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Effectiveness of two plant (Arta and Neem) extracts on larva and adult of *Tribolium confusum* (Coleoptera: Tenebrionidae).

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The confused flour beetle, *Tribolium confusum* is the most serious pests of stored cereals and induced high damage of cereals and their products. This study was carried out to evaluate different extracts (with ethanol or water) of two indigenous plants (arta and neem) with different concentrations on larvae and adults of *T. confusum.* The obtained results indicate that both plant extracts are more effective if extracted with ethanol than that of with water. The higher mortality was achieved for larvae or adults with arta extracted with ethanol. The mortality rates by arta extracted with ethanol (96.67% and 100 % for larvae and adult, respectively) were significantly higher than those by neem extracted with ethanol (66.67 % and 73.33 % for larvae and adult, respectively). These results revealed that the adults are more sensitive than larvae for all tested plant extracts. The LC_{50} value of arta extracted with ethanol (59.3%) was differed significantly from those of the other three extracts for larvae while there is no significance difference for LC_{50} between both neem (68.86%) and arta (54.46%) extracted with ethanol for adults. It could be concluded that the extract of arta with ethanol being more effective for controlling larvae and adults of *T.confusum* and it could be used as environmentally safe to prevent the hazardous pesticides for human in the stored products.

Keywords: stored products, insects, arta, neem, mortality, LC50

INTRODUCTION

Insect pests are causing high damage to stored grains and processed products by reducing their dry weight and nutritional value (Sinha and Watters, 1985). The confused flour beetle, *Tribolium confusum* is one of the most serious pests of stored cereals and processed cereal products worldwide (Champ and Dyte, 1976 and Hill, 1990). This insect damages are ranging from 5-30% of the world's total agricultural production (Mohammed, 2013).

The protection and control of stored grains from insect damage is currently dependent on synthetic pesticides. Botanical compounds are a promising source of pest control. The plant kingdom can be a rich source of a variety of chemicals with the potential for development as successful pest control agents (Rahman and Schmidt, 1999). Secondary compounds from plants including alkaloids, terpenoids, phenolics, and flavonoids can affect insects in several ways. They may disrupt major metabolic pathways and cause rapid death or accelerate development (Houghton, 1996). Insecticides those extracted from plants can successfully be employed as adequate alternative to the synthetic chemical insecticides (Rajkumar and Jebanesan, 2004). It has been found that the efficacy of insecticides synthesized from plant is attributed to the presence of bioactive chemicals, which inhibit growth development and metamorphosis of insects (Sukumar et al., 1991). In general, the employment of insecticides synthesized from plant extract has been proven to be a reliably efficient approach in insect control and they are environmentally-safe, readily available, less expensive, quick degradable (Nathan and Kalaivani, 2005). Plants can also be less toxic, readily biodegradable and of great economic interest both from the agronomic and preventive medicine points of view (Ojimelukwe and Adler, 1999). Over 2000 species of plants are known to possess some insecticidal activity, by containing either antifeedant, repellent or insecticidal compounds that enable the crude plant material or an extracted active compound to protect stored products (Bouda et al., 2001). The botanicals comprise only 1% of the pesticides market (Debashri and Tamal, 2012). These botanicals may become a potential solution to synthetic chemicals, and they do not have severe side effects as they are biodegradable and hence do not interfere with ecosystems (Rajendran and Sriranjini, 2008).

Arta, *Calligonum comosum* (Polygonaceae) is a plant of tropical and subtropical regions and is widespread in KSA. This plant have pharmacological and toxicological effect for many human diseases (Kamil et al., 2000).Soliman et al. (2018) assumed that the produced volatile oils are magnified when *C. comosum* plants grow in groups and these oils may play a role in protecting the plants from the attack of local insects and pathogens

All parts of neem plant have some biologically active components that are responsible for checking the insect population. Azadirachtin was isolated from neem fruit, has been proven to be the tree's main agent for combating against insects. Moreover, resistance does not develop in insect against neem (Vollinger, 1995).

Mohammed (2013) tested five indigenous plants from Iraq and indicated that 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.

Therefore, this study aimed to evaluate different extracts of two indigenous plants (arta and neem) with different concentrations on larvae and adults of *T. confusum.*

MATERIALS AND METHODS

Insect cultures.

The culture of the flour beetle *T. confusum* was maintained from the laboratory. This culture without exposure to insecticides over one year ago. The adults of *T. confusum* were confined in plastic boxes (30 cm length x 20 cm width x 20 cm high), with a fine mesh net as lids for ventilation.

The insect cultures were maintained on a basic diet consisting of wheat flour mixed with yeast (10 : 1 w.w) and kept in the dark at a constant temperature 30^0 C and 65 ± 5% R.H.(Haliscak and Beeman, 1983).

Plant materials

Fresh leaves of *Azadirachta indica* and *C. comosum* were collected from Jeddah and Dammam, respectively, Saudi Arabia. Leaves were washed with distilled water for cleaning, shade dried and well ground to be a fine powder in air sealed plastic containers at room temperature. Extracts were prepared with two different methods as follow according to (Mohammed, 2016).

Water extract: Ten grams of prepared powdered were dissolved in 100 ml water and heated for 10 min at 70ºC was applied. Furthermore, clear solution was obtained after mixture filtration through whatman filter paper (WHA1001125).

Ethanolic extract: Ten grams of powder were dissolved in 100 ml of (abslute or 70%) Ethanol and kept for 24 hours under shaking. Mixture was filtered through whatman candidate No. 1 (pore size 125 mm) and pure solution was obtained. Moreover, for alcohol evaporation, heating at 200º C was applied.

Mortality test

For the mortality rate of T. *confusum,* last instar larval (7^{th} instar) and one week old adults were tested. Different concentrations (25, 50, 75 and 100 mg/ml) were prepared from both extracts (Morallo, 2004). A mount of 0.5 ml from both extracts of *A. indica* and *C. comosum* at different concentrations was dropped with a pipette on filter paper whatman No. 1. Then, they were placed individually inside a petri dish (4 cm diameter) and filter paper was then air-dried for 5 minutes. For mortality test, 10 adults or 10 last larval instar were introduced into each petri dish, and the same number was also confined to filter papers treated with water and ethanol as control and kept under rearing conditions (sahaf, 2008). Three replications were made for each treatment. The mortality rates of larvae and adults were recorded after 24 and 48 hours of the treatments.

Statistical analysis:

To compare the mortality of four studied plant extracts at the same concentration as well as at different four concentration at the same extract, One Way ANOVA was used by SPSS program. LC_{50} values were calculated using probit analysis. Then, each character (LC50, Slope, Intercept and χ^2) was analysed by One Way ANOVA. Means were compared by the Duncan's test (P = 0.05). All the analyses (ANOVA, Probit analysis and Duncan's test) were conducted using SPSS program, version 23 (SPSS, 2015).

RESULTS AND DISCUSSION

The mortality was not corrected because there was no mortality in the control that was treated even with water or ethanol only. Results presented in Tables (1 and 2) indicates that both plant (neem and arta) extracts are more effective if extracted with ethanol than that of with water. Moreover, the mortality was significantly increased gradually with increasing the concentration of each extract. The higher mortality was achieved for larvae or adults with arta extracted with ethanol. The mortality rates by arta extracted with ethanol (96.67% and 100 % for larvae and adult, respectively) were significantly higher thanthose by neem extracted with ethanol (66.67 % and 73.33 % for larvae and adult, respectively). These results revealed that the adults are more sensitive than larvae for all tested plant extracts. Similar result was found by Mohammed (2013) who stated 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 finding was also found by Liu and Ho (1999)who reported that *T. castaneum* adults were significantly more susceptible to the fumigant toxicity of the essential oil of *Evodia rutaecarpa* (Fam: Rutaceae) than the larvae. In general, arta extract was significantly higher effective than neem extract. Microscopy-based investigations indicated that stems of *C. comosum* developed hard structures in its outer layers including sclerenchyma and cluster crystals of calcium oxalate (CaOx). Sclerenchyma and CaOx are difficult to be eaten by herbivores and insects and can harm their mouthparts Also, this plant has cuminaldehyde that is known as a toxic volatile substance that has a killing effect on mammalian cells, insects and microbes (Soliman et al., 2018). Al-Keridis (2017) found that the shoot extract of arta, *C. comosum* was effective against the second instar larvae of *Culex pipiens* mosquito as indicated by the total mortality rate after 15, 18 and 24 h corresponding to concentrations 100, 75 and 50%, respectively. Ghazanfar (1994) stated that the major plant phytochemicals of arta plant that serves as antioxidants with cytotoxic effect are the anthraquinones and flavonoids.

Table (1): Effect of four extracts (with four concentrations) of two plant species on mortality ((Mean ± SE)) of larvae of T. *confusum*.

Concentrations (%) / Plant Extracts	25	50	75	100	*F* values	*P*
Neem with water	$0.0{\pm}0.0^{B}_{C}$	$10.0{\pm}5.8\ ^{C}_{BC}$	$13.33{\pm}3.3^{D}_{AB}$	$23.33{\pm}3.3\ ^{C}_{A}$	6.667	0.014
Neem with ethanol	$16.67{\pm}3.3^{A}_{D}$	$30.0{\pm}5.8\ ^{AB}_{C}$	$46.67{\pm}3.3\ ^{B}_{B}$	$66.67{\pm}3.3\ ^{B}_{A}$	28.000	<0.001
Arta with water	$10.0{\pm}5.8\ ^{AB}_{C}$	$16.67{\pm}3.3\ ^{BC}_{C}$	$33.33{\pm}3.3\ ^{C}_{B}$	$63.33{\pm}3.3\ ^{B}_{A}$	33.944	<0.001
Arta with ethanol	$16.67{\pm}3.3^{A}_{C}$	$33.33{\pm}3.3^{A}_{C}$	$63.33{\pm}3.3\ ^{A}_{B}$	$96.67{\pm}3.3\ ^{A}_{A}$	111.583	<0.001
df	3, 11	3, 11	3, 11	3, 11		
***F* values**	4.467	5.458	40.250	81.583		
P	0.040	0.025	<0.001	<0.001		

Means within each column bearing different upper letters are significantly different according to Duncan test (P= 0.05).
Means within each row bearing different lower letters are significantly different according to Duncan test (P= 0.05).

Table (2): Effect of four extracts (with four concentrations) of two plant species on mortality ((Mean ± SE)) of adults of T. *confusum*.

Concentrations (%) / Plant Extracts	25	50	75	100	*F* values	*P*
Neem with water	$0.0\pm0.0^{B}_{C}$	$6.67\pm3.3^{B}_{C}$	$16.67\pm3.3^{D}_{B}$	$26.67\pm3.3^{C}_{A}$	16.333	.001
Neem with ethanol	$23.33\pm3.3^{A}_{C}$	$33.33\pm3.3^{A}_{C}$	$53.33\pm3.3^{B}_{B}$	$73.33\pm3.3^{B}_{A}$	44.250	<0.001
Arta with water	$6.67\pm3.3^{B}_{C}$	$13.33\pm3.3^{B}_{C}$	$33.33\pm3.3^{C}_{B}$	$66.67\pm3.3^{B}_{A}$	65.333	<0.001
Arta with ethanol	$23.33\pm3.3^{A}_{D}$	$36.67\pm3.3^{A}_{C}$	$66.67\pm3.3^{A}_{B}$	$100\pm0.0^{A}_{A}$	139.556	<0.001
df	3, 11	3, 11	3, 11	3, 11		
***F* values**	16.889	19.583	43.583	110.222		
P	.001	<0.001	<0.001	<0.001		

Means within each column bearing different upper letters are significantly different according to Duncan test (*P*= 0.05).
Means within each row bearing different lower letters are significantly different according to Duncan test (*P*= 0.05).

Table (3): Values of LC_{50} of four extracts of two plant species on larvae of T. *confusum*.

Treatments	LC_{50} ± SE (%)	Intercept± SE	Slope ± SE	χ^2 (df=2)
Neem with water	136.34±10.59 A	-2.75±0.570 B	0.021±0.006 B	9.49±4.19 A
Neem with ethanol	77.86±1.80 B	-1.46±0.119 A	0.019±0.001 B	5.44±1.08 A
Arta with water	89.28±1.65 B	-2.05±0.223 AB	0.023±0.003 AB	12.61±7.60 A
Arta with ethanol	59.30±2.69 C	-2.07±0.241 AB	0.035±0.004 A	10.35±2.16 A
df	3, 11	3, 11	3, 11	3, 11
***F* values**	34.416	2.495	3.890	0.443
P	<0.001	0.134	0.055	0.729

Means within each column bearing different letters are significantly different according to Duncan test (*P*= 0.05).

Table (4): Values of LC_{50} of four extracts of two plant species on adults of T. *confusum*.

Treatments	LC_{50} ± SE (%)	Intercept± SE	Slope ± SE	χ^2 (df=2)
Neem with water	125.11±8.77 A	-3.023±0.65 B	0.025±0.007 AB	5.033±1.92 B
Neem with ethanol	68.86±2.57 C	-1.267±0.06 A	0.018±0.001 B	4.123±3.02 B
Arta with water	86.96±2.25 B	-2.470±0.32 AB	0.028±0.003 AB	5.826±2.01 B
Arta with ethanol	54.46±2.47 C	-1.829±0.14 AB	0.034±0.002 A	17.083±3.34 A
df	3, 11	3, 11	3, 11	3, 11
***F* values**	39.454	4.277	2.977	5.301
P	<0.001	0.045	0.097	0.026

Means within each column bearing different letters are significantly different according to Duncan test (*P*= 0.05).

Results presented in Table (3 and 4) indicates the LC_{50} values of tested four extracts at 24 hours after treatment. The LC_{50} value of arta extracted with ethanol (59.3%) was significantly higher than those of the other three extracts (136.34, 77.86 and 89.28% for neem with water, neem with ethanol and arta with water, respectively) (*F*= 34.416, *P*<0.001) for larvae (Table 3). Meanwhile, there is no significance difference for LC_{50}between both neem (68.86%) and arta (54.46%) extracted with ethanol for adults (Table 4). While this difference is significant in between both neem (125.11%) and arta (86.96%) extracted with water for adults (Table 4). On the other hand, Zaka et al., (2018) found that abamectin and cypermethrin insecticides, neem essential oil and citrus plant extract also killed adults of *T. confusum* quicker as compared other essential oils, extracts and herbicides.

CONCLUSION

In conclusion, our results revealed that the extract of arta with ethanol being more effective for controlling larvae and adults of *T. confusum* . Moreover, it is environmentally safe and it could be used instead of highly hazardous pesticides for stored products' pests.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest".

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AUTHOR CONTRIBUTIONS

NMG designed and performed the experiments and also wrote the manuscript draft. LAA performed the extraction from the plants. SMS performed data analysis and reviewed the manuscript. All authors read and approved the final version.

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