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Repellency and toxicity of three plants leaves extraction against *Oryzaephilus surinamensis* L. and *Tribolium astaneum* Herbst.

S. S. Modarres Najafabadi[1], E. Beiramizadeh[2], R. Zarei[3]

[1]*Department of Entomology, Agriculture and Natural Resources Research Center, Arak, Iran*

[2]*Faculty of Ornamental Plants Research Institute, Mahallat, Iran*

[3]*Faculty of Payame Noor University, Mahallat Branch, Iran*

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Abstract

Screening of plant extracts from wild species of plants for insecticidal properties could lead to the discovery of new agents for pest control. Keeping this in view, the ethanolic extracts of three plants leaves Neem (*Azadirachta indica*), Mint (*Mentha longifolia*) and Datura (*Datura stramonium*) were tested against two stored grain pests viz., *Oryzaephilus surinamensis* L. and *Tribolium castaneum* Herbst. The results revealed that all of the tested materials had repellent and lethal effects against the tested pests as compared to untreated check. The plant extracts were mixed with grain 10mg/g of grains. Comparison of test plant extracts on *O. surinamensis* showed that the Mint extract was the most effective causing 48.30±4.01 mortality percent. Datura and Neem extracts with 35.26±3.21 and 25.60±2.33 mortality percent were the next levels. But, the plant extracts effect on *T. castaneum* revealed that Datura, Neem and Mint extracts were the most effective with 21.42±2.31, 16.66±1.54 and 15.95±1.89 mortality percent, respectively. Present study also revealed that all the treatments significantly deterred/repelled the tested insects. Datura extract showed maximum repellency of 33.09±2.35% against *O. surinamensis*. Mint extract showed maximum repellency of 59.52±4.12% against *T. castaneum*. The results of thin layer chromatography (TLC) also showed that four components were detected in Mint extract and three components in Datura and Neem were detected respectively in the ethanol extractive.

***Corresponding Author:** Seied Saeid Modarres Najafabadi ✉ s_modarres_705@yahoo.com

Introduction

Tribolium castaneum Herbst (Red Flour beetle) and *Oryzaephilus surinamensis* L. (Saw-Toothed grain beetle) are major and destructive stored grain pests of Iran. These are generally found in granaries, mills and warehouses. These pests not only cause economic loss, but are also responsible for 10% loss of world's cereal production (Wolpert, 1967). In Indo-Pak region, Farmers have inherited knowledge of mixing leaves, barks, seeds, roots and oils of some traditional plants with the stored grains for protection against insect pests during storage (Saxena *et al.*, 1988). Research reveals that extracts prepared from plants have a variety of properties including insecticidal activity, repellency to pests, antifeedant effects, insect growth regulation, toxicity to nematodes, mites and other agricultural pests, also antifungal, antiviral and antibacterial properties against pathogens (Prakash & Rao, 1997). Abubakr *et al.*, (2000) also reported repellent and antifeedant properties of *Cyperus articulatus* against *T. castaneum*. Jilani *et al.,* (2003) tested neem seed oil from five localities of Pakistan against red flour beetle as growth inhibitor and found significant reduction in the progeny at 250 ppm or higher rate in all the samples. Khanam*et al.,* (2006) reported toxic and repellent properties of sugarcane bagasse-based lignin against some stored grain insect pests including *T. castaneum*. Kumar *et al.,* (2007) evaluated the long-term efficacy of the protein enriched flour of pea (*Pisum sativum* L. var. Bonneville) in its toxicity, progeny reduction and organoleptic properties by combining it with wheat flour and testing the admixture against the red flour beetle, *T. castaneum* Herbst. The toxicity and progeny-reducing effects of the wheat flour treated with protein-enriched pea flour were stable for a period of 5 months when stored at 28°C with 75% r.h. Keeping in view the importance of plant extracts, the present study was carried out to test the efficacy of commonly used plant leaf extracts i.e., Neem (*Azadirachta indica*), Mint (*Mentha longifolia*) and Datura (*Datura stramonium)* on two major insect pests of stored grains viz., *Oryzaephilus surinamensis* and *Tribolium castaneum*.

Materials and methods

Local

Experiments were conducted in the Entomology Research Laboratory, Department of Plant Protection, Agriculture and Natural Resources Research Center, Markazi Province, Iran.

Insect's culture

Adults of *Tribolium castaneum* and *Oryzaephilus surinmensis* were drawn from laboratory mass cultures reared in glass jars (capacity 50ml) at temperature 25±1°C without controlling conditions. The *T. castaneum* and *O. surinmensis* used for experiment were about 2 months old (In very old age insect response to chemicals may not be normal) and reared on rice grain.

Plant material

The plants leaves used for extracts were *Azadirachta indica* (Neem), *Mentha longifolia* (Mint) and *Datura stamonium* (datura or jimson weed). The leaves were collected from Medicinal plants Research Station of Aliabad-Arak, Iran. The leaves were dried in the shade and ground to coarse powder with an electric blender.

Preparation of extract

For the extraction, Soxhlet Apparatus was used, about 20g powder of each plant leaves was extracted with 300ml ethyl alcohol. The extraction of each plant sample was done in about 12 hrs. After soxhlet extraction, the material was run on rotary evaporator. The extracts were concentrated on rotary evaporator by removing the excess solvent under vacuum. After evaporation of solvent with rotary evaporator there maining extracted material was kept on water bath for removing remaining solvent from the extracts. The extracts were stored at 4°C prior to application.

Apparatus used for experiment

Small plastic jars (capacity 50 ml) were used for the experiment, there was one set of two jars joined by clear plastic pipe of 1cm diameter at an angle of 180 degree for each replication. One jar of each set was

provided with 10g of grains given the name 'A' while the other jar was kept empty and given the name 'B'. In jar 'A', the grains treated with extracts were placed, while the jar 'B' remained empty. The jars used for experiment were disinfected with alcohol.

Bioassays

The extract was diluted with ethyl alcohol solvent (20mg/ml). The extracts were topically applied at doses of 10mg/g of grains (0.1gm of extract in 5ml of solvent). The solvent was allowed to evaporate at room temperature from treated grains. 10 adults of each species were released in the treated grain jars 'A'. The jars used for experiment were covered with pieces of cloth size of jars with rubber band. For control no extract was applied on grains, only the solvent of respective extract was applied on the grains and allowed to evaporate. There were three replicates for each treatment and control.

Repellency and toxicity observation

The treated jars either repelled the insects or forced them to move from treated jars 'A' to an empty jar 'B' through the plastic pipe or killed them indicating the insecticidal properties in both the situations. The ones found in plastic pipe were considered repelled individuals. The mortality and repellency data i.e. dead (in treated and untreated jars) and alive (in empty or untreated jars) were recorded for 14days at an interval of 24 hours for each observation.

Statistical analysis

The data were arranged in tabulated form and graph formats. The mortality (%) was corrected by Abbots's formula (1):

$$(1)\ \text{Pr} = \frac{P0 - PC}{100 - PC} \times 100$$

Where, Po = Observed mortality, Pc = Control mortality. The data was analysed using a one way (ANOVA) test, using graph pad Prism Version 4 for Windows, Graph Pad Software, San Diego California USA,*(www.graphpad.com)*. Results with $p<0.05$ were considered Statistically Significant.

Preliminary components detection by thin layer chromatography (TLC)

All the fine extractives were applied to pre-coated silica gel plate to perform TLC (Thin Layer Chromatography) with following mixture of solvents [distilled water, acetonitrile and ethanol (ratio1:1:8)]. The aim of this procedure is to identify the number of components present in each extract. Components were detected with ultraviolet light. After the spots were visualized and labeled, their retention factor (Rf value) were calculated. The Rf values were calculated according to the following formula (2):

$$(2)\ \text{Rf value} = \frac{\text{Distance travelled from the original point to the spot}}{\text{Distance travelled by solvent from the original point to front line}}$$

Results

Repelling effects of three different plant extracts

The percent repellency of *Oryzaephilus surinamensis* (Saw-Toothed Grain Beetle) against different plant extracts is shown in Table 1. It was obvious from the table that the tested plant extracts exhibited significant repellency on the target species by forcing it to move from treated jar to untreated jar through plastic pipe. Analysis of variance revealed that all the plant extracts differed significantly ($F=8.686$; df.,5; $p<0.0001$) from that of control. Mean repellency for Neem was 30.23±3.25%, Mint 24.75±1.95%, Datura 33.09±2.35% and control 1.90±0.62%. Hence, after 14th day of treatment, the maximum number of repelled alive insects were recorded in Datura leave extract. The plant extracts showed that the maximum repellency against *O. surinamensis* in the following ascending order of preference were Datura>Neem>Mint.

The percent Repellency of *Tribolium castaneum* (Red Flour Beetle) against different plant extracts is given in Table 1. *Tribolium castaneum* was significantly repelled from the applied plants extracts ($F=56.71$; df. 5; $p<0.0001$). Mean repellency for Neem was

55.23±4.01%, Mint 59.52±4.12%, Datura 48.33±3.25 and control 0.95±0.01%. Maximum number of repelled alive insects were recorded in Mint leaves extract. The plant extracts showed that the maximum repellency against *T. castaneum* in the following ascending order of preference were Mint>Neem>Datura.

Toxicity effects of three different plant extracts

Data with respect to mortality percent of *O. surinamensis* observed on different days (D) in plastic pipes, treated and untreated empty jars are shown in Table 2. Analysis of variance revealed that all the plant extracts differed significantly (F=11.72; df.,5; p<0.0001) from that of control. The means were further separated by LSD (Tukey's Multiple Comparison Test) at 5% level of significance. It is evident that the total mean mortality percent of *O. surinamensis* for treatments, Neem was 25.60±2.33%, Mint 48.30±4.01%, Datura 35.26±3.21% and control 0.71±0.02%. Maximum mortality was recorded for Mint, while minimum for Neem. Data table also revealed that Neem and Datura had no effect on first day of treatment but as number of days increased, the mortality percent also increased.

The data with respect to percent mortality of *T. castaneum* observed on different days (D) in plastic pipes, treated and untreated empty jars is shown in Table 2. Analysis of variance revealed that these plant extracts were found significantly different from control (F=6.827; df. 5; p<0.0001). The means were further separated by LSD (Tukey's Multiple Comparison Test) at 5 % level of significance. From the table it is evident that the total average percentage mortality of *T. castaneum* against extract of Neem was 16.66±1.54%, Mint 15.95±1.89%, Datura 21.42±2.31% and control 0.00%. Maximum mortality was recorded for Datura leave extract, while minimum for Mint. Table also reveals that the plant extracts have no effect on first day of treatment but as number of days increased the mortality percent also slightly increased. Datura showed mortality after 48 hrs of treatment.

Detection of components

Every extractive was dissolved in ethanol and applied to 1B2-F silica gel plate. The results of thin layer chromatography (TLC) showed that four components were identified from Mint extract. From Datura and Neem extract, three components from each were detected respectively in the ethanol extractive. Rf values for each components were calculated and given in Table 3.

Table 1. Repelling effects (Mean±SE) of three different plant extracts on *O. surinamensis* and *T. castaneum*

Time after treatment (days)	Alive repelled organisms (%)							
	O. surinamensis				*T. castaneum*			
	Neem	Mint	Datura	Control	Neem	Mint	Datura	Control
1	53.33±4.32ab	50.00±2.65 b	60.00±2.52 a	0.00±0.00 c	56.66±4.14 b	76.66±4.21 a	73.33±4.12 a	0.00±0.00 c
2	63.33±3.28 a	53.33±3.12 b	56.66±3.65 b	0.00±0.00 c	60.00±4.11 c	86.66±4.97 a	73.33±4.12 b	0.00±0.00 d
3	46.66±2.85 b	46.66±3.25 b	56.66±3.25 a	0.00±0.00 c	63.33±4.35 b	73.33±4.58 a	63.33±4.32 b	3.33±0.25 c
4	36.66±2.36 b	36.66±2.95 b	50.00±3.41 a	3.33±0.22 c	66.66±4.58 a	63.33±4.87 a	53.33±3.25 b	0.00±0.00 c
5	36.66±2.55 b	36.66±2.33 b	46.66±2.65 a	3.33±0.23 c	73.33±4.65 a	60.00±4.36 b	50.00±3.65 c	0.00±0.00 d
6	33.33±2.31 b	33.33±2.14 b	43.33±2.14 a	0.00±0.00 c	60.00±3.98 a	63.33±4.55 a	50.00±4.23 b	0.00±0.00 c
7	26.66±1.23 c	30.00±2.13 b	33.33±2.11 a	0.00±0.00 d	46.66±3.24 c	63.33±4.78 a	53.33±3.22 b	0.00±0.00 d
8	23.33±2.45 b	23.33±2.15 b	30.00±1.96 a	6.66±0.78 c	50.00±3.65 b	60.00±3.92 a	50.00±2.69 b	0.00±0.00 c
9	30.00±2.65 a	16.66±1.69 c	26.66±1.25 b	6.66±0.74 d	60.00±3.55 a	60.00±3.96 a	40.00±2.85 b	0.00±0.00 c
10	20.00±1.58 a	3.33±0.89 b	20.00±1.32 a	3.33±0.24 b	53.33±3.47 a	53.33±3.54 a	36.66±2.35 b	3.33±0.45 c
11	16.66±2.32 a	3.33±0.32c	13.33±1.23 b	3.33±0.36 c	53.33±2.89 a	53.33±3.25 a	36.66±2.58 b	0.00±0.00 c
12	13.33±2.12 a	3.33±0.65 b	13.33±1.12 a	0.00±0.00 c	40.00±2.13 a	43.33±2.54 a	36.66±2.14 b	0.00±0.00 c
13	13.33±1.32 a	6.66±0.99 b	6.67±0.55 b	0.00±0.00 c	46.66±2.15 a	43.33±2.13 a	30.00±2.17 b	6.66±0.78 c
14	10.00±1.85 a	3.33±0.78 c	6.67±0.48 b	0.00±0.00 d	43.33±2.89 a	33.33±2.02 b	30.00±2.23 c	0.00±0.00 d
Mean	30.23±3.25 b	24.75±1.95c	33.09±2.35 a	1.90±0.62 d	55.23±4.01 b	59.52±4.12 a	48.33±3.25 c	0.95±0.01 d

O. surinamensis: F = 8.686; d.f.,5 ; p<0.0001, *T. castaneum :* F = 56.71; d.f.,5 ; p<0.0001

Table 2. Effect of three different plant extracts on mortality% (Mean±SE) of *O. surinamensis* and *T. castaneum*

Time after treatment (days)	Mortality (%)							
	O. surinamensis				*T. castaneum*			
	Neem	Mint	Datura	Control	Neem	Mint	Datura	Control
1	0.00±0.00 b	3.33±0.98 a	0.00±0.00 b	0.00±0.00 b	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a
2	3.33±0.47 b	6.66±0.79 a	0.00±0.00 c	0.00±0.00 c	0.00±0.00 b	0.00±0.00 b	6.67±0.39 a	0.00±0.00 b
3	6.66±0.85 b	13.33±1.12 a	13.33±1.02 a	0.00±0.00 c	3.33±0.11 b	0.00±0.00 c	6.67±0.55 a	0.00±0.00 c
4	13.33±1.12 c	23.33±1.56 a	16.66±1.36 b	0.00±0.00 d	6.67±0.21 a	6.66±0.44 a	6.67±0.89 a	0.00±0.00 b
5	23.33±1.56 c	36.66±2.44 a	26.66±1.88 b	0.00±0.00 d	13.33±1.32 a	10.00±0.97ab	13.33±1.29 a	0.00±0.00 c
6	23.33±1.89 c	43.33±2.15 a	26.66±2.34 b	0.00±0.00 d	13.33±1.22ab	10.00±1.02 b	16.66±1.22 a	0.00±0.00 c
7	26.67±2.03 c	46.66±2.47 a	33.33±2.46 b	0.00±0.00 d	16.67±1.74 b	16.66±1.23 b	20.00±1.12 a	0.00±0.00 c
8	33.33±1.36 c	53.33±1.36 a	43.33±2.39 b	0.00±0.00 d	20.00±2.04 a	20.00±1.65 a	20.00±1.32 a	0.00±0.00 b
9	31.03±2.55 c	56.66±1.98 a	46.66±1.91 b	0.00±0.00 d	23.33±2.35 a	23.33±2.32 a	23.33±1.99 a	0.00±0.00 b
10	32.14±2.12 c	66.66±3.65 a	53.33±3.67 b	0.00±0.00 d	23.33±1.89ab	23.33±2.65ab	26.66±1.69 a	0.00±0.00 c
11	35.71±2.78 c	76.66±4.25 a	60.00±3.48 b	0.00±0.00 d	26.67±1.98 a	26.66±2.66 a	26.66±1.34 a	0.00±0.00 b
12	37.03±3.12 c	82.75±4.15 a	62.06±2.87 b	3.33±0.01 d	26.67±2.47 b	26.66±2.33 b	43.33±2.14 a	0.00±0.00 c
13	40.74±3.02 c	83.44±3.98 a	67.85±2.98 b	3.33±0.21 d	26.67±2.41bc	30.00±1.65 c	43.33±2.98 a	0.00±0.00 d
14	51.85±3.47 c	83.44±2.31 a	67.85±2.54 b	3.33±0.38 d	33.33±2.69 c	30.00±4.31bc	46.66±3.65 a	0.00±0.00 d
Mean	25.60±2.33 c	48.30±4.01 a	35.26±3.21 b	0.71±0.02 d	16.66±1.54 b	15.95±1.89c	21.42±2.31 a	0.00±0.00 d

O. surinamensis: F = 11.72; d.f.,5 ; p<0.0001, *T. castaneum :* F = 6.827; d.f.,5 ; p<0.0001

Table 3. Components detected in plants extracts.

Plant extracts	No. of components	Rf Values of components detected
Neem	1-A	0.96
	2-B	0.92
	3-C	0.78
Mint	1-A	0.97
	2-B	0.88
	3-C	0.78
	4-C	0.72
Datura	1-A	0.88
	2-B	0.92
	3-C	0.95

Discussion

Researchers in different parts of world have been using plants for controlling pests including stored grain pests. Previous studies revealed that different plant compounds were used in controlling pest and they proved effective and eco-friendly. Many researchers investigated the compounds in plants that have a variety of properties including insecticidal activity, repellence to pests, antifeedant effects, insect growth regulation, toxicity to nematodes, mites and other agricultural pests, also antifungal, antiviral and antibacterial properties against pathogens. Lawati *et al.,* (2002) reported the extracts of eight plants local to Oman, viz., Qarat *(Acacia nilotica),* Mustafal *(Annonas quamosa),* Neem (*Azadirachta indica),* Luban (*Boswellia sacra),* Kheshkhash *(Crotalaria juncea),* Zebrot (*Jatropha dhofarica),* Yas (*Myrtus communis)* and Suwwad (*Suaeda aegyptiaca)* in water and solvent (methanol or ethanol) were repellent and toxic causing mortality to *Callosobruchus chinensis*. They reported that high mortality was achieved from *A. nilotica, C. juncea, M. communis* and *S. aegyptiaca* in methanol and *B. sacra, J. dhofarica*, *S. aegyptiaca* and commercial Neem in ethanol. Extracts of *M. communis* in methanol were highly repellent to the beetles compared to other extracts. Khan & Marwat (2004) evaluated the leaves, bark and seeds of bakain (*Melia azadarach*) and Ak (*Calotropis procera*) powder against lesser grain borer (*R. dominica*). They tested that insect (*R. dominica*) was repelled from bakain's bark powder with 98.25% repellency followed by powder of Ak (*Calotropis procera*). Kundu *et al.*, (2007) evaluated the toxicity, repellent and residual effects of Bishkatali plant extracts in chloroform and ethyl alcohol solvents against the red flour beetle. It was reported that Bishkatali plant extracts in both

chloroform and ethyl alcohol had remarkable residual effects on *T. castaneum* by reducing the production of F1 progeny and/or by increasing the population mortality Moreira *et al.,* (2007) screened plants with insecticidal activity, in order to isolate, identify and assess the bioactivity of insecticide compounds present in plants, Basil (*Ocimum selloi* Benth.), Rue (*Rutag raveolens* L.), Lion's ear (*Leonotis nepetifolia* L.), Jimson weed (*Datura stramonium* L.), Baleeira herb (*Cordia verbenacea* L.), Mint (*Mentha piperita* L.), wild Balsam apple (*Mormodica charantia* L.), and Billy goat weed or mentras to (*Ageratum conyzoides* L.) against Coleoptera pests of stored products: *Oryzaephilus surinamensis* L., *Rhyzopertha dominica* F. In the present work repellent property was well depicted by all the treatments soon after their application.

The results of this research showed that maximum repellency against *Oryzaephilus surinmensis,* was found in extract of Neem after 48 hrs., i.e., 63.33±3.28% (Table 1). The results indicated that the least effective repellency was observed in Mint leave extract only for 10th day of treatment. It was also seen that the tested insect *Tribolium castaneum* strongly repelled from all the extracts till the termination of experiment. Mint showed maximum repellency i.e., 73.33±4.58% after 72 hrs of treatment but slightly decreased after passage of time and mean percent repellency was 59.52±4.12% (Table 1). Datura proved least effective repellent as compared to other extracts with an average of 48.33±3.25% repellency. The results also showed that the tested plant extracts proved to be effective in mortality of *Oryzaephilus surinmensis*. In case of lethal effects, the Mint gave maximum mortality and mean percent mortality was 48.30±4.01% (Table 2). The results showed that the *Tribolium castaneum* (red flour beetle) was a tolerant species. The tested plant extracts showed slight toxicity against *T. castaneum*. The most effective mortality was observed in extract of Datura followed by Neem. The average mortality was 21.42±2.31%and 16.66±1.54% found in Datura and Neem respectively. Mint extracts was least effective to cause mortality of *T. castaneum* with average of 15.95±1.89% (Table 2).The findings of present study are some what in conformity with those reported by the Saljoqi *et al.,* (2006) that the effect of ethanolic extracts of Bakain drupes (*Melia azdarach*), Habulas leaves (*Myrtus communis*), Mint leaves (*Mentha longifolia*), Bakain leaves, Harmal shoots and seeds (*Pegnum harmala*) and Lemon grass roots (*Cymbopogon citratus*) had repellent and lethal effects against rice weevil, *Sitophilus oryzae* L. The difference in this regard with the present study maybe due to difference in pest species, dose of the treatment, methodology of research, varietal and commodity variations, laboratory and environmental differences. The plants used in this study proved effective repellent and toxicant for the targeted post harvest grain pests. These plants have a range of chemicals which can be isolated and used for pest control. The test plants being medicinal would yield environmentally sound chemicals having no harmful effects on the non target organisms. From the present study, it is concluded that the ethanolic extracts of plant materials i.e., Neem leaves (*Melia azadiarachta*), Mint leaves (*Mentha longifolia*) and Datura leaves (*Datura stramonium)* possess toxic principles with significant insecticidal effects and could be a potential grain protectant against *Oryzaephilus surinamensis* (Saw-Toothed Grain Beetle) and *Tribolium castaneum* Herbst (Red Flour Beetle).

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