BIOLOGICAL CONTROL

Evaluation of Botanical and Synthetic Insecticide for the Control of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

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Avaliação de inseticidas botânicos e sintéticos para o controle de *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

RESUMO - Estudos foram realizados para avaliar a ação inseticida de dois produtos de origem vegetal e um inseticida sintético em um importante inseto praga de produto armazenado, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). As espécies de plantas estudadas foram, *Psidium guajava* (L.) (goiaba, folhas; Myrtaceae) e *Citrus reticulata* (Kinnow, casca e folhas; Rutaceae). Metopreno foi utilizado como insecticida sintético. Duas formulações viz., pó e extracto de etanol de cada planta e quatro concentrações (5, 10, 15 e 20%) de metopreno foram testadas. A repelência foi determinada utilizando-se o teste de papel de filtro, enquanto que, a mortalidade, a protecção na perda de peso e o potencial anti-alimentar de todos os tratamentos foram avaliadas usando grãos de trigo. Os resultados evidenciaram que todos os tratamentos testados tiveram efeitos significativos para todas as variáveis analisadas e o extrato de etanol foi significantemente mais eficiente do que a forma de pó da mesma planta. Além disso, folhas e cascas de *C. reticulata* não diferiram significativamente quanto à sua toxicidade aos adultos de *T. castaneum*, mas, mais eficiente do que *P. guajava*, enquanto que, a atividade de metoprene foi comparável ao de plantas em concentração de 20 ppm.

PALAVRAS-CHAVE - *Citrus reticulata*; *Psidium guajava*; metopreno; Rutaceae; Myrtaceae.

ABSTRACT - Research studies were carried out to evaluate insecticidal action of two plant products and a synthetic insecticide on a major stored-product insect, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). The plant species studied were, *Psidium guajava* (L.) (Guava, leaves; Myrtaceae) and *Citrus reticulata* (Kinnow, peel and leaves; Rutaceae). Methoprene was used as synthetic insecticide. Two formulations viz., powder and ethanol extract of each plant and four concentrations (5, 10, 15 and 20%) of methoprene were tested. Repellency was tested using the filter paper test whereas mortality, weight loss protection and anti-feedant potential of all treatments was evaluated by using whole wheat grains. Our results reported that all tested treatments had significant effects pertaining to all variables analyzed and ethanol extract was found to be remarkably more potent than powder form of same plant. Furthermore, leaves and peel of *C. reticulata* did not differ significantly pertaining to their toxicity against adult *T. castaneum* but stronger than *P. guajava* whereas, activity of methoprene was comparable to of botanicals at concentration of 20 ppm.

KEY WORDS - *Citrus reticulata*; *Psidium guajava*; methoprene; Rutaceae; Myrtaceae.

Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae) is primary pest of flour and other milled products of cereals and a secondary pest of stored wheat (LeCato 1975, Hameed & Khattak 1985, Irshad & Talpur 1993, Suresh & White 2001) causing severe damages to quantity and quality of these food grains (Smith *et al*. 1971). Besides these, members of genus *Tribolium* are reported to secrete certain toxic quinones of carcinogenic nature in stored commodities thus posing serious risks to human health (Ladisch *et al*. 1967, El-Mofty *et al*. 1989).

These losses could be prevented either by use of pesticides or by non-chemical methods. Chemical methods involve the use of synthetic insecticides in most parts of the world. However, at present there is an emphasis on the application of reduced risk insecticides such as methoprene. It is a juvenile hormone analogue, which interferes with normal development of immature insects and can have ovicidal and sub-lethal effects such as reduced adult fecundity in insects exposed as immature (Oberlander *et al*. 1997). The effectiveness of methoprene against many insect species, including *Rhizopertha dominica* (Fabricius), *Cryptolestes ferrugineus* (Stephens), *Oryzeaphilus surinamensis* (L.), and *T. castaneum* have been reported in literature (Nayak *et al*. 1998, Arthur 2004, Chanbang *et al*. 2008, Arthur 2008).

However, the use of synthetic insecticides for controlling stored product insects have been or may be banned globally due to problems such as their persistent toxicity in food grains, the subsequent development of resistance in insect populations, effects on non-target organisms and other adverse environmental impacts. In short, global ecology is facing severe threat from the use of pesticides so the search for ecologically safe methods to control insect pest of stored food products is an awe inspiring field of research. Of these, for sound management of stored cereals there is an increasing interest in biological control, which will prove eco-friendly with highly reduced negative effects on environment (Arbogast 1984, Guedes 1990, Brower *et al*. 1996). Use of plant based pesticides, either in crude form or by processing into different formulations, is one of the many possible avenues explored with regard to biological control.

Most of the plants thrive in rough environmental conditions so they have developed a multitude of defense mechanisms against natural enemies in the course of evolution. Among these are morphological and subtle chemical defense mechanisms against insects and other parasites that do not generally cause immediate death but interfere with their vital biochemical and physiological functions (Prakash & Rao 1997). Certain plant families, particularly plant products of Rutaceae and Myrtaceae had shown, in previous observations, repellent, insecticidal, anti-feedant, and growth regulatory properties against insect pests of stored commodities (Jacobson 1989, Isman 1995, Owusu 2001, Singh & Singh 2005, Prakash & Rao 2006, Kestenholz *et al*. 2007, Neoliya *et al*. 2007, Sankari & Narayanswamy 2007, Chayengia *et al*. 2010, Yankanchi & Gadache 2010). Some of the Citrus species (*C. sinensis, C. aurantifolia, C. reticulata, C. limon*) (Rutaceae) have been reported as a source of botanical insecticides (Ezeonu *et al*. 2001, Owusu 2001, Chayengia *et al*. 2010). A variety of these plants contain secondary metabolites that show insecticidal activity against several coleopteran and dipterans species (Salvatore *et al*. 2004, Shrivastava *et al*. 2010). Limonoids, extremely bitter chemicals present in citrus seeds, act as antifeedants or antagonize ecdysone action in many species of coleopteran including *T. castaneum* (Jayaprakaha *et al*. 1997). The bioactive compounds viz., limonene (46.7%), geranial (19.0%), neral (14.5%), geranyl acetate (3.9%), geraniol (3.5%), nerol (2.3%) etc. are extracted from *C. reticulata* peel and used to formulate medicines, pesticides and fungicides (Chutia *et al*. 2009). Nonetheless, a number of other candidate plant materials including seeds and peel of *C. reticulata*; leaves of *C. limon* have been used in stored product pest management (Owusu 2001, Chayengia *et al*. 2010, Krishnappa *et al*. 2011). However, the potential of *C. reticulata* leaves for the control of pests has not formally been reported.

Myrtaceae family has been an indispensable source of plants that are investigated as potential candidate materials for their insecticidal properties for millennia (Peterson *et al*. 2002; Clemente *et al*. 2003, Lee *et al*. 2004, Tapondjoua *et al*. 2005, Rahman & Talukder 2006, Negahban & Moharramipour 2007). Members of this family are usually highly aromatic e.g. *Eucalyptus* spp. Aromatic plants have monoterpenoids as bioactive principles with insecticidal properties. Due to their volatile and lipophilic nature, monoterpenoids can rapidly penetrate into insect's cuticle and cause their mortality by interfering with their vital physiological functions (Isman 2000). *Psidium guajava* is one of the aromatic plants belonging to family Myrtaceae.

The bioassays of leaves of *P. guajava* and rind of *C. reticulata* have potential to be used against certain insect pests of stored grains. These plant materials are processed to form various formulations viz., powders, water extracts, volatile oils and ethanol extracts to check their efficacy against differen insect pests (Chayengia *et al*. 2010). Though Sargodha (Punjab, Pakistan) is rich in flora diversity especially *P. guajava* and *C. reticulata*, perusal of literature reveals that reports on use of these plants as biological control agents are scanty. Hence, feeling the paucity of the work, the present study was designed to investigate toxic action of *C. reticulata* (peel and leaves), *P. guajava* (leaves) and methoprene against adults of *T. castaneum* regarding its repellent, antifeedant, fetal and weight loss reduction effects in wheat grains. The investigation are likely to be fruitful by adding a share in the knowledge about screening of phyto-chemicals and their processing into suitable bioassays to be used use as candidates for inclusion in the arsenal of weapons for pest management with special focus on most destructive, *T. castaneum*.

Materials and Methods

Procurement of raw materials. Different plant materials viz., *P. guajava* leaves and fruit peel and leaves of *C. reticulata* were collected from different places of Sargodha, Punjab, Pakistan and different formulations of each plant were selected for the study. Methoprene (100% pure, white solid in fine powdered form) procured from local market, was used in study. Untreated seeds of a common wheat cultivar of Pakistan viz. Sehar-2006 were used for different bioassays.

Preparation of powder. For the preparation of powder, the collected plant parts were washed with distilled water and shade dried at room temperature for one month. Thereafter, powders were prepared using domestic grinder followed by sieving through 60 mesh size sieve. Powders were kept in polythene bags at room temperature and properly sealed to prevent quality loss (Chayengia *et al*. 2010).

Preparation of ethanol extract. Shade dried fine powder of each plant part weighting 200 g was soaked in 400 ml of 90 per cent ethanol and placed for one week in an orbital shaking incubator at 80 rpm (revolution per minute) and 37°C temperature. Thereafter, it was filtered by using Oklahoma filter screen through Whatman No.1 filter paper and filtrate was concentrated with a rotary evaporator under reduced pressure at 50° C to afford crude ethanol extract that served as stock solution. Stock solution was refrigerated and fractionated into 100 ml ethanol to get a 10 % concentrated extract at time of application (Saljoqi *et al*. 2006).

Preparation of methoprene formulations. Solid, powdered methoprene weighing 0.1g was dissolved in one liter doubled distilled water to prepare a stock solution of 100 ppm strength and stored at 4ºC in a tightly sealed glass vial. Four concentrations viz., 5, 10, 15 and 20 ppm were prepared in order to perform the bioassays (Braga *et al*. 2005).

Repellent activity bioassay. The repellent action of powders of above mentioned plant components was tested in a choice bioassay method. Fine powder of each component weighing 5g was mixed with small quantity of distilled water to form slurry. Ten wheat grains were coated in slurry of each of the powder and left to stand for three hours for drying while control grains were treated with distilled water only. The treated and untreated grains were placed adjacent to each other with a space between them in a Petri dish (9 cm). Ten adult *T. castaneum* were introduced into the middle of Petri dishes. Each treatment was replicated three times and the number of insects present on control and treated grains were recorded after one hour and up to five hours (Udo 2000).

To determine repellency of ethanol extracts of plants and different concentrations (5, 10, 15 and 20 ppm) of methoprene solution, filter-paper circles of 9 cm in diameter were cut into two halves. One ml ethanol extract of each of the three components of *C. reticulata* and *P. guajava* were taken in separate pipettes and uniformly applied on one half of each of the filter-papers. Ethanol was applied on second halves that served as untreated half or control. Both the treated and untreated half circles were air-dried until the solvent was totally evaporated. The treated and the untreated half circles were joined by tape from lower side and placed on the Petri dishes. Ten *T. castaneum* were released in each dish at the centre of the two halves. *T. castaneum* present in each half circle were counted at hourly intervals for 5 h after treatment (Talukder & Howse 1993, 1994). Data from all treatments was converted to express percentage repulsion (PR) using the following formula:

$$PR\ (\%) = (Nc - 50) \times 2$$

where Nc is the percentage of beetles present in the control half. Positive values (+) indicated repellency and negative values (-) attractancy. Five replications were made of each treatment. Data from all treatments was subjected to non parametric Kruskall-Wallis test ($P \leq 0.05$). Means were compared using Analysis of variance (ANOVA). Mean values were classified according to the following scale:

Class Repellency rate (%)

0. >0.01 to < 0.1
1. 0.1 to 20
2. 20.1 to 40
3. 40.1 to 60
4. 60.1 to 80
5. 80.1 to 100

Mortality bioassay. Two doses (1 and 2 g) of powder of each plant component were mixed thoroughly with 50 g grains. The sample without powder served as control. Grains were then put into 250 ml plastic jars roofed with muslin cloth and tightened with rubber band. Ten adult *T. casteneum* were released in each jar. Three replications were maintained for each dose of the individual plant powder and each time interval.

Wheat grain samples weighing 50 grams were taken and laid out into a single kernel layer waxed paper. These were then sprayed with two doses viz., 1 and 2 ml of ethanol extract of each plant component and four concentrations (5, 10, 15 and 20 ppm) of methoprene solution. The control was treated with ethanol alone. After that each grain sample was hand tumbled for 30 seconds (Arthur 2004) in a plastic bag for uniform dispersion of the compounds in the grain sample. The treated and untreated samples were shifted to 250 ml plastic jars. Ten adult *T. castaneum* were released in all treatments and control samples. Observations were recorded on 3, 7, 14 and 21 days after the treatment. After completion of each interval, dead and live adults were counted and % mortality was calculated (Yankanchi & Gadache 2010). All observations were corrected by using the Abbott's (1925) formula. Corrected observations were subjected to statistical analysis, the one-way ANOVA and T-test.

Weight loss and feeding ratio bioassays. Five grams of wheat grains were placed in Plastic jars (50 ml). Two doses (1 and 2 g) of powder of each plant component were mixed thoroughly with grains. The sample without powder served as control. Jars were roofed with muslin cloth and tightened with rubber band. The jars were then infested with 20 previously starved adult insects which were allowed to feed for 30 days.

Five grams of wheat grain samples were laid out into a single kernel layer on a waxed paper. These were then sprayed with two doses viz., 1 and 2 ml of ethanol extract of each plant component and four concentrations of methoprene solution viz., 5, 10, 15 and 20 ppm. The control was treated with ethanol alone. The treated and untreated samples were shifted to plastic jars and infested with 20 previously starved adult insects which were allowed to feed for 30 days. After the completion of feeding period, pests were separated from samples and grains were reweighed. Feeding ratio (Fr) was calculated following the method as described by Owusu (2001):

$$Fr = 1 - FW/5$$

where, FW represents the final grain weight after the 30 days feeding period.

For grain weight loss calculation damaged and undamaged grains were separated, counted and weighted and following formula was used.

$$\%\ Weight\ Loss = (U\ Nd) - (D\ Nu) / U\ (Nd + Nu) \times 100$$

where: U= weight of undamaged grains; D= weight of damaged grains; Nu= number of undamaged grains; Nd= number of damaged grains.

Comparison analyses between control and treated samples were made using T-test.

Results and Discussion

Repellency Effects. Results of present study reported that among all the treatments tested, the ethanol extract of *C. reticulata* peel was the one with the strongest repellent effect on *T. castaneum* with mean % repellency of 70.66 (class 4) (Table 1). Other treatments with significant repellent activity were powder and ethanol extract of *C. reticulata* leaves, both having same mean % repellency of 66.66% (class 4) followed by 20 ppm methoprene (class 4), powder of *C. reticulata* peel (class 4), 15ppm methoprene (class 3), ethanol extract of *P. guajava* leaves (class 3), powder of *P. guajava* leaves (class 2), 10 ppm methoprene (class 2) and 5ppm methoprene (class 1). Analysis of variance indicated that fruit peel and leaves of the *C. reticulata* did not differ significantly pertaining to their repellent effect against *T. castaneum*. However, repellent effect of *C. reticulata* was extraordinarily stronger than *P. guajava* (Fig 1). In the present study, ethanol extract was found to be remarkably more potent than powder form of same plant. There was noteworthy increase in repellent effect of synthetic pesticide viz., methoprene with increase in concentration as indicated: 5 ppm (Class 1); <10 ppm (Class 2); <15 ppm (Class 3); <20 ppm (Class4).

All the plants products and methoprene tested in present study have been investigated earlier for their insecticidal potential using other parameters but no reference is accessible in literature regarding their repellent effects (except *C. reticulata,* peel). However, the findings of present study reflected that all treatments exhibited significant repellent effects against *T. castaneum*. The higher repellent activity of plant products in ethanol extract might be attributed to increasing solubility and thus repellent potential of some volatile toxic compound in this particular organic solvent. Some other interesting findings were reported by different scientists on repellency but they used either different plants or insect species.

Our findings are in accordance with those of Mishra & Tripathi (2011). They worked on repellent potential of *C. reticulata* and some other plants against *Sitophilus oryzae* L. and *T. castaneum* but they used essential oils. They reported that the oil extracts of all plants recorded significant repellency against both tested species however; *T. castaneum* was proved to be relatively resistant one.

Mishra *et al*. (2012) carried out studies on repellent efficacy of essential oils of *Eucalyptus globulus* (Myrtaceae) and *Ocimum basilicum* (Lamiaceae) leaves against adults of *T. castaneum* and *S. oryzae*. They reported that the repellent effects of *E. globulus* and *O. basilicum* were significantly

Table 1. Repellency of powder and ethanolic extract of chosen plant components and synthetic pesticide (methoprene) formulations on adults of *Tribolium castaneum,* using the filter paper test.

Treatment	Formulation	Dose	Repellency (%)[1] at;					Mean Repellency (%)	Class repellency
			1 h	2 h	3 h	4 h	5 h[2]		
C. reticulata (peel)	Powder	2g	60.00	73.33	73.33	13.33	80.00	59.99 aefghi	4
	Ethanol Extract	2ml	53.32	80.00	73.33	80.00	66.66	70.66 befghi	4
C. reticulata (leaves)	Powder	2g	66.66	73.33	60.00	66.66	66.66	66.66 cefgh	4
	Ethanol Extract	2ml	66.66	60.00	46.66	73.33	86.66	66.66 defgh	4
P. guajava (leaves)	Powder	2g	6.66	40.00	46.66	26.66	26.66	29.32 abcdej	2
	Ethanol Extract	2ml	80.00	33.33	20.00	40.00	46.66	43.99 abcdfj	3
Methoprene	Aq. Sol.[3]	5ppm	00.00	6.66	6.66	33.33	46.66	18.66 abcdgi	1
		10ppm	20.00	00.00	26.66	60.00	26.66	26.66 abcdhi	2
		15ppm	53.33	60.00	33.33	53.33	66.66	53.33 abi	3
		20ppm	46.66	60.00	80.00	73.33	66.66	65.33 efghj	4
Plant species									
C. reticulata (peel)								65.33 ac	
C. reticulata (leaves)								66.66 bc	
P. guajava (leaves)								36.65 abc	
Formulation									
Powder								51.99 ab	
Ethanol extract								60.43 ba	

Values followed by the same letter are significantly different according to the Kruskall-Wallis test ($P \leq 0.05$). [1] Percentage of repellency PR (%) = (Nc – 50)*2, where Nc is the percentage of beetles present in the control half; [2] Hours after treatment; [3] Aqueous Solution.

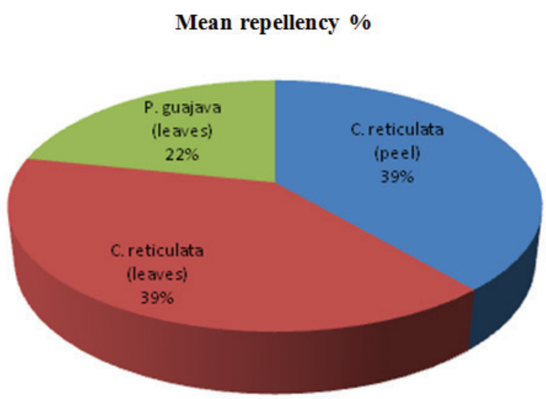

Figure 1. Comparison between repellent effects of plant products.

(P<0.05) higher even at very low concentration against *T. castaneum*. Novo *et al*. (1997) also recorded promising repellent activity of several crude extracts against *T. castaneum* while working on four native plants of Argentina.

Mortality Effects. The data with respect to percent mortality of *T. castaneum* reported that all treatments started showing fetal effects to test insects on first observation (3 days) except least doses of ethanol extract of *C. reticulata* leaves and methoprene. Furthermore, most of the tested treatments recorded significant effects on suppression of the pests' population after 21days when compared with control. In general, there was significant increase in cumulative insect mortality with an increase in the dose as well as exposure time of the treatments. Cent per cent mortality of adult beetles was only observed in all four concentrations of methoprene viz., 5, 10, 15 and 20 ppm after period of 14 days. Ethanol extract of *C. reticulata* leaves at dosage of 2 ml was the most effective among botanical treatments reaching up to 97.66% mean mortality at 21-day of exposure (Fig 2). Powder of *C. reticulata* leaves and ethanol extract of *P. guajava* leaves showed a trend of moderate % mortality after 21 days of release of insects to treated grains. Powder of *C. reticulata* (peel) at 1g was the one with least toxic effect against *T. castaneum* with percent mortality of 37.33 on the completion of experimental period. Adult beetles were significantly less susceptible to *P. guajava* (leaves) than peel and leaves of *C. reticulata*. Perusal of present data also revealed that ethanol extract of botanicals was significantly more toxic to test insects than powder formulation of same plant. All test

Table 2. Effect of chosen plant components and synthetic pesticide formulations on adult mortality (%) of *Tribolium castaneum*.

Treatments	Formulation	Dose	Mortality (%) at;			
			3 days	7 days	14 days	21 days
C. reticulata (peel)	Powder	1g	7.66±1.98	13.33±0.45	23.33±0.93	37.33±0.96
		2g	13.33±1.34	30.00±1.25	50.00±2.66	63.66±1.15
	Ethanol Extract	1ml	23.33±1.78	37.33±1.33	63.66±1.14	80.33±2.55
		2ml	30.00±0.67	60.00±1.56	70.33±1.86	93.33±1.44
C. reticulata (leaves)	Powder	1g	13.33±1.25	30.00±2.67	47.33±0.56	60.00±0.23
		2g	20.33±2.17	40.33±1.21	57.33±1.22	63.33±1.41
	Ethanol Extract	1ml	23.33±1.11	43.33±1.26	80.00±2.11	93.66±2.19
		2ml	40.00±0.56	67.66±1.45	87.66±2.17	97.66±1.71
P. guajava (leaves)	Powder	1g	00.00±1.64	10.33±1.21	20.33 ±1.68	40.00±1.65
		2g	07.33±1.41	13.33±1.42	33.66±1.84	57.33±0.91
	Ethanol Extract	1ml	17.33±1.78	37.33±2.11	50.00±1.86	53.33±0.82
		2ml	27.33 ±1.77	47.66±0.95	60.00±1.25	67.66±0.76
Methoprene	Aqueous solution	5ppm	00.00±0.00	30.00±1.34	100.00±0.66	-
		10ppm	10.00±0.93	50.00±2.09	100.00±0.98	-
		15ppm	20.00±0.86	70.00±1.45	100.00±0.67	-
		20ppm	30.00±0.91	90.00±1.56	100.00±0.91	-
Control	-	-	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00

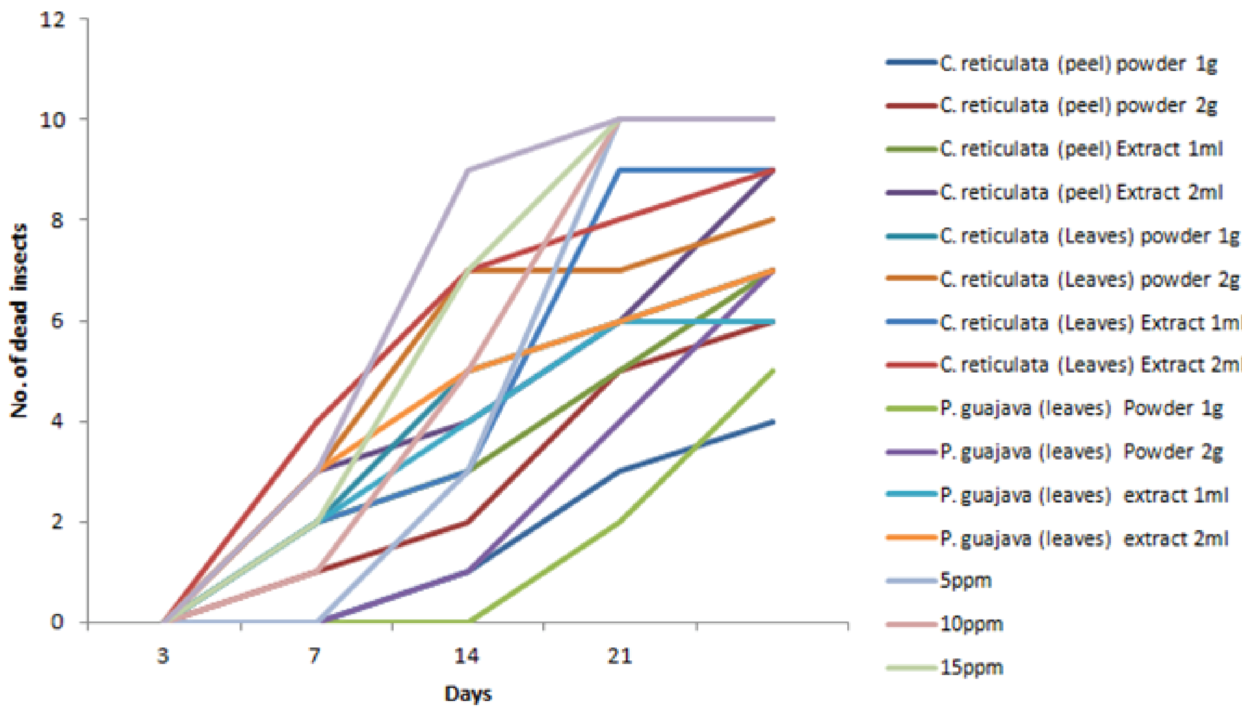

Figure 2. Biocidal activity of chosen plant materials and synthetic pesticide (methoprene) on adult *Tribolium castaneum.*

insects were found alive showing zero percent mortality in the control sample throughout the period of the experiment (Table 2).

The adult mortality might be attributed to contact toxicity or to the induction of some unknown physiological changes (Mathur *et al*. 1985). Effective adhesion of dust particles to spiracles of pest and their death due to suffocation might be one of the many possible reasons of adult mortality.

These findings were in confirmation with those of Chayengia *et al*. (2010) who evaluated efficacy of volatile oils, powders, ethanol extracts and water extracts of *Bihlongoni, Polygonum* (Leaf); *Psidium guajava* (Leaf); *Zingiber officinalae* (Leaf); *Curcuma longa* (Leaf); *Pogostemon cablin* (Leaf); *C. reticulata* (Rind); *Oxalis debilis* (Leaf); *Ipomoea aquatic* (Leaf); *Eichhornia crassipes* (Leaf) and *Acontium ferox* (Bark) against *S. oryzae*. They found that volatile oil of *C. reticulata* resulted in 100% mortality after 24 hours of exposure followed by *Curcuma longa* (90%). Among powder treatments, *C. reticulata* attained 6.67 per cent mortality of adult weevils after 72 hours of exposure which is more or less same as found in present study. This corroboration in results makes a sense that *C. reticulata* might be equally potent for controlling *S. oryzae* in stored rice as well as for *T. castaneum* in wheat. Furthermore, the use of *C. reticulata* peel powder against other insect pests in other susceptible merchandise might be induced from these findings.

Many species of citrus have been studied for their biocidal potential against insect pests. Zewde & Bekelle (2010) carried out studies on *Citrus sinensis* peel formulations using *Zabrotes subfasciatus* as test animal in stored haricot beans. Surprisingly, different solvents (Acetone, Ethanol, Petroleum Ether and Water extracts) used for experimentation did not show any insecticidal potentiality for this beetle. However, essential oil of *Citrus sinensis* was significantly potent that resulted in 67% accumulative mortality and 53.75% mean repellency against *Zabrotes subfasciatus* (Bohemann).

Our results demonstrated that most of the *P. guajava* treatments caused significantly ($P \leq 0.05$) higher mortality at 21 day of exposure when compared to untreated check. None of the treatments of *P. guajava* achieved hundred per cent mortality throughout the experimental period. As mortality was found to be directly proportional to exposure time and dosage so cent percent mortality might be attained by increasing either of both. The outcomes of present study illustrated the lethal effects of methoprene to adult beetles resulting in 100% mortality at 14 day of exposure even at low application rates. This is first hand information about lethality of methoprene to adult individuals of *T. castaneum* as most of the earlier scientists reported its development inhibitory action and reproductive sterility effects against *T. castaneum* (Oberlander *et al*. 1997, Chanbang *et al*. 2008, Tucker *et al*. 2010, Arthur & Fontenot, 2012). The results of all tested parameters demonstrated that efficacy of methoprene formulations increased gradually with increase in concentration with 20ppm being most effective among all the four (5ppm, 10ppm, 15ppm and 20ppm). This lethal activity of methoprene to adult *T. castaneum* might be attributed to unknown interference of this hormone mimic to some physiological function as hormones are complex molecules with multitude of functions.

These findings conform to several reports of earlier researchers. Arthur (2004) proposed that exposure of *R. dominica* adults to dust and liquid formulations of methoprene caused significant mortality. Tucker *et al*. (2010) made studies on the potential of methoprene for horizontal transfer from treated *T. castaneum* to untreated beetles. They observed that addition of larvae, pupae, or adults treated with methoprene to flour patches with untreated *T. castaneum* larvae, resulted in increased pupa and adult deformities and higher numbers of dead focal individuals (Single late stage untreated larvae of *T. castaneum*).

The results also indicated that leaves and peel of *C. reticulata* did not differ significantly with respect to their toxicity against adult *T. castaneum*. Firstly, *C. reticulata* fruit peel is used in artificial flavors, beverage, insecticide, cosmetics, soap, perfume, shampoo, lubricants, for fragrance in air fresheners, candles, and in aromatherapy by processing it in numerous ways (Thaman *et al*. 2000, Walter & Sam 2010). Secondly, it is only available for few months in a year. So use of leaves as its alternative is recommended due to their relative abundance and accessibility throughout the year. Further studies are undertaken for the isolation of individual components of *C. reticulata* leaves for their possible incorporation in natural insecticides on commercial scale.

Table 3. Effect of chosen plant components and synthetic pesticide formulations on feeding ratio and grain weight loss (%) caused by adult *Tribolium castaneum.*

Treatment	Formulation	Dose	Feeding Ratio	Weight loss (%)
C. reticulata (peel)	Powder	1g	0.020	1.07
		2g	0.000	0.66
	Ethanol Extract	1ml	0.026	0.33
		2ml	0.006	0.00
C. reticulata (leaves)	Powder	1g	0.010	1.12
		2g	0.000	0.84
	Ethanol Extract	1ml	0.006	0.00
		2ml	0.000	0.00
P. guajava (leaves)	Powder	1g	0.020	1.36
		2g	0.000	1.31
	Ethanol Extract	1ml	0.026	0.65
		2ml	0.000	0.00
Methoprene	Aqueous solution	5ppm	0.040	0.69
		10ppm	0.006	0.96
		15ppm	0.004	0.51
		20ppm	0.001	0.00
Control	-	-	0.066	2.64

Feeding ratio and weight loss protection effects. The treatments with the strongest antifeedant effect on *T. castaneum* were 2 g powders of *C. reticulata* (peel), *C. reticulata* (leaves), *P. guajava* (leaves) and 2 ml ethanol extracts of *C. reticulata* (leaves) and *P. guajava* (leaves) which exhibited total inhibition of diet consumption (Table 3). All the four treatments of standard control, methoprene gave important antifeedant effects with significantly lower feeding ratios of 0.002 at 20 ppm, 0.004 at 15 ppm, 0.006 at 10 ppm and 0.040 at 5 ppm when compared to untreated check (0.066). The analysis of antifeedant effect of each plant, regardless of the formulation used, revealed that *C. reticulata* (leaves) was stronger than *P. guajava* (leaves). However, non-significant differences were observed between *C. reticulata* (peel) and *P. guajava* (leaves). Statistical analysis (T-test) of the data depicted non-significant differences between antifeedant effects two formulations (powder and extract). There was observed marked decrease in feeding ratio with an increase in the dose of each tested treatment.

Among different botanicals evaluated for their efficacy against *T. castaneum* in stored wheat grains, ethanol extracts of *C. reticulata* leaves was found to be most effective by recording no weight loss of seed at both doses. Same results were recorded with ethanol extracts of *C. reticulata* (leaves), *C. reticulata* (peel) and *P. guajava* at 2 ml. Among four treatments of methoprene, only 20 ppm offered complete protection from *T. castaneum* without any loss in seed weight in comparison with botanicals. The least weight loss (0.33%) was observed in ethanol extract of *C. reticulata* (peel) at 1 ml. Significantly higher weight loss (2.64%) was recorded in untreated check followed by *P. guajava* (leaves) powder (1.36%) at 1 g. In general, all treatments were effective in controlling *T. castaneum* and weight loss protection increased in dose dependant manner. Comparisons between two different plant species showed that both components (peel and leaves) of *C. reticulata* were noticeably stronger in reducing weight loss than *P. guajava*. In addition, contrast between two formulations established ethanol extract as significantly ($P \leq 0.05$) more effective than powder form of same plant. The weight loss protection activity of synthetic pesticide methoprene was comparable to that of 2 ml ethanol extracts of botanicals at concentration of 20 ppm.

It is evident from our findings that efficacy of *C. reticulata* leaves and peel was more or less same. That might be due to presence of same concentration of active principles in either components or different but equally potent bioactive compounds. The results of our findings are in corroboration with those of Owusu (2001). The author worked on alcohol extracts of six Ghanaian plant materials to check their efficacy against *T. castaneum* and *S. oryzae*. He reported that leaves of *Ocimum viride* showed significant antifeedant and repellent effects against both but *T. castaneum* was found to be comparatively resistant.

Similar results of antifeedant effects on *T. castaneum* and

S. oryzae were observed by Talukder & Howse (1993, 1994) with four different extracts of *Aphanamixis polystachya*. Although, Chayengia *et al*. (2010) carried out studies on bioactivity of *P. guajava* along with some other plant components to protect stored rice from *S. oryzae* but no work has done pertaining to its efficacy against *T. castaneum*. Therefore, present study introduces an innovative approach to the use of guava plant for stored wheat protection. Use of powdered forms of both tested plants is recommended for small scale farmers of developing countries due to its simplicity of application, easy removal and non-toxic effects even if consumed by humans as both plants having medicinal properties.

Conclusion

In the light of the findings of present study, it could be stated that *Psidium guajava*, *Citrus reticulata* and methoprene shown promising effects of seed protection and insecticidal properties so these might be used in *T. castaneum* population managing in stored wheat. Further investigation to determine the exact mode of action of their active components and their effect on non-target organisms is suggested.

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