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Ismail Seham M
Department of Insect Population Toxicology, Central Agricultural Pesticide Laboratory, Agriculture Research Center, Dokki, Giza, Egypt

Sleem Fatma MA
Department of Insect Population Toxicology, Central Agricultural Pesticide Laboratory, Agriculture Research Center, Dokki, Giza, Egypt

Corresponding Author:
Sleem Fatma MA
Department of Insect Population Toxicology, Central Agricultural Pesticide Laboratory, Agriculture Research Center, Dokki, Giza, Egypt

Contact toxicity, repellent activity and residual effect of *Syzygium aromaticum* L. (Myrtaceae) and *Piper nigrum* L. (Pipeaceae) powders against *Sitophilus oryzae* L. (Coleoptera: Curculionidae)

Ismail Seham M and Sleem Fatma MA

Abstract
Weevils are important pest of several stored food and their products. Reducing post harvest losses is a resource-efficient way of increasing food availability. The contact toxicity, repellent activity and residual effect of clove and pepper powders were evaluated against rice weevils. Results showed that there were significantly differences between clove and pepper. The percentage mortality in clove was higher than in pepper powder. Whereas the LD_{50} values were 7.67 and 12.83% after 24 hours for clove and pepper, respectively. Clove was more toxic than pepper against the rice weevils after 15 days of exposure. But pepper was more persistence than clove, LT_{50} values were 52.5 and135 days for clove and pepper, respectively. Moreover, Pepper was more repellency than clove. The percentage repellency values of pepper were fluctuated at all concentrations and times of exposure. It can conclude that, clove and black pepper powders can be used as natural protectants of stored grains to reduce the usage of the synthetic insecticides.

Keywords: Contact toxicity, plant powder, repellent activity, residual effect, sitophilus

Introduction
Wheat is the most important food crop of Egypt. It is an important winter cereal. Wheat is the leading source of protein in human foods, having higher protein content than maize or rice, the other major cereal grains. In terms of total production tonnages used for food, it is currently second to rice as the main human food crop and ahead of maize [1]. Insect pests cause heavy post-harvest losses to stored grain globally but the problem is more devastating in developing countries [2]. In Egypt, the annual loss in wheat due to stored insects is estimated as equivalent to half a million tons of which 12% is caused by the rice weevil alone [3, 4]. Sitophilus weevils are well-known insect pests of stored grains worldwide. Pesticides worth more than 30 billion US dollar are intentionally released into the global environment every year. A high proportion of these is highly toxic and has immediate adverse effects on human health, wildlife, local food sources such as cattle or fish, beneficial insects and biodiversity [5]. Therefore, discover and develop botanicals as crop protection agents that will be environment- friendly and no harm to non-target organisms. Use of plant products as insecticide is one of the important approaches to the management of stored grain pests and it has shown many benefits over the use of synthetic chemicals [6]. Plant materials have been used as natural protectants of stored grains [7-15]. Furthermore, because of their natural origin, are biodegradable and normally do not leave toxic residues on the food commodities after application [16]. Plants powders are safer, more affordable, and easy to follow and the farmers can do by themselves [5]. Therefore, this research aims to assess the efficacy of two organic plants powders in controlling rice weevils in storage to reduce the usage of the synthetic insecticides.

Materials and Methods
1. Experimental Insect
Adult insects of the sitophilus weevil, *Sitophilus oryzae*, were obtained from already infested maize grains from farmers' stored corn grains in El-Mahala El-Kobra, Gharbia Governorate, Egypt. The culture was maintained in rice grains as growth medium throughout the study [17]. The weevils were reared for about three generations. Three hundred adult weevils were introduced into plastic jars containing 400g of rice.

These plastic jars were then covered with a muslin cloth with the help of rubber bands to prevent insects escaping and to allow ventilation. For obtaining adult weevils of same age, the insects were allowed to oviposit for two weeks then they were removed. After emergence the new adults were transfer to other jar containing fresh grains [18] and unsexed weevils, 2-4 weeks old, were used in bioassays.

2. Preparation of plant powder

Locally purchased black pepper seeds (*Piper nigrum*) (Pipeaceae) and dried flower buds of clove (*Syzygium aromaticum*) (Myrtaceae) were used. The powdered samples were prepared by pulverizing them with the help of an electric blender and sieved to obtain fine powder. Powders were kept in polythene bags at room temperature and properly sealed to prevent quality loss.

3. Contact toxicity (Effect of the plant powders on mortality)

Before actual experiments ad-hoc trials for each powder were done to have a clear idea of the dose-mortality response. About 10 g of rice grains were weighed into Petri dishes 9 cm mixed with 0.05, 0.1, 0.25, 0.5, 1 and 2g (corresponding; 0.5, 1, 2.5, 5, 10 and 20 %) and 0.1, 0.25, 0.5, 1 and 2g (corresponding; 1, 2.5, 5, 10 and 20 %) of *S. aromaticum* and *P. nigrum* powders in separate, respectively. The Petri dish contents (treatments and rice grains) were shaken thoroughly to ensure uniform distribution of the botanical powders. Ten unsexed adults of *S. oryzae* were then carefully introduced into the contents and exposed to treatments and maintained in the dark at a room conditions. Control containing untreated rice grains and ten unsexed adults of *S. oryzae* were set up [19]. Insect mortality was recorded at 24, 48 and 72 hours after treatment and converted it to percentage of mortality using the formula (dead insects/total insects treated *100). Insects which did not react or move when were probed gently with a needle or when no antennal movements were observed were considered dead and were discarded. In the final test, 4 mortality points ranging from 16 to 100 % were chosen from the preliminary test at 2.5, 5, 10 and 20 %. No mortality in control was observed, so no correction was considered necessary. The experiment was performed with three replicates. The data were reported as the mean ± the standard error (SE). Data were analyzed using 2-way ANOVA. If ANOVA results were significant, Fishers Protected Least Significant Differences ($p<0.05$) were used to separate the means. The lethal dose 50 (LD_{50}) values, the confidence upper and lower limits (CL), regression equations, the slope, the standard error (SE) of the slope and chi-square (χ^2) values were calculated [20].

4. Repellent bioassay

Repellent bioassay of *S. oryzae* adults with choice chamber was studied using two glass bottles, 15 cm length of glass bottle, (7 cm in height and 4 cm in diameter of bottleneck, 8 cm in height and 6 cm diameter of bottom of the bottle). About 10 g of rice grains were weighed into one of them and were mixed with 0.05, 0.1, 0.5, 1 and 2g (corresponding to 0.5, 1, 5, 10 and 20 %) and 0.1, 0.25, 0.5, 1 and 2g (corresponding to 1, 2.5, 5, 10 and 20 %) of *S. aromaticum* and *P. nigrum* powders in separate, respectively. The glass bottles contents (plant powder and rice grains) were shaken thoroughly to ensure uniform distribution of the botanical powders, and were fixed at the position A. About 3g of rice were weighed into the other to decrease chances of mortality due to starvation where the adults were starved for 4 hours before using them in the experiment. It was fixed at the position B, as control. Twenty unsexed adults *S. oryzae* were then carefully introduced into the contents and exposed to treatments. After that, the two glass bottles' mouths, A and B, were attached horizontally together by using cello-tape according to Saljoqi *et al*. [21] with some modifications. Bioassay was carried out in the dark at room conditions. The treatments were replicated three times. After 1, 2, 3, 4, 5, 6, 12 and 24 hours of treatment, the number of adults which found in control and in both of bottlenecks was recorded and the Excess Proportion Index (EPI) and Percentage repellency (Pc) were calculated [22].

$$EPI = Nt-Nc/Nt+Nc \qquad Pc = [1- Nt/Nt+Nc] *100$$

Where:
Nt: The number of insects present in the treated
Nc: The number of insects present in the control
Pc: Percentage repellency (i.e. percentage of insects trapped in control test side)
EPI: Excessive Proportion Index

5. Residual effect

Residual effect of clove and black pepper powders was assessed to find out the period of time which they could protect rice grains against infestation by *S. oryzae*. In this study, the concentrations at 5% of clove and 10 % of pepper powders were used, whereas these concentrations caused about 90% of mortality after 72 hours of exposure [23]. Ten unsexed of *S. oryzae* were introduced into the Petri dish contents (treatments and rice grains at 5% of clove and 10 % of pepper powders) and were exposed to each concentration after 15, 30, 45, 60, 90 and 180 days separately. The number of dead insects was recorded at 72 hours after each exposure time, and then, the life and dead insects were removed. The treatments were replicated three times. Percentage mortality was calculated after each exposure time for each concentration.

Results and Discussion

Contact toxicity (Effect of the plant powders on mortality)

The contact toxicity of black pepper seeds and dried flower buds of clove powders as insecticide against rice weevil, *Sitophilus oryzae* was established through mortality test. The average mortality percentages of *S. oryzae* which exposed to four concentrations 2.5, 5, 10 and 20% of tested powders and the control groups after 24, 48 and 72 hours of exposure period were showed in table (1). Generally, there was significant difference of the mean mortality between clove and pepper powder, the percentage mortality in clove was higher than in black pepper powder. Whereas there was no significant different between 2.5% of clove powder and 10% of pepper powder, but as for between the concentrations at 20% of both of them there was no significant difference. Moreover, there were significant differences among remain concentrations. The mortality percentages were increased with increasing of the time and the exposure time. However, it was evident that, 0% mortality was observed within 24 and 48 hours of exposure at 2.5% black pepper powder and not effective even after 72 hours, it was about 20%. On the other hand, the percentage mortality in clove was 16.67 and 66.67% after 24 and 48 hours at the same concentration. Noticeably,

in clove, 100% mortality was observed in all concentrations after 72 hours of exposure. The percentage mortality was about 33.33 and 83.33 at 5% after 24 and 48 hours, respectively. It was (56.67 and 90%) at 10 %, and (83.33 and 100%) at 20% after 24 and 48 hours of exposure, respectively. Fore black pepper powder, percentage mortality was about (6.67, 20 and 63.33%) at 5%, (33.33, 60 and 93%) at 10% and (76.67, 93.33 and 100%) at 20% after 24, 48 and 72 hours of exposure, respectively. Finally, it can be concluded on the basis of Table (2) that clove powder was more toxic than pepper powder. Whereas the LD_{50} values were 7.67 and 12.83% after 24 hours and 1.78 and 8.4% after 48 hours for clove and pepper powder, respectively, while the LD_{50} was 4.2% after 72 hours for pepper powder. Moreover, calculated χ^2 values in all the concentrations of plant powders tested were less than of table value 5.99 at probability (0.05), and degrees of freedom (n-2) suggesting that the adult population was homogenous.

Table 1: The average percentage mortality of *S. oryzae* exposed to four concentrations of *Piper nigrum* and *Syzygium aromaticum* powders at three times, 24, 48 and 72 hours

Plant powder	Conc. (%)	% Mortality (Mean± S.E)				
		24 hrs.	48 hrs.	72 hrs.	Mean**	Mean*
CLOVE *Syzygium aromaticum*	0.0	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00	00.00 a	77.5 A
	2.5	16.67 ± 3.33	66.67 ± 3.33	100.0 ± 0.00	61.11 d	
	5	33.33 ± 18.56	83.33 ± 3.33	100.0 ± 0.00	72.22 e	
	10	56.67 ± 12.02	90.00 ± 5.78	100.0 ± 0.00	82.22 f	
	20	83.33 ± 8.82	100.0 ± 0.00	100.0 ± 0.00	94.44 h	
Pepper *Piper nigrum*	0.0	0.00 ± 0.00	0.00 ± 00.00	0.00±00.00	00.00 a	47.22 B
	2.5	0.00 ± 0.00	0.00 ± 0.00	20.00±5.77	6.66 b	
	5	6.67 ± 3.33	20.00 ± 5.77	63.33±6.67	30.00 c	
	10	33.3 ± 3.33	60.00 ± 5.77	93.33±3.33	62.22 d	
	20	76.67 ± 12.02	93.33 ± 3.33	100.0±0.00	90.00 g	

Values followed by the same letter are not significantly different according to the Fisher's LSD test (*p≤0.05) (**P<0.01)
L.S.D0.05 between clove and pepper = 7.03 L.S.D0.05 among 0, 2.5, 5, 10 and 20%= 3.32

Table 2: Probit analysis of mortality for *S. oryzae* adults exposed to four concentrations of *Piper nigrum* and *Syzygium aromaticum* powders for three times 24, 48 and 72 hours

Plant powder	Time (hour)	LD_{50} %95 confidence limit	Regression equation	Slope ±SE	Chi square χ^2
CLOVE ***Syzygium aromaticum***	24	7.67 (0.58-1.26)	Y=2.09x+3.15	2.09± 0.2	1.05NS
	48	1.78 (0.68-1.24)	Y=1.99x+4.55	1.99± 0.28	4.46NS
	72	--	--	---	--
Pepper ***Piper nigrum***	24	12.83 (0.61-1.63)	Y= 3.68x+0.92	3.68 ± 0.4	0.16NS
	48	8.4 (0.83-1.01)	Y= 3.85x+1.45	3.85 ± 0.4	0.14NS
	72	4.22 (-0.13-0.05)	Y= 3.65x+2.69	3.65 ± 0.3	0.21NS

The contact effect of plant powders may due to cause abrasion of insect cuticle and lead to water loss [24], which may cause stress and eventual death. While Ofuya and Dawodu [25] showed that there is a direct relationship between particle size of plant powders and insect mortality in treated grains. Fine particle size aids even distribution of powders on the surface of seeds and the walls of the storage container thus increasing their possibility of making contact with the insects and killing them. Other earlier reports with similar trends to this study have shown that the insecticidal effects of various plants parts and plant products on *S. zeamais* showed varying degrees of success. While Devi and Devi [26] screened various spices to find out their insecticidal potential and anti ovipositional properties against *S. oryzae*, a serious stored food grain pest. Bioefficacy of powders and hexane extracts were determined by assessing the toxicity, effect on F1 progeny, contact toxicity persistence and seed viability. Responses varied with spices, dosage and exposure time. Mace and pepper, at 1% level resulted total mortality by one week followed by nutmeg and clove with 100% mortality and cinnamon and star anise with 90% mortality at concentration 5%. Moreover, Duke *et al.* [27] revealed that black pepper is one of the species which contain piperine whereas the piperine is more toxic to houseflies than pyrethrin. A mix of 0.05% piperine and 0.01 pyrethrins is more toxic than 0.1% pyrethrin. Also, piperine is synergistically insecticidal to rice weevils and cowpea weevils. While Vijay kumar *et al.* [28] determined biological activities of spices namely turmeric, chilli, coriander, fennel seeds, black pepper, ginger, fenugreek garlic and cumin against *T. castaneum*. All spices showed significant effect on adult mortality. Toxic effect followed- black pepper> cumin> garlic> fennel seed> ginger> fenugreek> untreated control. While Ashouri and Shayesteh [29] mixed black pepper seed powder and red pepper fruit powder in 20 g of wheat as direct admixture at five different doses 0.15, 0.2, 0.27, 0.37 and 0.5% (w/w) for black pepper and 0.0, 0.5, 1.5, 3 and 5% for red pepper, to assess mortality. Treated grains were infested with 20 adults. 100% mortality of *S. granarius* at 0.5% in first five days and *R. dominica* at 5% showed 100% mortality after 16 days. But red pepper did not cause complete mortality after 14 days.

Repellent bioassay

Percentage repellent and EPI values of *S. oryzae* treated with clove and pepper powders were illustrated in Table (3). Excessive Proportion Index (EPI) values give an idea about the repellency or attractant of chemical substance against tested insects whereas positive and negative values indicate attractant and repellent activity, respectively. There were no significant different between clove and pepper powder, where both of them had repellent activity. While there were significant differences among all concentrations of clove and pepper powder except 0.5 and 5% of clove powder, there was no significant different between them. Moreover, clove powder showed both attractant and repellent activity. In general, repellent increased with increasing the concentration

of clove powder and exposure time. At lower concentrations, 0.5 and 1 %, of clove powder showed attractant effect where EPI values ranged from 0.63 to 0.13 indicating percentage of repellent ranged from 18 to 43%, respectively after four hours. After that, the percentage of repellent gradually increased to 55, 58 and 65% after 5, 6 and 12 hours, respectively at concentration 0.5%, then decreased to 60% after 24 hours of exposure. While at concentration 1%, the percentage of repellent was fluctuated. It was 53, 48, 68 and 52% after 5, 6, 12 and 24 hours of exposure. In addition to data also showed that the value of EPI at concentrations 5 and 10 % ranged from 0.37 to 0.0 indicating the percentage of repellency ranged from 32 to 50%, respectively after four hours. It is also noted that the weevils exposed to concentrations 5 and 10% showed the lowest repellent compared to those treated with concentrations 0.5 and 1% of clove powder after 5, 6, 12 and 24 hours of observation. On the other hand, the highest concentration 20% of the plant powder manifested the highest percentage of weevil's repellency which was 47, 42, 62, 68, 67 and 77 % indicating EPI values of 0.07, 0.17, -0.23, -0.37, -0.33 and -0.53 after 1, 2, 3, 4, 5 and 6 hours of exposure, respectively, then decreased to 70 and 68 % indicating EPI values of -0.4 and -0.37 after 12 and 24 hours of exposure, respectively. Tripathi *et al.* [30] revealed that, clove powder at 1.5g/50g dose showed 100% repellent against adult of *T. castaneum*. While Salvadores *et al.* [31] showed that powders of *P. nigrum*, *Cinnammomum annum* and *Cinnammommum zeylanicum* had a repellent effect on *S. zeamais*.

Table 3: Percentage repellency and EPI values of *Sitophilius oryzae* treated with different concentrations of the powders of *Syzygium aromaticum* and *Piper nigrum* for 1, 2, 3, 4, 5, 6, 12 and 24 hours

Plant powder	Conc. (%)	Time of exposure (hours)																M
		1		2		3		4		5		6		12		24		
		EPI	PC	EPI	PC	EPI	PC	EPI	PC	EPI	PC	EPI	PC	EPI	PC	EPI	PC	
CLOVE *Syzygium aromaticum*	0.5	0.5	25	0.4	30	0.37	32	0.27	37	-0.1	55	-0.17	58	-0.3	65	-0.2	60	45 g
	1	0.63	18	0.57	22	0.2	40	0.13	43	-0.07	53	0.03	48	-0.4	68	-0.03	52	41 i
	5	0.37	32	0.13	43	0.17	42	0	50	0.07	47	0.07	46	0.17	42	0.03	48	44 h
	10	0.27	37	0.03	48	0.13	43	0.27	37	0	50	0.07	47	0.07	47	-0.03	52	45 g
	20	0.07	47	0.17	42	-0.2	62	-0.37	68	-0.33	67	-0.53	77	-0.4	70	-0.37	68	63 c
Pepper *Piper nigrum*	1	-0.2	60	0.17	42	-0.17	58	0	50	-0.27	63	-0.3	65	-0.47	73	-0.6	80	61 d
	2.5	0.07	47	-0.3	65	0.07	47	-0.13	57	-0.03	52	-0.37	68	-0.03	52	-0.23	62	56 e
	5	-0.33	67	-0.27	63	0	50	-0.47	73	-0.53	77	-0.7	85	-0.6	80	-0.7	85	73 a
	10	0.07	47	0	50	-0.07	53	0.03	48	-0.13	57	-0.07	53	-0.07	53	-0.07	53	52 f
	20	0.03	48	-0.7	85	0.07	47	-0.37	68	-0.27	63	-0.47	73	-0.4	70	-0.3	65	65 b

PC=percentage of repellency, EPI= excess proportion index, M= mean

Values followed by the same letter are not significantly different according to the Fisher's LSD test (*p≤0.05)

Repellent percentage and EPI values of *S. oryzae* treated with pepper powder were showed in Table (3). Generally, the repellent percentage values were fluctuated at all concentrations and all time of exposure. There were significant differences among all concentrations of pepper powder, the concentration at 5% was the highest repellent followed by 20, 1, 2.5 and 10%. Noticeably, the percentage repellency was the highest values at 20% of pepper powder after 2 hours and at 5 % after 6 and 24 hours, it was 85% indicating EPI value of -0.7 followed by at 5% after 12 and 5 hours of exposure it was 80 and 77% indicating EPI values -0.6 and -0.53, respectively however after that, it decreased to 73% indicating EPI value of -0.47 at 5 and 20% after 4 and 6 hours of exposure, respectively. Moreover, the percentage repellency at 1% was 60% indicating EPI value of -0.2 after 1 hour, and then decreased to 42% indicating EPI value of 0.17 after 2 hours, after that, it increased to 58% indicating EPI value of -0.17 after 3 hours, and then decreased again to 50% after 4 hours. Then, it increased more significantly. It was 63, 65, 73 and 80% indicating EPI values of -0.27, -0.3, -0.47 and -0.6 after 5, 6, 12 and 24 hours of exposure, respectively. While the repellent percentage at 2.5% was 47% indicating EPI value of 0.07 after 1 and 3 hours, and increased to 65 and 68% indicating EPI values of -0.3 and -0.37 after 2 and 6 hours, respectively, after that, it decreased again to 52% after 12 hours of exposure, however after that, it increased to 62% after 24 hours of exposure. Meanwhile, the percentage repellency at the concentration of 10% increased statically, it was 47, 50 and 53% after 1, 2 and 3 hours, respectively. After that, it decreased to 48% then increased to 57% after 4 and 5 hours of exposure, respectively. After this time until 24 hours of exposure, it was static; it was 53% indicating EPI value -0.07. These are in agreement with Ntonifer and Monah [32] who found that entire or powdered fruits of *Piper* have insecticidal and repulsive effect against many pests. Also, this result justified by Shayeshteh and Ashouri [33] who tested four powdered spices, black pepper (*P. nigum*), chilli pepper (*C. annuum*), cinnamon (*C. aromaticum*) and turmeric (*Curcuma longa*) against three stored-product insects *Rhyzopertha dominica*, *S. granarius* and *T. castaneum*. Wheat treated with cinnamon powder were most repellent to adult *S. granarius* 92.5% after 1hr, chilli pepper treatment for *T. castaneum* 72.5% after 6hr and black pepper treatment for *R. dominica* 58.75% after 24 hr. while Upadhyay and Jaiswal [34] said that *P. nigrum* L. at 0.2% concentration against *T. castaneum* (Herbst) showed repellent in filter paper test.

Residual effect

Data in table (4) revealed that there was significant different between clove and pepper powders whereas, pepper powder had the highest longing residual effect extend up to 30 days caused 73.33% mortality. The prolongation of time to 45 days revealed less mortality, (63.67%) followed by 60, 56.67 and 43.33% after 60, 90 and 180 days of exposure, respectively. The result also showed that clove powder was more toxic than pepper powder against the adults of rice weevil extend to 15 days, whereas mortality percentage was (100 and 93.33%) after 3 days and (86.67 and 80%) after 15 days of exposure, respectively. Although clove powder was more toxic than pepper powder against the rice weevils after 15 days of exposure, but the pepper powder was the highest toxicity, it was more persistence. Mortality percentage was 53.33, 56.67,

43.33, 30 and 13.33% for clove powder, while 73.33, 63.67, 60, 56.67 and 43.33% for pepper powder after 30, 45, 60, 90 and 180 days, respectively. Moreover, LT_{50} values were 52.5 and 135 days for clove and pepper powders, respectively. In previous study of Saxena (2016) [23], who said that, extracts of black pepper and clove buds gave residual efficacy against the adults of *T. castaneum*. Clove and black pepper were effective up to 60 days of post treatment and gave 50 and 46.67% mortality, respectively. While Meena *et al.* [35] found that 0.5% dose of black pepper seed powder against *Rhyzopertha dominica* exhibited maximum adult mortality 65 and 29.75% after 48 hours adults were released at 40 and 60 days after treatment, respectively.

Table 4: The residual effect of *Piper nigrum* and *Syzygium aromaticum* powders against *S. oryzae* exposed to seven time initial, 15, 30, 45, 60, 90 and 180 days

Time after exposure (days)	Mortality percentage ± SE		Mean
	S. aromaticum	***P. nigrum***	
Initial	100±0.0	93.33±3.3	96.67 NS
15	86.67± 3.3	80.0±5.7	83.33 NS
30	53.33±12	73.33±3.3	63.33 NS
45	56.67±8.8	63.67±3.3	60.00 NS
60	43.33±3.3	60.0±5.8	51.67 NS
90	30.0±5.7	56.67±3.3	43.33 NS
180	13.33±3.3	43.33±6.6	28.33 NS
Mean**	383.33 **A**	470 **B**	426.67
LT_{50} **(days)**	52.5	135	

Values followed by the different letter are significantly different according to the Fisher's LSD test (**P<0.01)
L.S.D0.01 between clove and pepper = 9.46

Conclusion

This study shows that black pepper and clove powders possess contact toxicity and repellent activity against adults of *S. oryzae*. The pepper powder was the highest toxicity, it was more persistence. Both of them have high longing residual effect extend up to 60 days. LT_{50} values were 52.5 and 135 days for clove and pepper powders, respectively. Hence, black pepper powder could serve as an alternative wherewithal to synthetic insecticides in controlling *S. oryzae* infesting stored grain.

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