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Repellent Activity of Extracts of Black Pepper, Black Seeds, Garlic and White Cumin Against Red Flour Beetle

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Abstract

Many insects are pests of standing crops and stored grains that cause lot of damage and economic losses every year. Commonly these are controlled by synthetic compounds that are unsafe for humans and environment. Certain parts of plants are used for their repellent activity against insect pests of stored grains. In this context, the present study was planned to investigate the repellent activity of extracts of black pepper, black seeds, garlic and white cumin against red flour beetle that cause a huge damage to stored food crops. Repellent activity was tested using multiple concentrations of these extracts. Results of the present study concluded that 2 plant extracts have shown effective repellent activity against red flour beetle. The most effective extract was garlic followed by black pepper in various concentrations. Both were more effective at 1.5 % concentration. It is concluded in this study that these plants are natural sources of repellent compounds and hence are a potential source of biological insect repellents.

Keywords: insect repellency; plants extracts; red flour beetle.

1. Introduction

Insect pests cause huge loss of stored grains, crop wastage and these also affect plants and trees causing significant economic losses [1, 2]. Common control measures include use of synthetic chemical compounds to deal with the pests. These chemical compounds may have their own harmful effects on the crops, crop storage and also on human health and environment. There are methods of biological control showing their own benefits like reduced losses, minimizes bad effects on crops and storage and less harmful for human health [3, 4]. Several plants and their various parts are historically used for repellency against insect pests of food crops and storage [5]. Extract of black pepper (*Piper nigrum*) are used for their insecticidal and larvicidal properties against mosquito larvae and many other insects [6, 7].

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Black seeds (*Nigella sativa*) is considered to be a miracle seed and are used in many traditional medicines in South East Asia. Their insecticidal potential is also tested and it was concluded that these have insecticidal properties [8]. White cumin (*Cuminum cyminum*) is also evaluated for its insect repellent properties and found to be effective both in air and in solution against *Sitophilus zeamais* [9].

Insect pests cause huge losses of crops and stored food material. Red flour beetle (*Tribolium castaneum*) is a common insect pest of stored food crops and a model organism for food safety studies. This inset is a common pest of stored foods causing huge losses [10]. It is one of the most important pest of legumes, corn and rice grain storage. Chemical based insecticides are used to deal with the pests which pose a risk to human health and environment [11].

Several plants and their parts are historically used by the farmers to protect their crops and stored food materials which demonstrate that these compounds might be tested for their efficacy as repellents [12].

The present study was planned in the context above to estimate and compare repellent activity of four plant extracts (black pepper, black seeds, white cumin and garlic) against red flour beetle.

2. Materials and Methods

2.1 Insect collection and identification

The insects (red flour beetles) were isolated from rice brought from a local shop. Animals were kept in rice grains at room temperature for the study period.

2.2 Plant Collection

Black pepper, black seeds, white cumin and ginger were purchased from local vendor in Badian Road, Lahore.

2.3 Extract preparation

Plant extracts were prepared using methods reported by Parekh and his colleagues 2005 and Onyeagba and his colleagues 2004 [13, 14]. Methodology was optimized to our lab conditions.

The plant materials (black pepper, black seeds, white cumin and garlic) were washed with water to remove any contamination. Then these were air dried. Two types of extracts were prepared; aqueous extract was prepared with the help of water and ethanolic extract with the using ethanol.

- a. Aqueous extract: Following method was used to prepare aqueous extract: Ten grams of crushed plant part was added in 100 ml of distilled water. This material was treated with slow heat for six hours. It was filtered through 8 layers of muslin cloth every 2 hours. and centrifuged at 5000 rpm for 15 minutes. and supernatant was collected. After 6 hours (3 centrifugations), supernatant was collected and concentrated to 1/4th of original volume. It was then autoclaved at 121°C (15 lb/in² pressure) and stored at 4°C.
- b. Ethanolic extract: Ten grams of crushed plant part was kept in 100 ml of ethanol for 24 hours. Thereafter, it was

filtered through 8 layers of muslin cloth and centrifuged at 5000 rpm for 15 minutes. The supernatant was collected. Then the solvent was evaporated to make the final volume $1/4^{\text{th}}$ of the original volume and stored in an air-tight bottle at 4°C.

2.4 Repellent Material Preparation

Repellency testing

For repellency testing filter paper disks were prepared following the method of MsDonald and his colleagues 1970 [15]. Whatman filter paper disks (9 cm in diameter) were prepared and divided into two halves. Extracts (1 ml) of specific concentrations were uniformly applied on the half disks and then air dried at room temperature. Other half was left blank. Both halves were joined with cellotape. The disks were placed in petri dishes. Then 5 insects were released on the strips carefully. Insects were free to move on both halves. Repellent behavior was observed after every hour for five (5) hours.

3. Results

Results showed that different plant materials demonstrate different repellent activity at various concentrations. Aqueous and ethanolic extracts proved to be acting in similar ways in repelling these insects.

Table 1: Repellency shown by red flour beetle by 0.5% concentration of aqueous and ethanolic extracts of blackpepper (Aq = aqueous, Et = ethanolic).

Extract % of	Nun	umber of insects repelled								
Original Extract	1hr		2hrs	1	3hrs	3hrs			5hrs	
Extract Solvent	Aq	Et	Aq	Et	Aq	Et	Aq	Et	Aq	Et
0.5%	2	2	2	4	1	3	1	2	2	1
0.5%	2	0	3	4	2	2	2	3	2	2
0.5%	2	2	3	3	2	2	2	3	1	3

Data in Table 1 shows that aqueous extract of clove at 0.5% concentration did not prove to be very effective in repelling red flour beetle. Insects kept moving away and revisiting the disk. Observations till 5th hours had shown the same result. Ethanolic extracts have shown similar result. Statistically significant difference were not found among aqueous and ethanolic extracts.

Table 2: Repellency shown by red flour beetle at 1 % concentration of aqueous and ethanolic extracts of blackpepper (Aq = aqueous, Et = ethanolic).

Extract % of	Nun	umber of insects repelled										
Original Extract	1hr		2hrs	;	3hrs		3hrs		4hrs		5hrs	
Extract Solvent	Aq	Et	Aq	Et	Aq	Et	Aq	Et	Aq	Et		
1%	1	1	3	3	1	1	2	3	3	3		
1%	3	3	3	2	2	2	3	3	3	1		
1%	2	0	2	3	3	3	3	4	2	3		

Table 2 shows that at 1 % concentration of the black pepper showed the similar results as of its 0.5%

concentration. Insects kept visiting the area but visits were less frequesnt than it was with of 0.5% concentration.

Table 3: Repellency shown by red flour beetle by 1.5 % dose of aqueous and ethanolic extracts of black pepper(Aq = aqueous, Et = ethanolic).

Extract % of	Num	Number of insects repelled								
	1hr		2hrs		3hrs		4hrs		5hrs	
Original Extract										
Extract Solvent	Aq	Et	Aq Et		Aq	Et	Aq	Et	Aq	Et
1.5%	3	2	3	3	3	2	3	3	3	3
1.5%	3	3	3	2	3	4	3	4	4	4
1.5%	3	3	3	4	3	3	2	4	3	4

Table 3 shows that with 1.5% concentration, more insects were repelled in comparison to 0.5% and 1% concentrations and much less revisits were seen. Statistically significant difference was not found between aqueous and ethanolic extracts.

Table 4: Repellency shown by red flour beetle at 0.5% concentration of aqueous and ethanolic extracts of blackseeds (Aq = aqueous, Et = ethanolic).

Extract % of	Nun	Number of insects repelled								
	1hr		2hrs	5	3hrs	5	4hrs	5	5hrs	5
Original										
Extract Solvent	Aq	Et	Aq	Et	Aq	Et	Aq	Et	Aq	Et
0.5%	0	0	0	0	0	0	0	0	0	0
0.5%	0	1	0	0	0	0	0	0	0	2
0.5%	0	0	0	0	0	0	0	0	0	0

Table 4 shows that extract of black seeds did not show repellent activity or negligible repellency at 0.5% concentration. Both aqueous and ethanolic extracts showed similar results.

Table 5: Repellency shown by red flour beetle at 1% concentration of aqueous and ethanolic extracts of blackseeds (Aq = aqueous, Et = ethanolic).

Extract % of	Nun	Number of insects repelled								
	1hr		2hrs	5	3hrs	5	4hrs	5	5hrs	
Original										
Extract Solvent	Aq	Et	Aq	Et	Aq	Et	Aq	Et	Aq	Et
1%	1	1	0	0	0	1	1	2	0	0
1%	0	2	0	0	0	0	0	1	0	3
1%	0	3	0	0	0	0	2	2	0	3

Table 5 shows that extract of black seeds did not show effective repellent power at 1% concentration. No significant difference was found between aqueous and ethanolic extracts.

Table 6: Repellency shown by red flour beetle at 1.5 % concentration of aqueous and ethanolic extracts of blackseeds (Aq = aqueous, Et = ethanolic).

Extract % of	Num	Number of insects repelled								
	1hr		2hrs		3hrs		4hrs		5hrs	
Original										
Extract Solvent	Aq	Et	Aq Et		Aq	Et	Aq	Et	Aq	Et
1.5%	2	2	0	0	0	0	2	2	0	4
1.5%	2	2	0	2	1	2	0	0	1	4
1.5%	1	3	0	3	0	3	1	3	1	5

Table 6 demonstrates that repellency by 1.5 % concentration of ethanolic extract of black seeds shows effective repellent activity after 5 hours of exposure. Aqueous extract however, did not show effective repellency.

Table 7: Repellency shown by red flour beetle due to different doses of aqueous and ethanolic extracts of garlic(Aq = aqueous, Et = ethanolic).

Extract % of	Nun	Number of insects repelled								
	1hr		2hrs	5	3hrs	5	4hrs	;	5hrs	1
Original										
Extract Solvent	Aq	Et	Aq	Et	Aq	Et	Aq	Et	Aq	Et
0.5%	2	0	1	2	2	2	3	2	2	2
0.5%	1	0	2	2	2	1	1	1	2	1
0.5%	0	0	1	3	3	1	3	2	2	2

Table 7 demonstrates repellency by 0.5 % concentration of garlic extract was not very effective in repelling insects. Insects kept revisiting the disk.

Table 8: Repellency shown by red flour beetle due to different doses of aqueous and ethanolic extracts of garlic

(Aq = aqueous, Et = ethanolic).

Extract % of	Nun	Number of insects repelled								
	1hr		2hrs	5	3hrs	5	4hrs	5	5hrs	;
Original										
Extract Solvent	Aq	Et	Aq	Et	Aq	Et	Aq	Et	Aq	Et
1%	1	1	3	2	2	1	2	2	3	3
1%	3	1	3	2	3	1	3	3	3	4
1%	3	1	3	2	3	3	2	4	3	4

Table 8 shows the repellency by 1 % concentration of garlic extract repelled the insects quite efficiently. Ethanolic extract was proved to be more effective against insects.

Table 9: Repellency shown by red flour beetle due to different doses of aqueous and ethanolic extracts of garlic(Aq = aqueous, Et = ethanolic).

Extract % of	Num	umber of insects repelled								
Original	1hr		2hrs		3hrs		4hrs		5hrs	
Extract Solvent	Aq	Et	Et Aq Et		Aq	Et	Aq	Et	Aq	Et
1.5%	3	2	3	2	4	2	3	2	3	3
1.5%	4	2	4	2	4	2	4	4	4	4
1.5%	3	1	4	1	4	3	4	3	4	5

Table 9 shows the repellency 1.5 % concentration of garlic extract demonstrated good efficiency against red flour beetle. Few insects kept revisiting but at hour 5, most of these were repelled. Both extracts shown similar activity.

Table 10: Repellency shown by red flour beetle at 0.5 % concentrations of aqueous and ethanolic extracts ofwhite cumin (Aq = aqueous, Et = ethanolic).

Extract % of	Nun	lumber of insects repelled								
	1hr		2hrs	5	3hrs	;	4hrs	5	5hrs	;
Original										
Extract Solvent	Aq	Et	Aq	Et	Aq	Et	Aq	Et	Aq	Et
0.5%	0	0	0	2	0	2	0	2	0	2
0.5%	0	1	0	3	0	1	0	3	1	1
0.5%	0	0	0	1	0	1	0	3	0	1

Table 10 shows that repellency by 0.5 % concentration of white cumin extract was negligible. With ethanolic extracts, insects initially were repelled but they kept revisiting. Aqueous extract did not show repellent activity against these insects.

Table 11: Repellency shown by red flour beetle at 1 % concentration of aqueous and ethanolic extracts of whitecumin (Aq = aqueous, Et = ethanolic).

Extract % of	Nun	Number of insects repelled								
	1hr		2hrs	5	3hrs	5	4hrs	5	5hrs	5
Original										
Extract Solvent	Aq	Et	Aq	Et	Aq	Et	Aq	Et	Aq	Et
1%	1	1	0	2	1	1	2	2	0	2
1%	0	1	0	2	0	2	1	1	2	2
1%	0	3	0	3	0	3	2	2	1	2

Table 11 shows that 1 % concentration of white cumin extract shows similar repellency as that of 0.5%. There is only a minor decrease in revisits by the insects.

Table 12: Repellency shown by red flour beetle 1.5 % concentration of aqueous and ethanolic extracts of white
cumin (Aq = aqueous, $Et = ethanolic$).

Extract % of	Number of insects repelled									
	1hr		2hrs		3hrs		4hrs		5hrs	
Original										
Extract Solvent	Aq	Et	Aq	Et	Aq	Et	Aq	Et	Aq	Et
1.5%	2	2	0	4	0	3	2	2	0	4
1.5%	2	2	2	3	2	3	0	5	1	5
1.5%	1	3	0	3	0	3	2	3	3	4

Table 12 shows the repellency by ethanolic extract of 1.5 % concentration was high as in 5th hour, most of the insects were repelled however with aqueous extract, insect kept revisiting even after 5 hours of exposure.

4. Discussion

In this study plant extracts were used in different concentrations, it was observed that all of these showed good repellent activity against red flour beetle in higher concentration, i.e., 1.5% in this case. Lower concentrations also have shown repellent activity but it was of lesser magnitude. These results are similar to those reported from India in particular which reported that many plant extracts showed promising repellent activity against destructive insect pests [16]. It was also reported by the same paper that ethanolic extracts were effective in controlling insects which also coincide with our study as our study also showed effectivity of ethanolic extracts against insects. It is an expected resemblance as India and Pakistan share same geographical location and environment. Ahmad and his colleagues 2019 also showed that many plant extracts have strong insect repellency against red flour beetle [17]. Among all the extracts used in this study, most effective extract was of garlic followed by black pepper. Garlic has shown highest repellency in this study and effectively repelled insects after 5 hours of exposure. Both aqueous and ethanolic extracts of ginger have shown the similar effect. This result coincides with Ahmad and his colleagues 2019 [17] that also reported higher insecticidal activity by garlic and ginger. The paper reported that application of garlic and ginger extracts reduced the growth of the beetle in gram pulse and rice effectively. In our study, black pepper extract has shown higher repellent activity considering both aqueous and ethanolic extracts while ethanolic extract of garlic was more effective. Ethanolic extract proved to be stronger than aqueous extracts in general among the plant extracts tested in this study. Black seeds proved to be least effective extracts in all concentrations. These results are also showed that the aroma probably is also an important factor as it was observed that strongest aroma led towards strongest repellency. It is also reported in a review that aroma is an important factor in insect repellency and a stronger odour is related with strong repellent activity and ethanolic extracts work better than aqueous ones [18]. Overall, ethanolic extracts were stronger in their repellent activity in comparison to aqueous extracts in this study.

5. Conclusion

It was observed concluded that many plant extracts have potential repellent activity against red flour beetle that is a common pest of stored crops. Garlic and black pepper had shown better repellent activity while black seeds proved to be least effective. 1.5 % concentration was the most effective concentration. One percent concentration was also effective for garlic. Overall, these plant extracts showed potential repellency. These should be further explored as a potential source of safe biological insect repellents.

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