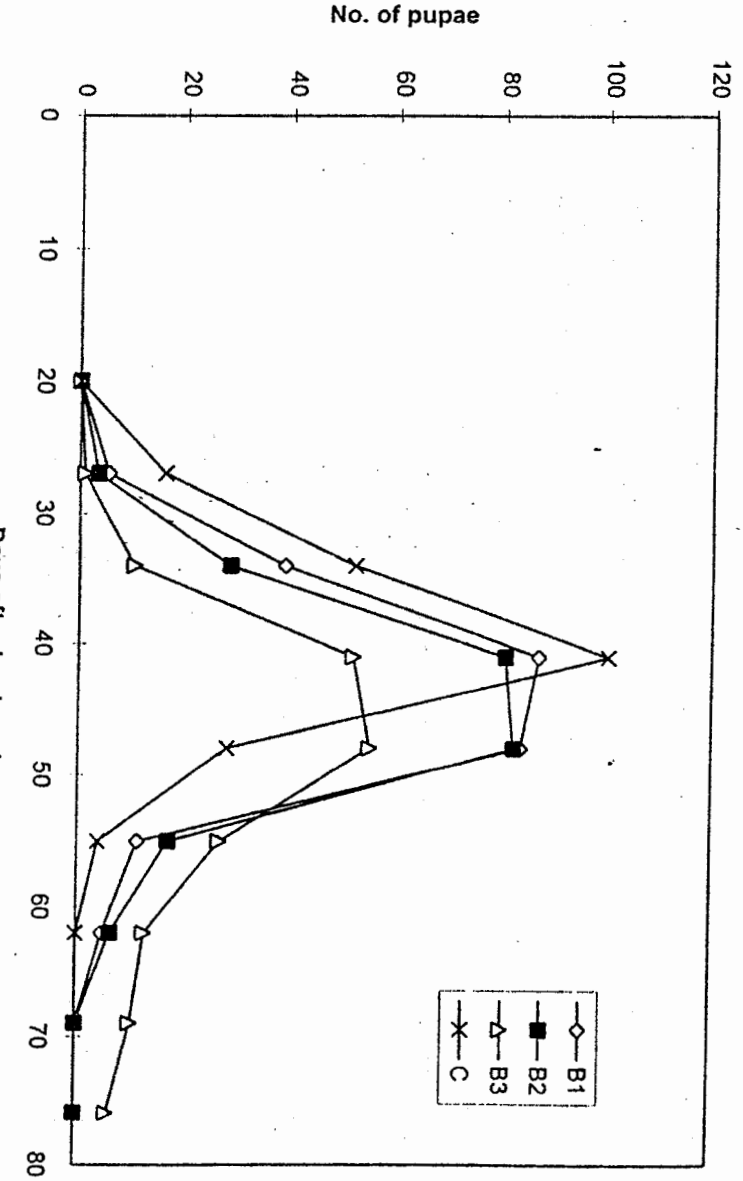


Fig. 2 : Botanical extract effects on the mean number of progeny (pupae) of F1 generation of *Tribolium confusum* Duval. treated as adults. B1, B2, B3 = *Pituranthos tortuosus* with concentrations of 0.5, 1.0 and 1.5%, respectively. C = Control.
N.B., no pupae produced in treatments of A1, A2 and A3.

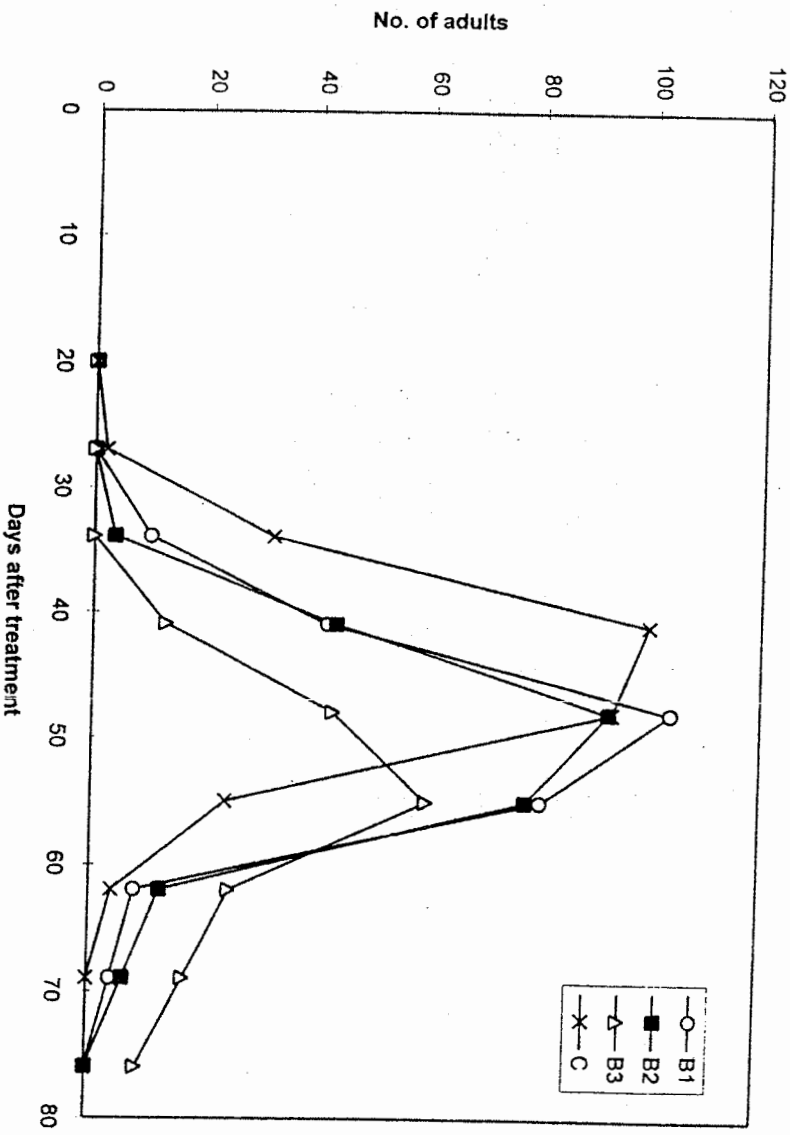


Fig. 3 : Botanical extract effects on the mean number of progeny (emerged adults) of F1 generation of *Tribolium confusum* Duval. treated as adults.
B1, B2, B3 = *Piturantbos tortuosus* with concentrations of 0.5, 1.0 and 1.5%, respectively. C = Control.
N.B., no adults emerged in treatments of A1, A2 and A3.

the reproduction, development and mortality of different stages of : *Sitophilus granarius* L., *Sitophilus oryzae* L., and *Callosobruchus chinensis* L. (El-Nahal *et al.*, 1989; Risha *et al.*, 1990; and Schmidt *et al.*, 1991). In spite of the above resistance of *Tribolium confusum*, it showed considerable susceptibility to the application of crude plant extract A and B, that might be important in the control of this harmful insect. Comparable data were obtained, where adults of three planthopper species reared on neem oil treated plants, showed significant retardation in the development of nymphs (Heyde *et al.*, 1984). When red flour beetles reared on wheat flour treated with turmeric oil, *Curcuma longa*; sweetflag oil, *Acorus calamus*; and neem oil, *Azadirachta indica* at 200 ppm, the resulting larvae, pupae and adults were decreased as compared with control group. Turmeric oil was the most effective as the reduction percentage of larvae, pupae, and adults were 42.64, 44.06, and 44.06% respectively compared with control (Jilani *et al.*, 1988).

The investigated plant extracts need further studies aiming at isolation of the active components, which may be promising in the insect control programmes.

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# التاثيرات الطاردة ، المانعة للغذاء ، والمانعة للنمو لمستخلصات كل من كليرودندرون انيرم ، وتورانسس تورتيوسس على خنفساء الدقيق المتشابهة ، ترايبوليم كونفيوزم (غمدية الأجنحة ، تينيبريونيدى)

طلعت السيد عمارة – عبادة أبوذكرى عصر
قسم علم الحيوان – كلية العلوم – جامعة المنوفية
شبين الكوم – مصر

تم اجراء دراسات معملية لمعرفة تأثير المستخلص الايثانولى لأوراق نبات *Clerodendron inerme* والأجزاء الهوائية لنبات *Pituranthos tortuosus* على خنفساء الدقيق المتشابهة *Tribolium confusum*. اظهرت المستخلصات المستخدمة تأثيرات طاردة repellent effects وتأثيرات مانعة للغذاء antifeedant effects وتزداد هذه التأثيرات بزيادة الجرعة المستخدمة . تم معاملة اليرقات عمر (١٦ ± ٢ يوم) بهذة المستخلصات واوضحت النتائج تأثيرا واضحا على نسبة التعذر pupation ونسبة خروج الطور اليافع adult emergence ، كما اثرت المستخلصات المستخدمة على النتاج (النسل) progeny الناتج من الحشرات البالغة adults التى تم معاملتها فى الطور اليرقى .

تم معاملة الحشرات البالغة عمر (اسبوعين) واظهرت النتائج انخفاض فى نمو و فى عدد النسل الناتج منها . وكانت هذه التأثيرات معتمدة على نوع المستخلص وعلى الجرعة المستخدمة . واوضحت النتائج ان مستخلص نبات *Clerodendron inerme* افضل من مستخلص نبات *Pituranthos tortuosus* حيث انة منع نمو اليرقات (الناتجة من الأباء المعاملة فى الطور البالغ) من التحول إلى طور العذراء .