Insecticidal activity of Spinosad and Emamectin and Their Mixture with Different Types of Inert Dusts against Khabra Larvae *Trogoderma granarium* Everts

Nabil M. Almallah* & Ahmed M. Jihad

Department of Plant Protection, College of Agriculture, University of Mosul, Iraq

*corresponding author: nbl_mstf@yahoo.com

Abstract: The study showed that the highest activation percentage of the insecticides (Spinosad, Emamectin and the mixture of the insecticides) were obtained when mixed with silica gel for the larvae grown on the corn (2.266, 1.255 and 1300 respectively), while the lowest activation percentage (Antagonism) was recorded for the mixture of each of the Spinosad and Emamectin separately with the gypsum of the larvae grown on the wheat giving 0.776 and 0.989, respectively. The highest of synergism percentage was for silica gel when mixed with each of Spinosad, Emamectin and the mixture of insecticide against the larvae which grown on corn reached 2.014, 1.178 and 1.256 respectively; the lowest of synergism percentage was for gypsum when mixed with the Spinosad and the insecticide mixture against larvae grown on the wheat, which reached 0.454 and 0.918 respectively, while the ratio was 0.841 for the Emamectin insecticide when mixed with the limestone against the larvae which grown on wheat. For the percentage of potentiation, the study showed that the treatment of larvae grown on wheat and corn which treated with a mixture (silica gel and Spinosad insecticide) gave the highest percentage of the potentiation of the pesticide reached 0.41 and 0.252 respectively, and the lowest potentiation percentage of larvae on wheat and maize was recorded for gypsum with the mixture of insecticides reaching 0.082 and 0.016 respectively.

Keywords: Activation, Emamectin, Inert dust, Khabra Larvae, Spinosad, Synergisim.

Introduction

Stored grain insects are one of the main causes of stored grain losses which may reach 9% in developed countries and 20% or more in developing countries (Phillips & Throne, 2010). Khabra beetle *Trogoderma granarium* Everts is one of the most important stored grain pests, the most

dangerous and resistant to insecticides (Mahmood *et al.*, 1996). It

adapts to various environmental conditions, where larvae can survive for 23 months without food and enter into dormancy until the food is available. Nowadays, Khabra beetle developed resistance to many insecticides, especially organophosphorus

compounds (Arthur, 1996). The increased need for pesticide residues free food has led to the search for new and less dangerous techniques of control, including the use of inert dust, biocides or natural extracts from plants or microorganisms that have been evaluated as alternative techniques to the traditional chemical insecticides (Vayias *et al*., 2009). Spinosad and Emamectin are among the most important promising bio-insecticides that are derived from microorganisms, which have a new effect on specific sites in the nervous system of the insect that are the receptors of nicotinic acetylcholine, and the chemical carrier GABA (Gama Amino Butyric Acid) which found in the musculoskeletal regions of the nervous system (Salgado, 1997).

The Spinosad insecticide is a safe pesticide for the environment and public health that affects a wide range of pests, including stored product pests (Fang *et al.*, 2002; Nayak *et al.*, 2005); also it has low toxicity to mammals (Fang *et al*., 2002; Subramanyam *et al*., 2003). For these reasons, Spinosad was recommended by the US Environmental Protection Agency for the protection of stored grain in 2005 (Subramanyam, 2006). Another promising and alternative way to conventional chemical insecticides is the use of inert dust because of its low toxicity to mammals (Subramanyam & Roesli, 2000). These materials are now well known to be scratching the fatty layer which found in the insect's cuticle. Many of these materials are commercially available for use in the protection of stored food and many have proved to be very effective in both field and laboratory tests (Athanassiou *et al*., 2003; 2004; 2005; Kavallieratos *et al*., 2005). On the other hand, the use of inert dusts has a

negative effect on the physical properties of grains, especially the bulk density (Korunic *et al*., 1998). The effectiveness of these substances in the protection of stored food requires high concentrations or doses more than 500 ppm (Vayias *et al*., 2006); this problem is the main constraint in the use of these substances. Also the bio-insecticides are characterized by their slow action compared to conventional chemical insecticides. The present study aimed at overcoming the negative effects or problems resulting from the use of inert dusts or bio-insecticides separately by combining them.

Materials & Methods

Source of Insects and Rearing

Trogoderma granarium Everts was obtained from an insect culture previously raised in the insect research laboratory of the Department of Plant Protection, Faculty of Agriculture and Forestry, University of Mosul. The insect was reared on healthy and cleaned wheat grain *Aestivum triticum* L. on incubator at 30 ± 1°C and 60 ± 5% relative humidity in 1 kg plastic cans. Its nozzle was covered with malleable cloth and sealed with rubber bands. The culture was cleaned from time to time to get rid of the moulting skin. The culture was renewed after each generation by taking the new insects out of the pupa stage to work for other cultures.

Bio-insecticides

Spinosad pesticide

inseticide equipped with a Suspension Concentrate (SC) containing 480 g of Spinosad (active ingredient) per a litre of commercial formulation, the trade name of

the inseticide is Tracer, produced by Dow Agrosciences (Copping & Duke, 2007).

Emamectin

Emulsifiable concentrate containing 2% Emamectin benzoate (effective ingredient) per a litre of commercial formulation, the brand name is EMAVAP produced by Vapco.

The materials used in the study

Silica gel:

It is fine white powder which obtained from the Branch of Basic Sciences, Faculty of Agriculture and Forestry, University of Mosul. Its chemical composition is H2F2S4 type 60.

Limestone Powder

It is a natural calcium carbonate deposit $(CaCO_3)$ in the form of calcite metal forming rocks known as limestone. It may be white or grey. which obtained from the Department of Earth Sciences at the Faculty of Science, University of Mosul.

Gypsum powder

It is a calcium sulphate dihydrate (CaSO4.2H2O) which is deposited naturally as rocks known as gypsum rocks. It was obtained from the Department of Earth Sciences, Faculty of Science, University of Mosul.

Laboratory experiments

All experiments were carried out in the Insect Research Laboratory, Department of Plant Protection during 2013-2014.

1- Effect of acute toxicity values (LC50) of insecticides and inert dust against larvae of Khabra beetle.

Five concentrations of 500, 1000, 1500, 2000 and 2500 ppm were used for the commercial formulation of spinosad and 250, 500, 750, 1000 and 1500 ppm of Emamactin were prepared to test on the third instar larvae with three replicates for each concentration. Each replicate included ten larvae which were placed in a Petri dish after treating with immersion method for two seconds with the above concentrations of each insecticide and 10 g of wheat grains were added to each replicator (the food host on which the insect was fed). The dishes were placed under laboratory conditions at an average temperature of 25 ± 5°C and relative humidity 60 ± 5%. The treatment of the control experiment was treated with water only, and the mortality rate was calculated after 24 and 48 hours of treatment. The experiments of the insecticide mixture were done in the same way after mixing the insecticides with 1: 1 and using five concentrations of 250, 500, 750, 1000 and 1500 ppm.

The values of LC50 for inert powders were calculated by exposing the larvae of the third instar to four concentrations of inert dusts (silica gel, limestone and gypsum) as follows (10, 30, 50 and 70) g. kg^{-1} grains. 10 g of wheat grains were placed with each concentration of inert dusts concentrations the treated material was placed in plastic containers of 50 ml and covered with a tapestry cloth and tied with a rubber band with three replicates per concentration. Ten larvae were transferred to each replicate of the experiment. Treatment of the control experiment was left without the addition of the inert dusts. The containers are placed under laboratory conditions at an average temperature of 25 ± 5°C. The humidity of the grain used in the

study was stabilized to no more than 14% using the HOH-EXPRESS.HE50 grain moisture meter and the death ratio was corrected using the Abbott (1925) equation. The LC50 values for each insecticide were calculated using Finney (1971), mentioned in Al-Mallah & Al-Jubouri (2012).

2- Effect of the values of the active components of inert powders with insecticides against the larvae of the Khabra beetle grown on the wheat and corn seeds

Ten grams of each food host (wheat and corn) was treated with LC10, LC25 and LC50 concentrations of Spinosad and Emamectin separately and their mixed which calculated from the first axis of the study by dipping for one minute with three replicates per treatment. The treated food was then placed on filter paper then transferred to plastic cans, ten third instar larvae were transferred to each replicate.

The cans were covered with mesh cloth and fixed with a rubber band. All treatments were tested under laboratory conditions at an average temperature of 25 ± 5 °C and relative humidity 30 ± 5%, the control treatment was treated only water.

The above-mentioned experiment was repeated for the three inert powders (silica gel, limestone, gypsum); the treated and non-treated wheat and corn grains with Spinosad and Emamectin individually and mixture were treated with LC10, LC25 and LC50 concentrations). Then all these treatments were treated with LC50 concentration for each of the three powders. The data of mortality rates after 1, 3, and 7 days of the treatment was calculated, as the ratio of synergy and activation of the above-mentioned transactions using the following equations mentioned in the Al-Mallah & Al- Jubouri (2012).

First: calculation of the total activation ratios: according to the Metcalf's equation:

$$\text{Total Activation Rate} = \frac{\text{LC50 value of the pesticide}}{\text{LC50 value of the mixture}}$$

Second: Calculation of the synergy ratio: Excluding the potentiation ratio, which represents the percentage of killing caused by the activated substance in the test animals. This method can be summarized in the following steps:

a. Find the corrected mortality ratios for both insecticides and inert dusts concentrations.

b. Find the corrected mortality ratios for insecticides and inert dust mixtures concentrations.

c. Correct the mortality ratio of the mixture using the Abbott equation mentioned in the Al-Mallah & Al- Jubouri (2012)

$$\% \text{ corrected kill for the mixture} =$$

$$\frac{\%Kill\ for\ the\ mixture - \%\ kill\ the\ activated\ substance}{100 - \%Kill\ the\ activated\ substance} * 100$$

d. Determine LC50 values for both insecticide and mixture

e. Calculation of the synergistic effect ratio using Metcalf's equation:

$$\text{Percentage of synergistic effect} = \frac{\text{LC50 value of the pesticide}}{\text{LC50 value for the corrected mixture}}$$

Third: Calculation of the potentiation ratio in activated substances: by using the following equation:

Potentiation ratio = Total activation ratio - Synergy ratio

Results & Discussion

Effect of food host species on the values of the activation constituents of the inert dusts with insecticides against the Khabra beetle larvae:

Table (1) shows that the activation ratios for mixtures of inert dust and insecticides varied according to the type of inert dust, the type of insecticide and the type of host. In general, it was found that when treated larvae reared on corn with a mixture (silica gel and spinosad) gave the highest rate of activation of the insecticide (2.266) and the lowest activation rate appeared when treated larvae reared on wheat with a mixture (gypsum and spinosad) reaching 0.776 (antagonist). It was also noted that the highest activation rate of the three insecticides (Spinosad, Emamectin and their mixture) were obtained when mixed with silica gel for larvae reared on corn at 2.266, 1.255 and 1300, respectively. While the lowest activation ratio was recorded for the mixture of both Spinosad and Emamectin separately with the gypsum of larvae reared on wheat, giving 0.776 and 0.989 respectively.

Table (1): Total activation ratio of silica gel, limestone and gypsum with both Spinosad, Emamectin and the insecticide mixture against the larvae of the Khabra beetle grown on wheat and corn.

Type of food host	Type of Inert dust additive	LC50 value of insecticides and their mixtures ppm			Total Activation Rate		
		Spinosad	Emamectin	Their mixture	Spinosad	Emamectin	Their mixture
Wheat	Without adding	17.087	15.603	13.683	-	-	-
	Silica Gel	13.986	14.175	11.263	1.221	1.100	1.214
	Limestone	17.143	14.758	13.267	0.996	1.057	1.031
	gypsum	21.992	15.771	13.681	0.776	0.989	1
Corn	Without adding	34.727	22.756	18.955	-	-	-
	Silica Gel	17.241	19.305	15.080	2.266	1.255	1.300
	Limestone	21.499	23.293	17.813	1.790	1.051	1.109
	Gypsum	36.045	23.174	17.807	1.101	1.013	1.080

This results were in agreement with Al-Mallah & Al-Sabaa (2005) who showed that LC50 values for insect growth inhibitors (EC 50% Match and EC 10% Dimilin and WP 75% Trigard) varied according to the type of food (date, figs) and the type of insect *Ephestia cautella* Walk. *Ephestia calidella* Guenee. Akbar *et al*. (2004) pointed out that the rustic flour beetle *Tribolium castaneum* did not respond to any of the concentrations used of *Beauveria bassiana*. and addition of diatomaceous earth (DE) powders did not significantly increase the mortality rate of these adults. While the use of DE against the larvae of this insect at concentration of 190 mg.kg^{-1} grains, had the activation effect which enhanced the effectiveness of the fungus *B. bassiana* and at all concentrations, ranging from 33 - 2700 mg of the conidia.kg^{-1}, The same authors confirmed that this ratio supported the hypothesis that the DE material enhanced the effect of *B. bassiana* against larvae *T. castaneum* especially in the affected part of insect cuticle.

Athanassiou & Korunic (2007) found that inert dust which enhanced with abamectin (DEA-p /wp and bitterbarkomycin (DEBBM-p /wp) were more effective against four of the stored beetles (*Sitophilus oryzae*, *Ryzopertha dominica*, *Tribolium castaneum*) as compared to preparations currently commercially available from DE .

Table (2) shows that the synergy ratio for the mixtures of insecticides and inert dust varied according to the type of food host to which the larvae were fed, the type of the inert dust and the insecticide.

Table (2): The synergistic effect of inert dusts (silica gel, limestone and gypsum) with both the Spinosad, Emamectin and the insecticide mixture against the larvae of the Khabra beetle grown on wheat and corn.

Type of food host	Type of Inert dust additive	LC50 value of insecticides and their mixtures after correction			Synergy rate		
		Spinosad	Emamectin	Their mixture	Spinosad	Emamectin	Their mixture
Wheat	Without adding	17.087	15.603	13.683	-	-	-
	Silica Gel	21.060	17.941	12.822	0.811	0.869	1.067
	Limestone	24.546	18.549	14.769	0.696	0.841	0.926
	Gypsum	37.605	17.935	14.905	0.454	0.869	0.918
Corn	Without adding	34.727	22.756	18.955	-	-	-
	Silica Gel	17.241	19.305	15.080	2.014	1.178	1.256
	Limestone	21.499	23.293	17.813	1.615	0.976	1.064

| Gypsum | 36.045 | 23.174 | 17.807 | 0.963 | 0.981 | 1.064 |

Table (3): Values of the activation components for each of the iner dusts (silica gel, limestone and gypsum) with the insecticides (Spinosad, Emamectin and the insecticides mixture) against the Khabra beetle larvae on wheat and corn.

The mixture	Total activation values	Synergy rate	Potentiation rate
Wheat			
Spinosad + Silcagel	1.221	0.811	0.41
Spinosad + Limestone	0.996	0.696	0.3
Spinosad + Gypsum	0.776	0.454	0.322
Emamectin + Silcagel	1.100	0.869	0.231
Emamectin + Limestone	1.057	0.841	0.216
Emamectin + Gypsum	0.989	0.869	0.12
Mixture of insecticides + Silcagel	1.214	1.067	0.147
Mixture of insecticides + Limestone	1.031	0.926	0.105
Mixture of insecticides + gypsum	1	0.918	0.082
Corn			
Spinosad + Silcagel	2.266	2.014	0.252
Spinosad + Limestone	1.790	1.615	0.175
Spinosad + Gypsum	1.101	0.963	0.138
Emamectin + Silcagel	1.255	1.178	0.077
Emamectin + Limestone	1.051	0.976	0.075
Emamectin + Gypsum	1.013	0.981	0.032
Mixture of insecticides + Silcagel	1.300	1.256	0.044
Mixture of insecticides + Limestone	1.109	1.064	0.045

Mixture of insecticides + gypsum	1.080	1.064	0.016

In general, the highest synergism was reached 2.014 for the silica gel and spinosad mixture with Khabra larvae feeding on corn, while the lowest synergism was obtained from larvae feeding on wheat which treated with gypsum and spinosad mixture (0.454).

The synergy ratio of silica gel with Emamectin and insecticide mixture were 1.178 and 1.256 respectively for larvae reared on corn. In a study of the synergistic effect of DE powders with the deltamethrin insecticide in the control of stored food insects and for the purpose of calculating the synergy by potentiation the mixture; Korunic & Rozman (2010) reported the co-toxicity and Co-efficiencies on studies insects which reached more than 100 (170-386) of *Sitophilus zeamais*, (188-601) for *Ryzopertha dominica* and *T. castaneum* (157- 285), which clearly indicate the synergistic effect of DE and deltamethrin.

Finally, it was found from the table (3) that the highest potentiation was from the silica gel and Spinosad mixture as it reached 0.41,

0.252 for larvae reared on wheat and corn respectively. While the lowest potentiation percentage was for the gypsum and insecticide mixture which reached 0.082, 0.016 for larvae reared on wheat and corn respectively. Chintzoglou *et al.* (2008) demonstrated the possibility of combined use of spinosad powder with DE but the effectiveness of this usage differed according to the target insect and the type of treated grains.

Vayias *et al.* (2009) indicated that low doses of DE (less than 600 ppm) with spinosad (less than 1 ppm) to control the adults, larvae of *Tribolium confusum* especially at a temperature greater than 25 °C. The results of the Wakil *et al.* (2013) on the effect of grain type showed an increase in the death rates for the insects fed to the treated wheat compared to rice or corn.

Conclusion

The type of food host (wheat, corn) used in insect rearing, the type of inert dust and the insecticide had played an important role for Khabra larvae response to insecticides and their mixture with inert dusts which is reflected on the values of total activation and ratios of synergy and potentiation. The highest activation and synergy of insecticides were achieved when mixed with the silica gel for the larvae reared on the corn, while the lowest activation rate (antagonist) was recorded at mix it with gypsum and for larvae reared on wheat.

Acknowledgments

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: all applicable national and international guidelines for the care and use of animals were followed.

References:

Abbott, W.B. (1925). A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265-267.

Akbar, W.; Lord, J.C.; Nechols, J.R. & Howard, R.W. (2004). Diatomaceous earth increases the efficacy of *Beauveria bassiana* against *Tribolium castaneum* larvae and increases conidia attachment. J. Econ. Entomol., 97(2): 273-280

Al-Mallah, N.M & Al-Jubouri, A.R. (2012). Practical applications in pesticides. Dar Al Ola Publ., Mosul: 537pp.

Al-Mallah, N.M & Al-Sabaa, R.R (2005). Effect of the type of food host and some of the growth inhibitors in the rate of loss of food and the rate of increase of fig leaf and raisins. Tikrit Col. Pure Sci., 10 (1): 25-29.

Arthur, F.H. (1996). Grain protectants: current status and prospects for the future J. Stored Prod. Res., 32: 293-302.

Athanassiou, C.G. & Korunic, Z. (2007). Evaluation of two new diatomaceous earth formulations, enhanced with abamectin and bitterbarkomycin, against four stored-grain beetle species. J. Stored Prod. Res., 43 (4): 468-473.

Athanassiou, C.G.; Kavallieratos, N.G. & Andris, N.S. (2004). Insecticidal effect of three diatomaceous earth formulations against adults of *Sitophilus oryzae* (Coleoptera: Curculionidae) and *Tribolium confusum* (Coleoptera: Tenebrionidae) on oat, rye and triticale. J. Econ. Entomol., 97: 2160-2167.

Athanassiou, C.G.; Kavallieratos, N.G.; Tsaganou, F.C.; Vayias, B.J.; Dimizas, C.B. & Buchelos, C.Th. (2003). Effect of grain type on the insecticidal efficacy of SilicoSec against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). Crop Prot., 22: 1141-1147.

Athanassiou, C.G.; Kavallieratos, N.G.; Economou, L.P.; Dimizas, C.B.; Vayias, B.J.; Tomanovic, S. & Milutinovic, M. (2005). Persistence and efficacy of three diatomaceous earth formulations against *Sitophilus oryzae* (Coleoptera: Curculionidae) on wheat and barley. J Econ. Entomol., 98: 1404-1412.

Chintzoglou, G.; Athanassiou, C.G. & Arthur, F.H. (2008). Insecticidal effect of spinosad dust, in combination with diatomaceous earth, against two stored-grain beetle species. J. Stored Prod. Res., 44: 347- 353.

Copping, L.G., & Duke, S.O. (2007). Natural products that have been used commercially as crop protection agents. Pest Manag. Sci., 63(6): 524-554.

Fang, L.; Subramanyam, B. & Arthur, F. H. (2002). Effectiveness of Spinosad on four classes of wheat against five stored product insects. J. Econ. Entomol., 95(3): 640-650.

Finney, D.J. (1971). Probit analysis. 3rd ed. London, Cambridge Univ. Press: 333pp.

Kavallieratos, N.G.; Athanassiou, C.G.; Paschalidou, F.G.; Andris, N.S. & Tomanovic, Z. (2005). Influence of grain type on the insecticidal efficacy of two diatomaceous earth formulations against *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae). Pest Manag. Sci., 61: 660-666.

Korunic, Z. (1998). Diatomaceous earths, a group of natural insecticides. J Stored Prod. Res., 34: 87-97.

Korunic, Z. & Rozman, V. (2010). A synergistic mixture of diatomaceous earth and deltamethrin to control stored grain insects. 10th Int. Working Conf. on Stored Product Protection. 27 June to 2 July 2010, Estoril, Portugal. Julius Kühn-Institut, Berlin, Germany.

Mahmood, T.; Ahed, M.S. & Ahmeed, H. (1996). Dispersion of stored grain insect pests in a wheat fill Silo. Int. J. Pest Mang. 42: 321-324.

Nayak, M.K.; Daglish, G.J. & Byrne, V.S. (2005). Effectiveness of spinosad as a grain protectant against resistant beetle and psocid pests of stored grain in Australia. J. Stored Prod. Res., 41: 455-467.

Phillips, T.W. & Throne, J.E. (2010). Biorational approaches to managing Stored-product insects. Anim. Rev. Entomol., 55: 375-397.

Salgado, V.L. (1997). The mode of action of spinosad and other insect control products. Down to Earth, 52(1): 35-43.

Subramanyam, Bh. & Roesli, R. (2000). Inert dusts.. In: Subramanyam, B. & Hagstrum, D.W. (Eds.). Alternatives to pesticides in stored-product IPM. Kluwer Academic Publishers, Dordreecht, pp.321-380.

Subramanyam, Bh., Toews, M.D. & Fang, L., (2003). Spinosad: an effective replacement for organophosphate grain protectants. Pp. 916–920. In: Credland, P.F.; Armitage, D.M.; Bell, C.H.; Cogan, P.M. & Highley, E. (Eds.). Proc. 8th Int. Working Conf. on Stored-Product Protection, 22-26 July 2002, York. CAB Int., Wallingford, Oxon.

Subramanyam, Bh. (2006). Performance of spinosad as a stored grain protectant. Pp: 730–740. In: Lorini, I., Bacaltchuk, B., Beckel, H., Deckers, E., Sundfeld, E., dos Santos, J.P., Biagi, J.D., Celaro, J.C., Faroni, L.R.D'A., de Bortolini, L.O.F., Sartori, M.R., Elias, M.C., Guedes, R.N.C., da Fonseca, R.G., Scussel, V.M. (Eds.). Proc. 9th Int. Working Conf. on Stored-Product Protection, 15-18 October 2006, Campinas. ABRAPOS, Rodovia.

Vayias, B.J.; Athanassiou, C.G.; Kavallieratos, N.G. & Buchelos C.T (2006). Susceptibility of different European populations of *Tribolium confusum* (Coleoptera: Tenebrionidae) to five diatomaceous earth formulations. J. Econ. Entomol., 99: 1899-1904

Vayias, B.J.; Athanassiou, C.G. & Buchelos, C.Th. (2009). Effectiveness of spinosad combined with diatomaceous earth against different European strains of *Tribolium confusum* du Val (Coleoptera: Tenebrion-idae): Influence of commodity and temperature. J. Stored Prod. Res., 45: 165-176.

Wakil, W.; Riasat, T. & Lord, J.C. (2013). Effects of combined thiamethoxam and diatomaceous earth on mortality and progeny production of four Pakistani populations of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) on wheat, rice and maize. J. Stored Prod. Res., 52. 28-35.