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RESEARCH ARTICLE

EFFECT OF SOME PLANTS EXTRACTS AGAINST RED FLOUR BEETLE (*Tribolium castaneum* (HERBST)

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ABSTRACT

Effects of ethanol extracts of *Curcuma longa* L., *Myristica fragrans* Houtt. and *Piper nigrum* L. on the mortality of red flour beetle were investigated. In the current study, ethanol plant extracts showed that the mortality rate increased with increasing concentration as well as the length of exposure times. On the other hand, results of this study point out that the three plants of this study might be useful as potent insect control agents. The results showed that *Myristica fragrans* had the best effect on the mortality of adults and Larvae (100%) in 8% concentration, followed by *Curcuma longa* and *Piper nigrum* extracts of mortality were achieved. After 24-hour exposure, the LD_{50} values of ethanol extract of *Piper nigrum*, *Curcuma longa* and *Myristica fragrans* against adult *Tribolium castaneum* were found to be 0.73, 0.79 and 0.45 mg/cm^2, respectively, while in larvae found 0.34, 0.27 and 0.12 mg/cm^2 respectively. No mortality was observed in control. The LD_{50} values for fifth instar larvae were 0.34, 0.27 and 0.12 mg/cm^2 in *Piper nigrum, Curcuma longa*, and *Myristica fragrans* respectively.

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INTRODUCTION

Tribolium castaneum (Herbst) (Coleoptera: Tenebrioidae) is a very important insect pest of food grains, it is considered as a major pest of stored grains (Howe, 1965). Stored-grain insect pests have been damaging food grains,according to FAO estimate, 10 to 25% of the world harvested food is destroyed annually due to insects and rodent pests and this percentage reaches 80% sometimes in the Third World as reported (Anonymous, 1980; Matthews, 1993; Dwivedi and Shekhawat, 2004). Synthetic insecticides using for control of these insects, relies heavily on the use of synthetic insecticides and fumigants. But their widespread use has led to some serious problems including great environmental hazards, toxic residues on stored grain and development of insect strains resistant to insecticides (Zettler and Cuperus, 1990; White, 1995; Ribeiro *et al*., 2003; Bakkali *et al*., 2008; Ayaz *et al*., 2010). So that, increased attention of the researchers towards local alternatives such as natural products which have few harmful effects, safe for humans and environment, cheaper and easily available way for controlling pests(Okonkwo and Okoye, 1996). The plants have been interesting pesticide properties against *T. castaneum*, the effect of solvent extracts (petroleum ether, acetone and ethanol extracts) of rhizomes *Curcuma longa* studied by Jilani and Su (1983), As well as a noticeable repellent activity of turmeric rhizomes and some other medicinal plantsagainst *Tribolium castaneum* studied by (Chander *et al*., 1992; Chander *et al*., 1994; Apisariyakul *et al.*1995; Chander *et al*., 2000; Abida *et al.,* 2010; Damalas, 2011; Tarigan *et al*., 2016). Matter *et al*. (2008) observed that petroleum ether and diethyl ether extracts of turmeric showed noticeable reduction F1 progeny of *T.castaneum.* Amin *et al*., (2012) studied the insecticidal activity of three plant extracts Helencha (*Enhydra fluctuans* Lour), Ghetu (*Clerodendrum viscosum* Vent) and Kalomegh (*Andrographis peniculata* Wall) were tested against stored grain pest *Tribolium castaneum* (Herbst). Pepper and Ginger extracts on *Tribolium castaneum* has been tested by five concentrations of 0.5, 1.0, 1.5, 2.0 and 2.5 ml for 7 days by (Mary and Durga, 2017), it is found that the *T.castaneum* mortality mean rate was recorded highest in 2.5 ml concentration. *Myristica fragrans* Houit (Myristicaceae) essential oil possesses insecticidal and antifeeding activities against *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* (Huang *et al*. 1997; Jang *et al*. 2005). In a preliminary experiment, a methanolic extract of the seeds from nutmeg, *Myristica fragrans* was shown to have potent insecticidal activity against adult females of *Blattella germanica*. In the present study, insecticidal and development inhibition activity of three medicinal plants have been reported against wheat grain pest *Tribolium castaneum* (Herbst) (Coleoptera Tenebrionidae) to control its infestation.

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MATERIALS AND METHODS

Rearing of test insect mixed population of *Tribolium castaneum* (Herbst) was collected from infected stored wheat for rearing in the laboratory. The insects were collected and kept in wide jars covered with muslin cloth. The insects were kept in the laboratory for two months for rearing. The adults of *Tribolium castaneum* were sieved and placed in breeding containers in the medium of uninfected wheat. The samples were placed in an incubator at 30± 2°C and 65± 5% relative humidity for 24 hours). Then, the insects were transferred from the jars to new containers. The media (wheat) in which the adults of *Tribolium castaneum* were initially retained for 72 hours contained enough eggs laid by females. In these jars, the first larvae appeared after four days and the highest number of larvae appeared after seven days. Same age and size progeny were removed from these stocks. It was further kept for another period of five days before testing (Hasan *et al*., 2006). Plant materials samples (rhizomes of *Curcuma longa*, andseeds of *Piper nigrum* L. and *Myristica fragrans* Houtt) were collected from different places Baghdad in Iraq (Table 1). All the identified plant materials were dried by using air for 6-7 days, while others were dried by using the oven at a temperature of 30°C for a period of 24 hours. The dried materials were macerated and powdered in blender machine type (Moulinex, France).

Table 1. Plants used against *Tribolium castaneum*

Plant	Family	Tissue used
Piper nigrum L.	Piperaceae	Seeds
Curcuma longa L.	Zingibaraceae	Rhizomes
Myristica fragrans Houtt.	Myristicaceae	seeds

Preparation of ethanol plant extracts

Extracts of seeds and rhizomes were prepared by mixing 25g from sample in 250 ml of ethanol in a flask. The flask was closed with a piece of cotton and aluminum foils and placed in rotary shaker at 30°C for 24 hours or soxhlet for extraction. The extract was filtered to obtain the stock solution.

From the stock solution 2, 4, 6 and 8 % concentrations were prepared. Seventy-twopetri dishes were taken, and filter papers were cut according to the size of petri dishes for both test solutions. Plant extract were spread uniformly on the filter papers (Whatman No. 1.7 cm diameter). Insect mortality was recorded after 3, 6, 9, 12 and 24 hours of treatment. Three replicates were calculated for each concentration. Abbott's formula (Abbott, 1925) was used to correct the mortality. Corrected mortality (PT) was calculated using the following formula:

$$PT = [(Po – Pc) / (100 - Pc)] \times 100$$

Where Po = observed mortality and Pc = controlled mortality.

Statistical Analysis

Statistical analysis of variance was carried out with the Statistical Package for Social Science (SPSS), using a factorial completely randomized design (CRD). Treatment means showing significant difference (P<0.01) were separated by using Least Significant Difference. The mortality data were subjected to probit analysis for the determination of LD_{50} values using the computer software SPSS of 14 version. Results with P < 0.05 were statistically significant.

RESULTS

Toxicity of ethanol plant extracts on *Tribolium castaneum* adult: The insecticidal activity of the ethanol extracts of seeds of *Myristica fragrans* and *Piper nigrum* and rhizomes of *Curcuma longa* were tested against *Tribolium castaneum*, using various concentrations had significant (P<0.01) contact toxicity on *Tribolium castaneum*. The data in a Table (2) and Figure (1) showed mean percent mortality of *Tribolium castaneum* after24 hours under the various treatments of the ethanol plant extract, the results showed that *Myristica fragrans* gave maximum mortality 100 % at a concentration8 %. followed by*Curcuma longa* and *Piper nigrum*extracts where 96.66%, 60% of mortality were achieved, respectively. Mortality percentage increased with exposure time of thesame concentration. *Myristica fragrans* caused the highest mortality of 40.10, 47.90, 80 and 100 per cent at 2,4,6 and 8 per cent concentrations respectively, *Piper nigrum* was the lowest lethal it was 30, 42.60, 45.25 and 60 per cent concentrations in same concentrations this was clearly seen from (Table 2).

Table 2. Toxicity of ethanol plant extracts on *Tribolium castaneum* adult

Plant	Concentration %	Mortality %					Mean	Mean Plant
		3	6	9	12	24		
Control	-	0	0	0	0	0	0	
Piper nigrum	2	3.33	6.66	6.66	13	30	11.93	24.97
	4	6.33	10	10	22.63	42.60	18.30	
	6	13.33	20	20	30	45.25	25.70	
	8	36.66	40	40	43.33	60	43.98	
Curcumalonga	2	3.33	10	10	21	37.5	16.36	34.35
	4	20	20	20	26.66	51	27.53	
	6	23.33	23.33	26.66	30	71	34.86	
	8	40	50	53.33	53.33	96.66	58.66	
Myristica fragrans	2	0	0	10	33.33	40.10	16.68	41.42
	4	10	16.66	18.33	26.66	47.90	23.91	
	6	36.66	43.33	46.66	46.66	80	50.66	
	8	66.66	66.66	70	70	100	74.44	
	Mean time	19.96	23.58	25.51	32.04	54.04		

RLSD 1% of concentration = 3.15
RLSD 1% of time= 6.91
RLSD 1% of plant extract = 9.16
RLSD 1% interaction between plant extract and concentration = 14.48

Toxicity of ethanol plant extracts on *Tribolium castaneum* larvae: The effect of plant extracts on the survival of

Tribolium castaneum fifth instar larvae is presented the (Table 3; Figure 2). The results clearly indicated that *Myristica fragrans* was the most toxic plant followed by *Curcuma longa* and *Piper nigrum* and this order of toxicity was clearly expressed in the LD_{50} studies (Figure3,4). *Myristica fragrans* caused the highest mortality of 53.33, 100, 100 and 100 per cent at 2,4,6 and 8 per cent concentrations respectively, *Piper nigrum* was the lethal 30, 30, 60 and 80 per cent concentrations in same concentrations this was clearly seen from (Table 3; Figure 2). The mortality values in different concentrations of each treatment were significantly different ($P < 0.05$).

Probit Analysis

The mortality (%) was recorded and statistical data regarding LD_{50}, 95% confidence limit and chi-square values were calculated and presented in the in the (Table 4, Figures 3, 4) showed the log-dose responses of *Tribolium castaneum* exposed to ethanolplant extract. Result indicated that the test insect is sensitive to *Myristica fragrans* extract as indicated by it is low $LD_{50}$was 0.12% for the 24 hours exposure period. The upper and lower limits of the LD_{50} of the ethanol extract of rhizomes of *Curcuma longa* and seeds of *Piper nigrum* and *Myristica fragrans* are shown in adults and larvae (Table 4; Figure 3,4).

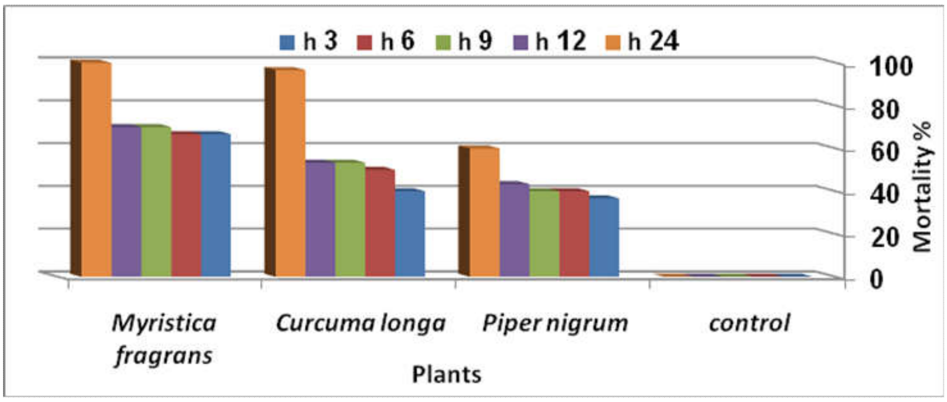

Figure 1. Toxicity of ethanol plant extracts on *Tribolium castaneum* adult after 24 h of treatment

Table 3. Toxicity of ethanolic plant extracts on *Tribolium castaneum* larvae

Plant	Concentration %	Mortality %					Mean	Mean plant
		3	6	9	12	24		
Control	-	0	0	0	0	0	0	
Piper nigrum	2	3.33	4.20	6.66	10	30	10.83	26.70
	4	10	10	13.33	13.33	30	15.33	
	6	20	23.33	26.66	30	60	31.99	
	8	26.66	30	36.66	60	80	48.66	
Curcuma longa	2	0	0	10	10	23.33	8.66	24.34
	4	6.66	6.66	10	13.33	36.33	14.59	
	6	10.66	23.33	26.66	30	60	30.13	
	8	23.33	26.66	36.66	40	93.33	43.99	
	2	20	20	26.66	28.33	53.33	29.66	53.51
Myristica fragrans	4	26.66	26.66	56.66	75.73	100	57.14	
	6	30	40	60	83.33	100	62.66	
	8	30	40	63.33	89.66	100	64.59	
		15.94	20.06	28.71	37.20	58.94		

Mean Concentration= 12.18
R.L.S.D. 1% of hour = 11.03
R.L.S.D. 1% of plant extract = 3.15
R.L.S.D. 1% Interaction between plant extract and concentration = 7.97

Table 4. Log-dose probit responses of *Tribolium castaneum* (adult and Larva) exposed to ethanolplants extract after 24 hours of exposure

Plants	LD_{50} (mg/ cm^2) in Adult insects	95% Confidence limits		Chi-square, χ2 (Degree of freedom)
		Lower	Upper	
Adult insects				
Piper nigrum	0.73	9.97	11.16	0.920(3)
Curcuma longa	0.79	0.516	4.60	6.421(3)
Myristica fragrans	0.45	0.10	3.50	11.003(3)
Larvae insects				
plants	LD_{50} (mg/ cm^2) in larvae insect	95% Confidence limits		Chi-square, χ2 (Degree of freedom)
	Larva	Lower	Upper	
Piper nigrum	0.34	3.06	7.48	0.941(3)
Curcuma longa	0.27	0.70	2.10	3.310(3)
Myristica fragrans	0.12	0.06	0.14	5.790(3)

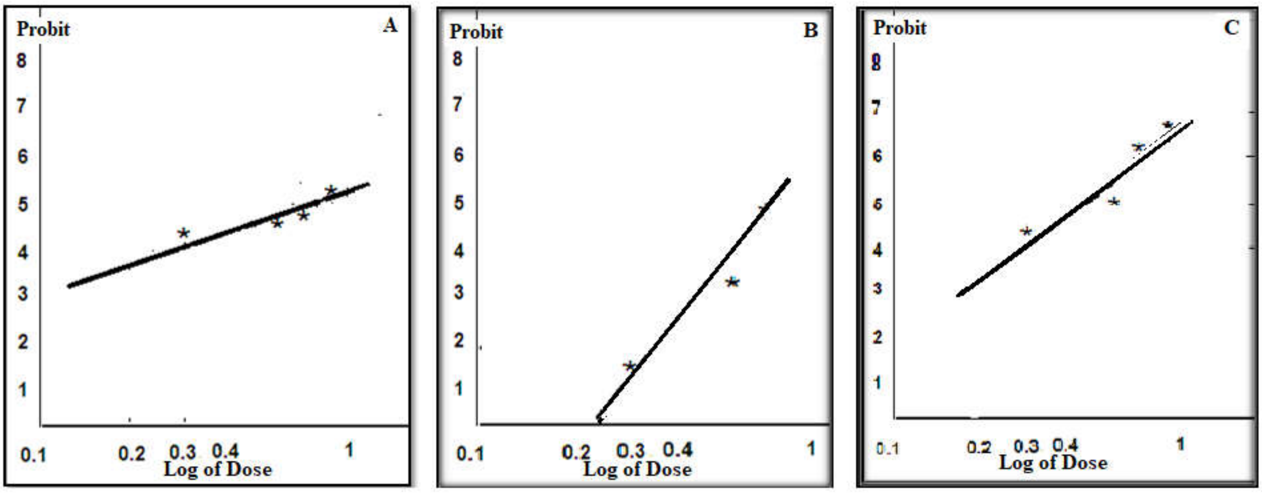

Figure 3. Log dose probit line of 24 hours response of *Tribolium castaneum* adult exposed to the plants extract A-*Piper nigrum* B- *Curcuma longa*C- *Myristica fragrans*

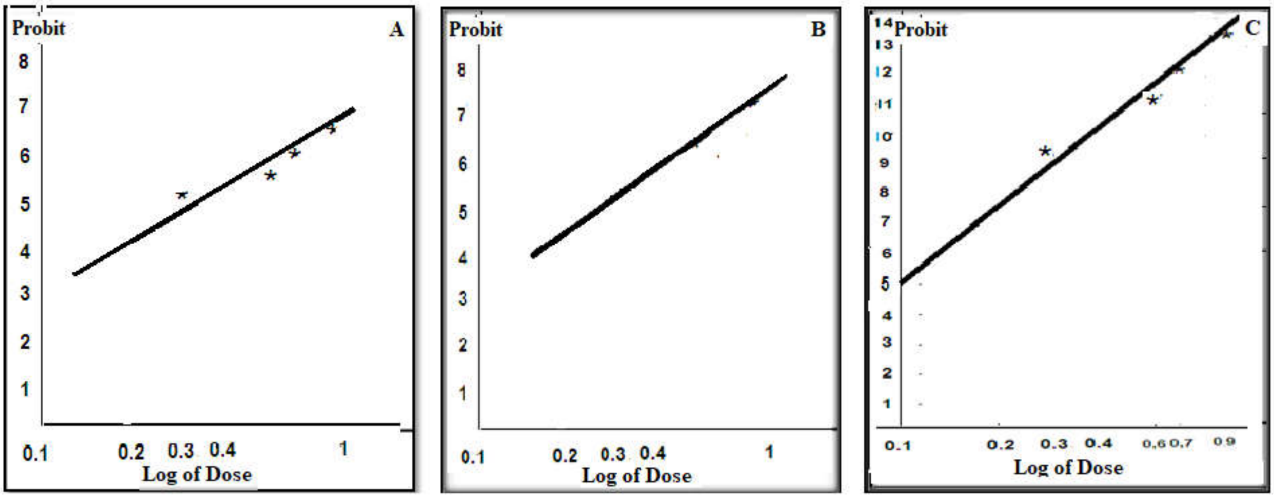

Figure 4. Log dose probit line of 24 hours response of *Tribolium castaneum* larva exposed to the plants extract A-*Piper nigrum* B- *Curcuma longa* C- *Myristica fragrans*

The LD_{50} values in 8% concentration of three ethanol extract after 24-hour exposure against adult *Tribolium castaneum* were found to be 0.73, 0.79 and 0.45 mg/cm^2, respectively, while in larvae found 0.34, 0.27 and 0.12mg/cm^2 respectively. No mortality was observed in control. The results of this study indicated that the mortality caused by each sample was increased with the increasing of exposure time. Based on 24-h LD_{50} values, the insecticidal activity of *Myristica fragrans* (0.45 mg/cm^2) was comparable with that of other two plants *Curcuma longa* and *Piper nigrum* were found to be (0.79, 0.73mg/cm^2) respectivily.

DISCUSSION

Our results have shown that *Myristica fragrans* possesses high insecticidal activity against adult *Tribolium castaneum* as compared with other two plants. Literature revealed that *Myristica fragrans* contains different quantity and quality of active compounds exhibited potent lethal activity against Tribolium castaneum such as phenolics, terpenoids, alkaloids and essential oils (dipentene, α-phellandrene, safrole, and α-terpineol, (*E*)-sabinene hydrate, camphor, citronellal, *α*-pinene, *β*-pinene) was the most toxic insecticide (Chakravarty, 1976; Miyakado *et al.,* 1983; Ahn *et al*., 1997; Isman 2001; Jang *et al*., 2005; Jung *et al*., 2007) which may be the direct reason of killing the insects and act insecticidal and antifeeding activities against Tribolium castaneum (Huang *et al.* 1997; Jung *et al*., 2007). Some chemical compounds effected on enzyme activity, and resulted reduction in enzyme activity. Chun *et al*. (2015) reported that *Myristica fragrans*consists of aromatic compounds such as myristicin which acts as a narcotic which interferes with acetylcholinesterase activity resulting in brain damage. The high insect mortality could be attributed to the presence of toxic secondary metabolites may act as insecticides or anti-feedants against insects (Isman, 2001). The second tested plants have insecticidal activity on *T. castaneum* was *Curcuma longa,* those findings have been supported by (Tripathi *et al*. 2002 and Venugopal and Saju, 1999) with the report of that curcuma oil exhibits excellent insect repellent. In addition, evaluated oviposition-deterrent and ovicidal actions of *C. longa* leaf oil against *T. castaneum*. As well as *C. longa* showed strong repellent activity against *T. castaneum* adults Thavara *et al.* (2007). Many researchers reported that the plant toxins such as terpenes, steroid, alkaloids, phenol, essential oils and glycosides have been explored for their insecticidal properties against stored grain pest *Tribolium castaneum*, and cause mortality (Duke, 1990; Okonkwo and Okoye, 1996; Baser *et al*., 1998; Isman, 2001; Rajendran and Sriranjini, 2008; Al-Jabr, 2006; Jbilou *et al*., 2006; Chaubey, M. K. 2012; Sagheer *et al.,* 2014). On the other hand, *Piper nigrum* have some chemical compounds flavonoids, reducing sugars, carbohydrates, anthraquinone, terpenoids, glycosides resin and alkaloid piperine, piperettine, tricostacine, peepuloidin, piplartin and trichonine which is exhibits insecticidal and larvicidal activities, these compounds have been demonstrated to be toxic against some insects fruit flies, cockroaches, *Callosobruchus chinensis*, *Acanthoscelides obtectus*, *C. cephalonica*, *Ephestia cautella* Hubn., *Oryzaephilus surinamensis* (L.), *Sitophilus zeamais* Mosteh, *Rhyzopertha dominica* (Fab.) and *Tribolium castaneum* Herbst. (Awoyinka *et al*., 2006; Dhanya *et al*., 2007; Fan *et al*., 2011; Ahmad *et al*, 2015; Mary and Durga, 2017).

Results agreed with Scott *et al*. (2003); Upadhyay and Jaiswal (2007) and Kraikrathok *et al*. (2013) which reported that a dose response relationship as the larval and adult mortality increased while the larval survival and adult emergence decreased with increase in the concentration of essential oil. Khani *et al*. (2012) reported that the *P. nigrum* extracts offer a unique and beneficial source of bio-pesticide material for the control of insect pests. The LD_{50} (lethal concentration) were used to estimate the levels of the plant toxicity. From the LD_{50} values it was clearly that *Piper nigrum* was a less toxic plant however caused 60 percent mortality at 8 percent concentration in the fifth instar larvae, LD_{50} values was higher in *Myrstica fragrans*100percentat the same concentration. In the fifth instar larvae, percentage mortality was found to be high in all the treatments (Table 1; Figure, 3,4). The order of toxicity' of the plant products to adult and fifth instar larvae*of Tribolium castaneum* can be written as *Myristica fragrans*>*Curcuma longa*>*Piper nigrum.* The results of this study indicated that the mortality and probit analysis caused by each sample was increased with the increasing of exposure time. This finding is quite like the previous research (Mondal, 1994; Osman *et al*., 2011) in which the exposure time played an important role in influencing susceptibility. Furthermore, In the present study, plant extract caused high mortalities higher concentrations (8%) and the LD_{50} value for fifth instar was 2 times in *Myristica fragrans* compared with other two plants tested.As well as the $LD_{50}$of plants tested was correspond to larval age, this trend was supported by (Abida *et al.,* 2010; Amin *et al*., 2012). The response of *T. castaneum* to different concentrations of ethanolic extract might have been due to the plants extract toxicity effect against *T. castaneum* larvae (Abida *et al.,* 2010). Finally, there are many factors effective on plants presence of chemical compounds in plants depends on the place of origin, weather, climatic conditions, application method, a period of extraction and plant parts (Latha and Ammini, 2000; Chun *et al*., 2015).

Conclusion

The insecticidal property of rhizomes of *Curcuma longa* and seeds of *Piper nigrum* and *Myristica fragrans* were clearly expressed in this study. *Myristica fragrans* and *Curcuma longa* were found to be the most effective bio-pesticides against *T. castaneum* adult and larval stages.plant extracts used in the present study act as adulticidal, larvicidal, and possess growth and emergence inhibiting against the *T. castaneum*. Furthermore, this study suggested that ethanol extracts of plants have toxic effects with significant insecticidal effect and could be a potential tool to protect stored grains against *T. castaneum*, and this may contribute to a reduction in the application of synthetic insecticides, which in turn increases the opportunity for natural control of various medically important pests by plant pesticides.

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