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Toxicological effects of *Melaleuca ericifolia* and *Taxodium distichum* extracts on adults rust-red flour beetle

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ABSTRACT

Petroleum ether and acetone extracts of leaves from swamp paper bark (*Melaleuca ericifolia*) and bald cypress (*Taxodium distichum*) were tested for their contact and stomach poison activity on adults *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). The results showed that, percentage of mortality increased with increasing the concentration of different extracts and exposure time. Extracts of Swamp paper bark gave higher mortality percent compared with that from bald cypress. Petroleum ether extract of swamp paper bark was found to be the most potent on *T. castaneum* adults with LC_{25} , LC_{50} and LC_{95} values (1.65%, 2.59% and 7.79% (w/w), respectively) at 48 hours exposure period. The bio-residual activity of LC_{95} of petroleum ether extract of swamp paper bark continued up to 13^{th} weeks in treated adults of *Tribolium castaneum*. On the other hand, treatment with LC_{25} and LC_{50} significantly reduced the number of deposited eggs with complete reduction (100%) in their hatchability and progeny. Treatment of *T. castaneum* adults with LC_{50} of petroleum ether extract of swamp paper bark caused significant increase in both carbohydrates and proteins content compared with control, while significant decrease in lipids content was noticed.

Petroleum ether extract of swamp paper bark at LC_{95} values, caused adverse effects on wheat grains germination at initial time and after storage period but water absorption was not affected.

Phytochemical screening of petroleum ether extract of swamp paper bark revealed that methyl eugenol was the major component. The presence of some of these compounds may be responsible for the insecticidal action of the extract of this plant.

Based on the results of this study, petroleum ether extract of swamp paper bark could serve as an alternative to synthetic insecticides for the protection of stored wheat from rust-red flour beetle.

Key words: Toxicological effects, *Melalleuca ericifolia*, *Taxodium distichum* , extracts, rust-red flour beetle, *Tribolium castaneum.*

INTRODUCTION

The red flour beetle, *T. castaneum* is one of the primary pests infesting dry stored produce worldwide (Garcia *et al.*, 2005). Adults are long lived for more than three years (Walter, 1990). They have a high rate of population increase because the adult female can lay large number of viable eggs throughout their life (Hill, 1990; Campbell and Runnion, 2003). Significant losses are caused by infestations due to direct consumption of produce, nutrients reduction and increase in temperature and moisture conditions that lead to accelerated growth of molds, including toxigenic species (Chijindu and Boateng, 2008). Problems associated with synthetic insecticides usage such as; insect resistance, pest resurgence, health hazards, residual toxicity, increasing costs of application and widespread environmental hazards have directed the need for effective, biodegradable pesticides although, synthetic insecticides have proved very effective in controlling these

beetles (Talukder and Howse 2000 , Elhag 2000).

Plant materials have been used over the years for the protection of field crops and stored commodities against insect attack (Golob and Webley, 1980). They are rich sources of bioactive compound which might deadly act on the insect physiological system (Kim *et al*. 2003; Daoubi *et al.,* 2005) and belonging to various classes (alkaloids, glycosides, tannins, proteic amino acids, steroids, phenols, flavonoids, glucosinolates, quinones, terpenoids etc.), which have behavioral and physiological effects on pests (Gonzalo, 2009).The extracts and oils of plant materials have been found to be alternatives to conventional synthetic insecticides to control stored product insect pests (Rees *et al*., 1993; Lale, 1995; Boeke *et al*., 2001) since the chemical fumigants used in stored products, for protection cause high mammalian toxicity, insect resistance and health hazard (Sighamony *et al*., 1986; Adedire and Ajayi, 1986 ; WMO, 1995).

Current researches showed that plant extracts had insecticidal activities against a wide range of insect pests to control and reduce damage, due to the possession of active insecticidal ingredients, availability, affordability and they are environmentaly save to man (Ivbijaroand Agbaje, 1986; Lale, 1994; Ogunwolu and Idowu, 1994; Ogunwolu and Odunlami, 1996; Adedire and Lajide, 2001; Ofuya and Dawodu, 2002 a,b; Ofuya and Salami, 2002).

Swamp paper bark (*Melaleuca ericifolia*) was found to have a high bioactivity against harmful pests stored insect (lee *et al*., 2001; Abdel-Salam, 2010). On the other hand, Sabbour (2003) assessed insecticidal efficiency of bald cypress (*Taxodium distichum*). The present study was carried out to evaluate the efficacy of petroleum ether and acetone extracts of leaves of both swamp paper bark and bald cypress leaves on the rust - red flour beetle , *T. castaneum* adults.

MATERIALS AND METHODS

T. castaneum culture

The rust - red flour beetle *T. castaneum* adults were obtained from Stored Grains pest Research Department, Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza, Egypt and reared on a crushed wheat grains (media) at 30 ±2°C, 65 ± 5% RH. Stock culture was set up by introducing adults pairs in glass jars containing sterilized media. Beetles were left for about two weeks to feed then infested media was sieved and the parent stocks were removed from jars. Media with eggs were returned again to the jars and left in incubator until new adults emerged. The adults (1-3) weeks old of mixed sex were picked out and used for the experiments (Rozman *et al*., 2007).

Preparation of plant extracts

Plants materials used

Leaves of *M. erciifoliae* and *T. distichum* were supplied from ornamental gardens of the Faculty of Agriculture Moshtohor, Kalubia farm , Egypt during Septemper 2013 .

Method of extraction

Plant extracts of leaves of *M. ericifolia* and *T. distichum* were prepared by the maceration method described by Salem *et al*. (2007). Leaves of *M. ericifolia* and *T. distichum* were ground to powder using a grinder prior to extraction and further sieved to pass through 1mm 2 mesh (Ileke and Bulus, 2012). The powders (250g) were successively extracted by soaking in petroleum ether (60-80%), filtered and the residues were extracted afterwards using acetone. The mixture was stirred for 3-4 hours daily using magnetic stirrer and extraction ended after 48 hrs.The homogenate was filtered and solvent was evaporated by using arotary evaporator (Labo-Rota C 311) at 50°C and reduced pressure. The crude extract obtained was stored in the refrigerator prior to use (Aina *et al*., 2009).

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Identification of chemical constituents of petroleum ether extract of swamp paper bark leaves

This was carried out by using GC/MS (Gas - chromatography - mass spectrometry) based on the retention time of each component (Rt) compared with those of the Wiley 9 and NIST08 mass spectra libraries NIST (2010).

Bioassay of botanical extracts toxicity

Laboratory bioassays to evaluate contact and stomach poison toxicity of *M. ericifolia* and *T. distichum* leaves extracts against *T. castaneum* adults were carried out according to Shemais *et al*. (2007): Five Concentrations (6, 5, 4, 3 and 2 % w/w) were evaluated for their insecticidal efficiency by adding 3 , 2.5 , 2 , 1.5 and 1 ml of the stock solution of each extract to 10 g of wheat grains in glass tube. These concentrations were derived from 20% (w/v) stock solutions obtained by dissolving 0.2 g of each plant crude gum in 1ml of appropriate solvent and kept at 4°C. Twenty five of *T. castaneum* adult beetles (1-3) weeks old were introduced into each tube after solvent evaporating. As a control, wheat grains were mixed with 1ml of the solvent. The tubes were kept in the incubator under constant conditions (30±2ºC, 65±10% RH). Three replicates of both treated and untreated grains were used. Mortality of adult beetles was monitored and recorded after 1, 2, 3, 5, 7, 14 and 21 days of treatment. The number of offspring was inspected (60) days post-treatment. Mortality was corrected according to Abbott's formula Abbott (1925) and statistically calculated according to Finney (1971) , plotted on log probability paper to obtain lethal concentrations and slope. The reduction percent (%) of emerged adults was determined using the equation of El-Lakwah *et al*. (1996).

Bioassay of residual activity of botanical extracts

The test was carried out according to Ahmed *et al*. (2008) with some modifications as following: tubes containing 10 g of wheat grains were treated with LC_{95} of petroleum ether extract of swamp paper bark. Then were divided into several groups and stored . Three tubes were selected every week and 25 *T. castaneum* beetles were introduced into each tube. This process was repeated weekly for 13 weeks (as storage period). Mortality count was carried out following 2^{nd} days of introducing beetles and corrected using Abbott's formula Abbott (1925). Similarly, three replicates of wheat grains mixed with 1ml of the solvent were used as control. The infested tubes with deposited eggs were kept under laboratory conditions and were observed for adult emergence. The reduction percent (%) of emerged adults was determined using equation of El-Lakwah *et al*. (1996). In most cases mortality was recorded until mortality percent was neglectful.

Effect of botanical extracts on some biological aspects of *T. castaneum* :

1-Number of deposited and hatched eggs:

Five grams of wheat grains treated with LC_{25} and LC_{50} of petroleum ether extract of swamp paper bark leaves were placed in tube. Ten couples of two weeks old *T. castaneum* beetles were introduced into each tube and covered with muslin then kept in the incubator set at 30 ± 2 ºC and 65 ± 10 % RH. Beetles were left for 7days for mating then removed and the number of deposited eggs in the grains was calculated. Also, the number of hatched eggs was counted. Three replicates, each treated with 1ml of petroleum ether were used for comparison (negative control) with three others without solvent (positive control). Percent of hatchability and the reduction in hatchability were determined according to Sokker (2013).

2-Percentage of adult emergence

Ten couples of two weeks old *T. castaneum* adults were placed into a tube containing 10 g of wheat grains treated with LC_{25} and LC_{50} of petroleum ether extract of swamp paper bark . Beetles were left for 7days for mating then removed . The infested treated grains bearing eggs were incubated and observed 60 days post-treatment. The reduction percent (%) of emerged adults was determined using equation El-Lakwah *et al*. (1996). Also, the total number of emerged female and male was detected.

Effect of botanical extracts on some biochemical parameters of *T. castaneum*

1-Preparation of samples for bio-chemical analysis:

Homogenate for biochemical assay was obtained from (1-3) weeks old of *T. castaneum* adults when they offered to crushed wheat grains treated with LC_{50} of the tested extract. The control was set for each experiment comprising the same number of adults reared on wheat grains mixed with the petroleum ether (negative control) and without solvent (positive control)**.** After 2hr of feeding, the insects were removed and a weight of 0.5 g of these beetles was homogenized represented by 1g body weight, in 1ml distilled water by using chilled glass Teflon grinder. The homogenate was centrifuged for 10 min at 7000 rpm at zero ^{o}C; the samples' solutions were filtered and the supernatant was used for the subsequent assay. Each was replicated 3 times.

2- Determination of the main metabolites

i. Total proteins

Total protein content of homogenate was determined by the method of Bradford (1976).

ii.Total carbohydrates

Total carbohydrate content of homogenate was estimated in acid extract of sample by the phenol-sulphoric acid reaction by Dubois *et al*. (1956). Total carbohydrates were extracted and prepared for assay according to Crompton and Birt (1967).

iii.Total lipids

Total lipids content of homogenate was estimated using phosphor vanillin reagent by the method of Knight *et al*. (1972)

Effect of botanical extracts on some viability of wheat grains

Effect of petroleum ether extract of swamp paper bark on wheat grains viability was detected by germination and water absorption tests at the initial time and the end of the considered storage period (13 weeks). Germination tests were carried out according to International Standard Methods Anonymus (1966). Water absorption was detected by treatment 5g of wheat grains with LC_{95} of petroleum ether extract of swamp paper bark. Treated and untreated grains were placed in tubes and submerged in water for 1, 4 and 24 hours according to Schoonhoven (1978). Water absorption was measured as percent in weight increase after the dryness of the wet grains or seeds surface with paper towels according to Yantai and Burkholder (1981)

Data Analysis

Percentage mortality of beetles treated with petroleum ether extract of swamp paper bark was calculated and corrected using Abbott's formula (Abbott, 1925) and probit analysis was statistically computed according to Finney (1971) using LDP line program (www.ehabsoft.com/ldp/line) to obtain the toxicity regression lines. The lethal concentrations LC_{50} and LC_{95} were determined. The other data obtained were statistically analyzed using Procs ANOVA which were performed using SAS statistical software packge 1998 (SAS Institute Inc Cary, NC, USA) and the variance ratios were calculated. Means

were detected using LSD in the same operation and compared by Duncan multiple range tests at 0.05 probability level Duncan (1955).

RESULTS AND DISCUSSION

Chemical constituents of petroleum ether extract of swamp paper bark

Table (1) represents the chemical composition of the petroleum ether extract from the leaves of swamp paper bark *M. ericifolia*. It was obvious that compounds, representing about 99.77% of extract composition were characterized. The major component was methyleugenol (88.83%) , in addition to other constituents such as 1,8 cineole (4.86%) , camphor (1.38%) and α-copaene (1.16%). In consistence with the present results , high concentration of methyleugenol in *M.linariifolia* oil as mentioned by Silva *et al*. (2010), *M. sthypheliodes* oil with a phenolic compound (91.1%) Amri *et al*. (2012) and in essential oil of *Pimenta pseudocaryophyllus* (Myrtaceae) used against *Sitophilus zeamais* (11.35%) with chavibetol (38.14%), and terpinolene (9.17%) as reported by Ribeiro *et al.* (2015). High proportion of monoterpenoids (mainly 1,8-cineole, terpinen-4-ol, terpinolene), phenyl-propanoids (mainly methyleugenol, (E)-methyl isoeugenol) , and sesquiterpenoids (mainly (E) -nerolidol, viridiflorol) in essential oils of *M. linarrifolia* was reported by Padalia *et al.* (2015). Monoterpenoid 1,8-cineole (77.5%) was the major component of the essential oil of *M. fulgens* used against *S. orayzae* with lower amounts of other mono and sequiter-penoids (Lee *et al*., 2004) and for *M. teretifolia* leaves and twigs,1,8-cineole (84.0%) with the other compounds, α-pinene, β-pinene , limonene ,terpinen-4-ol and α terpineol as demonstrated by Southwell *et al.* (2005) .

The insecticidal activity of petroleum ether extract of swamp paper bark could be related to its chemical composition. The isolated pheny-lpropanoid, methyleugenol showed contact toxicity to *S. zeamais* and *T. castaneum* (Huang *et al*., 1997 & 2002) and in the essential oil of *Acorus calamus* against booklice, with LD_{50} value of 103.22 μg/cm^2 (Liu *et al*., 2013). Yildirim *et al*. (2013) reported that some oxygenated monoterpenes were strong toxic compounds and among them, borneol, 1,8-cineole, camphor attained 100 % insecticidal effect against S. *zeamais*. Mahdi and Rahman (2008) referred the insecticidal potency of clove (*Syzygium aromaticum*) against *Callosobruchus maculates* to its smell due to eugenol .

Toxicity of botanical extracts

The results obtained from Tables (2&3) revealed obviously that adult mortality increased with increasing the concentrations and the exposure time. Generally, *M. ericifolia* extracts were more potent on *T. castaneum* adults compared with that of *T. distichum.* Petroleum ether extracts were more potent on *T. castaneum* compared with acetone ones for the two tested plants. Petroleum ether extract of swamp paper bark leaves evoked 94.7% mortality at the highest concentration of 6% (w/w) at 2nd day post–treatment, while complete mortality was recorded at 5th days post-exposure. When the concentration decreased to 2 % (w/w), 41.3% mortality was recorded at 2nd day, while complete mortality was obtained at 7th days post-exposure. In contrast to the previous results regarding to petroleum ether extract of swamp paper bark , it was found that its acetone fraction, petroleum ether and acetone extracts of bald cypress at 6% (w/w) gave 13.3%, 4% , 5.3% mortality, respectively at 2nd days post-exposure indicating low virulence towards *T. castaneum* adults. Similar findings were reported by Abd El-Salam (2010) who found that *M. alternifolia* essential oil gave 90.0% and 100% mortality at 8.0 and 16.0 μl /50 ml air, 24 hr of treatment for *S.*

oryzae and *C. maculates*, respectively. Mortality of *C. maculatus* caused by extracts of *S. aromaticum* increased with the increase in concentration and exposure period with percentages of 100, 90 , 70 and 44% for the tested concentrations at 5 days from the initial treatment as mentioned by Abd-El-Aziz (2002). Devi and Devi (2013) found that hexane extracts of clove (LD_{50} values of 0.517, $\mu l/cm^2$) showed 92% mortality against *S. oryzae* after 5 days. High potency of hexane extract of *Eugenia aromatica* on *S. oryzae* and *C. maculatus* than ethanol one when mixed with wheat grains and cowpea seeds was reported by Arab *et al.* (2004). Higher larval mortality percent was obtained by *Nigella sativa* followed by *T. distichum* against *S. granarius* for all the concentrations of the three tested oil extracts as mentioned by Sabbour and Abdel-Raheem (2015 Ethanolic extract from *P. pseudocaryophyllus* leaves (Myrtaceae), did not have a significant effect on adult survival, F_1 progeny, or damage caused by *S. zeamais* when added to the diet (Ribeiro *et al*., 2015).

Table (1): Chemical components of petroleum ether extract of swamp paper bark leaves.

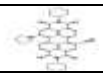
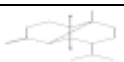
Class	No	Common name	R.T.	Area %	M.W	M.F.	Chemical structure
Dyes	1	Besmide dyes	10.59	0.59	648	$C_{41}H_{36}N_4O_4$	
Heterocyclic compounds	2	4hydroxy3(2oxo2h1oxa3 phenanthryl)2(1h) quinolinon E.	12.20	0.65	355	$C_{22}H_{13}N\,O_4$	
	3	7hydroxy5,6,7,8tetrahydr oindolizine.	13.56	0.25	137	$C_8H_{11}NO$	
Oxygenated monoterpenes	4	1,8Cineole	14.33	4.86	154	$C_{10}H_{18}O$	
	5	Camphor	18.62	1.38	152	$C_{10}H_{16}O$	
	6	Endo-Borneol	19.58	0.25	154	$C_{10}H_{18}O$	
	7	Iso-safrole	28.21	0.21	178	$C_{10}H_{10}O_3$	
Sesquiterpenes-hydrocarbons	8	α-Bourbonene	27.0	0.09	204	$C_{15}H_{24}$	
Oxygenated sesquiterpenes	9	α –copaene	30.14	1.16	204	$C_{15}H_{24}$	
	10	Y-Elemene	30.58	0.26	204	$C_{15}H_{24}$	
	11	Trans- calamenene	31.39	0.77	202	$C_{15}H_{22}$	
Phenyln-propanoids	12	Methyl eugenol	27.79	88.83	178	$C_{11}H_{14}O_2$	
Triterpenes	13	Squalene	60.69	0.47	410	$C_{30}H_{50}$	
Total	99.77%						

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Table (2): Percentage mortality of adults *T. castaneum* after exposure for different periods to swamp paper bark leaves extracts.

Extracts	Conc (w/w) %	Accumulative adult mortality(%) after indicated days							Mean number of the offspring	Reduction in emerged adults %
		1	2	3	5	7	14	21		
Petroleum ether	6	52±2.3	94.7±1.3	96±2.3	100±0	—	—	—	0	100
	5	38.7±0	84±2.3	88±2.3	100±0	—	—	—	0	100
	4	25.3±0	68±2.3	81.3±1.3	100±0	—	—	—	0	100
	3	21.3±0	52±2.3	62.7±1.3	100±0	—	—	—	0	100
	2	5.3±1.3	41.3±1.3	60±2.3	98.7±1.4	100±0	—	—	0	100
	Control								52	
Acetone	6	6.7±1.3	13.3±1.3	14.7±1.4	17.3±1.4	24±2.3	36.5±1.4	50±1.4	0.7	97.6
	5	5.3±1.3	6.7±1.3	12±2.3	16±2.3	20±2.3	35.1±2.3	47.2±1.4	1	96.6
	4	1.3±1.3	4±2.3	8±0	13.3±1.3	17.3±1.3	33.8±2.5	40.3±1.4	2.3	92.1
	3	0±0	0±0	5.3±1.3	8±2.3	10.7±1.4	25.7±1.5	36.1±1.4	3.7	87.2
	2	0±0	0±0	1.3±1.3	5.3±1.3	8±0	9.5±2.3	25±1.3	9	69
	Control								29	

Each datum represents the mean of three replicates.

Petroleum ether extracts of *Annona squamosa* seeds offered the highest mortality, while acetone caused the least mortality between all the tested extracts against *T. castaneum* as reported by Khalequzzaman and Sultana (2006). Mahfuz and Khanam (2007) revealed that petroleum ether extract of different plants exhibited higher toxic effect than acetone and methanol extracts against the *T. confusum* beetles at all the intervals. Chowdhury *et al*. (2009) arranged nishinda, *Vitex negundo* extracts according to the degree of solvent toxicity against *T. castaneum*, methanol > petrolium spirit > acetone > ethylacetate.

Reduction percent in emerged adults was 100% at all the tested concentrations of petroleum extract of swamp paper bark, while it ranged from 69%- 97.6% , 85.4%-100% and 51.5% - 83.9% for acetone extract of swamp paper bark and petroleum ether and acetone extracts of bald cypress, respectively.

In the harmony with the current results, El-Naggar *et al*. (2012) mentioned that the reduction in F_1 progeny increased by increasing the concentration of the plant extract, the lowest percent (45.3%) of adult emergence of *Spodoptera littoralis* larvae at 10% concentration of *T. distichum* extract, while the highest percentage (72.15%) was at 0.625% concentration. The oil of *S. aromaticum* prevented adult emergence of the *S. oryzae* weevil at all concentrations as revealed by Ileke *et al*. (2014). Similarly, significant reduction against *S. zeamais*, *C. maculatus* and *T. castaneum* at all levels by ethanolic extract of *E. aromatica* essential oil was reported by Olotuah (2014).

Table (3): Percentage mortality of adults *T. castaneum* after exposure for different periods to bald cypress extracts.

Extracts	Conc. (w/w) %	Accumulative adult mortality(%) after indicated days							Mean number of the offspring	Reduction in emerged adults %
		1	2	3	5	7	14	21		
Petroleum ether	6	2.7±1.3	4±0	8±2.3	9.5±1.4	20.3±3.6	40.5±2.3	81.9±1.4	0	100
	5	1.3±1.3	2.7±1.3	6.7±1.3	8.1±1.4	16.2±1.4	35.1±2.3	59.7±1.4	0	100
	4	1.3±1.3	1.3±1.3	5.3±1.3	6.8±0	10.8±2.3	29.7±1.4	40.3±1.4	1.7	95.9
	3	0±0	1.3±1.3	2.7±1.3	4.1±1.4	9.5±2.7	24.3±1.4	36.1±1.4	4.7	88.6
	2	0±0	0±0	1.3±1.3	2.7±0	8.1±1.4	17.6±1.4	29.2±2.4	6	85.4
	Control								41	
Acetone	6	2.7±1.3	5.3±1.4	9.3±1.4	10.7±1.3	17.6±2.3	31.9±1.4	46.5±1.4	5.3	83.9
	5	1.3±1.3	4±0	5.3±1.3	8±0	9.5±1.4	19.4±1.4	32.4±2.4	8	75.8
	4	0±0	2.7±1.3	4±0	6.7±1.3	8.1±1.4	16.7±2.4	31.1±1.4	10.7	67.6
	3	0±0	1.3±1.3	2.7±1.3	5.3±1.3	6.8±2.3	11.1±2.5	28.2±2.4	12	63.6
	2	0±0	0±0	1.3±1.3	4±2.3	5.4±1.4	9.7±1.4	21.2±1.4	16	51.5
	Control								33	

Each datum represents the mean of three replicates.

The estimated toxicity values of lethal concentrations and slopes for petroleum ether extract of *M. ericifolia* against *T. castaneum* adults are presented in Table (4). The results showed that the lethal concentrations were exposure period dependant i.e the higher the exposure period, the lower the LC values. Based on probit analysis, toxicity values represented in LC_{25}, LC_{50} and LC_{95} were 1.65 , 2.59 and 7.79% (w/w), respectively. In agreement with the present results , Ko *et al*. (2009) showed that the LD_{50} values of leaf essential oil of *M. cajuputi* against *S. zeamais* and *T. castaneum* in contact toxicities were 0.062 and 0.143µL insect. Lee *et al*. (2004) reported that 2 *Melaleuca* species namely *M. armillaris* and *M. fulgens* had fumigant toxicity to *T. castaneum* with the LD_{50} equaling 30.6 and 28.6 µL L^{-1}, respectively. Adedire and Akinkurolere (2005) determined that *E. aromatica* ethanol extract was the most potent with the least LT_{50} value against *T. castaneum* adults .

Table (4): LC values of petroleum ether extract of swamp paper bark against *T. castaneum* adults at 2nd days post-exposure.

Lethal concentrations (w/w) %		Slope of Regression line
LC_{25}	1.65	
LC_{50}	2.59	3.44±0.377
LC_{95}	7.79	

Residual activity of botanical extracts

The residual toxic effect of petroleum ether extract of swamp paper bark at LC_{95} level (Table 5) showed that the effect of that extract was relatively stable up to 9th weeks of storage with 93.3% mortality at 2nd days post - treatment. Persistence failed gradually to 74.7% mortality after 13 weeks of storage. Petroleum ether extract of swamp paper bark caused 100% reduction in emerged adults and succeed in preventing emergence of F_1 progeny during tested storage period indicating that it can be used as a protectant for 13 weeks with zero production of progeny. In the harmony with this reseults, Abd-El-Aziz (2001) reported that essential oils of clove (*E. aromaticum*) had promising toxicity in suppressing *C. maculatus* and protecting cowpea seeds from beetles' infestation for 4 months during storage. Arab *et al.* (2004) mentioned that reduction percentages of progeny of *S. oryzae* and *C. maculates* caused by hexane extract of *E. aromatica* were 63.0% and 35.0% and 79.8% and 58%, respectively after 4 and 5 months. Adedire and Akinkurolere (2005) revealed that ethanol extract of *E. aromatica* was the most potent of four plant materials tested against adult *S. zeamais, T. castaneum, C. maculatus, Oryzaephilus mercato*r and *Lasioderma serricorne* with being the percentage grain damage by insects nil in treated grains stored for 90 days at 2.0% (v/w).

Effect of botanical extracts on some biological aspects of *T. castaneum*

Number of deposited and hatched eggs

The results in Table (6) showed that the mean number of deposited eggs of treated *T. castaneum* beetles with LC_{25} and LC_{50} levels of swamp paper bark petroleum extract was 18.3 and 4.3 eggs / female compared with 29.6 and 29 eggs/female for negative and positive controls, respectively. Regarding to hatchability, there were no hatched eggs at LC_{25} and LC_{50} levels compared with 27.3 and 27.7 eggs for negative and positive controls, respectively.

Table (5): Residual activity of LC_{95} of petroleum ether extract of swamp paper bark against *T. castaneum* adults for 13 weeks.

Storage time (weeks) S	Mortality (%)of exposed insects after							Reduction in emerged adults%
	1day	2days	3 days	5 days	7 days	14 days	21 days	
Initial	100	—	—	—	—	—	—	100
1	100	—	—	—	—	—	—	100
2	100	—	—	—	—	—	—	100
3	100	—	—	—	—	—	—	100
4	100	—	—	—	—	—	—	100
5	100	—	—	—	—	—	—	100
6	92	100	—	—	—	—	—	100
7	81.3	98.7	100	—	—	—	—	100
8	78.7	97.3	100	—	—	—	—	100
9	74.7	93.3	96	100	—	—	—	100
10	72	84	89.3	100	—	—	—	100
11	69.3	78.7	84	87.5	100	—	—	100
12	68	76	81.3	86.3	100	—	—	100
13	66.7	74.7	80	82.7	98.7	100	—	100

Toxicological effects of *Melalleuca ericifolia* and *Taxodium distichum* extracts on adults rust-red flour beetle

Table (6): Effect of LC_{25} and LC_{50} of petroleum ether extract of swamp paper bark leaves on the number of deposited and hatched eggs of *T. castaneum* adults.

Tested extracts	Conc.	Mean no. of eggs / female			
		Total no. of eggs	Hatched eggs	Hatchability	Reduction in hatchability
		No.	No.	No.	No.
swamp paper bark	LC25	18.3±0.9 b	0±0	0	100
	LC50	4.3±0.7 c	0±0	0	100
Negative control	—	29.6±0.3 a	27.3±0.3 a	92.1	7.9
Positive control	—	29±0.6 a	27.7±0.9 a	100	—

Means with different letters within each column are significantly different ($P \leq 0.05$)

Percentage of adult emergence

Results in Table (6) regarding to hatchability confirmed the results in Table (7). Complete reduction in emerged adults was recorded at both LC_{25} and LC_{50} levels compared with 43 and 47 adults for negative and positive controls, respectively. Sex ratio was 1:1 in negative and positive controls.

Statistical analysis from Table (8) maintained the effects of all factors affected the number of emerged adults of *T. castaneum*. All factors including treatment and solvents were highly significant ($p \leq 0.05$) except for LC .

In interpretation for the previous results, (Adedire, 2002; Akinkurolere *et al*., 2006; Ileke 2008; Oni and Ileke, 2008; Adedire *et al*. 2011; Ileke and Oni, 2011; Ileke and Olotuah, 2012) reported that no adult emergence were observed in all treated seeds with extracts and this could be referred to high mortality of adult insects, inhibition of locomotion that disrupt mating and sexual communication preventing females from laying eggs and complete suppression of the developmental stages. Iqbal *et al*. (2015) found that between the tested extracts of sweet flag, petroleum ether and acetone extracts caused the maximum inhibition ratio of resulting *T. castaneum* adults at 1000 µg/g dosage.

Statistical analysis from Table (8) maintained the effects of all factors affected on number of deposited and hatched eggs by *T. castaneum* . All factors including treatment, LC and solvents were highly significant ($p \leq 0.05$) except for LC in case of the number of hatched eggs. The obtained results are consistent with Adjalian *et al*. (2015) who reported that essential oil of *M. leucadendron* caused significant reduction or inhibition in egg laying of *Sitotroga cerealella* female compared to control groups. Abd-El-Aziz (2001) proved that the essential oils from clove (*E. aromaticum*) and eucalyptus (*Eucalyptus globules*) as surface protectants had promising oviposition deterrency and suppressing *C. maculatus* egg deposition and adult emergence. Khani *et al*. (2012) mentioned that petroleum ether extract of black pepper [*Piper nigrum*] and physic nut (*Jatropha curcas*) showed strong inhibition on egg hatchability and adult emergence of *Corcyra cephalonica* at the lowest concentration. Kaur and Saivastava (2014) found that extracts of *Peganum harmala*

different parts at the highest dose concentration of 10% were the most effective in increasing adult mortality, retarding developmental rate, reducing egg laying and adult emergence of *T. castaneum.*

Table (7): Effect of both LC_{25} and LC_{50} of petroleum ether extract of swamp paper bark on percentage of adult emergence of *T. castaneum* .

Tested extracts	Conc.	Progeny emergence					
		Total No. (F1 emerged adults)	Reduction in emerged adults %	Female No.	Male No.	Sex ratio (%)	
						Female	Male
Swamp paper bark	LC25	0±0	100	0±0	0±0	0	0
	LC50	0±0	100	0±0	0±0	0	0
Negative control	—	43±0.6 b	8.5	21±0.6	22±1.2	48.8	51.2
Positive control	—	47±1 a	0	24.7±1.2	22.3±0.9	52.5	47.4

Means with different letters within each column are significantly different ($P \leq 0.05$).

Table (8): Factorial analysis for different factors affecting the number of eggs deposited and hatched and emerged adults of *T. castaneum*.

Factor	Level	Deposited eggs			Hatched eggs			Emerged adults		
		No	Mean		No	Mean		No	Mean	
Treatment	swamp paper bark	6	11.33	b	6	0.00	b	6	0.00	b
	Control	12	29.33	a	12	27.50	a	12	45.00	a
LC	25	9	25.67	a	9	18.33	a	9	30.00	a
	50	9	21.00	b	9	18.33	a	9	30.00	a
Solvents	Petroleum ether	12	20.50	b	12	13.67	b	12	21.50	b
	—	6	29.00	a	6	27.67	a	6	47.00	a

Means with different letters within each column are significantly different ($P \leq 0.05$) .

Effect of botanical extracts on biochemical parameters of *T. castaneum*

- **Effect of petroleum ether extract of swamp paper bark on main metabolites**

Data summarized in Table (9) showed that the treatment of *T. castaneum* beetles's with LC_{50} of petroleum ether extract of swamp paper bark leaves induced a significant increase in the whole body homogenate of total protein and carbohydrate, however a significant decrease was observed in case of lipid contents compared with negative and positive controls ($p<0.05$). Mean of increase in protein and carbohydrate content was 56.93 and 47.7 mg/ml compared with 41, 40.5 mg/ml and 31.7, 30.1 mg/ml, respectively. Mean decrease of lipid content was 6.3 mg/ml, compared with 7.6 and 7.8 mg/ml for negative and positive controls, respectively.

Statistical analysis from the obtained results in Table (10) maintained

the interaction among the various factors affected the main metabolites of *T. castaneum* beetles. All factors including treatment and solvent were found to be highly significant ($P \leq 0.05$).

Total proteins

The total protein content of *T. castaneum* adults was increased after treatment with LC_{50} of swamp paper bark extract. Similar findings were reported by Abo El Makarem *et al*. (2015) who mentioned increase in total proteins content of basil and anise oils treated *S. granarius* adults , they suggested that this increase could be as the result of an elevation in tissues metabolic activity to compensate the stress caused by the essential oils.

Total carbohydrates

The total carbohydrates content of *T. castaneum* adults was increased after treatment with LC_{50} of swamp paper bark extract. This was in agreement with Al-Moajel (2004) who reported that total carbohydrates content of *S. oryzae* adults increased after treatment with acetone extract of mesquite plant (*Prosopis juliflora*) seeds. Also, it agrees with the results of Abo el makarem *et al*.(2015) in case of *S. granaries* adults treated with sub lethal doses of *Pimpinella anisum* (anise) and *E. aromatic* (clove), *Ocimum basilicum* (basil) essential oils. They reffered that increase to the inability of insects to assimilate the food thereby increasing the level of carbohydrate in their tissues or, due to increase synthesis of transaminase enzymes (GOT and GPT) in insects hemolymph that are responsible for metabolizing amino acids and obtaining energy under the stress of toxic oils. The same interpretation was recorded for Sharma *et al*. (2011) who suggested that stress induced by anise and clove oils might have enhanced glycogenolysis leading to the hyperglycemia.

Table (9): The main metabolites in the whole body homogenate of *T. castaneum* adults after treatment with LC_{50} of swamp paper bark extract.

Plant extracts	Total proteins content (mg/ml)	Total carbohydrates content (mg/ml)	Total lipids content (mg/ml)
	Mean ±SE	Mean ±SE	Mean ±SE
swamp paper bark	56.93±0.6 [a]	47.7±0.4 [a]	6.3±0.3 [b]
Negative control	41±0.5 [b]	31.7±0.6 [b]	7.6±0.3 [a]
Positive control	40.5±0.3 [b]	30.1±0.6 [b]	7.8±0.4 [a]

Means with different letters within each column are significantly different ($P \leq 0.05$)

Table (10): Factorial analysis for different factors affecting *T. castaneum* main metabolites.

Factor	Level	Proteins		Carbohydrates				Lipids		
		No	Mean	No		Mean		No	Mean	
Treatment	Swamp paper bark	3	56.93	a	3	47.66	a	3	6.28	b
	Control	6	40.77	b	6	30.88	b	6	7.73	a
Solvents	Petroleum ether	6	48.97	a	6	39.67	a	6	6.96	b
	—	3	40.53	b	3	30.10	b	3	7.83	a

Means with different letters within each column are significantly different ($P \leq 0.05$) .

Total lipids

The total lipids content of *T. castaneum* adults was decreased after treatment with LC_{50} of swamp paper bark extract. Similar decrease in total lipids content in *T. castaneum* larvae treated with essential oil of *Agastache foeniculum* was reported by Ebadollahi *et al*. (2013) and *S. granaries* adults treated with clove oil (Abo El Makarem *et al*., 2015). Moreover,

Arulprakash and Veeravel (2007) reported a reduction in *C. maculatus*, *S. oryzae* and *T. castaneum* total lipids after treatment with *Calotropis gigantean*. Significant reduction in lipids hemolymph of *Hippodamia variegatal* larvae treated with spirodiclofen was reported by Alimohamadi *et al.* (2014). Decreasing the total lipids content may be attributed to the mode of action of clove oil in inhibition of lipid biosynthesis (Abo El Makarem *et al*., 2015) or strong deterrence effect of *A. foeniculum* oil Ebadollahi *et al*. (2013). Moreover, such decrease may be attributed to increases lipase enzyme activity and consequently lipid metabolism resulting in decreased lipid content as reported by Alimohamadi *et al*. (2014) or due to several mechanisms including formation of lipoproteins which are utilized for repair of damaged cell and tissue organelles, also for energy requirements in cells as suggested by Steele (1985); Lohar and Wright (1993).

Effect of botanical extracts on some viability of wheat grains

Treatment of wheat grains with LC_{95} of petroleum ether extract of swamp paper bark leaves showed adverse effect on grains germination, following treatment as shown in Table (11). Statistical analysis of data from Table (12) maintained the interaction among the various factors affected the germination of wheat grains. All factors including treatments, time (at initial and after storage period) and solvents were highly significant ($P \leq 0.05$).

Water absorption values of treated grains with LC_{95} of petroleum ether extract of swamp paper bark leaves shown in Table (13) generally , were appeared to be equal to the control by the end of submerge period (after 24 hr) especially, at the initial treatment indicating that slight effect on water absorption with some extracts at some intervals of submergence. This slight effect could be an indicative that treatments did not negatively affect the permeability of the grain testa and thus water absorption of seeds was not affected as suggested by Akinkurolere *et al*. (2006). Statistical analysis of data from Table (14) maintained the interaction among the various factors affected the water absorption of wheat grains. All factors including treatments, time (at initial and after storage period) and intervals (1,4 and 24 hr) were highly significant ($P \leq 0.05$). The current results are in consistence with Amri *et al*. (2012), who found that the *Melalleuca* species oils of *M. armillaris*, *M. styphelioides* and *M. acuminate*, each tested for their phytotoxicity at the concentrations of 0.062 µg/mL, 0.125 µg/mL, 0.25 µg/mL, 0.625 µg/mL, 1.25 µg/mL and 2.5 µg/mL did not affect germination, but they affected the radicle elongation of the *Triticum durum* seeds at the highest dose. As well as, hexane extracts of clove used to control *S. oryzae* at 1,000 ppm had no inhibitory effect on wheat seed germination as revealed by Devi and Devi (2013). Kassis (2002) mentioned that *E. aromatic* extracts at LC_{50} could be used without any harmful effect on the viability of wheat grains. Negligible effect on water absorption for treated grains with pet-ether, chloroform and acetone seed extracts of *P. juliflora* used against *R. dominica* was demonstrated by Al-Moajel and Ahmed (2005), while there was a significant reduction (14.3-21.6%) in germination of treated grains compared with control. In another study, Ahmed *et al*. (2001) reported that petroleum ether extract of *Brassica rapa* [*B. campestris*] used against *C. maculatus* infestation had an adverse effect on cowpea seeds germination, while water absorption percentage was slightly affected.

Table (11): Effect of LC_{95} of petroleum ether extract of swamp paper bark on wheat grains germination at the initial time and after storage period (90 days).

Plant extracts	LC95 (w/w) %	Initial time		After storage period (90 days)	
		Germination%	Reduction%	Germination%	Reduction%
swamp paper bark	9.1	66.3±2.7 c	33.7	52.4±2.3 c	47.6
Negative control	—	99±1 b	1	98.9±1.2 b	1
Positive control	—	100±1.7 a	—	100±1.1 a	—

Means with different letters within each column are significantly different ($P \leq 0.05$)

Table (12): Factorial analysis of different factors affecting germination of wheat grains.

Factor	Level	Germination		
		No	Mean	
Treatment	swamp paper bark	8	59.38	b
	Control	16	96.50	a
Time (Days)	Zero-Time	12	86.10	a
	90 days	12	82.15	b
Solvents	Petroleum ether	16	76.2	b
	—	8	100.0	a

Means with different letters within each column are significantly different ($P \leq 0.05$).

Table (13): Effect of LC_{95} of petroleum ether extract of swamp paper bark on wheat grains water absorption at the initial time and after storage period (90 days).

Tested extracts	LC95 (w/w) %	Water absorption% at initial time			Water absorption% after storage period		
		1h	4h	24h	1h	4h	24h
Swamp paper bark	9.1	9.5±0.04 b	18.8±0.08 a	42.6±0.09 a	9.2±0.01 b	18.5±0.02 b	40.4±0.03 b
Negative control	—	14.3±0.06 a	23.2±0.08 a	42.2±0.1 a	5.9±0.01c	16.7±0.01 c	33.6±0.03 c
Positive control	—	12.9±0.02 b	23.3±0.06 a	47.8±0.14 a	9.03±0.02 a	20.7±0.01 a	41.3±0.02 a

Mean with different letters within each column are significantly different ($P \leq 0.05$).

Table (14): Factorial analysis of different factors affecting water absorption of wheat grains.

Factor	Level	Water absorption		
		No	Mean	
Treatments	Swamp paper bark	18	6.17	b
	Positive control	18	6.13	b
	Negative control	18	6.29	a
Time (days)	Zero-time	27	6.23	a
	90 days	27	6.16	b
Intervals (hr)	1	18	5.51	c
	4	18	6.02	b
	24	18	7.07	a

Means with different letters within each column are significantly different ($P \leq 0.05$)

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التأثيرات السمية لمستخلصات الميلالوكا والتاكسوديوم علي حشرة خنفساء الدقيق الحمراء

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المستخلص

تم اختبار التأثير السام بالملامسة وعن طريق المعدة لمستخلصات الايثيرالبترولي والأسيتون لأوراق كلا من نباتي الميلالوكا والتاكسوديوم علي الحشرات البالغة لخنفساء الدقيق الحمراء (رتبة غمديات الأجنحة : تينيبريونيدي) . ولقد لوحظ من خلال النتائج بأن نسبة الموت ازدادت بزيادة كلا من تركيز المستخلص وفترة التعريض له ، مع تسجيل نسب موت أعلي لمستخلصات الميلالوكا مقارنة بنظيرها للتاكسوديوم .

أظهرمستخلص الايثيرالبترولي لأوراق نبات الميلالوكا أنه الأكثر فاعلية علي حشرة خنفساء الدقيق الحمراء بتركيزات ١,٦٥ و ٢,٥٩ و٧,٧ ٩% (وزن :وزن) المميته لنحو ٢٥ و ٥٠ و ٩٥% من الحشرات علي التوالي بعد فترة ٤٨ ساعة من التعريض. وعند دراسة مدى بقاء هذا المستخلص وجد أن التركيز المميت لنحو ٩٥% من الحشرات أدي الي تأثير متبقي استمر حتي الأسبوع الثالث عشر . بينما ظهر خفض معنوي في عدد البيض وخفض كامل لفقسه وبالتالي النسل الناتج وذلك بعد معاملة الحشرات بالتركيزات المميتة ٢٥ و ٥٠ % للحشرات علي التوالي . كما ظهرت زيادة معنوية في محتوي كلا من الكربوهيدرات والبروتين مقارنة بالتجربة الضابطة مع وجود نقص معنوي في محتوي الدهون وذلك عند المعاملة بالتركيز ال مميت٥٠ % من الحشرات .

لم يكن للمعاملة بالتركيز المميت لنسبة ٩٥% من الحشرات لمستخلص الايثير البترولي للميلالوكا تأثيرا يذكر على معدل امتصاص حبوب القمح للماء سواء مع بداية التجربة أو بعد فترة التخزين، إلا أنها أظهرت تأثيرا سلبيا علي معدل الانبات للحبوب . التحليل الكيميائي لمستخلص الايثير البترولي لأوراق الميلالوكا أوضح أن مركب الميثيل ايوجينول هو المركب الرئيسي المكون للمستخلص. وبالتالي من الممكن أن يكون وجود هذه المركبات هو المسؤل عن التأثير الفعال لمستخلص هذا النبات .

ويتضح من نتائج هذه الدراسة أن مستخلص الايثير البترولي لاوراق نبات الميلالوكا يمكن ان يستعمل كبديل للمبيدات من أجل حماية القمح المخزون من الاصابة بخنفساء الدقيق الحمراء.