Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

JEZS Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2015; 3 (1): 87-91

Received: 04-01-2015
Accepted: 28-01-2015

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Efficacy of extracts of some Egyptian plants against economically important stored grain pest *Sitophilus oryzae* L.

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Abstract
Stored grain IPM involves replacing some or all insecticide application with insect control practices such as sanitation, aeration and the use of natural resources as alternatives to pesticides. The aim was to study the efficacy of Egyptian wild plants namely, Gerba (*Farsetia aegyptia*), Egyptian mint (*Mentha pulegium*) and Halama, (*Moltkiopsis ciliate*) against Rice weevil *Sitophilus oryzae* L, for their insecticidal properties. Based on the solvents polarity separating funnel was used to fractionate the ethanolic extracts of each plant material (petroleum ether (fraction I), chloroform (fraction II), ethyl acetate (fraction III) and n-butanol (fraction IV)). The positive response of targeted insect toward treatments was indicating insecticidal and repellency properties. The results data revealed that high percent mortality of 52.0% was found in the fraction III (ethyl acetate) obtained from *M. ciliate* crude extract on D_6 of treatment time. And the maximum number of repelled alive insects was recorded in the extract fraction III (chloroform) of *M. pulegium* with percent repellency 30.0% on D_2 and increased to 42.0% on D_4. From the results; its highly recommend the tested plants to be used as efficient safe alternative source for rice weevil control.

Keywords: Alternatives, pesticides, *Farsetia aegyptia, Mentha pulegium, Moltkiopsis ciliate, Sitophilus oryzae,* control, mortality, repellency.

1. Introduction
Wheat and rice suffer heavy losses during storage due to insect pests infection. According to the FAO estimate 10 to 25% of the world's harvested food is destroyed annually by insects and rodent pests [1]. Insect pests cause damage to stored grains and processed products by reducing their dry weight and nutritional value [2]. Additionally, insect infestation-induced changes in the storage environment may cause warm moist "hotspots" that provide suitable conditions for storage fungi that cause further losses [3]. The protection of stored grain and seeds against insect pests has been a major problem due to the adverse and hazardous effects of the conventional pesticides/insecticides on the human health, animals and environment [4]. Moreover, the wide spread use of synthetic insecticides has led to the development of insect strains resistant to insecticides [5]. Currently and under the umbrella of sustainability the researchers work hard for investigation and determining the efficacy of ecofriendly safe chemicals that may work as alternatives to the conventional chemicals used [6]. Plants may provide potential secondary metabolites (alkaloids, terpenoids, phenolics, and flavonoids) alternative to currently used insect-control agents because they constitute a rich source of bioactive green chemicals. Aromatic plants are among the most efficient insecticides of botanical origin and essential oils often constitute the bioactive fraction of plant extracts [7].
The present investigation was undertaken to study the efficacy of Egyptian wild plants namely, Gerba (*Farsetia aegyptia*), Egyptian mint (*Mentha pulegium*) and Halama, (*Moltkiopsis ciliate*) against Rice weevil *Sitophilus oryzae* L, for their insecticidal properties.

2. Materials and Methods
2.1 Insect Culture and Biology
The adults of rice weevils *S. oryzae* (Egyptian strain), were obtained from the existing culture in the Entomology laboratory, Zoology Department, Faculty of Science, Mansoura University, Mansoura, Egypt. This strain has been continuously reared free of insecticidal contamination for several years. Clean un-infested and sterilized rice grains were used to rear the insects inside 1 litre jars covered with muslin cloth. The jars were kept at 28±2 °C and 75±5 % RH in

insect cages and the culture was maintained by replacing the devoured grains with fresh uninfested ones.

2.2 Plant Materials and Extraction
Three wild aromatic plants were collected from Egypt. *F. aegyptia* Turra (Fam: Brassicaceae) was collected during its blooming time (late March, 2014) from wadi Tangol, Cairo-Siwiz desert way. While, *M. pulegium* L. (Fam: Lamiaceae) was collected during late September (2014) from the irrigation canal of Dakahia Fields. and *M. ciliate* (Fam: Boraginaceae) was collected from the coastal area of Gamasa city, Dakahlia Governorate during its blooming time at the end of March, 2014. Identification of the plant materials was done kindly by specialists of plant ecology and flora Prof. Dr. Ibrahim A. Mashaly, and Dr. Ahmed Mohamed Abd El-Gawad, Lecturer of Plant Ecology, Botany Department, Faculty of Science, Mansoura University, Egypt.

The leaves of *F. aegyptia*, *M. pulegium*, and *M. ciliate* were washed with distilled water and air dried for six days and macerated using domestic blender. The powdered materials were separately subjected to 95% ethanol extraction with Soxhlet apparatus for 15-18 hrs. Crude extracts were passed through Whatman (No. 1) filter paper and concentrated by a rotatory evaporator under low pressure. Chlorophyll removal from the dark-green residue was done by using activated charcoal. The chlorophyll free filtrate was then concentrated, and the solvent totally evaporated. The dried residue was suspended in distilled water and partitioned with petroleum ether (fraction I), chloroform (fraction II), ethyl acetate (fraction III) and n-butanol (fraction IV) by separating funnel. Each extract was dried over anhydrous sodium sulphate, filtered and evaporated to dryness. The obtained fractions were stored in glass vials and maintained in a refrigerator (4 ºC) until further use.

2.3 The Bioassay Test
The crude extracts and isolated fractions were diluted to 10% using acetone solvent. All of these extracts were used at 0.5 ml per 20 g of sterilized wheat grains. The bioassay was done according to Saljoqi *et al.* [8] with some modifications. 100 ml capacity plastic vials were sterilized with 90% alcohol 24 hours before inoculation. For each treatment a control containing no extracts was fixed. The repellency bioassay test was designed by joining two vials using clear plastic pipe of 1 cm diameter at an angle of 180 degrees forming one set for each replication. One vial of each set was provided with 20 g of wheat grains, and fixed at the position A, while the other vial was kept empty, and given the name B or fixed at the position B. Before filling with grains and in all replications, vials A were sprayed with tested materials, while empty vials (B) were not treated. Ten adults of *S. oryzae* of the same age were released in vials A.

The assumption was that either the treated vials would repel the insects and force them to move to empty vials through the plastic pipe or get killed indicating insecticidal properties of the product in both the situations. The mortality or repellency data i.e. dead and alive insects in the vial B were recorded for 6 days at an interval of 48 hrs for each observation. The ones found alive in the plastic pipes were considered repelled individuals. In total, there were 16 treatments including the control experiment. Each treatment was replicated 5 times. The data recorded and calculated for percent of mortality and repellency after correction using *Abbott's* formula [9]. Recording the data for repellent efficacy of the treatments was done through the observation of insects in the plastic pipes and in untreated empty vials during the time of observation. The percent mortality and repellency calculated by dividing the number responded insects by the total number of tested insects, then multiply by 100. The experiments were done during September, 2014 at incubation conditions of 28±2 °C and 75±5% RH.

2.4 Analysis of Data
All the data obtained were subjected to ANOVA and the mean values were separated on the basis of LSD pf the 0.05 probability level using the COSTAT 6.3 program.

3. Results and Discussion
3.1 Percent Mortality of *S. oryzae*
The data revealed that tested plant extracts and fractions shown significant toxic effect against *S. oryzae* observed on different days (D) of treatment. And the data in Table 1 shows that tested materials have reflective effect on suppression/dominance of the pests' population. High percent mortality of 52.0% was found in the fraction III (ethyl acetate) obtained from *M. ciliate* crude extract on D_6 of treatment time. The mortality percent 28.0% in *M. ciliate* crude extract was recorded on the 2nd day (D_2) of observations lower than the mortality percent 36% in *M. ciliate* ethyl acetate extract (fraction III) and during further observation with increasing the time of exposure till D_6 the mortality was found increased. This may be due to the antagonistic effect of some chemicals in the contents of the crude extract leading to lower toxicity effect than the toxicity effect of ethyl acetate fraction of the same plant material.

Similarly, the mortality in treatment of *F. aegyptia* fraction II (chloroform) ranged from 32.0% on D_2 to 48.0% on D_6 with a total average of 20.7%. Lastly, the percentage of mortality in the insect commodity treated with *M. pulegium* was the least significant value compared with the rest of tested plant materials. *F. aegyptia* has no literature data about its insecticidal activity against *S. oryzae* and this study may considered the first record of *F. aegyptia* insecticidal activity against rice weevil pest and it is of significance to mention the study on cytotoxicity effect of new flavonoid compound isolated from *F. aegyptia* [10]. The study was to discover the anticancer activity of the phenolic compound isolated from terrestrial Egyptian plants, the EtOH extract of the aerial parts of the Egyptian medicinal plant *F. aegyptia* (Forssk.). The phenolic-rich fraction of the EtOH extract was subjected to further fractionation, which led to the isolation of new flavonoid (kampferol 7-8 diglucoside); low polar fractions revealed the isolation of other known compound which were identified spectroscopically. Furthermore new flavonoid compound give high cytotoxicity against Hela and MCF-7 cell lines, also cytotoxicity of the isolated known compounds were studied. The study evidenced the safety of *F. aegyptia* to be used as stored grains insecticide.

Our result findings are somewhat in line with Odeyemi [11] who found that at 0.7 ml oil per 50 g maize *Cymbopogon citratus* increased mortality of the maze weevil, *Sitophilus zeamais* as compared to no treatment (control). Ofuya and Okuku [12] reported the insecticidal activity of acetone extract of *C. citratus* against *Aphis craccivora* and caused significant nymphal mortality and inhibited reproduction.

3.2 Percent Repellency of *S. oryzae*
The data with respect to percent repellency of *S. oryzae* observed on different days ($D_{2\text{-}6}$) of exposure time in plastic pipes and untreated empty vials are presented in Table 1. The tested materials played significant role on the target adult insect species by forcing it to move from treated vials to

untreated vials through plastic pipe. The maximum number of repelled alive insects was recorded in the extract fraction III (chloroform) of *M. pulegium* with percent repellency 30.0% on D_2 and increased to 42.0% on D_4. While observation on D_6 indicted non-significant increase of repellency effect against the tested insects. The total mean repellency by this treatment was determined as 19.0% till termination of the experiment.

In some cases like *M. ciliate* fraction II (chloroform) the maximum number of repelled alive insects was recorded on D_4 as 32.0% while observation on D_6 shown non-Significant decrease in the percent of repellency in the insect commodity. However, the total repellency averaged to 14.3%. The explanation of the decreased repellency effect may be due the gradual decrease with the passage of time, of the quick effect at initial exposure to the pest. Fraction IV (n-butanol) as general in all tested plant materials was found comparatively least effective on the basis of quick action but was non-significantly effective of its total effect in cases of fraction IV from *M. ciliate* and *M. pulegium,* within percent repellency 2.0 % on D_4 and D_6. The average repellency was recorded as 0.7% for both plant materials.

Studies on the insecticidal, antifeedant, and repellent effects of other plant extracts against *S. oryzae* have been documented earlier. For example, Park *et al.* [13] reported that *Acorus gramineus* (Araceae) rhizome-derived extracts elicited 70 and 90% mortality against *S. oryzae* adults at 0.064 and 0.255 mg cm^{-2} at 4 d after treatment. Insecticidal and fumigant activities of EO obtained from dry ground leaves of *Artemisia scoparia* (Asteraceae) against *S. oryzae, C. maculatus,* and *T. castaneum* (Herbst) have been demonstrated [14]. Similarly, the fumigant and repellent effects of *Ocimum gratissimum* (Labiatae) oil and its constituents against *S. oryzae, T. castaneum, Oryzaephilus surinamensis* (L.), and *Rhyzopertha dominica* (F.) have also been reported. The results of this study indicated the efficacy of *O. gratissimum* oil that influenced positively by concentration and duration of exposure after treatment [15]. Omar *et al.* [16] showed that extracts from the bark of *Lansium domesticum* (Meliaceae) had caused significant feeding inhibition of *S. oryzae* under laboratory conditions. In this study, the repellence of *X. aethiopica* or *D. tripetala* EO against *S. oryzae* is also in agreement with Yoon *et al.* [17]. who previously reported the repellent efficacy of *Carum carvii* (Umbelliferae) and *Citrus paradise* (Rutaceae) oils against *S. oryzae* in a T-tube olfactometer. Essential oil from the seed of *C. carvii* also exhibited toxic fumigant activity against *S. oryzae* at a dose of 1 pL mL^{-1} of volume on 2 cm filter paper disc [18].

3.3 Toxic and Repellent efficacy of Tested plants

Comparatively, among all the tested plant species *M. pulegium* was the highly significant of repellent efficacy against *S. oryzae*. While the toxic effect of the same plant extract and its fractions was significantly the lowest observed on different days (D) of treatment (Figure 1, 2, and 3). Strong repellency action of *M. pulegium* may be due to the volatile (flavonoids and terpenes) chemicals that expected to be separated in fraction II. This assumption and results are in agreement with Zekri *et al.* [19] study, that shown the fumigant effect of Leaves and flowers of *M. pulegium* L. against *S. oryzae* L. adults. And phytochemical tests on pennyroyal aerial parts revealed the presence of gallic tannins, flavonoids, alkaloids, sterols and triterpenes and saponins.

Our results, to some extent, similar to the strong antifeedant efficacy of petroleum ether and chloroform extracts from *P. nigrum* and petroleum ether extract from *J. curcas* against rice weevil, *S. oryzae* L. Furthermore, F1 adults were suppressed at the lowest concentration (2 µl/g) and no F1 was produced in all treatments. The results of this study showed that *P. nigrum* and *J. curcas* extracts were able to protect stored grain [20]. Also, ethanol extract of *Clerodendrum inerme* L. (Verbenaceae), *Withania somnifera* L. (Solanaceae), *Gliricidia sepia* L. (Fabaceae), *Cassia tora* L. (Caesalpiniaceae) and *Eupatorium odoratum* L. (Asteraceae) were evaluated for their efficacy on mortality and progeny production of rice weevil, *S. oryzae* L. Results indicated that *C. inerme* and *W. somnifera* extracts were more effective than *G. sepia*, *C. tora* and *E. odoratum* against adult insects [21].

Table 1: Comparative mortality and repellency efficacy of different treatments on the rice weevils *S. oryzae* L., infesting wheat grains.

Plant materials		Mortality %*				Repellency %*			
		D_2 exposure time	D_4 exposure time	D_6 exposure time	Mean Average	D_2 exposure time	D_4 exposure time	D_6 exposure time	Mean Average
F. aegyptia	- Crude extract	24.0 ^{cd}	32.0 ^{cd}	42.0 ^{bc}	16.3	4.0 ^{de}	6.0 ^{d}	2.0 ^{ef}	2.0
	- fraction I	20.0 ^{de}	36.0 ^{d}	46.0 ^{cd}	17.0	4.0 ^{de}	8.0 ^{d}	8.0 ^{ef}	3.3
	- fraction II	32.0 ^{abc}	44.0 ^{a}	48.0 ^{ab}	20.7	12.0 ^{cd}	14.0 ^{c}	14.0 ^{de}	6.7
	- fraction III	26.0 ^{bcd}	32.0 ^{cd}	36.0 ^{cd}	15.7	14.0 ^{c}	18.0 ^{c}	16.0 ^{cd}	8.0
	- fraction IV	8.0 ^{f}	16.0 ^{f}	22.0 ^{fg}	7.7	0.0 ^{a}	0.0 ^{d}	0.0 ^{f}	0.0
M. ciliate	- Crude extract	28.0 ^{abcd}	34.0 ^{cd}	38.0 ^{c}	16.7	6.0 ^{cd}	12.0 ^{c}	10.0 ^{cd}	4.6
	- fraction I	26.0 ^{bcd}	32.0 ^{cd}	36.0 ^{cd}	15.7	12.0 ^{b}	16.0 ^{b}	16.0 ^{ab}	7.3
	- fraction II	34.0 ^{ab}	38.0 ^{bc}	48.0 ^{ab}	20.0	24.0 ^{a}	32.0 ^{a}	30.0 ^{a}	14.3
	- fraction III	36.0 ^{a}	42.0 ^{ab}	52.0 ^{a}	21.7	4.0 ^{a}	8.0 ^{b}	8.0 ^{c}	3.3
	- fraction IV	10.0 ^{f}	14.0 ^{f}	16.0 ^{gh}	6.7	0.0 ^{cde}	2.0 ^{c}	2.0 ^{de}	0.7
M. pulegium	- Crude extract	6.0 ^{f}	12.0 ^{fg}	14.0 ^{gh}	5.3	4.0 ^{cd}	12.0 ^{c}	10.0 ^{bc}	4.3
	- fraction I	12.0 ^{ef}	26.0 ^{e}	28.0 ^{ef}	11.0	14.0 ^{b}	26.0 ^{cd}	26.0 ^{a}	11.0
	- fraction II	14.0 ^{ef}	28.0 ^{cd}	32.0 ^{de}	12.3	30.0 ^{a}	42.0 ^{a}	42.0 ^{a}	19.0
	- fraction III	14.0 ^{ef}	24.0 ^{cd}	28.0 ^{c}	11.0	18.0 ^{b}	34.0 ^{ab}	34.0 ^{a}	14.3
	- fraction IV	0.0 ^{f}	0.0 ^{g}	2.0 ^{h}	0.3	0.0 ^{c}	2.0 ^{c}	2.0 ^{de}	0.7

***** Different superscripted letters indicate values significantly than respective control (P≤ 0.05). similarity in the value's superscripted letters means there are no significant variation and as letters as far away from each other indicates high significant variation.

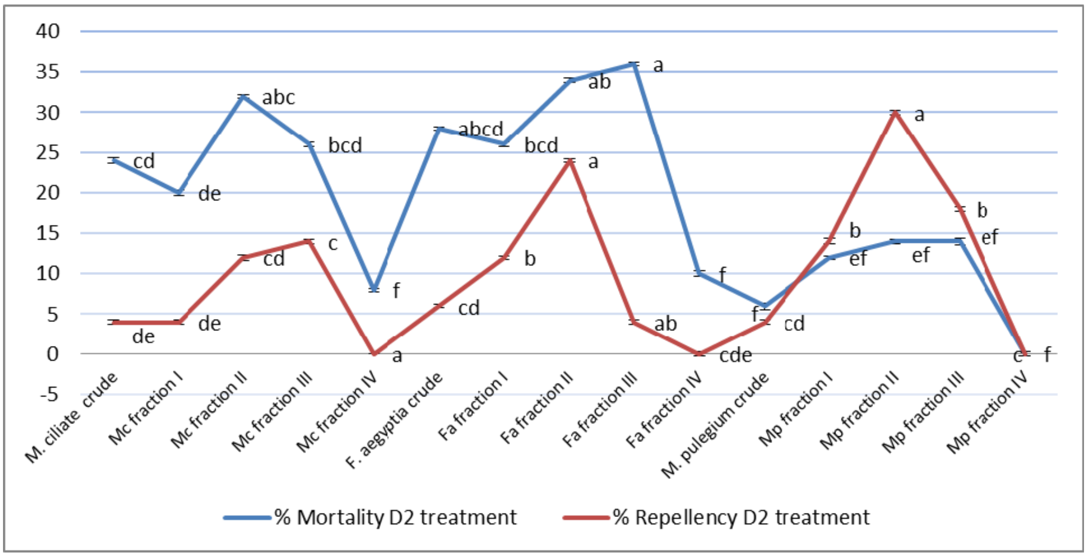

Fig 1: Comparative mortality and repellency efficacy of different treatments on the rice weevils *S. oryzae* L., on D_2 observation.

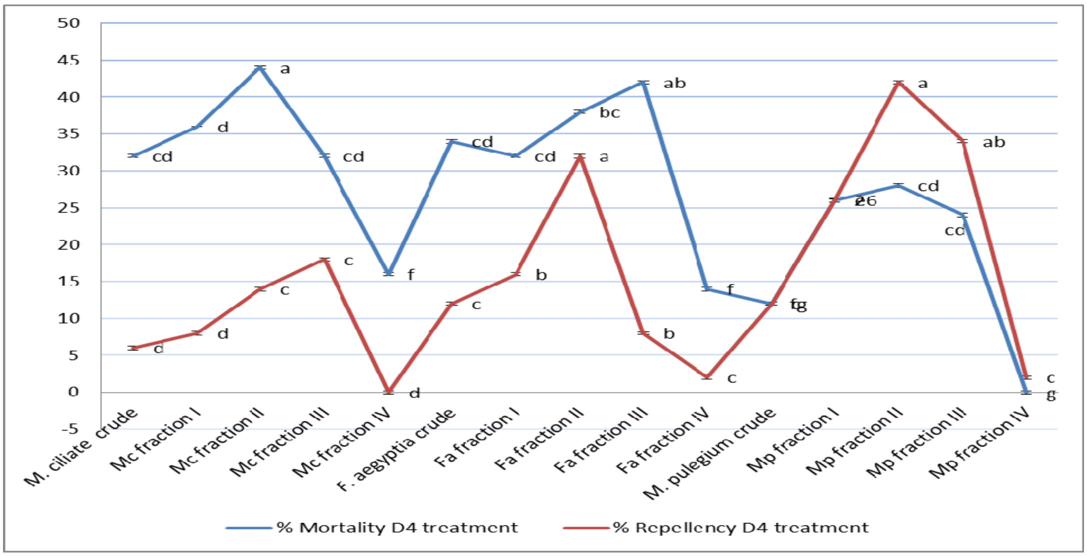

Fig 2: Comparative mortality and repellency efficacy of different treatments on the rice weevils *S. oryzae* L., on D_4 observation.

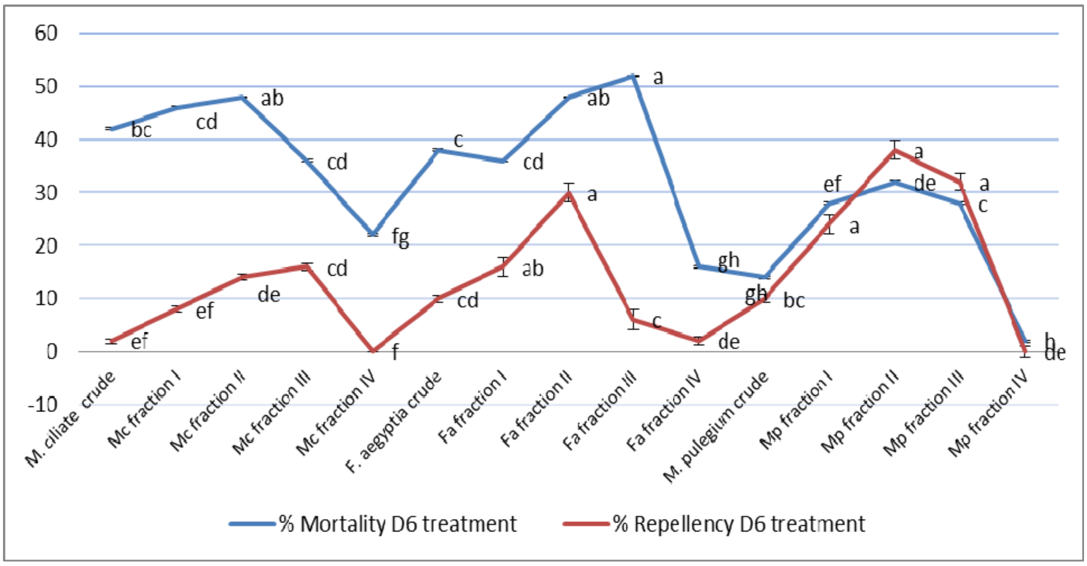

Fig 3: Comparative mortality and repellency efficacy of different treatments on the rice weevils *S. oryzae* L., on D_6 observation.

4. Conclusion

The above data indicate that leaf extract and fractions of *F. aegyptia* gave the highest mortality effect followed by *M. ciliate* against *S. oryzae* as a main index. However, the significant repellent effect was recorded in case of *M. pulegium* specially fraction II (chloroform). The study support the concept of "back to nature" and represent the role of alternative natural resources for pest control.

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