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Repellency of Ethanolic Extract of Some Indigenous Plants Against Tribolium *confusum* (du val))(Coleoptera: Tenebrionidae)

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Abstract: *Repellency of ethanolic extracts of five plants extract viz. Eucalyptus gluaca, Melia azedarach, Mentha arvensis, Olea europaea leaves and pericarp of Punica granatum was tested against adult and larvae of the confused flour beetle Tribolium confusum using a choice test with treated filter paper. The plant extracts were applied at four concentrations which (2.5%, 5%, 7.5% and 10%) for each adult and larvae. All the plant extracts were found to be effective repellents and showed more than 20 % average repellency. The M.arvensis extract had potent repellent activity against T. confusum adults with 100 % repellency values with 2.5% concentration at 1h of exposure time for adult stage, while for larval stage it was observed that E. glauaca induce 100% repellency with concentration 7.5% at 2h after exposure. These naturally occurring plant extracts could be useful to protect the grains from the damages caused by of T.confusum.*

Keywords: *Repellency, Tribolium confusum, plants extract, Indigenous Plants.*

I. Introduction

Tribolium confusum (Coleoptera: Tenebrionidae) is the most widespread and destructive major insect pest of stored cereals through out the world. The insect damages are ranging from 5-30% of the world's total agricultural production. The reasons for their widespread presence range from evolutionary adaptations (morphological, physiological and behavioral) to the actions of humans who transport them throughout the world and offer a protected habitat within stored food stuffs [1]. Generally, the protection of stored grain and seeds against insect pests has been a major problem from the development of agriculture. To control these pests, synthetic insecticides have been widely developed and are extensively used because of their effectiveness and easy application and storage. The widespread use of synthetic pesticides has led to several adverse effects such as food, soil, ground water, and air contamination with toxic residues, which have side effects on non-target insects and other organisms [2]. To overcome these problems, it is necessary to seek safe, convenient, environmental, and low-cost alternative pest control methods. Considerable efforts have focused on plant-derived materials that are potentially useful as commercial insecticides. Plant derivatives are less toxic or nontoxic to mammals, other vertebrates, and invertebrates. Plant products have several uses in insect control. These products have also been studied for acute toxicity, antifeedant, or repellent, attractant, and fumigant effects, as well as inhibiting reproduction of many pest species [3; 4], products from several floral species have been demonstrated to act as repellents, toxicants and antifeedants against a number of Coleoptera that attack stored products [5] conducted insect repellency assay using extracts of different plants on stored product pests. Oils of many plants have also been reported as repellent and toxic chemicals for the management of stored grain insect pests [6]. Very little information is available on the use of plant extracts on the confuse flour beetle *T. confusum*. Therefore the present study was initiated to find and recommend possibly the most effective repellent plant extracts against *T. confusum* in the stored wheat grains.

II. Materials and Methods

Tested insects

Mass culture of *T. confusum* was maintained in our laboratory over 2 years without exposure to insecticides and reared on wheat flour mixed with yeast (10: 1, w/w) in an incubator at 30^{o}C temperature.

Plant material

Fresh leaves of the plants *Eucalyptus gluaca, Melia azadrach, Mentha arvensis, Olea europaea* and pericarp of *Punica granatum* were collected during (2010-2011) from Erbil city in Iraq. The mentioned plants were washed with rinsed water, shade dried and well ground to a fine powder with an electric blender. The extracts of plants were prepared according to the methods described by [7, 8]. 100 gram of plant powder was dissolved separately in 300ml of ethanol 70% (w/v) ratio at room temperature. The mixtures were stirred for 45 minutes in an ultrasonic bath at constant temp of 25^{o}C, left to stand for 72 hour and shacked several times at

certain intervals. All extracts were filtered through muslin cloth and then the solution were dried by vacuum rotary evaporator at >40^{o}C [9].

Repellency test

The repellent effects of selected plant extracts against *T. confusum* adults and last larval instar (7th instar) were evaluated. The residue dissolved in distilled water at concentration 25, 50, 75, and 100 mg/ml [10].. The repellency test was conducted according to the method of [11]. Filter papers (Whatman 40.) of 9 cm in diameter were cut in half. 0.2 milliliter solution of each extract was applied to one half of the filter paper (treated half) and on the other half 0.2 milliliter of distilled water was applied. The treated filter paper was then air dried. The treated and the untreated half-circles were placed contiguously on the Petri dishes. Ten unsexed newly emerged adults and 7th larval instars were put into each Petri dish. Petri dishes were subsequently covered. The treatments were replicated 3 times. Insects that settled on each half of the filter paper disc were counted at 1 hour interval for 5 hours.

Statistical Analysis

The data were expressed as percentage of repulsion (PR) using the following formula:
PR = (Nc – 50) × 2. Where, Nc = % of insects present in the control half. Positive values expressed repellency and negative values attractancy. The averages were then assigned to different classes using the following scale [12, 13]: class 0 (PR<0.1%), class I (PR=0.1–20%), class II (PR=20.1–40%), class III (40.1–60%), class IV (60.1–80%) and class V (80.1–100%).
Factorial CRD with two factors using different plant species, different exposure period and application rates was made. Mean separation of repellent effect of the different extract and comparisons analyses between plant species, exposure period and application rate were made using least significant differences (LSD) at (P <= 0.05).

III. Result and Discussion

The percent repellency value for the tested plant extract on adult and larvae of *T. confusum* was shown in Table (1). The result showed that the tested plant extract exhibited repellent effect on target species by move from treated part to untreated part through the Petri dish. The percent repellency for *M.arvensis* was 97.66% followed by *E.gluaca* 92.33%, *O.euorpea* 90.33%, *M.azedarch* 89.69% and *P.granatum* 82.3% for adult stage, while for larval stage the percentage repellency was 94.1% with *E.gluaca* , followed by *O. europea* 79.96%, *M. arvensis* 78.8%, *M. azedarch* 67.3% and *P. granatum* 66.86%. The analysis of variance of each plant extract species, regardless of the concentration used and exposure period shows that the *E. gluaca, M. azedraech* and *O. europea* did not differ significantly for adult stage, but there are significant differences between the plants for larval stage. The biological activity of the plants might be revealed that the various compound present in the extracts have repellent effect.

Table (2) shows effect of different concentrations of tested plant extract against adult and larval stage of *T.confusum*. At concentration 2.5% there were significant differences recorded with the rest concentrations for adult stage, in contrast there's significant difference were shown only with 5% for larval stage. At 5%, there's significant differences were shown with the rest concentrations for adult stage, while there's no significant differences was shown between 5% and 1% for larva. Concentration 7.5% showed significant differences with 2.5 % and 5% for adult stage and only with 5% for larva. Finally at concentration 1% significant differences were noticed with concentration 2.5% and 5% for adult stage only.

Result in Table (3) show general trend of gradual increase of repellency with time. The highest repellency was 99.03% and 98.97% for adult and larval stage respectively at 5th hour; this can be due to the volatilization of those active components increasing with time for definite period. Statistically the first hour differ significantly from the rest for adult, while the fifth hour differ significantly from the rest for both adult and larva, but there's no significant differences shown between 2nd,3rd and 4th hours for both adult and larva.

Percent repellency against adult and larval stages of *T. confusum* with different concentration during the exposure period is given in Table (4). In case of *E. gluaca* for adult stage the highest repellency value was 100% with concentration 2.5% at the third hour. Statistically there were significant differences shown only at concentrations 2.5% and 5%.For concentration 2.5% there's significant differences shows between the first hour and the remaining hours, while concentration 5% shows significant differences at the first and second hours with the rest. The larval stage shows high repellency 100% at the second hour with concentration 7.5%. Statistically there are no significant differences between all treatments for tested plant at the exposure period. The present work inconformity with the result of [14, 15] who reported that the main component present in essential oils of *Eucalyptus species* are 1-8 cineole which showed different insecticidal properties against insect pests.

The *M. azedarach* extract showed repellency 100% against *T. confusum* with concentration 7.5% at 1hour. There is not defined behavior of insect against the used concentration at exposure period. However the repellency of the extract of all concentration for adult stage was slightly higher than of the larval stage of the same plants as well as of the same concentration. Analysis of variance showed that the used concentrations for both adult and larval stages were differing significantly at different hours. The present result supports the finding of [16] that showed the repellent activity of *Melia azedarach* drupes against *Sitophilus oryza*e which induce the maximum repellency 72%. [17] Reported that *Tribolium castaneum* was significantly repelled from the applied *Melia azedarach* with mean repellency 55.24 %

The repellency in case of *M.arvensis* stronger than the other extracts for adult stage. At 7.5%, 1 hour repelled all insects 100%. The strong effect of *M.arvensis* could be attributed to the mixture of compound detected by *T. confusum.* Statistically there is no significant difference between all tested concentration for different exposure period, but there's significant differences observed between most treatment for larval stage . These findings are supported by the results of [18] who showed the repellent effect of *Mentha piperitaa* oils on *T. confusum* adult were high compared to the larvae, and agree with [19], they found that the essential oil of leaf of *M. arvensis* showed repellency against larvae and adults of *T. castaneum*, they strongly repels *T. castaneum* even at low concentration. The essential oil of *M. longifolia* had significant high repellency to *T. confusum* [20].

The *O.euorpea* extract induce rapid maximum repellency100% at 2hour with concentration 1%. Statistically there were significant differences shown only at concentration 2.5% and 5% between the first hour and the rest for adult stage. For larval stage there was significant differences shown at all concentrations between most treatments at different exposure period.

The *P. granatum* extract at concentration 2.5% showed repellency ranging from 20-100 at 2 to 5 hour after release for adult stage, as the concentration progress the repellent increase. There were significant differences shown between the treatments only at concentration 2.5% and 5% for adults, however therewere significant differences showed at all concentrations between the treatments for larval stage. Among all plants the *p. granatum* showed the lowest over all repellent effect with concentration 5% for both adult and larval stage. The concentration 2.5%, 7.5% and 1% were laid in class IV, while 5% were laid in class III. This can be explained by the fact that the constituent of the crude ethanolic extract are low volatility. The obtained result agreement with [21] crude methanol extract of *D. grandiflora* (Lythraceae) are high molecular weight compounds with low volatility.

It is evident from this experiment that the repellent response of *T. confusum* is higher for adult than that of larvae, this may be attributed that the chemoreceptor of adult is well developed than larvae. Similar result was also found by [22] who reported that *T. castaneum* adults were significantly more susceptible to the fumigant toxicity of the essential oil of *Evodia rutaecarpa* (Family: Rutaceae) than the larvae.

IV. Tables

Table (1) Repelled effect of plant extract on *T. confusum* adult and larvae

Plants	Mean% Repellency	
	Adult	Larvae
E. gluaca	92.33	94.10
M. azadrach	89.70	67.30
M. arvensis	97.67	78.83
O.europaea	90.33	79.97
P. granatum	82.33	68.87
LSD	4.20	6.86

Table (2) Repelled effect of various application rates on *T. confusum* adult and larvae

Concen.	Mean% Repellency	
	Adult	Larvae
0.025	87.20	81.28
0.05	82.69	73.17
0.075	96.27	81.07
0.10	95.73	75.73
LSD	3.75	6.13

Table (3) Repelled effect of exposure period on *T. confusum* adult and larvae

Time hour	Mean% Repellency	
	Adult	Larvae
1	80.33	67.73
2	88.67	71.13
3	93.00	72.93
4	92.33	78.30
5	99.03	98.97
LSD	4.26	6.86

Table (4) Repellency of different plant extracts concentrations on *T. confusum* Adult and last instar Larvae at different exposure period

Plants	Concen.	Adult							Larvae						
		Time					Over All Mean	Repel. Class	Time					Over All Mean	Repel. Class
		1	2	3	4	5			1	2	3	4	5		
E. gluaca	2.5%	73.3	93.3	100	100	100	93.3	V	92.7	80.0	86.0	85.3	98.0	88.4	V
	5%	80.0	80.0	93.3	86.7	100	88.0	V	98.7	92.7	98.7	92.7	99.3	96.4	V
	7.5%	93.3	93.3	86.7	100	100	94.7	V	92.7	100	92.7	92.0	98.7	95.2	V
	10%	86.7	93.3	93.3	93.3	100	93.3	V	98.0	86.0	99.3	99.3	99.3	96.4	V
M. azadrach	2.5%	86.7	93.3	60.0	73.3	93.3	81.3	V	79.3	86.0	86.0	46.7	99.3	79.5	IV
	5%	93.3	86.7	100	100.0	93.9	94.8	V	40.0	60.0	53.3	60.0	99.3	62.5	IV
	7.5%	100	100	100	80.0	100	96.0	V	53.3	79.3	53.3	60.0	98.7	68.9	IV
	10%	60.0	80.0	100	100.0	93.3	86.7	V	46.7	40.0	66.0	40.0	98.7	58.3	III
M. arvensis	2.5%	100.0	100.0	93.3	100	100	98.7	V	66.0	60.0	86.0	98.7	99.3	82.0	V
	5%	100.0	86.7	100	100	100	97.3	V	69.3	73.3	46.0	99.3	98.0	77.2	IV
	7.5%	86.7	86.7	100	100	100	94.7	V	72.7	60.0	60.0	98.0	99.3	78.0	IV
	10%	100	100	100	100	100	100.0	V	60.0	59.3	73.3	98.0	100	78.1	IV
O.europaea	2.5%	66.7	86.7	100	100	100	90.7	V	73.3	66.7	80.0	80.0	98.7	79.7	IV
	5%	46.7	86.7	93.3	46.7	100	74.7	IV	40.0	80.0	79.3	72.7	99.3	74.3	IV
	7.5%	93.3	93.3	100	100	100	97.3	V	79.3	60.0	86.7	99.3	99.3	84.9	V
	10%	93.3	100	100	100	100	98.7	V	60.0	86.7	66.7	92.7	98.7	80.9	V
P. granatum	2.5%	20.0	86.7	66.7	86.7	100	72.0	IV	66.7	66.7	60.0	92.0	98.7	76.8	IV
	5%	33.3	26.7	60.0	73.3	100	58.7	III	46.7	66.0	40.0	26.7	98.0	55.5	III
	7.5%	93.3	100	100	100	100	98.7	V	59.3	66.7	86.0	79.3	100	78.3	IV
	10%	100	100	100	100	100	100.0	V	60.0	53.3	59.3	53.3	98.7	64.9	IV
LSD	18.8								30.7						

V. Conclusion

The results of these investigations suggest that all the test plants have potential for repellent action. This means that test plants contained those active components which were completely extractable with high solvent polarity solvent (ethanol) and might be useful as potent insect control agent. This study showed that repellency of different plant extract against *T.confusum* adult and larvae depended on several factors including chemical constituents of the extract, application rate and exposure time. This should encourage the breeding or selection of plant varieties that produce such compounds in greater amounts.

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