Dust Efficacy of Clove Buds Powder, *Syzygium aromaticum* Alone and in Combination with Spinosad and Abamectin against the Adults of Granary Weevil, *Sitophilus granaries* (L.)

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Abstract: The present investigation is conducted to evaluate the dust efficacy of clove buds powder alone and as a carrier to abamectin and spinosad insecticides against the granary weevil, *Sitophilus granaries.* The objectives of the research also include scanning of the effective compounds existed in the tested plant by using GC-Mass equipment and estimating the effect of the tested plant on one of the digestive enzymes, amylase of the granary weevil, *S. granaries.* Efficacy of clove powder was assessed on wheat at four dosages of clove powder gm/100 gm of grain wheat (corresponding to 0.125, 0.25, 0.5 and 1%). The highest tested concentration of clove powder that is 1 gm/100 gm of wheat grains achieved 20, 78 and 100% mortality percent against adults of granary weevil after 1, 3 and 7 days post treatment, respectively. From the calculated toxicity parameters found that LC_{50} levels were 10.4, 0.34 and 0.23% of clove gm/100 gm of wheat grains after 1, 3 and 7 days post treatment, respectively. The lowest concentration of abamectin, which caused potentiation with clove powder was (5 ppm) and caused co-toxicity factor (32.3). The co-toxicity values increase with increasing the concentration of abamectin above 5 ppm. It was noted that, the highest tested concentration (Clove at 0.25% + 15 ppm of abamectin) achieved highest potentiation value of co-toxicity factor (144). The values of the co-toxicity factor were increased dependent on spinosad concentrations. The highest tested concentration of spinosad (140 ppm), showed a value of 108 co- toxicity when spinosad mixed with clove powder. Effects of clove powder at concentration of LC_{50} on amylase activity of granary weevil, *S. granarius* at different temperature degrees, different time (min.) of inhibition reaction and the effect of pH on amylase activity in both treated and untreated insects were illustrated. It concluded that clove powder can be used to control granary weevils alone or when mixed with insecticides, spinosad or abamectin.

Keywords: Granary weevils, *Sitophilus granaries*, clove powder, spinosad, abamectin, co-toxicity and amylase activity

INTRODUCTION

Over years, insect damage to stored grains, pulses and cereals products has been of great concern. Quantitative and qualitative post-harvest losses of stored grains in stores, silos, and granaries ranged from 10-40% of overall production (Manandhar *et al*., 2018). Percentage losses had increased dramatically in the last years, because most storage insects are able to increase in numbers drastically within a relatively short time (Abbas *et al*., 2014). Insects of grains in granaries are controlled mainly by direct application of contact insecticides, known as grain protectants. These compounds include many insecticides of the synthetic organophosphorus (OPs) that might cause environmental pollution along with the presence of their residue in the grain, which pose health concerns to human (White and Leesch, 1995; Arthur, 1996). Use of insecticides in stored products is facing restrictions such as a high mammalian toxicity and expensive costs. In addition, pest populations are developing resistance to chemical insecticides (Phillips *et al*., 2000). Though there remains a need to continue to investigate safe and effective chemical control strategies for controlling insects on stored products. In this respect special attention is paid to insecticides with natural origin, such as spinosad and abamectin. This biopesticide has been reported to have fewer hazards for the environment and mammals than many other currently available pesticides (Thompson *et al*., 2000). Higher plants are a rich source of novel insecticides (Dev and Koul, 1997). Plant materials with insecticidal properties have been used traditionally for generations throughout the world (Belmain *et al.,* 2001). Botanical insecticides compared to synthetic ones may be safer for the environment. Further, these insecticides are less expensive, easily processed and used by farmers and small industries (Belmain *et al.,* 2001). These insecticides are often active against a limited number of species biodegradable to nontoxic products, and are potentially suitable for use in integrated pest management for controlling insects on stored products. They could lead to the development of new classes of safer insect control agents (Kim *et al.,* 2003).The present investigation shed light on the dust efficacy of Clove buds powder against the granary weevil, *Sitophilus granaries.* Further, use the tested plant powder as carrier materials for some insecticides (abamectin and spinosad) against the granary weevil, *S. granaries* and calculate Co-toxicity factor for the combination of plant powder with the tested insecticides. The objectives of the research also include scanning of the effective compounds existed in the tested plant by using GC-Mass equipment and estimating the effect of the tested plant on one of the digestive enzymes, amylase, on the granary weevil, *S. granaries*.

MATERIALS AND METHODS

Insect used: The tested insect was granary weevil, *Sitophilus granaries* (L.) (Coleoptera: Curculionidae). Adult insects were obtained from already existing culture in the Research Laboratory, Institution of Plant

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Protection, EL-Dokii - Giza. Sixty pairs of *S. granarius,* sexed following the reports of Odeyemi and Daramola (2000) and Adedire (2001) were introduced into 1 L plane glass jar containing 400 g of weevil - susceptible wheat grains and covered with muslin cloth. The jar was placed at ambient temperature of 30±3°C and 70±5% relative humidity.

Plant materials: The plant used in this study was Clove buds, *Syzygium aromaticum*. The materials of clove buds were sourced from the market. These plant materials were dried in an open laboratory and ground into very fine powder using an electric blender. The powders were packed in plastic containers with tight lids and stored in a refrigerator at 4°C prior to use.

Sampling of wheat grains: Wheat grains, *Triticum aestivum* hard red winter variety were obtained from newly stock grains free of insecticides from Ismailia Agricultural Research Station. Firstly, the grains were cleaned and disinfested by keeping at -5°C for 7 days to kill all hidden infestations. This is because all the life stages, particularly the eggs are very sensitive to cold (Koehler, 2003). The disinfested wheat grains were placed inside an oven at 40°C for 4 h (Jambere *et al*., 1995) before they were stored in plastic containers with tight lids disinfested by swabbing with 90% alcohol.

Toxicity of plant powders: Portions of 0.25, 0.5, and 1 g of clove pods powder completed to 100 gm of clean undamaged and uninfected wheat grains in 250 ml glass jars to produce 0.25, 0.5 and 1% w/w concentration for the treated grains and in the control containers with no plant powders were added to the wheat grains. The containers with their contents were gently shaken to ensure thorough admixture of the wheat grains and treatment powders. Ten adults of a 10- day old adults *S. granarius* were introduced to each of the containers and covered. Three replicates of the treatments and untreated insects were laid out in Complete Randomized Design. The adult mortality was assessed after 1, 3 and 7 days of exposure. Adults were considered dead when probed with sharp objects and there were no responses.

Tested Insecticides:

a. Abamectin - Trade name: Abamectin® - Chemical class: Avermectin Chemical name: 5-O-demethylavermectin A1a (i) mixture with 5-O-demethyl-25-de(1-methylpropyl)-25-(1-methylethyl) avermectin A1a (ii).

b. Spinosad - Trade name: tracer ® - Chemical class: Spinosyn - Chemical formulation: $C_{41}H_{65}NO_{10}$ (spinosyn A); $C_{42}H_{67}NO_{10}$ (spinosyn D).

Toxicity of insecticides carried on plant powder:

The following concentrations 0.5, 1, 5, 10 and 15 ppm of abamectin and 20, 60, 100, 120 and 140 ppm of spinosad were tested after preparing these concentrations in acetone solvent and loading them on 0.25 gm of the clove buds powder. These preparations were mixed with 100 gm of wheat grains at a concentration of 0.25% for each replicate. The containers with their contents were gently shaken to ensure thorough admixture of the wheat grains and treatment powders. Ten adults of a 10- day old adults *S. granarius* were introduced to each of the containers and covered. Three replicates of the treatments and untreated insects were laid out in complete randomized design. The adult mortality was assessed after 1, 3 and 7 days of exposure. Adults were considered dead when probed with sharp objects and there were no responses. The mortality was observed after 1, 3 and 7 days of exposure. Co-toxicity factor which has been calculated according to the equation given by Mansour *et al.* (1966) as follows:

Co-toxicity factor =

$$\frac{\text{Observed mortality \% - Expected mortality \%}}{\text{Expected mortality \%}} \times 100$$

This factor was used to differentiate the results into three categories, a positive factor of (+20) or more is considered potentiation or synergism, a negative of (-20) or more means antagonism and intermediate values more than (-20) and less than (+20) indicate only additive effect.

Preparation of insects for enzyme analysis: The insects were prepared as described by bold. They were homogenized in distilled water (50 mg/1 ml). Homogenates were centrifuged at 8000 r.p.m. for 15 min at 2ºC in a refrigerated centrifuge. The deposits were discarded and the supernatants, which is referred as enzyme extract, can be stored at least one-week without appreciable loss of activity when stored at 5°C.

General method for determination of amylase activity: Generally, 20 µl of diluted enzyme solution was incubated for 10 min at 30°C with 250 µl 1% starch (soluble potato starch, Lintner grade, Sigma Chemical Co.) in 50 mM acetate buffer pH 5.0 containing 20 mM NaCl and 0.1 mM $CaCl_2$. The reaction was stopped by adding 250 µl DNS reagent to each tube in boiling water for 5 min. Samples were cooled, diluted with 2.5 ml H_2O and read at 550 nm on Spectronic 1201 (Beckman, USA). Glucose was used as a standard. Appropriate dilutions of enzyme supernatant were used to obtain a linear production of glucose equivalents. Generally, for each test, amylase activity was determined from triplicate analyses of three groups of seedlings. The enzyme activity was expressed as µg glucose released /min/gm fresh weight. Bernfeld (1955) and Al-Qodah (2007).

Determination of amylase activity at different reaction times: Measuring the enzyme activity at different reaction timeswith fixing the rest of the reaction conditions and according to the general method for estimating the enzyme activity, 20 µl of diluted enzyme solution was incubated for 5, 10, 20 and 30 minat 30°C with 250 µl 1% starch (soluble potato starch, Lintner grade, Sigma Chemical Co.) in 50 mM acetate buffer pH 5.0 containing 20 mM NaCl and 0.1 mM $CaCl_2$

Determination of amylase activity at different temperature degrees: 20 µl of diluted enzyme solution was incubated for 10 min at 15, 25, 35, 50 and 60°C with 250 µl 1% starch (soluble potato starch, Lintner grade, Sigma Chemical Co.) in 50 mM acetate buffer pH 5.0 containing 20 mM NaCl and 0.1 mM $CaCl_2$ according to the general method for amylase activity determination.

Determination of amylase activity at different pH levels:

Diluted enzyme solution was incubated with 250 µl 1% starch (soluble potato starch, Lintner grade, Sigma Chemical Co.) for 10 min at 30°C in 50 mM acetate buffer pH 4,5,7 and 8 individual reactions then complete the reaction and the measurement according to the general method for amylase activity determination.

Assessment the effect of clove powder at LC_{50} level at the different tested conditions on amylase enzyme activity:

The activity of amylase enzyme was measured at different conditions of temperature, reaction times and pH levels in untreated adults of granary weevil and treated adults with LC_{50} of clove buds powder gm/100 gm of wheat grain.

Identification of chemicals of clove buds by GC/MS:

Plant extraction, clean up and the identification by GC-MS was performed in the Central for analyses of pesticides, Ministry of Agriculture. Agilent 6890 gas chromatograph equipped with an Agilent mass spectrometric detector, with a direct capillary interface and fused silica capillary column PAS-5 ms (30 mm × 0.25 um film thickness). Samples were injected under the following conditions. Helium was used as carrier gas at approximately 1 ml/min., pulsed split less mode. The solvent delay was 3 min. and the injection size was 1.0 ul. The mass energy of 70 e.v. scanned from m/z 50 to 500. The ion source temperature was 230°C and the quadruple temperature was 150v above auto tune. The instrument was manually tuned using perfluoro tributyl amine (PFTBA). The GC temperature program was started at 60°C for 3 min. then elevated to 280°C. at rate of 8°C/min, and 15 min. hold at 280°C the injector temperature was set at 280°C. Wiley and Nist 05 mass spectral data base was used in the identification of the separated peaks.

RESULTS AND DISCUSSION

Toxicological studies of clove buds powder:

This part of the investigation was intended to evaluate the efficacy of clove buds powder, *Syzygium* against the adults of granary weevil, *S. granaries*. Mortality was assessed after 1, 3 and 7 days of exposure and calculated LC_{25}, LC_{50} and slopes of the regression lines as toxicity parameters. The mortality and toxicity of clove buds powder are tabulated in Table (1). The results clearly showed that there is an effect of Clove buds powders on adults of granary weevil. This effect is evident from the mortality and toxicity parameters during the first, third and seventh days after treatments. It is observed that the concentration 0.25 gm of cloves was the least concentration of the plant that had an effect against granary weevil adults, so this concentration was chosen to load pesticides on it and this will be explained in the next point of the investigation. The highest tested concentration of clove powder that is 1 gm/100 gm of wheat grains achieved 20, 78 and 100% mortality percent against adults of granary weevil after 1,3 and 7 days post treatment, respectively. From the calculated toxicity parameters found that LC_{50} levels were 10.4, 0.34 and 0.23% of gm clove/100 gm of wheat grains after 1, 3 and 7 days after treatment, respectively.

Table (1): Effect of clove buds powder, *S. aromaticum* against granary weevil at different intervals of exposure

Concentrations*	%, Mortality		
	1day	3 days	7 days
Control	0.0	0.0	0.0
0.125 gm	5	15	15
0.25 gm	12	34	57
0.5 gm	15	76	86
1 gm	20	78	100
Toxicity			
Slope± SE	0.885±0.2205	2.19±0.22	3.5±0.36
Regression equation			
Y = a + b X	Y= 4.12+0.88X	Y=6+2.19X	Y=7.2+3.5X
LC_{25} (% gm/100gm)	1.47	0.17	0.15
LC_{25} Limits	(0.8 – 11.9)		(0.12 – 0.17)
LC_{50} (% gm/100gm)	10.4	0.34	0.23
LC_{50} Limits	(2.9 – 17.18)		(0.21 – 0.26)

*gm of clove buds powder/ 100 gm of wheat grains

The effectiveness of cloves on stored seed pests which has been previously studied as a fumigation formula of clove essential oils by Yang *et al.* (2003) and Formisano *et al.* (2008). They discussed the contact toxicity of the clove oil and the safety of this oil on non-target organisms including mammals. Also, the efficacy of clove oil against *Sitophilus oryzae* demonstrated by Mishra *et al.* (2012) that *S. aromaticum* essential oil has more insecticidal activity against *S. oryzae* than the other essential oils. Plata-Rueda *et al.* (2018) evaluated toxic effects, repellency and respiration rate caused by terpenoid constituents of cinnamon and clove essential oils against *Sitophilus granarius* and concluded that cinnamon and clove essential oil, and their terpenoid constituents were toxic and repellent to adult *S. granarius* and, therefore, have the potential to prevent or retard the development of insecticide resistance. The influence of different plant dried materials (as powder added to the products the pests feed on) on the cereal and flour pests has been widely researched (Blazejewska and Wyrostkiewicz, 1998). The use of spices is also less costly, easily available, safer and don't do any hazard using in the stores (Aslam *et al.,* 2002; Mahdi and Rahman, 2008).

According to Ashouri and Shayesteh (2010), who studied the activity of certain plants powder on adults of *Sitophilus granaries* concluded that plants powders have potential in protecting wheat against the tested species of tested insects. Regarding the side effects of synthetic pesticides, the study demonstrates that these plant powders can play an important role in protection of wheat from insect invasion during storage. This technology is cheap, safe, environmentally friendly and easy to adopt by small-scale farmer. Also, Khanal *et al.* (2021) evaluated the botanical Powders of certain plants for the management of rice weevil (*Sitophilus oryzae*) and found that botanical materials such as *A. sativum*, clove powder, *A. calamus*, rhizome dust and *N. tabacum*, leaf dust can be considered as excellent alternatives over the toxic pesticides for the management of rice weevil in wheat grain storage.

Joint action of tested insecticides carried on clove buds powder and the co-toxicity factors of the mixtures:

In practice, using the mixture of various classes of insecticides is an important approach of insecticide application to increase the control effect and avoid the insect developing resistance against a given pesticide. A similar strategy is also found and applied in the area of medicine. However, the interaction of different insecticides usually has three results: addition, antagonism and synergism. Whether synergism is influenced by many factors such as the pest species and class of insecticide used. Biochemically, the penetration, absorption, detoxification and target proteins of insecticides determine their interaction effects. Generally, synergistic action is preferably found in the mixture of insecticides have different mechanisms of action (Rozman *et al.*, 2001). This part of research was interested in studying the effect of abamectin and spinosad against the granary weevil, *S. granarius* but by indirect application by loading the concentrations of these insecticides on the powder of clove buds and calculate the co- toxicity factors.In this investigation the combination effect of the mixtures (abamectin and spinosad with clove powder) was calculated only after 3 days of treatment, because the mortality percent of granary weevils by clove powder was close to 100% after seven day of the treatment, so the value of the co-toxicity factor cannot be calculated after 7 days of application. It is worth noting that the treatment of wheat grains with the tested concentrations of the insecticides alone did not give any toxicity against the granary weevils. The combination effects of clove buds powder at the concentration of 0.25% of wheat grains with abamectin and spinosad at different concentrations against granary weevil after three days of exposure are shown in Table (2). The mortality percentage of granary weevil from the concentration 0.25 gm of clove/100 gm of wheat grains after 3 days of treatment were 34%, so the expected mortality was 34% to calculate the co-toxicity factor by the equation of Mansour *et al.* (1966). Results in Table (2) showed the observed toxicity and expected toxicity for all tested mixture concentrations. It is clear that the low concentrations of abamectin (0.5 and 1 ppm) have no effect against granary weevil and this is evident in the co-toxicity factor that was -26.4 and 2.9, respectively. The lowest concentration of abamectin, which caused potentiation with clove powder was (5 ppm) and caused co-toxicity factor (32.3). The co-toxicity values increase with increasing the concentration of abamectin above 5 ppm. It was noted that the highest tested concentration (Clove at 0.25% + 15 ppm of abamectin) achieved highest potentiation value of co-toxicity factor (144). We concluded from Table (2) thatis better to load low concentrations of pesticide abamectin or spinosad on the clove powder, which reduces the effect of the pesticide residues on exposed grains and provide the necessary protection for grains from granary weevil, *S. granarius*. According to the results obtained from Andrić *et al.* (2011) that for high efficacy of spinosad and abamectin against *S. oryzae* adults exposure period of 14-days is necessary. This can be explained by significantly slower action of natural insecticides compared to pyrethroids and organophosphates Hertlein *et al.* (2011). But from the results of our research we concluded that the combination between natural insecticides as abamectin and spinosad reduced the control period from 14 days to 7 days almost. Most of the published studies involved the use of spinosad as a liquid formulation, but it is also available as a dust. The use of spinosad dust formulation may be more advantageous than a liquid formulation in some cases because the dust can be removed from the treated grain before it is milled according to (Chintzoglou *et al.,* 2008). Moreover, there is evidence that liquid spinosad is not equally effective among different grains (Fang *et al.,* 2002; Subramanyam, 2006). The results shown in Table (2) indicated that the lowest concentration of spinosad can cause potentiation in the mixture with clove powder against granary weevil was approximately 100 ppm. The values of the co-toxicity factor were increased dependent of spinosad concentrations. The highest tested concentration of spinosad (140 ppm), showed a value of 108 co-toxicity when spinosad mixed with clove powder. There is an important research in the field of joint effect that demonstrated by Adarkwah *et al.* (2017) to determine the toxicity of powders of *Eugenia aromatic* and *Moringa oleifera* alone or combined with enhanced diatomaceous earth (Probe-A®DE, 89.0% SiO_2 and 5% silica aerogel) to adult *Sitophilus granarius, Tribolium castaneum* and *Acanthoscelides obtectus* and concluded that botanicals caused significant reduction of F1 adults compared to the control. Moreover, discussed combined action of botanical insecticides with DE as a grain protectant in an integrated pest management approach.

Table (2): Toxicity combination of abamectin and spinosad with Clove powder at 0.25% of wheat grains against granary weevil after three days of exposure

Mixtures concentrations	Observed mortality %	Expected mortality %	*Co-toxicity factor
Clove at 0.25% + 0.5 ppm of abamectin	25	34	-26.4
Clove at 0.25% + 1 ppm of abamectin	35	34	2.9 Ad
Clove at 0.25% + 5 ppm of abamectin	45	34	32.3 P
Clove at 0.25% + 10 ppm of abamectin	65	34	91.2 P
Clove at 0.25% + 15 ppm of abamectin	83	34	144 P
Clove at 0.25% + 20 ppm of spinosad	27	34	-20.5
Clove at 0.25% + 60 ppm of spinosad	40	34	17.6 Ad
Clove at 0.25% + 100 ppm of spinosad	58	34	70.6 P
Clove at 0.25% + 120 ppm of spinosad	67	34	97.1 P
Clove at 0.25% + 140 ppm of spinosad	71	34	108.8 P

***Co-toxicity factor** = $\frac{\text{Observed mortality \% - Expected mortality \%}}{\text{Expected mortality \%}} \times 100$.

* (P) = potentiation since co- toxicity factor ≥ +20
(Ad) = addition effect when Co-toxicity factor between +20, - 20

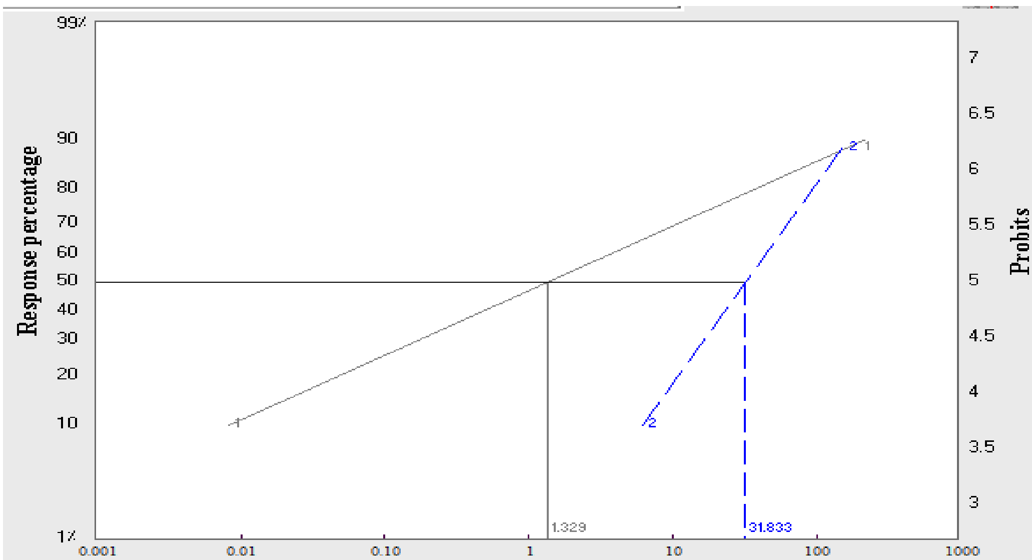


Fig. (1): Regression lines of spinosad and abamectin carried on clove powder at concentration of 0.25% against adults of granary weevil after 7-days exposure period
Concentrations 1= abamectin 2= spinosad

Effect of clove powder on amylase activity of granary weevil:

This part of the investigation which aims to study the activity of amylase enzyme under different conditions of temperature, pH and reaction times and Theoretical Maximum Velocity (Vmax) and Dissociation Constant (Michaels constant) (Km) also, study the effect of clove plant on amylase enzyme in adults of granary weevil under different conditions of temperature, pH and reaction times. Many insects, especially those feeding on grain products rich in starch depend on amylase for survival. Animals depend on digestive amylases to break down and utilize the starch in their food sources, and insect larvae that develop in starch-containing seeds require these digestive amylases for their development and survival. An excellent illustration of this principle comes from the work on the genetic engineering of peas and azuki beans with the amylase inhibitor aAI-1 from the common bean, *Phaseolus vulgaris* (Shade *et al.,* 1994; Ishimoto *et al.,* 1995; Schroeder *et al*., 1995). When amylase inhibitor is expressed in the seeds of pea (*Pisum sativum*) or azuki bean (*Vigna angularis*), the seeds are resistant to several species of bruchid beetles (*Callosobruchus maculatus* F., *Callosobruchus chinensis* L. and *Bruchus pisorum* L.). The inhibitor prevents larval development presumably because it inhibits the digestive amylases of the larvae (Ishimoto and Kitamura, 1989) found that amylase inhibitor extracted from fruits of china berry*, M azedrach* results in significant in vivo inhibition of amylase activity in *S. oryzae*. Moreover, the same authors revealed that significant mortality was observed in adults of *S. oryzae* treated with the purified amylase inhibitor from china berry. Use of a genetic engineering strategy to make insect-resistant plants requires characterization of the a-amylases of the target insect, the identification of suitable inhibitors from plants or other sources, and the cloning of genes of those inhibitors (Valencia *et al.,* 2000). Amylase enzyme activity was determine at different conditions of temperature, reaction times and pH on adults of granary weevil, *S. granarius* and illustrated in (Table 3). The results of Table (4) shows kinetic parameters terms of amylase in the granary weevil at 35°C and pH 7, Theoretical Maximum Velocity (V max) is 5.55 mg - glucose/min/mg protein (Michael's constant) (Km) is 0.l mole starch hydrolysis to glucose/min/mg/protein. Effects of clove powder at concentration of LC_{50} on amylase activity of granary weevil, *S. granarius* at different temperature degrees, different time (min.) of inhibition reaction and the effect of pH on amylase activity in both treated and untreated insects are illustrated in (Table 5). It is clear that the highest enzyme activity in the untreated insects and treated insects is recorded at a temperature of 35°C, however low inhibition of the enzyme activity 11% is deserved in clove powder treated insects. The most striking observation is the fact that enzyme activity in untreated insects is temperature - dependent till 35°C then decreased. However, inhibition of enzyme activity is fluctuated with highest inhibition is recorded at 15°C and the lowest inhibition is observed at 35°C. It is clear that enzyme amylase activity is time-dependent with the highest level is recorded after 30°C for untreated insects. However, inhibition of amylase activity is time-dependent with the inhibition level is recorded after 20 min then reduced. As for the effect of pH on amylase activity in both treated and untreated insects, results revealed that the enzyme activity is reached the maximum at PH 7.0 in both treated and untreated insects. However, the inhibition of amylase at pH 7.0 is found to be 26% then reduced at pH 8 to become 0.7%.

Table (3): Amylase enzyme activity of granary weevil adults at different conditions of temperature, reaction times and pH levels

Different conditions		**Enzyme Activity (mg glucose/min/mg protein)**
Temperature Degree °C.	15	4.92
	25	6.52
	35	8
	50	5.86
	60	4
Time (min.)	5	2.02
	10	4.12
	20	6.40
	30	7.56
pH	4	6.47
	5	8.14
	7	8.49
	8	4.43

Table (4): Kinetic Terms on amylase enzyme activity of granary weevil*, S. granarius* at 35°C pH 7

Kinetic Parameters	**Enzyme activity (mg glucose/min /mg protein)**
Theoretical Maximum Velocity (Vmax)	5.55
Dissociation Constant (Michaels constant) (Km)	0.1 M 0.l mole of starch hydrolysis to glucose/min/mg protein

Table (5): Effects of clove powder at concentration of LC_{50} on amylase activity of granary weevil, *S. granarius* at different temperature degrees, different time (min.) of inhibition reaction and the effect of pH on amylase activity in both treated and untreated insects

Different conditions		Enzyme Activity (mg glucose/min/mg protein)		Inhibition percent %
		Untreated insects	Treated insects with clove powder at LC_{50} level	
Temperature Degree °C.	15	4.92	2.36	52
	25	6.52	4.96	23
	35	8	7.12	11
	50	5.86	4.40	24
	60	4	3	25
Time (min.)	5	2.02	2.00	1
	10	4.12	3.01	26
	20	6.40	4.09	36
	30	7.56	5.89	22
pH	4	6.47	4.54	29
	5	8.14	6.10	25
	7	8.49	6.22	26
	8	4.43	4.11	7

The reduction of α-amylase activity by plant extracts could be due to the plant-defense compounds, including inhibitors that act on insect gut enzymes as hydrolases, α-amylases and proteinases (Ryan, 1990; Franco *et al*., 2002). Some molecules of plants inhibit the activity of α-amylase in vitro (Chen *et al*., 1992; Mendiola-Olaya *et al.,* 2000; Prashanth *et al.,* 2001; Franco *et al.,* 2002) as well as other digestive enzymes of insects (Jing *et al*., 2005; Senthil Nathan, 2006). The reduction of this enzyme activity could also be due to a cytotoxic effect of different extracts on epithelial cells of mid gut that synthesize α-amylase. Cytological studies showed clearly a very marked cytotoxicity and disorganization of the mid gut epithelial cells of *Tribolium castaneum* larvae, after ingestion of *P. harmala* extract or pure harmaline. The cytotoxicity of the mid gut epithelial cells and the inhibition of a-amylase activity could explain the observed developmental adverse and mortality of larvae of *T. castaneum* (Jbilou *et al.,* 2008).

Identification of chemical constituents of clove flower, *S. aromaticum* by using GC/MS technique:

Chemical constituents of clove flowers, *S. aromaticum* by using GC/MS technique are tabulated in Table (6), where some phenolic compounds are found with the major compound is Phenol, in addition to 2methoxy3(2propenyl) with retention time 23.91 min and peak, area of 45.56. In Gas Chromatography Mass Spectrophotometry (GC/MS) analysis, it was found that eugenol was the major component of the oil of *S. aromaticum* (Mercy Bastine *et al.,* 2013). It may be responsible for fumigant toxicity, oviposition deterrent, and inhibition of adult development of test insect. The insecticidal activity of the essential oil would be dependent on the active chemical constituents and the gross sensitivity of the target pest to the active chemical principles (Obeng-Ofori *et al.,* 1997). Eugenol is widely used in agricultural applications to protect food from microorganisms during storage, which might have an effect on human health, and as a pesticide and fumigant (Mahdi and Rahman, 2008).

Table (6): Chemical constituents of clove buds, *S. aromaticum* by using GC/MS technique

Peak	Component name	R_t (min)	Area %	Molecular Weight	formula
1	3Allyl6methoxyphenol	23.01	1.67	164	$C_{10}H_{12}O_2$
2	5amino1Hbenzimidazole- 3oxide	23.12	0.47	149	$C_7H_7N_3O$
3	Phenol,2methoxy4(2propenyl) (CAS)	23.28	12.45	164	$C_{10}H_{12}O_2$
4	2Pentyl3phenyl2propenal	23.43	13.40	202	$C_{14}H_{18}O$
5	Phenol, 2methoxy3(2propenyl)	23.91	45.56	164	$C_{10}H_{12}O_2$
6	ThujopseneI3 or Widdrene	24.81	22.49	204	$C_{15}H_{24}$
7	Eugenol	27.10	1.21	164	$C_{10}H_{12}O_2$

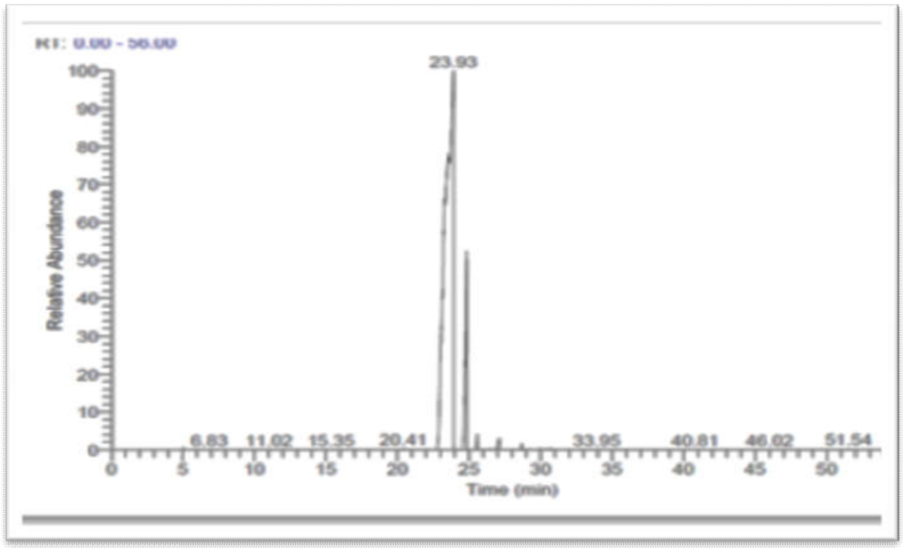
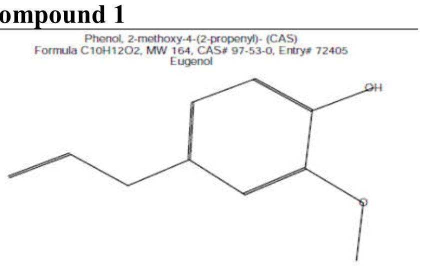


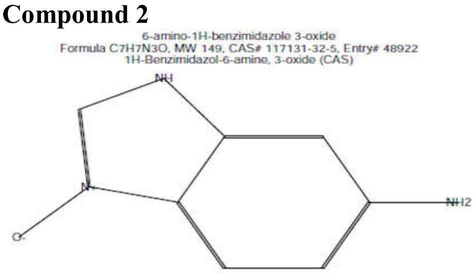
Fig. (2): Identification of clove flowers, *S. aromaticum* by using GC/MS technique

Table (7): Chemical structure of clove flower, *S. aromaticum* components by using GC/MS technique

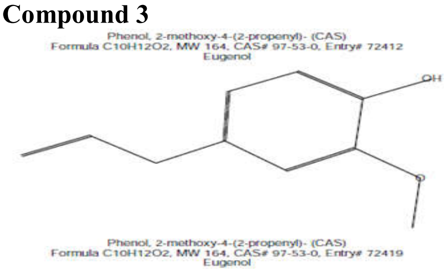
Compound 1



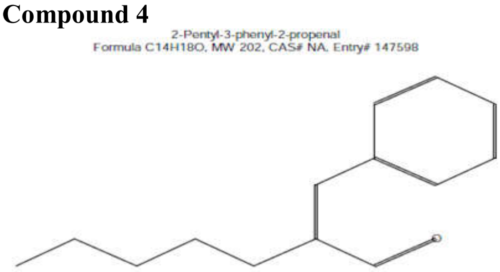
Compound 2



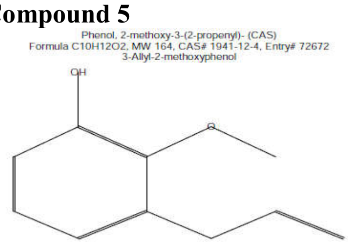
Compound 3



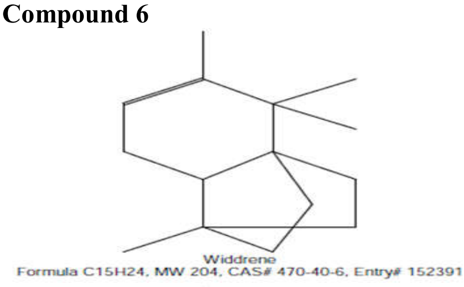
Compound 4



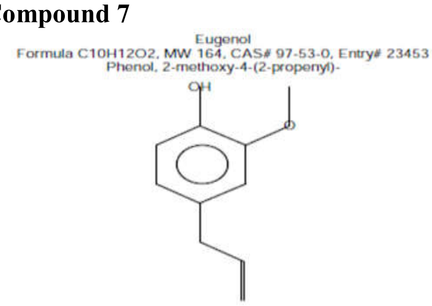
Compound 5



Compound 6



Compound 7



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التأثير التعفيرى لمسحوق براعم القرنفل بمفرده وعند الخلط مع كلا من مبيد الابامكتين و الاسبينوساد ضد الحشرة البالغة لسوسة القمح

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تمت هذه الدراسة بهدف تقييم فاعليه التعفير بمسحوق براعم القرنفل بمفرده أو عند خلطه مع مبيدات الحشرات الابامكتين والاسبيوساد ضد الحشرات البالغة لسوسة القمح. قياس تأثير مسحوق القرنفل على احد الإنزيمات الهاضمة في الحشرات وهو إنزيم الاميليز ثم التعرف على المركبات الموجودة في براعم القرنفل التي يرجع إليها تأثيره السام. كانت التركيزات المختبرة من مسحوق براعم القرنفل (٠.١٢٥ ، ٠.٢ ، ٠.٥ و ١٪) جم مسحوق القرنفل/ ١٠٠ جم من حبوب القمح. وكانت النسبة المئوية لموت الحشرات المعاملة بأعلى تركيز مختبر من مسحوق القرنفل (١%) هي ٢٠ و ٧٨ و ١٠٠٪ بعد ١و٣و٧ أيام من المعاملة. وكانت التركيزات القاتلة لـ ٥٠ % من الأفراد المعاملة هي ١٠.٤ ، ٠.٣٤ و ٠.٢٣٪ بعد يوم ،٣ و ٧ أيام من المعاملة، على التوالي. وجد من نتائج السمية المشتركة عند خلط مبيد الابامكتين مع مسحوق القرنفل أن اقل تركيز من مبيد الابامكتين يمكن أن يحدث تقويه (٥ جزء في المليون) وعامل السمية المشتركة (٣٢.٣). تزداد قيم السمية المشتركة مع زيادة تركيز أبامكتين فوق ٥ جزء في المليون. لوحظ أن أعلى تركيز تم اختباره (القرنفل عند ٠.٢٥٪ + ١٥ جزء في المليون من أبامكتين) حقق أعلى قيمة تقوية لعامل السمية المشتركة (١٤٤).ظهرت زيادة في قيم عامل السمية المشتركة اعتمادًا على تركيزات السبينوساد أيضًا. أعلى تركيز تم اختباره من سبينوساد (١٤٠ جزء في المليون) ، أظهر قيمة سمية مشتركة ١٠٨ عند خلط سبينوساد مع مسحوق القرنفل. تم فى هذا البحث أيضا توضيح تأثير درجات الحرارة المختلفة ومستويات الأس الهيدروجينى وتأثير التغيير من زمن التفاعل على نشاط إنزيم الاميليز المستخلص من الحشرات البالغة لسوسة القمح. ويمكن الاستخلاص من هذا البحث إمكانية مكافحة سوسة القمح بمسحوق القرنفل تعفيرا بمفرده أو زيادة تأثيره بخلطة مع مبيدات الحشرات الابامكتين والاسبينوساد.