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# Entomocidal, repellent, antifeedent and growth nhibition efects of different plant extracts against *Tribolium castaneum* (Herbst) (Tenebrionidae: Coleoptera)

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#### ABSTRACT

In present investigation, toxic, repellent, antifeedent and growth inhibition effects of five different plant extracts: *Melia azedarach, Pegnum hermala, Salsola baryosma, Azadirachta indica* and *Zingiber officinale*, were evaluated on different life stages of *T. castaneum*. The highest mortality (10.14%) was observed with *A. indica* and the minimum mortality (0.67%) was invoked by *Z. officinale* treatment. Similarly, *A. indica* showed highest repellent effect compared to rest of the plants. Feeding deterrence was highest (90.15%) with *S. baryosma* treatment, followed by *P. hermala* (84.85%), *M. azedarach* (80.19%), *A. indica* (73.48%) and *Z. officinale* (57.58%). The extracts inhibited the growth of *T. castaneum*. Inthe case of *A. indica*, the lowest numbers of larvae (32.67), pupae (16.33) and adults (11.33) emerged at 15% concentration, while the highest emergence of larvae (80.33), pupae (75.00) and adult (71.00) were observed for *Z. officinale*. The other three plant extracts had moderate regrowth inhibition on the beetle. Overall, *A. indica* extract was found to be the most effective while, *Z. officinale* extract was least effective against the beetle. This study can be very helpful in future when the use of plant extracts become common and available to the farmers as an alternative to synthetic pesticides.

Key words: Mortality, repellency, antifeedant effect, growth inhibition, plant extracts, T. castaneum

#### Introduction

Food security is an emerging threat to world's expanding population. Stored grain insect pests are severely damaging our valuable products like wheat. These stored grain pests are responsible for about 10% loss of cereals all over the world (Danahaye *et al.*, 2007). According to Matthews, (1993) 10-40% losses per annum have been estimated globally due to the infestation of these insects. These losses can reach 9% in developed and up to 20% in the developing countries throughout the world (Phillips and Throne, 2010).

Among the stored product insect pests, *T. castaneum* is the pest of economic importance throughout the world which cause serious damage to stored products (Arbogast, 1991). It is one of the major pests of wheat flour (Howe, 1965). Both larvae and pupae are responsible for the losses. In case of severe destruction, flour may be converted into greyish and moldy produce with pungent off-smell. Thus, the commodity becomes unfit for human use (Atwal and Dhaliwal, 2002). It can cause economic loss of 40% of wheat flour (Ajayi and Rahman, 2006).

Widespread and indiscriminate use of pesticides is posing a serious threat to the environment and human health (Subramanyam and Hagstrum, 1996). Consequently, the use of biopesticide for the control of stored commodity pests has received considerable attention throughout the world (Faraz

et al., 2015). Bio pesticides have potential to replace the use of conventional insecticides (Regnault-Roger et al., 2002). Plant extracts contain several compounds which are responsible for the control of insect pests of stored grain (Rahman and Schmidt, 1999). Several plant derivatives have been investigated to have mortality effect on stored product pests (Mukheriee and Joseph, 2000) and a number of plants of Pakistan have been known for their repellency to stored grain pests (Verma et al., 2000). Some of the plants show repellency and growth inhibition effects (Kanvil et al., 2006). The five plants which were tested in this research are abundantly available and possess numerous medicinal properties (lgbal et al., 2010). Azadirachta indica and M. azedarach are two closely related species of Meliaceae and are known in India and Pakistan for more than 2000 years. It is best known to have various medicinal properties including toxic and repellent effect. *Melia azedarach* (dhrek) has been found to have insecticidal, antifeedant, growth regulatory activities (Nakatani et al., 2004; Al-Rubae, 2009). They contain azadirachtin, meliacarpinin, bakayanin and triterpenoids (Vishnukanta, 2008). The pharmacological effects of Peanum hermala are found to be due to the presence of alkaloids which include:  $\beta$ -carboline, harmine, harmaline, harmalol, harman, peganine, dipeganine, isopeganine, deoxypeganine and quinazol (Fathizad et al., 2007; Asgarpanah and Ramezanloo, 2012). Zingiber officinale (ginger) has beautiful fragrance and bitter taste (Jean, 1995). The compounds which are responsible for bitter taste are gingerol, zingiberene, zingerone and shogaol. Kaempferol and quercetin are the flavonoids that are present in the various parts of Salsola baryosma(khar booti) (Kaur and Bains, 2012).

The current study was conducted to assess the toxicity repellency, the antifeedent behavior and the growth inhibitory action of five different plant extracts namely *Azadirachta indica, Melia azedarach, Pegnum hermala, Salsola baryosma* and *Zingiber officinale*) against *T. castaneum* using acetone as solvent.

## **Materials and Methods**

### Collection and Rearing of Insects

The heterogeneous population (larvae and adults) of *Tribolium castaneum* was collected from grain market, poultry litter and stores of Rehman Farms and J. K. Agricultural Farms located at Faisalabad District, Punjab, Pakistan. The insects were reared in plastic jars (1.5 kg capacity) containing wheat flour which had been sterilized at  $65^{\circ}$ C for 30 minutes before putting into rearing jars. In the rearing jars, 50 adults of *T. castaneum* were released on wheat flour. After 5 days the parent adults were removed from the rearing jars and culture medium containing eggs of the beetles were kept in incubator (Sanyo M.I.R 254) at optimum conditions ( $30\pm2^{\circ}$ C and  $65\pm5\%$  r.h.) for getting homogenous population.

#### **Collection and Preparation of Plant Materials**

Fresh leaves of Dhrek, *Melia azedarach*, and Neem, *Azadirachta indica*, seeds of Hermal, *Pegnum hermala*, stem of Khar booti, *Salsola barysoma* and rhizome of Ginger, *Zingiber officinale* were collected from different farms and fields located at District Faisalabad. The different plant parts were washed and after drying in shade for 5 days the leaves, seeds, stems and rhizomes were ground using electric grinder (Pascall Mortar grinder, Machine no. 20069) into fine powder. The powder was used to prepare extract using acetone as a solvent. In 250 ml flask, 50 gram of plant powder was soaked in 100 ml of acetone. The flasks were placed on rotary shaker at 220rpm for 24hr and then filtered. The filtrate thus obtained was used to make different concentrations for the various experiments. Three concentrations (5, 10 and 15%) of plant extracts were prepared in acetone (solvent). To make 5% solution, 5 ml of stock solution was added to 95 ml acetone and for 10% solution 10ml stock solution was added to 90 ml acetone and for 15% solution 15 ml stock solution was added to 85 ml acetone.

Toxicity and Progeny Emergence of Plant Extracts

The diet incorporation method was used to estimate the efficacy of the different plant extracts in terms of beetle mortality and growth inhibition. The three concentrations of plant extracts were applied to 100 g of wheat flour along with an untreated check. About 30 adults of *T. castaneum* were put into the vials and the treatments were replicated three times using Completely Randomized Design. The mortality of the beetles was recorded after 24, 48 and 72 hrs. The survivors of *T. castaneum* from the above experiment were released on fresh wheat grains to determine progeny emergence. The number of beetles that emerged was recorded after 30 and 60 days. Corrected mortality was calculated using Abbot's formula given below:

Corrected Mortality (%) =  $(Mo-Mc)/(100-Mc) \times 100$ 

Where, Mo = observed mortality

Mc = control mortality

Percent inhibition rate of progeny was calculated using following formula:

Percent Inhibition Rate (%) =  $(Cn-Tn)/Cn \times 100$ 

Where,

Cn = no. of progeny in control jars

Tn = no. of progeny in treated jars

**Repellent Effect of Plant Extracts** 

The repellent effect was determined using area preference method. After cutting the filter papers into two equal halves, one half of each paper was treated with plant extracts. After evaporation of excess solvent, the two halves were joined together and placed in petri dishes. 50 adults of *T. castaneum* were released in the center of treated filter papers. Repellency was recorded after 12, 24 and 48 hrs.

Percent repellency was calculated following the formula used by Guruprasad and Pasha (2014).

Percent Repellency (%) = (Nc-Nt)/(Nc+Nt)×100

Where,

Where,

Nc = No. of insects in control half

Nt = No. of insects in treated half

# Antifeedant Effect of Plant Extract

The jars containing 20 g of treated grains were kept in open environment for 24 hrs in order to allow the excess acetone to evaporate. After 24 hrs, thirty insects were released in each jar. The jars were weighed to take the initial reading. After 7, 14 and 21 days' interval the weight loss was recorded to check the antifeedant effect of the extracts.

Feeding Deterrence Index (%) =  $((C-T))/((C+T)) \times 100$ 

C = food consumed in control jars

T = food consumed in treated jars

# Growth Inhibitory effect of Plant Extracts

Thirty adults of mixed sexes of *T. castaneum* were released into the jars containing wheat flour treated with the different concentrations of the five plant extracts. The number of larvae emergence, pupae transformation and adult emergence was recorded after 15 days for larvae, 25 days for pupae and 40 days for adult.

#### Statistical Analysis

Data were subjected to Analysis of Variance using STATISTICA 6.0 as one factor complete randomized design. Means were compared using Tukey's HSD test at 0.05 probability level, to check the significant difference among treatments.

#### Results

#### Toxicity of Plant Extracts against Tribolium castaneum

In general, all the extracts showed low toxicity to *T. castaneum* as the highest percent mortality of 10.14% was recorded in the diet treated with extract of *A. indica* (Table 1). In all the diets treated with each of the five plant extracts, percent mortality was time and dose dependent as it increased with the increase in concentration and exposure time (Table 1). In diets treated with the extracts of *A. indica*, *M. azedarach*, *S. baryosma*, *P. hermala* and *Z. officinale*, percent mortality ranged from 1.33-10.14%, 1.33-9.46%, 0.00-8.79%, 0.00-6.76% and 0-6.08%, respectively (Table 1). For example, in diets treated with the extract of *A. indica*, the highest mortality 10.14% was recorded at maximum concentration of 15% after 72hrs while the lowest mortality of 1.33% was recorded at 5% after 24 hrs of exposure time.

Time	Conc.		Mortality (%) ± S.E.			
(hrs)	(%)	A. indica	M. azadirach	S. baryosma	P. hermala	Z. officinale
	5	1.33±0.66e	1.33±0.66e	0.00±0.66e	0.00±0.66d	0.00±0.66c
24	10	2.67±0.67de	2.00±0.65de	1.33±0.66de	0.67±0.66d	0.00±0.67c
	15	3.33±0.67cde	3.33±0.66cde	2.00±0.67cde	1.33±0.65d	0.67±0.66c
	5	3.35±0.65cde	2.68±0.67de	2.01±0.67cde	1.34±0.67d	1.34±0.67bc
48	10	5.37±0.65bc	4.69±0.67bcd	3.35±0.67bcd	3.35±0.67c	2.01±0.67bc
	15	7.38±0.66b	6.04±0.66bc	4.02±0.66bc	5.37±0.66ab	3.35±0.66b
	5	4.73±0.68cd	3.38±0.68cde	3.38±0.68bcd	3.38±0.68c	2.03±0.67bc
72	10	7.44±0.68b	6.76±0.68ab	5.41±0.68b	4.73±0.68bc	3.38±0.68b
	15	10.14±0.67a	9.46±0.65a	8.79±0.67a	6.76±0.67a	6.08±0.68a

Tab. 1 Comparison of mean mortality of Tribolium castaneum treated with different plant extracts

Repellency of Plant Extracts against Tribolium castaneum

All the plant extracts were highly repellent to *T. castaneum* and repellency was dose and time dependent as it increased with the increase in concentration and exposure time. (Table 1). Percent repellency ranging from 50.67-89.33%, 56.00-85.33%, 58.67-89.67%, 60.00-89.33% and 56.00-82.67% were recorded in diets treated with the extracts of *A. indica. M. azedarach, P. hermala. S. baryosma* and *Z. officinale,* respectively. (Table 2). The highest percent repellency of 89.67% was invoked by the extract of *P. hermala* and the lowest by *M. azedarach* at the highest concentration of 15% and 48 hours exposure time (Table 2).

Tab. 2 Comparison of (%) mean repellent effect of different plant extracts against Tribolium castaneum

Time	Conc.		Repellency (%) ± SE			
(hrs)	(%)	A. indica	M. azadirach	S. baryosma	P. hermala	Z. officinale
	5	50.67±1.33g	56.00±1.31f	60.00±1.33f	58.67±1.33f	56.00±1.30f
12	10	58.67±1.30f	60.00±1.30f	70.67±1.30e	68.00±1.33e	64.00±1.28e
	15	66.67±1.37e	73.33±1.28cd	81.33±1.27bcd	74.00±1.31cd	72.00±2.67cd
	5	60.00±1.35f	60.00±1.33f	72.00±1.27e	60.67±1.35f	68.00±1.33de
24	10	72.00±1.35d	68.00±1.33e	78.67±2.67cd	76.67±1.37bc	72.00±1.33cd
	15	80.00±1.33bc	76.00±1.31bc	85.33±1.33ab	80.33±1.40b	77.33±1.35ab
	5	76.00±1.33cd	70.67±1.31de	76.00±1.33de	70.00±1.41de	74.67±1.33c
48	10	81.33±1.31b	78.67±1.48b	82.67±2.67bc	78.00±2.67bc	77.33±1.33ab
	15	89.33±1.30a	85.33±1.41a	89.33±2.67a	89.67±2.67a	82.67±2.67a

Progeny Inhibition and Feeding Deterrence Effect of Plant Extracts against Tribolium castaneum

Both percent inhibition and feeding deterrence were time and dose dependent as they increased with the increase in concentration and exposure time (Tables 3 & 4). Percent inhibition ranging from 59.08-89.70%, 52.30-81.62%, 48.36-79.10%, 3.76-75.15% and 49.80-80.06% were recorded in diets treated with the extracts of *A. indica. M. azedarach, S. baryosma, P. hermala. d. officinale,* respectively. (Table 3). The highest percent inhibition of 89.70% was recorded at the highest *A. indica* extract concentration of 15% after 60days and the lowest percent inhibition of 75.15% was invoked by the extract of *P. hermala.* 

Similarly, percent feeding deterrence effect ranged from 51.45-73.48%, 53.31-85.61%, 66.39%-90.15%, 43.98-84.85% and 25.30-57.58% in diets treated with extracts of *A. indica. M. azedarach, S. baryosma, P. hermala*.and *Z. officinale*, respectively. (Table 4). The highest percent feeding deterrence of 90.15%) was recorded at the highest concentration of 15% of *S. baryosma* after maximum exposure period of 21days and the lowest percent deterrence of 57.58% was recorded in diets treated with the same concentration and exposure period (Table 4).

The results depict that the highest inhibition of larvae, pupae and adults was observed at the highest concentration of 15% (Table 5). The highest number of larvae (111.00) emerged in control treatment. Lower numbers of larvae (32.67, 38.00, 52.67, 57.33 and 71.67) emerged in diets treated with *A. indica, M. azedarach, S. baryosma, P. hermala and Z. officinale,* respectively (Table 5). Similarly, lower numbers of pupae (16.33, 14.67, 32.67, 39.00 and 57.33) emerged in diets treated with *A. indica M. azedarach, S. baryosma, P. hermala* and *Z. officinale* respectively compared with control in which 110 pupae emerged (Table 5). In the case of adults the highest number (110 adults) emerged in the control treatment compared with lower number of adults (11.33, 10.67, 26.00, 32.00 and 47.33) that emerged in diets treated with extracts of *A. indica, M. azedarach, S. baryosma, P. hermala* and *Z. officinale* respectively (Table 5).

Time	Conc.	Population Inhibition (%) ± SE				
(days)	(%)	A. indica	M. azadirach	S. baryosma	P. hermala	Z. officinale
	5	59.08±0.21f	52.30±0.21e	48.36±0.22f	43.76±0.21f	49.89±0.21f
30	10	62.36±0.22e	55.58±0.22d	51.42±0.22e	45.29±0.21e	51.42±0.22e
	15	65.43±0.22d	59.30±0.21c	54.27±0.21d	47.92±0.22d	59.74±0.21d
	5	86.83±0.05c	79.52±0.10b	75.75±0.05c	72.04±0.06c	76.35±0.05c
60	10	88.08±0.06b	80.06±0.10b	77.96±0.06b	74.13±0.06b	78.32±0.05b
	15	89.70±0.05a	81.62±0.06a	79.10±0.06a	75.15±0.05a	80.06±0.06a

Tab. 3 Comparison of (%) mean population inhibition of *Tribolium castaneum* treated with different plant extracts

Tab. 4 Comparison of (%) mean feeding deterrence effect of different plant extracts against *Tribolium* castaneum

Time	Conc.	Deterrence Effect (%) ± SE				
(days)	(%)	A. indica	M. azadirach	S. baryosma	P. hermala	Z. officinale
	5	51.45±0.93f	53.31±0.99f	66.39±0.93d	43.98±1.86g	25.30±0.93f
7	10	58.92±0.94de	60.78±0.94e	68.25±0.90d	52.38±1.86f	33.71±0.94e
	15	63.59±0.93cd	64.52±0.93de	73.86±0.95c	62.65±0.93d	42.11±0.94d
	5	57.80±0.86e	65.55±0.86d	74.16±0.86c	57.80±0.93e	37.12±0.86e
14	10	64.69±2.27c	72.27±0.68c	77.61±0.83bc	65.55±0.86d	43.15±0.87d
	15	70.71±1.86ab	80.19±0.86b	79.33±0.83b	72.44±0.86c	49.18±0.83bc
	5	67.42±0.76bc	78.03±0.75b	80.30±0.77b	70.45±0.75c	46.97±0.75c
21	10	70.45±0.75ab	80.30±0.77b	87.12±0.73a	77.27±0.77b	52.27±0.77b
	15	73.48±0.76a	85.61±0.77a	90.15±0.71a	84.85±0.74a	57.58±0.77a

Growth Inhibitory Effect of Different Plant Extracts against Tribolium castaneum

Plants	Conc. (%)	Larvae No.	Pupae No.	Adults No.
	5	53.00±0.57h	32.67±0.88h	26.67±1.00h
	10	45.67±0.57i	25.67±0.88i	20.00±0.66i
	15	32.67±0.33k	16.33±0.33k	11.33±0.67j
A. indica	0	111.00±0.33a	110.00±0.33a	110.00±0.33a
	5	54.00±0.57h	38.67±0.57g	34.33±1.00g
	10	46.33±0.33i	23.67±0.33j	23.67±0.66h
	15	38.00±0.33j	14.67±0.331	10.67±0.33j
M. azadirach	0	111.00±0.57a	110.00±0.88a	110.00±0.33a
	5	62.33±0.57f	47.33±0.88f	42.67±1.00f
	10	57.33±0.33g	40.67±0.33g	35.33±0.33g
	15	52.67±0.33h	32.67±0.33h	26.00±0.33h
S. baryosma	0	111.00±0.31a	110.00±0.33a	110.00±0.57a
	5	73.00±0.51d	59.33±0.88d	55.00±1.00d
	10	66.33±0.57e	50.67±0.33e	43.67±0.57f
	15	57.33±0.33g	39.00±0.33g	32.00±0.33g
P. hermala	0	111.00±0.33a	110.00±0.57a	110.00±0.57a
	5	80.33±0.57b	75.00±0.88b	71.00±1.00b
	10	77.00±0.33c	69.33±0.58c	61.33±0.57c
	15	71.67±0.57d	57.33±0.33d	47.33±0.33e
Z. officinale	0	111.00±0.33a	110.00±0.33a	110.00±0.33a

**Tab. 5** Comparison of mean growth inhibitory activities of different plant extracts
 against Tribolium

 castaneum
 castaneum
 castaneum



Fig. 1 Effect of different concentrations of plant extracts on the growth parameters of T. castaneum



Fig. 2 Effect of different plant extracts on the growth parameters of T. castaneum

## Discussion

The plant products have growth regulatory effects on different pests particularly red flour beetle, *T. castaneum* (Joseph *et al.*, 1994; Haque *et al.*, 2000). The most effective plant was *A. indica* in our study. It is known that *A. indica* contains bioactive compounds like azedarachtin. This compound affects the growth behavior and reproduction of stored grain insect pests (Pascual *et al.*, 1990; Mordue (Luntz) and Blackwell, 1993; Singh, 1993). *A. indica* interrupts the normal development of larvae and adults as well as metamorphosis of insects (Koul *et al.*, 1987). *A. indica* also significantly reduces the emergence of adults of *T. castaneum* and weight of the adults (Anonymous, 1992). The emergence of larvae, pupae and adults is affected by the change in dose rate of the plant extracts (Sagheer *et al.*, 2011). Reduced growth of the *T. castaneum* was observed with the use of different plants including *P. heramala* (Jbilou and Sayah, 2006). *Melia azedarach* has been found to have insecticidal, antifeedant, growth regulatory activities (Nakatani *et al.*, 2004; Al-Rubae, 2009)

In our study, all the plant extracts showed very low toxicity against *T. castaneum*. According to Fang *et al.* (2002) *Tribolium* sp. are the most tolerant among all stored grain insect pests so they are more difficult to control. Plant extracts contain several compounds which are responsible for the control of insects (Rahman and Schmidt, 1999). Several plant derivatives have been investigated to have mortality effect on stored product pests (Su, 1990; Desmarchelier, 1994; Schmutterer, 1995; Mukherjee and Joseph, 2000). Plant extracts reduce the release of phosphate ions for the production of energy during transphosphorylation reactions (Senthil-Nathan *et al.*, 2006). Anita *et al.* (2012) reported the dose-dependent insecticidal activity of *Eucalyptus globulus* against *T. castaneum*.

In the present study, neem extract was proved most effective in term of mortality. Neem possesses toxic effect against *T. castaneum* (Ahmed *et al.*, 2000). Many compounds in neem have been reported for their toxic effects the most potent is azadirachtin (Mordue and Blackwell, 1993; Lin-er *et al.*, 1995). As a polyphagous pest, *T. castaneum* has been controlled successfully by the use of different insecticides (Okonkwo and Okoye, 1996; Islam and Talukdar, 2005). However, this pest have developed resistance against these pesticides (Guedes *et al.*, 1997). Some plants have toxic effect against *T. castaneum* (Tripathi *et al.*, 2000). The efficacy of neem against insect pests varies with the change in the storage atmospheres and storage period. Reduced activity of neem as a bio pesticide has been reported when it was stored in sun. Hence, the bioactivity of neem was higher when it was stored for two weeks than when it was stored for 4-6 weeks (El Shafie and Almahy, 2012). The activity of the neem increased 10 times at low temperature (Kabaru and Mwangi, 2000). To use the plant extracts as bio pesticides, farmers should be well aware of its production as well as application (Hellpap and Dreyer, 1995).

A number of plants of Pakistan have been known for their repellency against grain pests (Jilani *et al.*, 1991, 1993; 2000; Verma *et al.*, 2000). Few plants show repellency and growth inhibition effects (Kanvil *et al.*, 2006). Neem possesses repellent, antifeedant, oviposition inhibition and deterrent effects (Lale and Abdulrahman, 1999; Weathersbee and Tang, 2002; Liang *et al.*, 2003; Hou *et al.*, 2004). Jilani *et al.*, (1984) reported the repellent effects of 30 plant extracts including *P. harmala* against *T. castaneum*. Diwivedi and Kumari (2000) reported the repellent action of different plant extracts prepared in acetone and petroleum solvents against *C. chinensis*.

Trematerra and Sciarretta (2002) reported that the mortality of the insects by plant extracts is due to its repellent and feeding deterrent effects. They also reported that the alkaloids present in the plants affect the chemoreceptors of insects thus inhibiting food consumption. Azadirachtin excites the deterrent cells of chemoreceptors and inhibits the sugar receptors thereby preventing the insects from feeding (Mordue-Luntz *et al.*, 1995).

Neem also significantly reduce the emergence of adults of *T. castaneum* and weight of the adults (Anonymous, 1992). The emergence of larvae, pupae and adults is affected by the change in dose rate of the plant extracts (Sagheer *et al.*, 2011). Reduced growth of the *T. castaneum* was observed with the use of different plants including *P. heramala* (Jbilou and Sayah, 2006).

The results of the study has shown that plant extracts can be used as a component of an IPM strategy for the control of insects both in the crops growing in the field and stored commodities. The use of plants as an insect control strategy can be a better substitute to synthetic insecticides.

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# Toxicity and repellence of *Citrus jambhiri* Lush rind essential oil against maize weevil (*Sitophilus zeamais* Motschulsky 1855) (Coleoptera: Curculionidae)

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#### Abstract

Rind of matured fruits of *Citrus jambhiri* Lush was hydro-distilled to obtain essential oil (EO) which was subjected to Gas chromatography-Mass spectrometry (GC-MS) analysis. The EO was evaluated for fumigant toxicity (at 27-107 $\mu$ L/L air) and repellence against maize weevil (*Sitophilus zeamais* Motschulsky). Area preference methodology was used to evaluate the repellence of the EO at 0.15-0.9  $\mu$ L/cm<sup>2</sup>, while isopropanol served as control for both bioassays. The experiments were set up in a completely randomized design and data were subjected to analysis of variance and probit analysis. Fifty-two compounds were identified in the EO with the