Research Article



Toxic and Repellent Characteristics of Some Plant Extracts used against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) Improve the Grain Quality of Stored Wheat

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Abstract | Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae) is one of the key insect pests of stored grains. Due to export regulations and zero-tolerance for alive insects in trade commodities, synthetic pesticides are often used to eliminate pest infestation in consignments. Phosphine is currently one and the only acceptable chemical used in stored grains, but due to their overuse, stored grain pests become resistant to this fumigant. There is, therefore, a great need to find alternatives. In this study insecticidal efficacy of different plant extracts viz. Ginger (Zingiber officinale), Neem (Azadirachta indica), Clove (Syzygium aromaticum) and Tobacco (Nicotiana tabacum) was compared against T. castaneum infesting stored wheat. The results have suggested that mortality and repellency in T. castaneum increased as the dose rate of crude plant extracts and exposure interval increased. The maximum mortality (86.67%) was achieved with the highest dose of crude extract of Z. officinale (80mg per 7.5g wheat) at 10 days exposure interval. Similarly, the highest dose of Z. officinale extract (80ml) was responsible to cause 100% repellency at 2 hours exposure interval. Results also suggested that grain damage and grain weight loss due to the feeding of T. castaneum decreased as the dose rate of crude plant extracts increased, while an increasing trend was found in these parameters with the increase in exposure interval. The maximum grain damage and grain weight loss (10.27% and 0.346%, respectively) were recorded at the lowest dose of *A. indica* crude extract at 10 days exposure interval. The result of Feeding Deterrence Index suggested that it increased as the dose rate of crude plant extracts increased while a decreased trend was found with the increase in exposure interval. The maximum feeding deterrence index (86.48%) was recorded at the highest dose of Z. officinale crude extract at 5 days exposure interval. The results of this study supported the use of Z. officinale to reduce the population of T. castaneum by its toxic and repellent characteristics which ultimately improve the grain quality of stored wheat.

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Keywords | Tribolium castaneum, Plant extracts, Toxicity, Repellency, Grain quality, Stored wheat

Abbreviation | DEI: Days Exposure Interval; HEI: Hours Exposure Interval; FDI: Feeding Deterrence Index; n: Insect population; T: Treated; Co: Control; Nc: Number of insects in control half; Nt: Number of insect in treated half; N_d: Number of damaged grains; N: Total number of grains in sample; W_u: Weight of undamaged grains; N_u: Number of undamaged grains; W_d: Weight of damaged grains; N_d: Number of damaged grains; C: Weight loss of control grains; T: Weight loss of treated grains; ANOVA: Analysis of Variance

1. Introduction

C afe storage of grains and food products is a major Oproblem in preventing damage from insect pests (Haq et al., 2005; Atta et al., 2020). Under favorable climatic and storage conditions, around 9% of global grain production is reduced through insect pests and mites (Adams and Schulten, 1978; Fields, 2006; Rahman et al., 2009). The red flour beetle, Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae), is one of the most important stored grain pests that have been reported to damage a variety of commodities such as grains (Shafique et al., 2006; Atta et al., 2020), flour (Campbell and Runnion, 2003; Naseri et al., 2017), peas (Pretheep-Kumar et al., 2007), beans (Abdullahi et al., 2018), nuts (Pires et al., 2017), dried fruits (Sarwar, 2015) and spices (Tripathi et al., 2009). Both larvae and adults feed on the grains destroyed by other pests (Mamun et al., 2009).

Preventive and curative control measures are in practice to control stored grains pests. Among these, highly toxic synthetic chemicals have been used for many years. However, these chemicals have some serious drawbacks on public health and the environment and insecticide resistance (Madkour et al., 2012). Therefore, it is necessary to find other sources that are readily available, affordable, less toxic to human and safe to the environment (Udo, 2005; Atta et al., 2019; Ayub et al., 2019a, 2019b, 2019c; Rizwan et al., 2019a, 2019b, 2019c, 2019d; Atta et al., 2020).

The use of plant material as traditional protectors of the stored products is an old practice that is used all over the world (Aslam et al., 2002). The protection of stored products usually involves the mixing of grains with the herbal mixture (Tapondjou et al., 2002). Efforts have been made in many countries to reduce the use of harmful pesticides through the use of native plant products, the implementation of integrated pest management methods, and the use of biodegradable products to protect stored grains (Khattach and Hameed, 1986). The control of stored grain pests using materials of natural origin is a topic of great importance today. Plants such as Azadirachta indica, Cassia fistula, Calotropis procera, Lantana camara and Chrysanthemum coronarium have shown insecticidal, growth-regulating antifeedant, repellant and properties to insects (Singh and Singh, 2005; Neoliya et al., 2007; Sankari and Narayanswamy, 2007). Such botanical products have several advantages over synthetic insecticides such as environmental safety, less hazardous, economic and easy availability (Gupta and Pathak, 2009; Tembo et al., 2018).

The present study was conducted to investigate the toxic and repellent characteristics of some plant extracts (*Z. officinale, A. indica, S. aromaticum* and *N. tabacum*) used against *T. castaneum* for the improvement of grain quality in stored wheat. The results could help devise environment-friendly management practices for the control of *T. castaneum*.

2. Materials and Methods

2.1 Experimental site

The study was conducted at the Entomology Laboratory, Rice Research Institute, Kala Shah Kaku, Punjab, Pakistan (31° 43′ 17" N and 74° 16' 14" E).

2.2 Acquisition and rearing of tribolium castaneum

The adults of *T. castaneum* were acquired from Akbari Grain Market Lahore, Pakistan (31.5807° N,74.3256° E) and were sub-cultured by rearing on synthetic food made from whole wheat flour and bakers' yeast with the proportion of 19:1 under laboratory conditions (Morgan et al., 2003; Amin et al., 2012; Qasim et al., 2013; Atta et al., 2020) of 28±2 °C, 60 ± 5% R.H. and 12:12 D:L photoperiod (Shafique et al., 2006; Safavi and Mobki, 2012; Qasim et al., 2013; Perez-Mendoza et al., 2014; Sami et al., 2018; Atta et al., 2020).

2.3 Plant materials

Fresh leaves of *A. indica*, *Z. officinale*, *S. aromaticum* and *N. tabacum* were collected from the trees planted under the boundaries of Rice Research Institute, Kala Shah Kaku, Pakistan, to conduct the study.

2.4 Preparation of plant extracts

For the preparation of plant extracts, the fresh leaves of *A. indica, Z. officinale, S. aromaticum* and *N. tabacum* were washed sufficiently with distilled water. After drying under the shade, the leaves were ground to a fine powder with an electrical grinder. For investigating the toxic effect of these plant extracts, four different doses of plant powders were weighed (20mg, 40mg, 60mg and 80mg) and placed these in conical flasks separately. For investigating the repellent effect of these plant extracts, plant powder (10g) was placed in conical flasks (250ml) along with 100ml of distilled water and placed on heating (60°C) and shake with a magnetic stirrer (AM4, Velp Scientifica, Italy) for



6 hrs to dissolve the leaf powder properly. The solid residues were removed using muslin cloths, and plant extracts were then filtered (Whatman No. 1). After evaporation by using a rotary evaporator at 60°C under vacuum, the dried plant extracts were brought to constant volume, using a hot air oven (60°C). These extracts were stored under 4°C till used as a stock solution. Four concentrations of *A. indica, Z. officinale, S. aromaticum* and *N. tabacum* extracts were prepared separately (20ml, 40ml, 60ml and 80ml) by dilution with distilled water from this stock solution. For investigating the impact of plant extracts on grain quality, four different doses (20mg, 40mg, 60mg and 80mg) were prepared by following similar procedure was adopted in toxic effect investigation.

2.5 Toxic characteristic of plant extracts against tribolium castaneum

Wheat grains (7.5g) were weighted and filled in plastic jars. Four concentrations of crude plant extracts (20, 40, 60, 80mg) were mixed in wheat grains separately. Twenty adults of *T. castaneum* were collected from rearing jars with the help of aspirator and released in jars separately and covered with the muslin cloth. The experiment was replicated thrice and placed in a dark room to enhance the activity of *T. castaneum*. The mortality was corrected and calculated after 5 and 10 days according to the formula (Henderson and Tilton, 1995).

 $\label{eq:corrected mortality} \mbox{(\%)} = (1 - \frac{n \mbox{ in } C_o \mbox{ before treatment } \times n \mbox{ in } T \mbox{ after treatment }}{n \mbox{ in } C_o \mbox{ after treatment } \times n \mbox{ in } T \mbox{ before treatment }} \times 100$

Where: n= Insect population; T= Treated; Co= Control

2.6 Repellent characteristic of plant extracts against tribolium castaneum

The test was started by taking filter paper cutting it according to the size of the Petri dish taken. Half of the filter paper dipped in four different doses of plant extracts separately for 5 min and the other half into distilled water. The experiment was replicated thrice. Twenty adults of *T. castaneum* were released in each replication in the center of the Petri dishes. The experiment was placed in a dark room to enhance the activity of *T. castaneum*. The data of repellency was recorded after 1 and 2 hrs by using the formula (Gillenwater and Mc Donald, 1975).

Repellency (%) =
$$\frac{Nc - Nt}{Nc + Nt} \times 100$$

Where: Nc= Number of insects in control half; Nt= Number of insect in treated half.

2.7 Assessment of grain quality parameters due to tribolium castaneum exposed to crude plant extracts

Wheat grains (7.5g) were weighted, counted and filled in plastic jars. Four concentrations of crude plant extracts were mixed in wheat grains separately. Twenty adults of *T. castaneum* were collected from rearing jars with the help of aspirator and released in jars separately and covered with the muslin cloth. The experiment was replicated thrice and placed in a dark room to enhance the activity of *T. castaneum*. After 5 and 10 days, damaged and undamaged wheat grains were counted and recorded the data by using the formula (Atta et al., 2020).

Grain damage (%) =
$$\frac{N_d}{N} \times 100$$

Where: N_d = Number of damaged grains; N= Total number of grains in sample.

After 5 and 10 days, treated grains were weighed to check the weight loss in wheat grains and recorded the data by using the formula (Atta et al., 2020).

$$\label{eq:Grain weight loss (%)} \text{Grain weight loss (%)} = \frac{(W_u \times N_d) - (W_d \times N_u)}{W_u (N_d + N_u)} \times 100$$

Where: W_u = Weight of undamaged grains; N_u = Number of undamaged grains; W_d = Weight of damaged grains; N_d = Number of damaged grains.

Feeding Deterrence Index (FDI) was calculated by using the formula (Brari and Kumar, 2019).

$$FDI (\%) = \frac{C - T}{C + T} \times 100$$

Where: C= Weight loss of control grains; T = Weight loss of treated grains.

2.8 Statistical analysis

Data was subjected to two-way analysis of variance (ANOVA) using statistical software Statistix[®] (Version 8.1). Treatment means were separated by Bonferroni test at $\alpha = 0.05$.

Toxic and Repellent Characteristics of Plant Extracts

3. Results and Discussion

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3.1 Toxic characteristic of plant extracts against tribolium castaneum

The main effects and interaction of various crude plant extracts and their doses are highly significant (P < 0.05) on percent mortality of *T. castaneum* at two different exposure intervals (Table 1). Percent morality of *T. castaneum* exposed to different crude extracts mixed in wheat grains was gradually increased as the exposure interval increased. The highest and lowest percent mortality (55.83±2.17% and 29.17±1.65%) was recorded in *Z. officinale* and *A. indica* treated wheat after 10 DEI, respectively, while no mortality was recorded in control treatment at both exposure intervals. Crude extracts caused mortality with the trend of *Z. officinale* < *S. aromaticum* < *N. tabacum* < *A. indica* (Figure 1). A similar trend of increase in mortality was observed in the case of doses of crude extracts as in Figure 1. The highest dose (80mg per 7.5g wheat) and lowest dose (20mg per 7.5g wheat) of crude extracts caused 51.33±6.37% and 15.33±2.43%

Table 1: Main effects and interaction of various plant extracts and their doses in relation to mortality (%) and repellency (%) of *Tribolium castaneum*, and grain quality parameters (%) due to *Tribolium castaneum* at different exposure intervals.

Factors	df	Fvalue	Fvalue							P value		
		Mortality (%)		Repellency (%)		Grain damage (%)		Grain weight loss (%)		Feeding Deter- rence Index (%)		-
		5 DEI	10 DEI	1 HEI	2 HEI	5 DEI	10 DEI	5 DEI	10 DEI	5 DEI	10 DEI	-
Plant extracts	4ª/59 ^b	53.35**	73.68**	67.96**	113.78**	1379.61**	3160.79**	748.00**	1461.76**	820.10**	335.32**	< 0.05
Doses	3ª/59 ^b	47.90**	47.93**	31.10**	30.62**	292.17**	462.21**	173.14**	304.01**	301.08**	110.40**	< 0.05
Plant extracts × Doses	12ª/59 ^b	3.19**	3.54**	2.54**	2.60**	22.37**	34.91**	14.67**	19.88**	19.86**	7.84**	< 0.05

^a: Treatment degree of freedom; ^b: Error degree of freedom; *df*: degree of freedom; DEI: Days exposure interval; HEI: Hours exposure interval; ^{**}: highly significant at α < 0.05.

Plant ex-	Mortality (%)			Repellency (%)				
tracts	Doses (mg per 7.5 g wheat)	5 DEI	10 DEI	Doses (ml)	5 DEI	10 DEI		
Zingiber officinale	20	23.33±5.09CDEFGH	30.00±6.67cdef	20	43.33±5.09BCDEFG	70.00±6.67abc		
	40	36.67±5.09BCDEF	46.67±3.85bcd	40	60.00±6.67ABCD	73.33±5.09abc		
	60	50.00±6.67ABC	60.00±6.67ab	60	73.33±5.09AB	100.00±0.00a		
	80	70.00±3.33A	86.67±5.09a	80	86.67±5.09A	100.00±0.00a		
Syzygium aromaticum	20	16.67±5.09EFGH	23.33±5.09defg	20	26.67±5.09DEFGH	43.33±8.39cd		
	40	30.00±5.77CDEFG	43.33±5.09bcde	40	36.67±11.71CDEFG	70.00±6.67abc		
	60	46.67±6.94ABCD	50.00±6.67bcd	60	70.00±6.67ABC	86.67±5.09ab		
	80	63.33±8.39AB	63.33±7.70ab	80	76.67±5.09AB	100.00±0.00a		
Nicotiana tabacum	20	10.00±3.33FGH	16.67±1.92efg	20	20.00±5.77EFGH	26.67±6.94de		
	40	20.00±3.33DEFGH	26.67±3.85defg	40	26.67±8.39DEFGH	53.33±10.18bcd		
	60	33.33±5.09CDEF	40.00±6.67bcde	60	46.67±8.39BCDEF	63.33±6.94bc		
	80	46.67±6.94ABCD	56.67±6.94bc	80	56.67±8.39ABCD	73.33±5.09abc		
Azadirachta indica	20	3.33±1.92GH	6.67±1.92fg	20	10.00±0.00GH	23.33±5.09de		
	40	10.00±3.33FGH	23.33±5.09defg	40	13.33±5.09FGH	40.00±6.67cd		
	60	30.00±5.77CDEFG	36.67±5.09bcde	60	26.67±8.39DEFGH	50.00±6.67cd		
	80	43.33±3.85ABCDE	50.00±3.33bcd	80	50.00±6.67BCDE	66.67±abc		
Control	-	0.00±0.00H	$0.00 \pm 0.00 g$	-	0.00±0.00H	0.00±0.00e		

Table 2: Mortality (%) and Repellency (%) (Mean \pm SE, n = 3) of *Tribolium castaneum* exposed to various plant extracts and their doses mixed in wheat grains at different exposure intervals.

DEI: Days exposure interval; HEI: Hours exposure interval; Means with different letters are significantly different (P < 0.05), Bonferroni test, comparisons across all treatments.



mortality, respectively after 10 DEI. Doses of crude extracts caused mortality with the trend of 80mg per 7.5g wheat < 60mg per 7.5g wheat < 40mg per 7.5g wheat < 20mg per 7.5g wheat (Figure 2). Percent mortality of *T. castaneum* in different doses (20-80mg per 7.5g wheat) of *Z. officinale* treated wheat grains ranged from 23.33-70.00% at 5 DEI and 30.00-86.67% at 10 DEI, *S. aromaticum* treated wheat grains ranged from 16.67-63.33% at 5 DEI and 23.33-63.33% at 10 DEI, *N. tabacum* treated wheat grains ranged from 10.00-46.67% at 5 DEI and 16.67-56.67% at 10 DEI, and *A. indica* treated wheat grains ranged from 3.33-43.33% at 5 DEI and 6.67-50.00% at 10 DEI (Table 2).

3.2 Repellent characteristic of plant extracts against tribolium castaneum

The main effects and interaction of various plant extracts and their doses are highly significant (P <0.05) on percent repellency of T. castaneum at two different exposure intervals (Table 1). The maximum number of T. castaneum showed repellent behavior to the control half, when half filter paper was exposed to different plant extracts and their doses. T. castaneum exposed to Z. officinale extract at the highest dose (80mg per 7.5g wheat) showed maximum repellent behavior as compared to other extracts (S. aromaticum, N. tabacum and A. indica) and doses (20mg, 40mg and 60mg per 7.5g wheat) (Figure 3). Percent repellency of T. castaneum exposed to different plant extracts was gradually increased as the exposure interval increased. The highest and lowest percent repellency (85.83±4.10% and 45.00±4.54%) was recorded in Z. officinale and A. indica treated wheat after 2 HEI, respectively, while no repellency was recorded in control treatment at both exposure intervals. Plant extracts caused repellency with the trend of Z. officinale < S. aromaticum < N. tabacum < A. indica (Figure 1). A similar trend of increase in repellency was observed in the case of doses of extracts as in Figure 1. The highest dose (80 ml) and lowest dose (20ml) of extracts caused 68.00±8.19% and 32.67±5.19% repellency, respectively after 2 HEI. Doses of extracts caused repellency with the trend of 80ml < 60ml < 40ml < 20ml (Figure 2). Percent repellency of T. castaneum in different doses (20-80ml) of Z. officinale extract ranged from 43.33-86.67% at 1 HEI and 70.00-100.00% at 2 HEI, S. aromaticum extract ranged from 26.67-76.67% at 1 HEI and 43.33-100.00% at 2 HEI,

N. tabacum extract ranged from 20.00-56.67% at 1 HEI and 26.67-73.33% at 2 HEI, and *A. indica* extract ranged from 10.00-50.00% at 1 HEI and 23.33-66.67% at 2 HEI (Table 2).



Figure 1: Mortality $(\%)^{(a)}$ and Repellency $(\%)^{(b)}$ (Mean ± SE, n = 3) of *Tribolium castaneum* exposed to various plant extracts (*Zingiber officinale*, *Syzygium aromaticum*, *Nicotiana tabacum* and *Azadirachta indica*) at different exposure intervals. Plant extracts are on x-axis and measured variables are on *y-axis*. DEI = Days exposure interval; HEI = Hours exposure interval; Means with different letters are significantly different (P < 0.05), Bonferroni test, comparisons across all treatments.



Figure 2: Mortality $(\%)^{(a)}$ and Repellency $(\%)^{(b)}$ (Mean ± SE, n = 3) of *Tribolium castaneum* exposed to various doses (20mg, 40mg, 60mg and 80mg) of plant extracts (*Zingiber officinale*, *Syzygium aromaticum*, *Nicotiana tabacum* and *Azadirachta indica*) at different exposure intervals. Extract doses are on x-axis and measured variables are on *y-axis*. DEI = Days exposure interval; HEI = Hours exposure interval; Means with different letters are significantly different (P < 0.05), Bonferroni test, comparisons across all treatments.

3.3 Assessment of grain quality parameters due to tribolium castaneum exposed to crude plant extracts

Grain damage (%): The main effects and interaction of various crude plant extracts and their doses are highly significant (P < 0.05) on percent grain damage due to the *T. castaneum* at two different exposure



intervals (Table 1). Percent grain damage due to the T. castaneum exposed to different crude extracts mixed in wheat grains was gradually increased as the exposure interval increased. The highest and lowest grain damage (12.40±0.42% and 7.00±0.56%) was recorded in A. indica and Z. officinale treated wheat after 10 DEI, respectively, while grain damage in control treatment ranged from 15.33-18.13% at both exposure intervals. T. castaneum exposed to different crude extracts caused grain damage with the trend of A. indica < N. tabacum < S. aromaticum < Z. officinale (Figure 4). Grain damage due to T. castaneum decreased as the dose rate of crude plant extracts increased. The lowest dose (20mg per 7.5g wheat) and highest dose (80mg per 7.5g wheat) of crude extracts caused 13.31±0.63% and 9.97±1.00% grain damage, respectively after 10 DEI. Doses of crude extracts caused grain damage with the trend of 20mg per 7.5g wheat < 40mg per 7.5g wheat < 60mg per 7.5g wheat < 80mg per 7.5g wheat (Figure 5). Percent grain damage due to T. castaneum exposed to different doses (20-80mg per 7.5g wheat) of A. indica treated wheat grains decreased from 12.80-8.93% at 5 DEI and 14.27-10.27% at 10 DEI, N. tabacum treated wheat grains decreased from 11.20-7.20% at 5 DEI and 12.67-9.20% at 10 DEI, S. aromaticum treated wheat grains decreased from 9.60-5.07% at 5 DEI and 11.60-7.33% at 10 DEI, and Z. officinale treated wheat grains decreased from 8.27-2.53% at 5 DEI and 9.87-4.93% at 10 DEI (Table 3).



Figure 3: Repellent behavior of total number of *Tribolium castaneum* on treated half of filter paper exposed to various plant extracts and their doses [(a) 20 ml, (b) 40 ml, (c) 60 ml, (d) 80 ml] and control half of filter paper at different exposure intervals.



Figure 4: Grain damage $(\%)^{(a)}$, Grain weight loss $(\%)^{(b)}$ and Feeding Deterrence Index $(\%)^{(c)}$ (Mean ± SE, n = 3) due to *Tribolium castaneum* exposed to various crude plant extracts (*Zingiber officinale*, *Syzygium aromaticum*, *Nicotiana tabacum* and *Azadirachta indica*) at different exposure intervals. Plant extracts are on *x*-axis and measured variables are on *y*-axis. DEI = Days exposure interval; Means with different letters are significantly different (P < 0.05), Bonferroni test, comparisons across all treatments.

Grain weight loss (%): The main effects and interaction of various crude plant extracts and their doses are highly significant (P < 0.05) on percent grain weight loss due to the feeding of T. castaneum at two different exposure intervals (Table 1). Percent grain weight loss due to the feeding of T. castaneum exposed to different crude extracts mixed in wheat grains was gradually increased as the exposure interval increased. The highest and lowest grain weight loss (0.462±0.024% and 0.230±0.024%) was recorded in A. indica and Z. officinale treated wheat after 10 DEI, respectively, while grain weight loss in control treatment ranged from 0.528-0.697% at both exposure intervals. T. castaneum exposed to different crude extracts caused grain weight loss with the trend of A. indica < N. tabacum < S. aromaticum < Z. officinale (Figure 4). Grain weight loss due to the feeding of T. castaneum decreased as the dose rate of crude plant extracts increased. The lowest dose (20mg per 7.5g wheat) and highest dose (80mg per 7.5g wheat) of crude extracts caused 0.493±0.029% and 0.319±0.046% grain weight loss, respectively after 10 DEI. Doses of crude extracts caused grain weight loss with a trend of 20mg per 7.5g wheat < 40mg per 7.5g wheat < 60mg per 7.5g wheat < 80mg per 7.5g wheat (Figure 5). Percent grain weight loss due to feeding of T. castaneum exposed to different doses (20-80mg per 7.5g wheat) of A. indica treated wheat grains decreased from 0.427-0.151% at 5 DEI and 0.568-0.346% at 10 DEI, N. tabacum treated wheat grains decreased from 0.340-0.110% at 5 DEI and 0.482-0.245% at 10 DEI, S. aromaticum treated wheat grains decreased from 0.256-0.084% at 5 DEI and 0.381-0.192% at 10

Atta *et al*.

Table 3: Grain damage (%), Grain weight loss (%) and Feeding Deterrence Index (%) (Mean \pm SE, n = 3) exposed to various crude plant extracts and their doses mixed in wheat grains in the presence of *Tribolium castaneum* at different exposure intervals.

	Doses (mg per	Grain damage (%	6)	Grain weight loss ((%)	Feeding deterrence index (%)		
extracts	7.5 g wheat)	5 DEI	10 DEI	5 DEI	10 DEI	5 DEI	10 DEI	
Zingiber officinale		8.27±0.42GH	9.87±0.36ef	0.184±0.014FG	0.335±0.018ef	48.94±1.45EF	35.45±0.95efg	
	40	6.53±0.36I	7.60±0.47h	0.119±0.013GHI	0.270±0.018gh	64.34±2.14BCD	44.80±1.36cde	
	60	4.40±0.31J	5.60±0.31i	0.084±0.011IJ	0.203±0.019i	73.76±2.23B	55.98±2.22bc	
	80	2.53±0.16K	4.93±0.36i	0.041±0.007J	0.113±0.015j	86.48±1.83A	73.33±2.43a	
Syzyg-	20	9.60±0.31EF	11.60±0.41d	0.256±0.018DE	0.381±0.014de	35.43±1.27GH	29.28±0.24fgh	
ium	40	8.13±0.42GH	9.60±0.47ef	0.175±0.016FGH	0.315±0.022fg	51.33±1.91EF	38.49±1.59def	
aromati- cum	60	6.93±0.36I	8.67±0.36g	0.120±0.008GHI	0.263±0.020gh	63.45±1.09BCD	45.97±1.67cde	
	80	5.07±0.16J	7.33±0.32h	0.084±0.011IJ	0.192±0.027i	73.63±2.21B	58.77±3.55b	
Nico- tiana tabacum	20	11.20±0.31C	12.67±0.42c	0.340±0.019C	0.482±0.020c	22.01±0.78I	18.33±0.33hij	
	40	10.13±0.36CDE	11.47±0.42d	0.208±0.012EF	0.411±0.025d	43.88±0.76FG	26.36±1.32fghi	
	60	9.07±0.36EFG	9.87±0.36ef	0.152±0.010FGHI	0.340±0.024ef	55.79±0.83CDE	35.17±1.65efg	
	80	7.20±0.21HI	9.20±0.37fg	0.110±0.010HI	0.245±0.018hi	66.13±1.45BC	48.68±1.58bcd	
Aza- dirachta indica	20	12.80±0.47B	14.27±0.36b	0.427±0.018B	0.568±0.020b	10.57±0.28J	10.20±0.10jk	
	40	10.80±0.37CD	12.93±0.36c	0.284±0.017CD	0.509±0.028c	30.45±0.80HI	16.04±1.18ij	
	60	10.00±0.31DEF	12.13±0.42cd	0.203±0.014EF	0.424±0.024d	45.14±1.27FG	24.73±0.99ghi	
	80	8.93±0.32FG	10.27±0.36e	0.151±0.008FGHI	0.346±0.022ef	55.66±0.86DE	34.18±1.34efg	
Control	-	15.33±0.36A	18.13±0.42a	0.528±0.023A	0.697±0.024a	0.00±0.00K	$0.00 \pm 0.00 k$	

DEI: Days exposure interval; Means with different letters are significantly different (P < 0.05), Bonferroni test, comparisons across all treatments.

DEI, and *Z. officinale* treated wheat grains decreased from 0.184-0.041% at 5 DEI and 0.335-0.113% at 10 DEI (Table 3).



Figure 5: Grain damage $(\%)^{(a)}$, Grain weight loss (%)^(b) and Feeding Deterrence Index $(\%)^{(c)}$ (Mean ± SE, n = 3) due to *Tribolium castaneum* exposed to various doses (20mg, 40mg, 60mg and 80mg) of plant extracts (*Zingiber officinale*, *Syzygium aromaticum*, *Nicotiana tabacum* and *Azadirachta indica*) at different exposure intervals. Extract doses are on *x-axis* and measured variables are on *y-axis*. DEI = Days exposure interval; Means with different letters are significantly different (P < 0.05), Bonferroni test, comparisons across all treatments.

Feeding deterrence index (FDI) (%): The main effects and interaction of various crude plant extracts and their doses are highly significant (*P* < 0.05) on percent FDI

Journal of Innovative Sciences June 2020 | Volume 6| Issue 1 | Page 7

due to *T. castaneum* at two different exposure intervals (Table 1). Percent FDI due to T. castaneum exposed to different crude extracts mixed in wheat grains was gradually decreased as the exposure interval increased. The highest and lowest FDI (52.39± 4.07% and 21.29± 2.62%) was recorded in Z. officinale and A. indica treated wheat after 10 DEI, respectively, while zero FDI in control treatment at both exposure intervals was recorded. T. castaneum exposed to different crude extracts caused FDI with the trend of Z. officinale < S. aromaticum < N. tabacum < A. indica (Figure 4). FDI due to T. castaneum increased as the dose rate of crude plant extracts increased. The lowest dose (20mg per 7.5g wheat) and highest dose (80mg per 7.5g wheat) of crude extracts caused 18.65± 2.85% and 43.00± 5.59% FDI, respectively after 10 DEI. Doses of crude extracts caused FDI with the trend of 80mg per 7.5g wheat < 60mg per 7.5g wheat < 40mg per 7.5g wheat < 20mg per 7.5g wheat (Figure 5). Percent FDI due to T. castaneum exposed to different doses (20-80mg per 7.5g wheat) of Z. officinale treated wheat grains ranged from 48.94-86.48% at 5 DEI and 35.45-73.33% at 10 DEI, S. aromaticum treated wheat grains ranged from 35.43-73.63% at 5 DEI and 29.28-58.77% at 10 DEI, N. tabacum treated wheat grains ranged from 22.01-



66.13% at 5 DEI and 18.33-48.68% at 10 DEI, and *A. indica* treated wheat grains ranged from 10.57-55.66% at 5 DEI and 10.20-34.18% at 10 DEI (Table 3).

Results of the present study have revealed that natural plant extracts have the potential to control *T. castaneum* population in stored wheat. *Z. officinale* has proved to be effective against the *T. castaneum* as compare to other tested plant extracts, which have more toxic and repellent characteristics, results in less wheat grain damage due to *T. castaneum*. These findings are in accordance with the earlier studies where *Z. officinale* has been proved to be an effective natural insecticidal against *T. castaneum* (Epidi and Odili, 2009; Chaubey, 2011). Ahmad et al. (2019) reported that *Z. officinale* found more effective against *T. castaneum* which result in 15 times higher mortality of adults and 4-5 times reduction in rice grain weight losses due to this insect pest.

Several plant species produce numerous chemical compounds that could be repellent or deterrent or even toxic for insect pests. Some of these compounds are also toxic to the plant itself, and consequently, they are stored in special organs such as flowers and seeds. These chemicals are aimed directly against plant-feeding insect pests (Shojaaddini et al., 2008). Few species of Zingiberaceae plants have been used for medicinal purposes and some species have been reported to have biologically active compounds (Grainge and Ahmed, 1987; Ohsawa et al., 1994).

Z. officinale contains a sesquiterpenes hydrocarbon; and the pungent odor appears to be responsible for its toxic and repellent effect on the insect pests (Purseglove, 1972). Many reports have been published on the insecticidal activity of the essential oils and other products from Zingiber species (Nugroho et al., 1996; Agarwal et al., 2001; Zhang et al., 2004; Owolabi et al., 2009). Anti-feedant and insect growth disruption activity of crude Z. officinale extractives have been already reported against the larvae of Spodoptera litura (Sahayaraj, 1998). Z. officinale oil or extract repels the Myzus persicae (Hori, 1999); Bemisia argentifolii (Zhang et al., 2004), Sitophilus zeamais (Ukeh et al., 2009). Z. officinale oil also has insect growth regulatory and antifeedant activity against larvae of the arctiid Spilosoma oblique (Agarwal et al., 2001).

Conclusions and Recommendations

Fumigants are being widely used management tactics

to rid of the insect pests related issues in stored products which also are responsible for ecological and medical issues due to persistent of toxicity in the environment as well as in the grains as carcinogenic and teratogenic. So, natural plant extracts are the alternatives for said issues. Control of *T. castaneum* with plant extracts is readily available, affordable, less toxic to mammals and safe to the environment. The results of present study indicated that *Z. officinale* is an effective natural plant for the management of *T. castaneum*.

Authors' Contributions

BA, MR and AMS conceptualized the study; BA and B recorded the data; AMS, MuhammadS and MDG statistical analyzed the data; MR, FA and MehranS wrote Introduction section of the manuscript; BA, B, FA and MehranS wrote methodology section of the manuscript; BA, MR and MDG wrote Results and Discussion section of the manuscript; BA edited the format of the graphs and Tables according to the format of this journal; AMS, MuhammadS and MDG reviewed the manuscript and gave suggestions and comments for its improvement. The final manuscript was ultimately perused, scrutinized and approved for final submission by all the authors.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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Atta *et al*.

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