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Activity of alkaloid compounds extract of tobacco on adults of the red flour beetles, *Tribolium castaneum*

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Abstract
The aim of this study was to see how effective natural extracts of tobacco (*Nicotiana tabacum*) were as botanical insecticides in controlling red flour beetles, *Tribolium castaneum*, as a safe alternative to synthetic insecticides. Blended tobacco leaves were extracted with ethyl alcohol (99%) solvent, as well as alkaloid compounds were extracted from ethanolic tobacco extract, and then bioassay experiments were conducted for both ethanolic and alkaloid compounds tobacco extracts on adults of *T. castaneum* as a contact poison using thin film residue and as a repellent agent, at concentrations of 20, 15, 10, & 5% for ethanolic extract and 6, 3, 1.5, and 0.75% for alkaliods extract. The mortality Adults and repellency percentage were calculated 24 hours after exposure. The LC50 values indicated that tobacco alkaloids extract exhibited the highest toxicity on adults of *T. castaneum* was 1.29%, while it was 13.24% for ethanolic extract. The toxicity index also indicated that the relative potency of the alkaloid extract was ten times that of the ethanolic extract. With regard to repellency activity, the two extracts of tobacco were strongly repellent to *T. castaneum* where the high concentrations of both of them maintained the highest class (V) of repellency over a period of 24 hours, with an advantage for the alkaloids extract, which is to obtain the highest repellency with lower concentrations than the ethanolic extract. In addition to the appearance of a nervous effect on insects during the experiment for alkaloid extract. Finally, tobacco extracts, especially alkaloids extract, are a good alternative synthetic insecticide for controlling flour beetles.

Keywords: tobacco, *Nicotiana tabacum*, leaf extract, alkaloids extract, *Tribolium castaneum*, botanical insecticides

Introduction

Many species of insects stored can be found in grocery stores at different times of the year. The bulk of these pests gain access to our homes through contaminated food. Every year, this problem results in the loss of millions of dollars in stored groceries and other supplies. If left unused and exposed, practically every food item in the kitchen or pantry will be infested by insects, which have the ability to multiply quickly and create numerous generations per year (Koehler, P. G. and Cha, 2003). Flour beetles are global insects that feed on a variety of stored foodstuffs (Popović *et al*., 2013) [24]. *Tribolium castaneum* is a dangerous insect pest that attacks stored products. It can live for 3 years and destroy 15% of the weight of seeds (Neethirajan *et al*., 2007; Singh, 2017) [21]. As a result, it is vital to protect stored items from insect attacks. To combat storage pests, insecticides and other fumigants are usually applied, but the problem is the accumulation of toxic residues on food products as well as the development of resistance to nearly all pesticides (Benhalima *et al*., 2004; Hafiz *et al*., 2018; Wakil *et al*., 2021) [3, 10, 37]. As a result of the foregoing, there is a demand for plants that can serve as viable replacements for currently employed insecticides because they contain a large number of bioactive chemicals (Rajashekar *et al*., 2012) [25]. Some plant molecules have been demonstrated to have insect toxicity similar to some chemical insecticides Some plant molecules have been demonstrated to have insect toxicity similar to some chemical insecticides against stored product insects. (Rajendran and Sriranjini, 2008) [26]. Botanical pesticides have been shown to have a variety of effects, including contact, fumigant, antifeedant, repellent, and lethal effects on a variety of stored grain pests. They also delay the developmental stages of stored grain insects, as well as inhibit the development of eggs and immature stages (; Liu *et al*., 2002; Sankari and P. Narayanasamy, 2007; Sule and Ahmed, 2009; Bueno-Marí, 2013 and Singh *et al*., 2021) [17, 27, 33, 4, 30]

Due to their easy accessibility, biodegradability, and protection against seed damage, botanicals are commonly utilize as stored grain bio preservatives (Ahmed and Grainge, 1986; Dwivedi and Garg, 2003) [1, 6]. Plant insecticides can be developed commercially and are safe and highly efficient alternatives to protect crops not only in the field but also post-harvest (Isman, 2006) [13]. Nicotine is a botanical pesticide, a poisonous ingredient found in *Nicotiana tabacum*. It's both a nerve and a contact toxin. Its route of action is demonstrated by its binding to receptors at nerve synapses. It is proven to be effective against many insects, such as sucking and piercing pests (Shimomura *et al*., 2006; Tomizawa *et al*., 1999) [29, 36].

Materials and Methods

In the laboratory of the Plant Protection Department, Faculty of Agriculture, Fayoum University, Egypt, this study was performed on adults of red flour beetles *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae).

Source and Culture of the Red Beetles

Tribolium castaneum, were obtained from a small market in Beni Suef, Egypt. The samples were placed in a large container and cultured on whole wheat flour mixed with 2% of dry yeast under laboratory conditions (Plant Protection Department, Faculty of Agriculture, Fayoum University, Egypt).

Extracts Preparation

Tobacco leaves *Nicotiana tabacum* Linnaeus (Solanaceae) used in this research are blended leaves (rolling tobacco) from Captain Black Virgina Company and were purchased from cigarette shops in Fayoum Governorate, Egypt.

According to the producing company (Captain Black Virginia), the tobacco stems are removed by hand and the tobacco leaves are aircured and darkfired in Kentucky.

Ethanol Extract: The tobacco was extracted by maceration with ethyl alcohol (99%) as the solvent for 48 hrs. In a flask, 10 gms of tobacco leaves were inserted, and 300 ml of ethanol was added. The flask was wrapped in aluminium foil and covered well, then left to extract for 48 hours at laboratory temperature (20 ± 2°C). The extract was filtered using Whatman filter paper No. 1 when the extraction period was completed. A rotary evaporator was used to concentrate the filtrate at a temperature of 45–40 °C. The resulting crude extract was kept at 4 °C in an airtight dark bottle. The extraction process was repeated many times.

Crude Alkaloids Compounds Extract: the crude ethanol extract was dissolved in 5 ml of ethyl alcohol and added to the extract alcohol (30 ml of sulfuric acid), then Wagner's test was performed on a portion of this solution sample. To check the presence of alkaloids, the test gave an eddish-brown precipitate (formed when 2 drops of reagent were added to the extract), after that, a suitable amount of NH_4OH is added at a concentration of 10% to achieve a pH of (9). The base solution was placed in a separating funnel, It was then added 10 ml of chloroform, shook several times, and allowed to separate into two layers.I too found the lower layer (containing the alkaloid compounds dissolved in chloroform) and the upper layer neglected and returned. The last procedure was performed three times, each time removing the lowest layer. The accumulated solution was dried in the oven at a temperature of 45–40 °C and. The dry material was kept in an opaque dark bottle in the refrigerator at 4 °C until it was needed. (Harbome, 1973) [12].

Bioassay of Contact Toxicity

Thin film residue was used to test the contact toxicity of *T. castaneum* adults. In a petri dish, the residue film was created (9 cm). Concentrations were (20, 15, 10, and 5%) for ethanolic tobacco and (6, 3, 1.5, and 0.75%) for alkaloids tobacco; all concentrations were diluted in acetone; three replicates of each concentration were carried out; and only acetone was used as a control. The dilutions were distributed uniformly across the whole surface of the petri dish. For each replicate, 20 adults were inserted into each petri-dish after the acetone solvent had evaporated for 10 minutes, leaving a thin film on the dishes' floors.

After 24 hours of post-exposure, the mortality percentages for completely dead adults were counted, while the adults were considered alive (non-responsive) in the case of movement in the antenna or legs.

Bioassay of Repellency Activity

The area preference technique is used by (Tapondjou *et al*., 2005) [35] to determine repellency. 7 cm Whatman filter paper disc No. 1 cut in half. The acetone was used to preparing successive concentrations of tobacco ethanol extract 20, 15, 10, and 5% and 6, 3, 1.5 & 0.75 % for tobacco alkaloids extract. Half of the filter paper discs (the tested half) were handled with 0.7 ml of acetonic solutions, while using 0.7 ml of acetone only for the other half of the filter paper discs (un tested half) as control. For ten minutes, the half's were left to dry. Half of the 9-cm-diameter glass Petri dish was covered with half-tested filter paper, while the other half was covered with half-untested filter paper. *T. castaneum* adults, both sexed, were placed in the centre of each Petri dish. Each concentration had three replicates, resulting in a rate of 60 adults per concentration. The experiment was conducted in a laboratory conditions (28 2°C). The number of adults on the two halves of paper discs were counted five times after 1, 4, 8, 12 and 24 hours from the start of the experiment to calculate the percentage repellency using the equation below.

$$\text{Percentage Repellency (PR)} = [NC\text{-}NT/\ NC+NT] \times 100$$

Adult No. is NC in the untested region, and Adult No. is NT in the tested area. The results were then divided into 0-V repellency classes, with Class 0 equaling 0.1, Class I = 0.1-20, Class II = 20.1-40, Class III = 40.1-60, Class IV = 60.1-80, and Class V = 80.1-100 percent, respectively (McGovern *et al*., 1977) [18].

Analyze Data

Probit analysis (Finney, D.J., 1971) was used to compute lethal concentrations (LC50 and LC90) with their 95% fiducial limits by the probit analysis of a computer programme (Lpd line).
Toxicity indexes and relative potency were calculated according to (Sun, 1950) Equations:

Toxicity Index= [LC50 of the standard material / LC50 of tested material] × 100

Relative Potency = LC50 of lowest toxic material / LC50 of tested material

Using the SPSS programme software version (21), ANOVA (one-way analysis of variance) and the Duncan test for significant differences post hoc tests were performed on the data of repellence activity to determine significant (P 0.05) differences across concentrations.

Results and Discussions

The yield of solvent ethanol extract from tobacco leaves is 7.86% w/w and the alkaloid compounds extracted from ethanol extract is 36.36% w/w.

Tobacco Bioassay for Contact Toxicity

Table 1 data shows the mortality percentages of T. castaneum adults after being exposed to various doses of botanical extracts (*N. tabacum*) for 24 hours using contact toxicity (thin film residue). *Nicotiana tabacum* has a variety of mode of action. It's possible that it's a nerve toxin (Suiter *et al*., 2008) [32], It is also a stomach poison and has a repellent effect (Narayanasamy, 2002).
Adult mortality was found to be 68.33% in tobacco ethanolic extract at the highest concentration (20%), and the lowest mortality (15%) was found at a 5% concentration. (Sarker and Lim, 2018) [28] Nicotiana tabacum was shown to have the highest mortality (92.0%) against first instar Oriental fruit moth larvae *Grapholita molesta* (Lepidoptera: Tortricidae) at a dose of 2 mg/ml (20%), also highly toxic towards adults and causing reduced oviposition. In field cages and open fields, water extracts of *N. tabacum* efficiently controlled the cabbage aphid, Brevicoryne brassicae, and the diamondback moth, Plutella xylostellain. Moreover, it is equivalent to the recommended synthetic pesticides, and even outperforms them, and it is also safer for predators (Amoabeng *et al*., 2013) [2]. The aqueous extract of *N. tabacum* exhibited comparable efficacy to synthetic pesticides towards cowpea beetles, *Callosobruchus* spp (Kawuki *et al*., 2005) [15].
On the other hand, the highest percentage of mortality shown in tobacco alkaloids extract was 96.67% at a concentration of 6%, while 36.67 % of mortality was observed at a concentration of 0.75 %. With increasing concentrations of both extracts, the percentage of mortality in *T. castaneum* was increased. There was no observed mortality in the control groups.
The toxicity index table (Table 2) and Figure 1 show that after 24 hours of exposure, the lethal concentration LC50 for tobacco ethanolic and tobacco alkaloids extracts against adults of *T. castaneum* was 13.24 and 1.29 %, respectively, and 42.85 and 5.79 % for LC90. It was also obvious that the alkaloid extract had 10 times the relative potency of the ethanolic extract. (Febriyanti *et al*., 2013) [8] refered that nicotine is a strong and safe insecticide against the Carambola fruit fly, *Bactrocera carambolae*.
In comparing the slopes of dose-effect lines, ethanolic extract has the maximum slope of 2.51, with a slight and non-significant increase over the alkaline extract, whose slope value is 1.97 (Table 2). The high slope means that the least increase in botanical extract dose will lead to high mortality. While the steep slope enhances toxicity, it also encourages the rapid evolution of resistance. As a result, the alkaline extract becomes more stable. Tobacco leaf extracts, using different solvents, proved highly effective as adulticides on rice weevils, *Sitophilus oryzae*, especially chloroform and acetone extracts, this could be due to the fact that the majority of the compounds in these two solvents are alkaloids, which could reflect their efficiency (Kanmani *et al*., 2021) [14]. (Nenaah, 2011) [22] found that glycoalkaloids extracted from *Solanum tuberosum* and *Lycopersicon esculentum* are poisonous to both *Tribolium castaneum* and *Sitophilus oryzae*.

Table 1: Mean replicates of the mortality percentages for different concentrations of tobacco ethanolic and tobacco alkaloids extracts towards the red flour beetles, *T. castaneum* after 24 hours exposure.

Tobacco ethanolic extract	
20	68.33 ± 1.67 a
15	55 ± 5 a
10	36.67 ± 6 b
5	15 ± 2.89 c
Control	0
Duncan test "F"	29.68**
Tobacco alkaloids extract	
6	96.67± 3.33 a
3	66.67 ± 4.41 b
1.5	53.33± 1.67 c

0.75	36.67 ± 1.67 d
Control	0
Duncan test "F"	102.63**

Table 2: Toxicity of tobacco ethanolic and tobacco alkaloids extracts against to adults of *T. castaneum* using thin film residue after 24 hour post –exposure

Extracts	**$\chi 2$**	**Slope**	**LC_{50} ppm**	**confidence limits 95%**		**LC_{90} ppm**	**confidence limits 95%**		**Toxicity index**	**Relative potency**
				Lower ppm	**Upper ppm**		**Lower ppm**	**Upper ppm**		
Tobacco alkaloids	6.47	1.97 ± 0.28	1.29	-	-	5.79	-	-	100	10.26
Tobacco ethanolic	0.091	2.51 ± 0.41	13.24	11.34	15.87	42.85	30.49	80.78	9.74	1

Table 3: Percentage repellency (PR) of tobacco ethanolic extract against adult of *T. castaneum* at different exposure times during one day.

Tobacco ethanolic extract										
Conc	1 Hour		4 Hours		8 Hour		12 Hour		24 Hour	
	% PR	Class	% PR	Class	% PR	Class	% PR	Class	% PR	Class
20	100 ± 0 a	V	100 ± 0 a	V	96.67 ± 3.33 a	V	100 ± 0 a	V	93.33 ± 6.67 a	V
15	93.33 ± 3.33 a	V	96.67 ± 3.33 a	V	96.67 ± 3.33 a	V	93.33 ± 6.67 a	V	90 ± 0 a	V
10	90 ± 0 a	V	90 ± 0 a	V	90 ± 0 a	V	100 ± 0 a	V	73.33 ± 12.02 ab	IV
5	76.67 ± 13.33 a	IV	86.67 ± 8.82 a	V	90 ± 5.77 a	V	100 ± 0 a	V	56.67 ± 13.33 b	III
Duncan test "F"	2.039		1.67		1.067		1.00		3.11*	

Table 4: Percentage repellency (PR) of tobacco alkaloids extract against adult of *T. castaneum* at different exposure times during one day.

Tobacco alkaloids extract									
Conc	1 Hour			4 Hours			8 Hours		
	% PR	Class	% unconscious	% PR	Class	%unconscious	% PR	Class	% unconscious
6	100 ± 0 a	V	65 ± 0	100 ± 0 a	V	80 ± 5.0	34.26 ± 16.69 b	II	51.67 ± 6.0
3	100 ± 0 a	V	41.67 ± 6.26	92.59 ± 3.71 ab	V	13.33 ± 3.33	82.98 ± 3.52 a	V	
1.5	73.33 ± 3.33 b	IV		73.33 ± 14. 53 b	IV		80 ± 10 a	IV	
0.75	73.33 ± 3.33 b	IV		70 ± 0 b	IV		76.67 ± 6.67 a	IV	
Duncan test "F"	42.67**			3.79*			4.84*		

Conc	12 Hours			24 Hours		
	% PR	Class	% unconscious	% PR	Class	% Total mortality
6	83.82 ± 8.09 ab	V	15 ± 2.89	100 ± 0 a	V	13.33 ± 1.67
3	90 ± 10 a	V		86.67 ± 6.67 ab	V	
1.5	80 ± 10 ab	IV		56.08 ± 16.08 bc	III	
0.75	53.33 ± 8.82 b	III		36.67 ± 8.82 c	II	
Duncan test "F"	3.046*			8.69**		

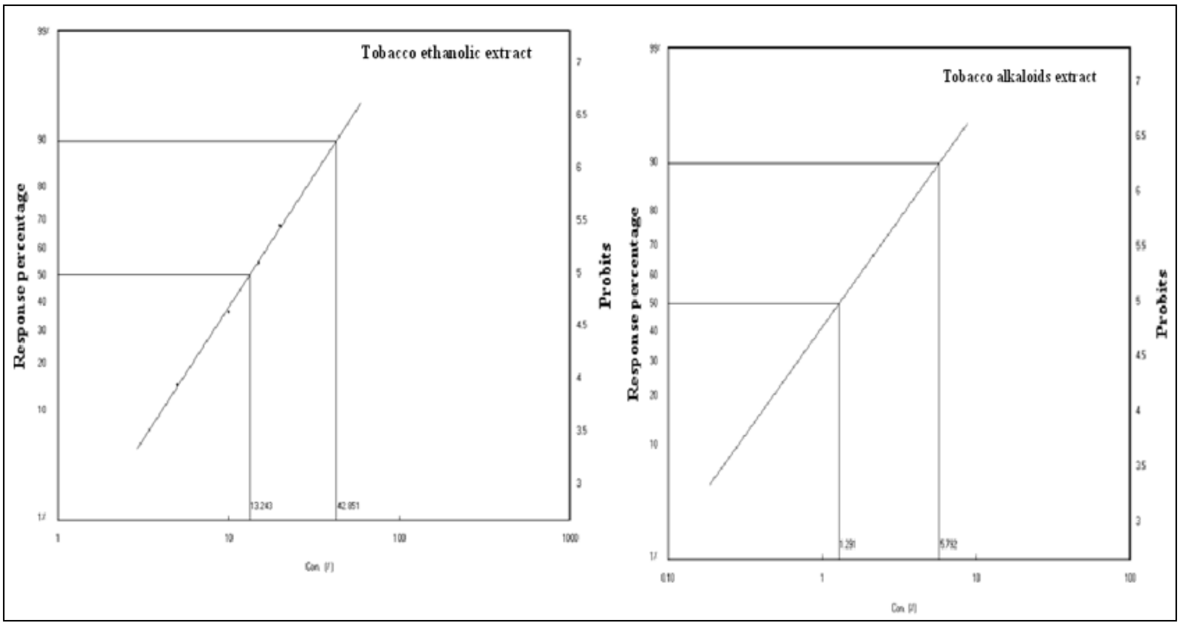

Fig 1: Toxicity of tobacco ethanolic extract and tobacco alkaloids extracts against to adults of *T. castaneum* using thin film residue after 24 hour post –exposure.

Repellent Activity

The two extracts of tobacco were strongly repellent to *T. castaneum*. Various concentrations of both extracts caused different responses in (Table 3 & 4). The concentration applied had a substantial impact on the repellence action which increased when adults were exposed to high doses.

Repellent Activity of Tobacco Ethanolic Extract

The various responses of *T. castaneum* adults are shown in Table 3 for the tobacco ethanolic extract during five times of exposure at four-hour intervals. PR values ranged from 100 to 56.67 % for all concentrations and time periods studied.

During the four exposure periods, the 20% concentration produced the highest percentage of repellence towards *T. castaneum* adults, with 100% after one hour of exposure and 93.33 % after 24 hours, keeping the highest percentage of repellence (class, V). Following that, a concentration of 3% in the same PR class V with no significant difference from a 20% concentration, with a PR range of 96.67 to 90%. On the other hand, after one hour of exposure, the percentage of repellence of ethanolic tobacco extract at a concentration of 10% was 90%, and after one day of exposure, it was 73.33%, putting it in second place (class IV). The 5% concentration was in the last PR class, as its repellence declined sharply from 93.33 to 56.67 % after 24 hours, putting it in the third category (class III). The highest significant F-test value that was recorded at 24 hours was 3.11, followed by 2.039 after 1 hour of the test. Our results are in agreement with his findings (Hanif *et al*., 2016) [11]. They noted that tobacco extracts are more toxic and effective than bitterapple and neem against *Tribolium castaneum*, with mortality percentages of 54, 56, and 54 after 24 hours at different concentrations of 5%, 10%, and 15%, and a good repellency with mean of repellency 23.89% after 24 hours.

Repellent Activity of Tobacco Alkaloids Extract

The adults of *T. castanaeum* recorded strong responses to different concentrations of 6, 3, 1.5 and 0.75 % of the tobacco alkaloid compounds extract. (Chowański *et al*., 2016) [5] It has been confirmed and proved that alkaloids extracted from Solanaceae can affect insects at all levels of physiological processes by changing redox equations, neuronal signalling, propagation, hormonal regulation, and causing toxic and repellent effects.

The alkaloids compounds of the tobacco in some high concentrations exceeded the effect of repellency, as shown in Table 4, high percentages of adults appeared in a state of doze (unconscious) for a long periods that ended with mortality in the higher concentration 6%, which indicates the strong effect of the alkaloid extract on the nervous system of insects, unlike the alcoholic extract completely. It gives an impression of the good effects of the alkaline extract when used as a fumigant.

The PR value of 100% is the highest class V recorded by the 6% concentration after both time periods, 1 hour and 24 hours of exposure, followed by a concentration of 3% in the same class (V), recording a repellency percentage that ranged from 100% after 1 hour to 86.67% after 24 hours under class V of repellency, but with a slight significant difference from the 6% concentration.

1.5 % of tobacco alkaloids extract recorded a medium repellency percentage that started at 73.33 percent, placing it in the fourth category (IV) in terms of repellence efficiency, and remained constant over three exposure periods of 1, 4, 8, and 12 hours, while the repellence percentage dropped to 56.08 % after 24 hours, falling to the third class (III).

On the other hand, the concentration of 0.75% recorded the lowest percentage of repellency after 1 hour of exposure with a percentage of 73.33 and it decreased sharply during the successive periods until the percentage of repellence reached 36.67% with high a significant difference from the highest concentration of 6%, occupying the second class (II).

It is worth noting that the highest significant value of the F-test was 42.67 after 1 hour of exposure and 8.69 after 24 hours.

The adults unconscious, at the first inspection (after 1 hour) for the repellent activity experiment of tobacco alkaloid extract, a big number of adults were found near death in the petri dish centre, their legs and antennae not moving.. This state results from the acute activity of alkaloids in the beetles, (Wickham *et al*., 1974) [38] noticed this situation and labelled it as such. Knockdown is a state of poisoning and partial palsy that frequently occurs before mortality.

They were left in place and were recorded and followed up carefully as shown in Table 4. This was observed only in 6 and 3% concentrations. After one hour of the experiment, the percentage of adults unconscious was 65 and 41.67% for both concentrations, respectively. After 4 hours, the percentage of unconscious adults increased to 80% at a 6% concentration, while the number decreased to 13.33 at a 3% concentration. After 8 hours of treatment, there were no unconscious insects at 3% concentration, while the number decreased at 6% concentration to 51.67% and then to 15% after 12 hours, at the end of the treatment, overall mortality for adults of *T. castaneum* was 13.33% at a concentration of 6%. It has the potential to be a nerve poison. Nicotine, an alkaloid compound extracted from tobacco leaves, is joined by two other compounds, nornicotine and anabasine, which operate as synaptic poisons that simulate chemical signals (acetylcholine) and it can cause spasm, paralyzed and death quickly in insects (El-Wakeil, 2013). Nicotine suppresses the action of acetylcholinesterase, which regulates synaptic transmission, resulting in a neurological system that is out of balance, synaptic blockage, also central nerve collapse and mortality. Their mode of action Similar to organophosphates and carbamate insecticides (Niño *et al*., 2006 and Mohamed *et al*., 2017) [23, 17].

Conclusion

It was concluded that all concentrations of both tobacco extracts were shown to be very effective as toxicants and repellents against the red flour beetle, *Tribolum castaneum*. Tobacco alkaloid extract as contact poison is better than tobacco ethanolic extract; the LC50 was 1.29%, while it was 13.24% for the ethanolic extract, and the relative potency was ten times that of the ethanolic extract. In contrast, the repellency activity of both extracts was strong over a full day, but tobacco alkaloids extract is more effective with a low concentration than ethanolic, and it also showed an effect on the nervous system, where insects appeared in a state of unconsciousness (knockdown) during the repellent experiment in the highest concentrations of 6 and 3%. Finally, synthetic insecticides can be replaced with botanical insecticides in the control of the red flour beetle, *T. castaneum*.

Recommendation

It is recommended to use tobacco alkaloid compounds extract in the control of red flour beetles, *Tribolum castaneum*, in stores and silos.

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