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Toxicity and Residual Effect of *Annona squamosa* L. and *Piper nigrum* L. Seeds Extracts against *Tribolium castaneum* and *Sitophilus oryzae*

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Abstract: ***Giving the importance of maintaining the inventory materials of the loss as a result of insect infestation, this research aims to evaluate the activity of Piper nigrum L. and Annona squamosa L. seeds extracts against Tribolium castaneum and Sitophilus oryzae. Bioassay tests were conducted by using the waved filter paper in plastic Petri dishes 9cm and residual film in glass Petri dishes 5 cm with four concentrations, 0.25, 0.5, 0.75 and 1% of P. nigrum petroleum ether extract and A. squamosa ethanol extract. LC_{50} values were 0.23 and 0.31% after 24 h of exposure of P. nigrum and A. squamosa, respectively against S. oryzae, and they were 0.16 and 0.35 % after 24 h, against T. castaneum. Moreover, P. nigrum was more repellency than A. squamosa against the both insects. Noticeably, the repellency effect increased with increasing the time of exposure of P. nigrum and A. squamosa against T. castaneum. While, it decreased after 10 h against S. oryzae. The A. squamosa extracts was more toxicity and persistence than P. nigrum extract with the prolongation of time to 30 days. Whereas, the mean mortality percentages were 80 and 51.67%, respectively. We can conclude that, Annona squamosa and Piper nigrum extracts will be used to control stored product insects.***

Keywords: *Piper nigrum*, *Annona squamosa*, *Tribolium castaneum*, *Sitophilus oryzae*, Repellency, Seed extracts

1. Introduction

The stored products are vulnerable to be attacked by many coleopteran insects as *Rhyzobertha dominica, Oryzaephilus surinamensis, Sitophilus spp.* [1]. *Sitophilus* oryzae (Linnaeus) is one of five primary insects caused the most damage to stored grains, *S. granarius*, *S. zeamaise*, *S. cerealella* and *R. dominica*. It can be found in all parts of the world. *T. castaneum* is a secondary pest, both adults and larvae surface feeder. They feed on dust or broken surfaces of grains [2]. There are negative impacts from infested the stored products by insects as the contamination by bodies fragments and toxins (methoxybenzoquinone, ethyl-1, 4-benzoquinone and methyl-1, 4-benzoquinone) [3]. These quinones caused skin irritation [4]. In addition to, loss of weight and quality [5]. Moreover, Fungal contamination and aflatoxins B1, B2 were detected in infested flour with *T. castaneum* [6].

Control of stored product insects depend strongly on the use of synthetic pesticides, which has led to several harmful impacts [7]. Nowadays, studies have been done for use eco-friendly materials as plant extracts. Where, the plant extracts were obtained by Egyptians and Persians since over 6000 B. C, by dry distillation [8]. *Piper nigrum* L. belongs to Piperaceae, has antioxidant and antimicrobial potentials [9], [10], insecticidal activity [11]. Also, Annonaceae has insecticidal activity [12]-[14] against *Tribolium castaneum* [15]. *Annona muricata* has an antimicrobial activity [16]-[18], nemato-toxic potential [17]. *Annona squamosa* showed anti-inflammatory, antioxidant and antidiabetic drugs [19], [20]. The present study aims to evaluate the activity of *P. nigrum* L. petroleum ether extract and *A. squamosa* L. ethanolic seeds extract against *T. castaneum* and *S. oryzae* and their residual effect to protect the stored grains.

2. Materials and Methods

2.1. Insect culture

Adults of *Tribolium castaneum* and *Sitophilus oryzae* were collected from infested cowpea seeds and wheat grains from local market and separately rearing under room conditions. Adults of *Tribolium castaneum (*Herbst) (Coleoptera: Tenebrioidae) was reared on crush cowpea seeds in organza bags as they are very easy to collect and handle. While, *S. oryzae* was reared on wheat grains in plastic jars sealed with muslin to prevent the escaping of insects. Mixed-sex adults were used at 1-2 weeks.

2.2. Seeds extraction

Piper nigrum L. seeds and *Annona squamosa* L. fruits were purchased from local market. Seeds of Anna fruits were washed and air-dried. One hundred gram of *Piper nigrum* and *Annona squamosa* seeds were fined to powder by using an electric blinder, separately. One hundred gram of *Piper nigrum* and *Annona squamosa* seeds were fined to powder by using an electric blinder, separately. One hundred and fifty ml of petroleum ether 99% and 150 ml of ethanol (99.9%) were added to 100g of *Piper nigrum* and *Annona squamosa* powder in conical flask coated with foil, separately. Two flasks were stirring occasionally to three weeks. then, the extracts were filtered. The cakes of each plant was re-extracted with the same solvent and steps. Without using heating, the solvents were evaporated from collected filtrates by exposing to air at room conditions [21]. 13.7 and 17 % (v/w) of *Piper nigrum* and *Annona squamosa* extracts were obtained. The stock solutions for two extracts were prepared in acetone (99.9%).

2.3. Determination of lethal concentrations of extracts

An ad-hoc bioassay was applied before the actual experiments to choose the concentrations caused from 20 to 100% mortality. Four concentrations, 0.25, 0.5, 0.75 and 1% were chosen. The residual film of one ml of each concentration was applied in 5 cm Petri dishes three times as replicates. One ml of acetone was used as control. After completely evaporation of acetone from treated and control, ten adults of *S. oryzae* and *T. castaneum* were released in Petri dishes [22]. The mortality percentages were recorded after 12, 24, 36 and 48 h of exposure. LC_{50} and LC_{90} values were calculated by using Ldp-Line software [23] using Probit analyses according to Finney, 1971 [24].

2.4. Evaluation of repellent efficacy of extracts

The repellent activity of *P. nigrum* and *A. squamosa* against *S. oryzae* and *T. castaneum* was studied by using waved filter paper as described McDonald et al, 1970 [25] modified with Sleem, 2020 [21]. Where, the filter paper was divided into two halves then waved. One of the treated with 0.5 ml acetone as control, and the other was treated with 0.5ml of tested plant extract. After completely evaporation of solvent, both of them were fixed in plastic Petri dish as completed circle. Ten adults of *S. oryzae* and *T. castaneum* were released on the center of waved filter paper. Numbers of tested insects in control were recorded and transformed to percentage (Nc) after 1, 2, 3, 4, 5, and 10 h, the percentages repellency (PR) were calculated, PR= (Nc-50) × 2, and assigned to 6 repellence classes from 0 to V [26].

2.5. Residual effect

Residual effect of *P. nigrum* and *A. squamosa* seeds extracts against *S. oryzae* and *T. castaneum* was estimated to study the persistence of tested plant extracts against tested insects. The concentration 0.75% of both of tested plant extract was used, whereas these concentrations caused above 90% of mortality after 72 hours of exposure [27]. Ten unsexed of *S. oryzae* and *T. castaneum* were released in Petri-dishes and were exposed after 15, and 30days replicated three times. The mortality percentages were calculated at 72 hours for each exposure time.

2.6. Statistical analysis

The ANOVA was performed to test the significant differences between treatments with statistical software SPSS computer program, ver.16.0. Means and their interaction were separated using Fisher's LSD test ($p \leq 0.05$).

3. Results and Discussion

3.1. Contact Toxicity

The mortality percentages of adults of *T. castaneum* exposed to four concentrations, 0.25, 0.5, 0.75 and 1 % of *P. nigrum* and *A. squamosa* seeds extracts at four times, 12, 24, 36 and 48 h were showed in Table (1). The results revealed that, there was significant different between *P. nigrum* and *A. squamosa*. Where, *P. nigrum* extract was more toxicant than *A. squamosa* extract. The mean mortality percentages were 85.58 and 77.71%, respectively. Moreover, there was no significant different among 0.75 and 1% of *P. nigrum* and 1% of *A. squamosa*, and between 0.75% of *P. nigrum* and 0.5% of *A. squamosa*. They were 97.5, 100 and 95.83%, and 85.83 and 90.83%, respectively. Noticeably, the concentration 0.5 % was the highest effect for both tested plant extracts after 36 h. It was 97 and 93% of *P. nigrum* and *A. squamosa*, respectively. While, 0.25% of *A. squamosa* was the lowest effect. Also, there was no significant between the effect of *P. nigrum* at 36 and 48 h after exposure to *P. nigrum* extract and 48 h after exposure to *A. squamosa* extract. There were 94, 96 and 98%, respectively. In generally, the mortality was increased with increasing of the time of exposure of both of tested plants extracts. LC_{50} values were 0.27 and 0.495 % after 12 h of exposure and 0.16 and 0.35 % after 24 h, while LC_{90} were 0.7 and 1.38 at 12 h and 0.44 and 1.04% at 24 h after exposure of *P. nigrum* and *A. squamosa*, respectively against *T. castaneum* Table (3).

Table 1: The mortality percentage of *T. castaneum* exposed to four concentrations of *P. nigrum* and *A. squamosa* seeds extracts at four times, 12, 24, 36 and 48 h

Plant extract	Conc. (%)	% Mortality (Mean± S. D)				Mean Plant*Conc.		
		12 h	24 h	36h	48 h			
P. nigrum	0.25	47±12 ^{h}	70±10 ^{ef}	80±20 ^{cd}	83±21 ^{bcd}	70 ^{c}		
	0.5	77±5.8 ^{de}	90±10 ^{b}	97±5.8 ^{a}	100±0.0 ^{a}	90.83 ^{b}		
	0.75	93±12 ^{a}	97±5.77 ^{a}	100±0.0 ^{a}	100±0.0 ^{a}	97.5 ^{a}		
	1	100±0.0 ^{a}	100±0.0 ^{a}	100±0.0 ^{a}	100±0.0 ^{a}	100 ^{a}		
Mean *P. nigrum**Time		79 ^{d}	89 ^{c}	94 ^{ab}	96 ^{a}	85.58^{A}		
A. squamosa	0.25	23±11.5 ^{i}	40±0.0 ^{h}	70±10 ^{e}	90±17 ^{b}	55.83 ^{d}		
	0.5	43.33±15 ^{h}	56.67±21^{g}	93±5.8 ^{a}	100±0.0 ^{a}	73.33 ^{c}		
	0.75	66.7±11.5 ^{f}	77±15 ^{d}	100±0.0 ^{a}	100±0.0 ^{a}	85.83^{b}		
	1	86.67±12 ^{bc}	96.67±5.7 ^{a}	100±0.0 ^{a}	100±0.0 ^{a}	95.83 ^{a}		
Mean *A. squamosa**Time		55 ^{f}	67 ^{e}	90 ^{bc}	98 ^{a}	77.71^{B}		
Control		00.00 ^{j}	00.00 ^{j}	00.00 ^{j}	00.00 ^{j}	00.00 ^{C}		
Mean Time		44.72 ^{d}	52.22 ^{c}	61.67 ^{b}	64.44 ^{a}	55.76		
Mean Conc. *Time	0.25%	23.33 ^{f}	36.67 ^{e}	50.0 ^{d}	57.78 ^{bc}	Mean Conc.	0.25	41.94 ^{d}
	0.5%	40.0 ^{e}	48.89 ^{d}	63.33 ^{a}	66.67 ^{a}		0.5	54.72 ^{c}
	0.75%	53.33 ^{cd}	57.78 ^{bc}	66.67 ^{a}	66.67 ^{a}		0.75	61.11 ^{b}
	1%	62.22 ^{ab}	65.56 ^{a}	66.67 ^{a}	66.67 ^{a}		1	65.28 ^{a}

Values followed by the same letter are not significantly different according to the Fisher's LSD test ($p \leq 0.05$)
L. S. $D_{0.05}$ among *P. nigrum*, *A. squamosa* and control = 2.33; L. S. $D_{0.05}$ among 12, 24, 36 and 48 h= 2.687;
L. S. $D_{0.05}$ among 0.25, 0.5, 0.75 and 1%= 2.687; L. S. $D_{0.05}$ between plant*Time= 4.65; L. S. $D_{0.05}$ between Conc. *Time= 5.37
L. S. $D_{0.05}$ among plant*Conc. = 4.65; L. S. $D_{0.05}$ among plant*Time*concentration= 9.3

Also, there was significant different between *P. nigrum* and *A. squamosa* seeds extracts against *Sitophilus oryzae*, where *P. nigrum* was more toxicant than *A. squamosa* extract Table (2). The mean mortality percentages were 86.67 and 78.34 %, respectively. Noticeably, *P. nigrum* and *A. squamosa* seeds extracts had the same effect against *S. oryzae*. Where, 0.75 and 1% of *P. nigrum* and 1% of *A. squamosa* had no significant different against *S. oryzae*. The mean mortality percentages were, 96.67, 100 and 99.17 %, respectively. The lowest effect was at 0.25% of *A. squamosa*. Also, there was no significant between the effect of *P. nigrum* at 36 and 48 h after exposure and 48 h after exposure of *A. squamosa*. They were 92.5, 95 and 92.5%, respectively. Moreover, there was no significant different between 0.5% of *P. nigrum* after 24 h and 0.5% of *A. Squamosa* after 48 h. The mean mortality percentages were 93 and 100%, respectively. The concentration at 0.5 % was the highest effect after 24 h followed by 0.75% after 12 h for both tested plant extract. LC_{50} values were 0.35 and 0.51 % after 12 h and 0.23 and 0.31% after 24 h, while LC_{90} were 0.72 and 0.9 at 12 h, and 0.49 and 0.65 % at 24 h after exposure of *P. nigrum* and *A. squamosa*, respectively against *S. oryzae* Table 3.

Table 2: The percentage mortality of *S. oryzae* exposed to four concentrations of *P. nigrum* and *A. squamosa* L. seeds extracts at four times, 12, 24, 36 and 48 h

Plant extract	Conc. (%)	% Mortality (Mean± S. D)				Mean Plant*Conc.		
		12 h	24 h	36h	48 h			
P. nigrum	0.25	26.67±5.7 ^{g}	53±15.3 ^{f}	70±20 ^{e}	80±10 ^{cd}	57.5 ^{d}		
	0.5	76.67±5.8 ^{de}	93±11.5 ^{a}	100±0.0 ^{a}	100±0.0 ^{a}	92.5 ^{b}		
	0.75	90±10.0 ^{b}	96.6±5.8 ^{a}	100±0.0 ^{a}	100±0.0 ^{a}	96.67 ^{a}		
	1	100±0.0 ^{a}	100±0.0 ^{a}	100±0.0 ^{a}	100±0.0 ^{a}	100.0 ^{a}		
*P. nigrum**Time		73.33 ^{d}	85.83 ^{b}	92.5 ^{a}	95 ^{a}	86.67 ^{A}		
A. squamosa	0.25	06.67±11.5 ^{h}	33.33±11.5 ^{g}	46.67±15 ^{f}	70±17.3 ^{e}	39.17 ^{e}		
	0.5	46.67±5.77 ^{f}	86.7±24.99 ^{bc}	100±0.0 ^{a}	100±0.0 ^{a}	83.33 ^{c}		
	0.75	76.67±5.8 ^{de}	90±10 ^{b}	100±0.0 ^{a}	100±0.0 ^{a}	91.67 ^{b}		
	1	96.67±5.77 ^{a}	100±0.0 ^{a}	100±0.0 ^{a}	100±0.0 ^{a}	99.17 ^{a}		
*A. squamosa**Time		56.67 ^{e}	77.5 ^{c}	86.67 ^{b}	92.5 ^{a}	78.34 ^{B}		
Control		00.00 ^{h}	00.00 ^{h}	00.00 ^{h}	00.00 ^{h}	00.00 ^{C}		
Mean Plant*Time		43.33 ^{d}	54.44 ^{c}	59.72 ^{b}	62.5 ^{a}	55		
Mean Conc. *Time	0.25%	11.11^{f}	28.89^{e}	38.89^{d}	50^{c}	Mean Conc. (%)	0.25	32.22^{d}
	0.5%	41.11^{d}	60^{ab}	66.67^{a}	66.67^{a}		0.5	58.61^{c}
	0.75%	55.56^{b}	62.22^{ab}	66.67^{a}	66.67^{a}		0.75	62.78^{b}
	1%	65.56^{a}	66.67^{a}	66.67^{a}	66.67^{a}		1	66.39^{a}

Values followed by the same letter are not significantly different according to the Fisher's LSD test ($p \leq 0.05$)
L. S. $D_{0.05}$ among *P. nigrum*, *A. squamosa* and control = 2.68; L. S. $D_{0.05}$ among 12, 24, 36 and 48 h= 2.188;
L. S. $D_{0.05}$ among 0.25, 0.5, 0.75 and 1%= 2.188; L. S. $D_{0.05}$ between plant*Time= 3.79; L. S. $D_{0.05}$ between Conc. *Time= 8.75;
L. S. $D_{0.05}$ between plant*Conc. = 3.79; L. S. $D_{0.05}$ among plant*Time*concentration= 7.08

Table 3: Probit analysis of mortality for *T. castaneum* and *S. oryzae* adults exposed to four concentrations of *P. nigrum* and *A. squamosa* seeds extracts for four times 12, 24, 36 and 48 hrs.

Plant extract	Insect	Time (hour)	LC_{50} %95 confidence limit	LC_{90} %95 confidence limit	Slope ±SE	χ^2
P. nigrum	*T. castaneum*	12	0.27 (0.17-0.34)	0.7 (0.54-1.28)	3.14±0.79	0.297
		24	0.16 (0.04-0.24)	0.44 (0.34-0.68)	3.03±0.95	0.26
	S. oryzae	12	0.35 (0.28-0.41)	0.72 (0.58-1.07)	4.1±0.81	0.20
		24	0.23 (0.15-0.29)	0.49 (0.396-0.73)	4.04±0.98	0.49
A. squamosa	*T. castaneum*	12	0.495 (0.39-0.6)	1.38 (1.01-2.59)	2.89±0.58	1.64
		24	0.35 (0.24-0.44)	1.04 (0.79-1.84)	2.73 ±0.57	3.84
	S. oryzae	12	0.51 (0.44-0.58)	0.9 (0.78-1.15)	5.16 ±0.77	0.88
		24	0.31 (0.23-0.37)	0.65 (0.53-0.96)	3.94±0.79	1.5

3.2. Repellent activity

The repellency effect of *P. nigrum* and *A. squamosa* seeds extracts against *T. castaneum* and *S. oryzae* was illustrated in Tables 4 and 5. Where there was significant different between *P. nigrum* and *A. squamosa* extracts. *P. nigrum* was more repellency than *A. squamosa*. The mean repellency percentages were 88.33 and 73.61% against *T. castaneum*, while they were 81.11 and 71.39% against *S. oryzae*, respectively. Noticeably, the repellency effect increased with increasing the time of exposure of *P. nigrum* and *A. squamosa* against *T. castaneum*. There was no significant between 5 and 10 hrs. They were the highest repellency. The mean repellency was 90 and 89.17%, respectively followed by 2, 3 and 4 h, there was no significant different among them. They were 77.5, 80.83 and 80.84%, respectively. While at 1 h was the lowest repellency effect. It was 67.5%. While the repellency effect of both tested extracts against *S.*

oryzae, there was no significant different among all times except 10 hrs. it was the lowest effect. The mean repellent percentages were 83.33, 78.33, 78.33, 73.33, 68.33 and 46.67% after 1, 2, 3, 4, 5 and 10 hrs., respectively. Moreover, there was no significant different among 0.5, 0.75 and 1 % of *P. nigrum* against *T. castaneum*. The mean repellency percentages were 87.8, 90 and 92%, respectively indicated class V. While, there was no significant different between 0.75 and 1% against *S. oryzae*. They were 96.67 and 94.44%, respectively indicating class V.

Table 4: Percentage repellency of *P. nigrum* and *A. squamosa* seeds extracts against *T. castaneum* treated with different concentrations for 1, 2, 3, 4, 5, and 10 hrs.

Plant extract	Conc. (%)	Time after application (h)						Mean Plant*Conc.	Repellent Class
		1	2	3	4	5	10		
P. nigrum	0.25	20^{i}	80^{bcd}	100^{a}	100^{a}	100^{a}	100^{a}	83.3^{bcd}	V (80.1-100%)
	0.5	60^{efg}	80^{bcd}	86.67^{abc}	100^{a}	100^{a}	100^{a}	87.8^{abc}	V (80.1-100%)
	0.75	73.3^{cde}	86.7^{abc}	93.33^{ab}	86.67^{abc}	100^{a}	100^{a}	90^{ab}	V (80.1-100%)
	1	66.7^{def}	100^{a}	93.33^{ab}	93.33^{ab}	100^{a}	100^{a}	92.2^{a}	V (80.1-100%)
*P. nigrum**Time		55.0^{e}	86.7^{abc}	93.33^{ab}	95^{a}	100^{a}	100^{a}	88.33^{A}	
A. squamosa	0.25	73.33^{cde}	53.3^{fgh}	40.0^{h}	46.67^{gh}	80^{bcd}	80^{bcd}	62.2^{f}	IV (60.1-80%)
	0.5	86.67^{abc}	80^{bcd}	66.67^{def}	80^{bcd}	86.67^{abc}	93.33^{ab}	82.2^{cd}	V (80.1-100%)
	0.75	66.67^{def}	80^{bcd}	86.67^{abc}	53.33^{fgh}	73.33^{cde}	66.67^{def}	71.1^{e}	IV (60.1-80%)
	1	93.33^{ab}	60^{efg}	80^{bcd}	86.67^{abc}	80^{bcd}	73.33^{cde}	78.9^{d}	IV (60.1-80%)
*A. squamosa**Time		80^{c}	68.33^{d}	68.33^{d}	66.67^{d}	80.0^{c}	78.33^{c}	73.61^{B}	
Mean Time		67.5^{C}	77.5^{B}	80.83^{B}	80.84^{B}	90^{A}	89.17^{A}	80.97	
Mean Conc. *Time	0.25	46.67^{h}	66.67^{g}	70^{fg}	73.33^{ef}	90^{abc}	90^{abc}	Mean Conc.	0.25 72.78^{c}
	0.5	73.33^{efg}	80^{cde}	76.67^{defg}	90^{ab}	93.33^{ab}	96.67^{a}		0.5 85^{ab}
	0.75	70^{fg}	83.33^{bde}	90^{abc}	70^{fg}	86.67^{abcd}	83.33^{bcde}		0.75 80.56^{b}
	1	80^{cdef}	80^{cdef}	86.67^{abcd}	90^{abc}	90^{abc}	86.67^{abcd}		1 85.56^{a}

Values followed by the same letter are not significantly different according to the Fisher's LSD test ($p \leq 0.05$)
L. S. $D_{0.05}$ between *P. nigrum*, and *A. squamosa* = 3.55; L. S. $D_{0.05}$ among 1, 2, 3, 4, 5 and 10 h= 6.15;
L. S. $D_{0.05}$ among 0.25, 0.5, 0.75 and 1%= 5.02; L. S. $D_{0.05}$ between plant*Time= 8.7; L. S. $D_{0.05}$ between Conc. *Time=12.3;
L. S. $D_{0.05}$ between plant*Conc. = 7.1; L. S. $D_{0.05}$ among plant*Time*concentration= 17.4

Table 5: Percentage repellency of *P. nigrum* and *A. Squamosa* seeds extracts against *S. oryzae* treated with different concentrations for 1, 2, 3, 4, 5, and 10 hrs

Plant extract	Conc. (%)	Time after application						Mean Plant*Conc.	Repellent Class
		1	2	3	4	5	10		
P. nigrum	0.25	73.33^{bcde}	86.67^{abc}	66.67^{cde}	93.33^{ab}	73.33^{bcd}	33.33^{f}	71.1^{bc}	IV (60.1-80%)
	0.5	73.33^{bcde}	80^{abcd}	73.33^{bcde}	73.33^{bcd}	73.33^{bcd}	0.0^{g}	62.22^{c}	IV (60.1-80%)
	0.75	100^{a}	86.67^{abc}	100^{a}	100^{a}	100^{a}	93.33^{ab}	96.67^{a}	V (80.1-100%)
	1	100^{a}	86.67^{abc}	100^{a}	100^{a}	100^{a}	80^{abc}	94.44^{a}	V (80.1-100%)
*P. nigrum**Time		86.67^{ab}	85^{abc}	85^{abc}	91.67^{a}	86.67^{ab}	51.67^{e}	81.11^{A}	
A. squamosa	0.25	73.33^{bcde}	73.33^{bcde}	73.33^{bcde}	73.33^{bcde}	60^{de}	33.33^{f}	64.44^{c}	IV (60.1-80%)
	0.5	60^{de}	66.67^{cde}	93.33^{ab}	73.33^{bcde}	53.33^{ef}	53.33^{ef}	66.67^{c}	IV (60.1-80%)
	0.75	100^{a}	86.67^{abc}	73.33^{bcde}	73.33^{bcde}	93.33^{ab}	33.33^{f}	76.67^{b}	IV (60.1-80%)
	1	100^{a}	86.67^{abc}	73.33^{bcde}	73.33^{bcde}	66.67^{cde}	66.67^{cde}	77.78^{b}	IV (60.1-80%)
*A. squamosa**Time		83.33^{abc}	78.33^{bcd}	78.33^{bcd}	73.33^{cd}	68.33^{d}	46.67^{e}	71.39^{B}	
MeanTime		85^{A}	81.67^{A}	81.67^{A}	82.5^{A}	77.5^{A}	49.17^{B}	76.25	
Mean Conc. *Time	0.25	73.33^{cdef}	80^{bce}	70^{def}	83.33^{bcd}	66.67^{ef}	33.33^{g}	Mean Conc.	0.25 67.78^{b}
	0.5	66.67^{ef}	73.33^{cdef}	83.33^{bcd}	73.33^{cdef}	63.33^{f}	26.67^{g}		0.5 64.44^{b}
	0.75	100^{a}	86.67^{abc}	86.67^{abc}	86.67^{abc}	96.67^{ab}	63.33^{f}		0.75 86.67^{a}
	1	100^{a}	86.67^{abc}	86.67^{abc}	86.67^{abc}	83.33^{bcd}	73.33^{cdef}		1 86.11^{a}

Values followed by the same letter are not significantly different according to the Fisher's LSD test ($p \leq 0.05$)
L. S. $D_{0.05}$ between *P. nigrum*, and *A. squamosa* = 4.78; L. S. $D_{0.05}$ among 1, 2, 3, 4, 5 and 10 h= 8.27
L. S. $D_{0.05}$ among 0.25, 0.5, 0.75 and 1%= 6.75; L. S. $D_{0.05}$ between plant*Time= 11.696; L. S. $D_{0.05}$ between Conc. *Time= 16.54;
L. S. $D_{0.05}$ between Plant*Conc. = 9.55; L. S. $D_{0.05}$ among plant*Time*concentration= 23.39

3.3. Residual effect

The results in Table 6 revealed that there was significant different between *P. nigrum* and *A. squamosa* extracts. Although the *P. nigrum* extract at 0.75% more toxic than *A. squamosa* extracts at initial time, after 3days of exposure, where the mean mortality percentages were 100 and 83.33%, respectively. *A. squamosa* extracts was more toxicity and persistence than *P. nigrum* extract with the prolongation of time to 30 days. Whereas, the mean mortality percentages were 80 and 51.67% for *A. squamosa* and *P. nigrum* extracts. While, there was no significant different among initial time, 15 and 30 days after exposure of 0.75% of *A. squamosa* extracts. The mean mortality was 83.33, 81.67 and 75%, respectively. For *P. nigrum* extract, there was significant different between 15 and 30 days after exposure. The mean mortality percentages were 55 and 0.0 %, respectively. Moreover, both tested plant extracts were more toxic against *T. castaneum* than *S. oryzae* with the prolongation of time. The mean mortality percentages were 71.7 and 60%, respectively.

Table 6: The residual effect of *Piper nigrum* and *A. squamosa* seeds extract against *S. oryzae* and *Tribolium castaneum* exposed to three times, initial, 15 and 30 days

Plant extract	Insect	% Mortality			Mean plant*Insect		
		Initial	15 days	30 days			
P. nigrum	*S. oryzae*	100 ^{a}	76.67^{cd}	0.0^{f}	58.89^{c}		
	T. castaneum	100 ^{a}	33.33^{e}	0.0 ^{f}	44.44^{d}		
Mean *P. nigrum**Time		100^{a}	55^{c}	0.0^{d}	51.67 ^{B}		
A. squamosa	*S. oryzae*	90 ^{ab}	86.67^{bc}	76.67^{cd}	84.44^{a}		
	T. castaneum	76.67^{cd}	76.67^{cd}	73.33^{d}	75.56^{b}		
Mean *A. squamosa**Time		83.33 ^{b}	81.67 ^{b}	75 ^{b}	80.0 ^{A}		
Mean		91.67^{a}	68.33^{b}	37.5^{c}	65.83		
Mean Insects*Time	*S. oryzae*	95 ^{a}	81.67^{b}	38.33^{d}	Mean Insect	*S. oryzae*	60^{b}
	T. castaneum	88.33 ^{ab}	55 ^{c}	36.67 ^{d}		*T. castaneum*	71.7^{a}

Values followed by the same letter are not significantly different according to the Fisher's LSD test ($p \leq 0.05$)
L. S. $D_{0.05}$ between *P. nigrum*, and *A. squamosa* = 5.32; L. S. $D_{0.05}$ among 0, 15 and 30 days=6.51;
L. S. $D_{0.05}$ between *S. oryzae* and *T. castaneum*= 5.3; L. S. $D_{0.05}$ between plant*Time= 9.21;
L. S. $D_{0.05}$ between Plant*insect= 7.52; L. S. $D_{0.05}$ among plant*Time*Insect= 13.02;
L. S. $D_{0.05}$ between Insect*Time= 9.21

The results revealed that *P. nigrum* and *A. squamosa* seeds extracts had insecticidal activity against *T. castaneum* and *S. oryzae* agreement with previous studies such as, Rao et al., 2005 [28] who revealed that, the LC_{50} values were 1195.41, 305.36 and 1446.32 ppm for *Annona squamosa* seed hexane, ethyl acetate and methanol extracts, respectively, for *Trogoderma granarium* neonate larvae; 5805, 1300 and 5815 ppm for 7 days old larvae. They showed that *A. squamosa* seed ethyl acetate extract was the best against Khapra beetle. While, Khalequzzaman and Sultana, 2006 [15] studied the insecticidal activity of petroleum spirit, ethyl acetate, acetone and methanol seed extracts of *A. squamosa* L. against four strains of the red flour beetle, *T. castaneum* (Herbst). For larval bioassay, petroleum spirit extract was the highest toxicity (LD_{50}= 0.03µg cm^{-2}) in Raj strain, while methanol extract was the lowest (LD_{50}=15.697µg cm^{-2}) in FSS II strain. In adults, petroleum spirit extract was the highest toxicity (LD_{50}= 58.697µg cm^{-2}) in CTC 12 strain and the lowest toxicity (LD_{50}=22004.710µg cm^{-2}) was for acetone extract in CR 1 strain. Bodroža-Solarov et al., 2008 [5] investigated the insecticidal activities of different doses of plant extracts obtained from *Piper nigrum*, *Carum carvi* and *Sesamum indicum* against rice weevil, *S. oryzae* L. in wheat. The extract of *P. nigrum* was found to be the most efficient causing the highest mortality rate. It was shown by the baking tests that bread made from the treated wheat did not absorb the aroma of plant extracts. And, Khani et al., 2012 [29] found that LC_{50} values were 287.7 and 530.5 µL/L air after 72 h of *P. nigrum* essential oils against *S. oryzae* and *C. cephalonica* larvae, respectively. Al-Saadi, 2017 [30] found that LD_{50} values of ethanol extract of *P. nigrum* against adult *T. castaneum* was found to be 0.73 mg/cm^2. While in larvae was 0.34 mg/cm^2. Lajitha and Aravind, 2018 [31] tested the toxic effect of *A. indica*, *C. gigantea*, *C. asiatica*, *P. nigrum* and *H. alternata* leaf extracts on *S. oryzae*, *T. castaneum* and *C. chinensis*. They found that *C. gigantea*, *C. asiatica*, and *H. alternate* possess strong contact toxicity against these pests. While *A. indica* and *P. nigrum* possess weak contact toxicity. Hidalgo et al., 2018 [32] revealed that, there were annonacin, *cis*-annonacin, *cis*-annonacin-10-one, asimicin, rolliniastatin-2, cherimolin-1, cherimolin-2, almuñequin, and two *β*-OH acetogenins: laherradurin and itrabinin *Annona squamosa*, *A. muricata* and *A. montana* seeds. Also, some acetylated and methoxy methylated ACG derivatives were synthesized and evaluated: annonacin (3 OAc), annonacin (4 OAc), asimicin (3 OAc), rolliniastatin-2 (3 OAc), rolliniastatin-2 (MOM), laherradurin (3 OAc) and itrabin (3 OAc). The natural acetogenin rolliniastatin-2 (100 µg·g^{-1} of diet) was the most toxic causing 100% mortality of early instar larvae. Derivatization of ACG yielded compounds that produced nutritional alterations. The incorporation of rolliniastatin-2 (3 OAc) and rolliniastatin-2 (3 MOM) (100 µg·g^{-1}) into the artificial diet of *S. frugiperda* displayed the strongest antifeedant effects causing marked decreasing in larval growth and adult lethal malformations. In addition to, Sharma et al., 2019 [33] evaluated the mortality of *T. castaneum* (Herbst) after the treatment of leaves extract of *A. squamosa* in aqueous solvent. LD_{10} and LD_{50} Values were 1.416 and 3, 951 ml/kg, respectively. Nenotek and Ludji, 2020 [34] revealed that LC_{50} and LC_{95} were 0.07% and 2.07% of mixture of *A. squamosa* and *T. vogelii* seeds extracts against *H. amigera* larvae, respectively. Ismail and Sleem, 2020 [35] showed that, the LD_{50} values were 7.67 and 12.83% after 24 h for *S. aromaticum* and *P. nigrum* powders, respectively. *S. aromaticum* powder was more toxic than *P. nigrum* powder against the *S. oryzae* after 15 days of exposure. But *P. nigrum* was more persistence than *S. aromaticum*, LT_{50} values were 52.5 and 135 days for *S. aromaticum* and pepper powders, respectively. In addition to, *S. aromaticum* and *P. nigrum* powders had repellent activity against *S. oryzae*. *P. nigrum* powder was more repellency than *S. aromaticum*. Moreover, Choden et al., 2020 [36] found that *Piper nigrum* oil at 5% and 10% at 96 hrs. caused 100% mortality and the high repellency was 84% at 10% of oil against *S. zeamais*. Also, Irwan et al., 2021 [37] evaluated the effect of *A. Squamosa* and *A. muricata* seed extracts on crickets and mealworm. The mixture of extracts of *A muricata* and *A. squamosa* induced high mortality rate to mealworm and cricket compared to extracts of *A. squamosa* and extract of *A. muricata* at all concentrations tested. Sleem, 2020 [21] studied the effect of *P. nigrum* and *Prunus cerasus* seeds exracts against *R. dominica*. She found that the mortality percentages were 53.33 and 100% within 24 and 48 h of exposure at 0.25% of *Prunus cerasus* extract. Moreover, the petroleum ether extract of *P. nigrum* was more repellency than *P. cerasus* extract on *R. dominica*. Where, 2% of *P. nigrum* was the strongest repellent effect with mean percentage repellency 76% followed by 1%, it was 42.6%. While they were 26.66,

36.67 and 56.67% after 24, 48 and 72 h at 0.25% of *P. nigrum* extract, respectively. Nisar et al., 2021 [38] investigated the impact of plant extracts, *Moringa oleifera*, *Allium sativum* and *Piper nigrum* on biological parameters of house fly. There were significant differences for larval duration. Maximum larval duration was for garlic followed by *P. nigrum* and *M. oleifera*, respectively. They noticed that, garlic extract was the highest repellency for house fly followed by black pepper and moringa after 30, 60 and 90 min. Alves et al., 2021 [14] revealed that *A. squamosa* are very efficient, and are effective in the issue of mortality rates of *C. brevis.*

4. Conclusion

In conclude, *Piper nigrum* L. and *Annona squamosa* L. seeds extracts had insecticidal activity against *Tribolium castaneum* and *Sitophilus oryzae. Piper nigrum* seeds extract was more toxic and repellency than *Annona squamosa.* While, *Annona squamosa* seeds extract was more persistence than *Piper nigrum* seeds extract. These activities suggested that, *Piper nigrum* and *Annona squamosa* seeds extracts have the potential to be candidate as alternative and effective natural method to control *Tribolium castaneum* and *Sitophilus oryzae*

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