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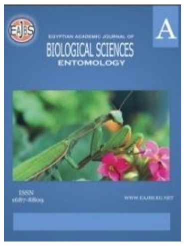

Effects of Various Salts on The Efficacy of *Bacillus thuringiensis* against the Larval Instar of Fall Armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae)

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ABSTRACT

This research aims to study the efficacy of some chemical additives along with commercial compounds for *B. thuringiensis* (Agrien) to increase its ability to control the Fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). Hence, the simi-filed experiment was carried out to investigate the impact of five inorganic salts: Zinc Sulfide (ZnS), Potassium Chloride (KCl), Calcium Oxide (CaO), Sodium Bicarbonate ($NaHCO_3$) and Potassium Sulfate ($K2SO_4$) on the potency of *Bacillus thuringiensis* (Bt) formulation against *Spodoptera frugiperda* larvae. Three concentrations of each salt (0.1, 0.25 and 0.5%) mixed with the recommended dose of Agrien were assayed against the 2nd instar larvae of fall armyworm. The obtained results indicated that the Bt+KCl 0.5% recorded the highest corrected mortality percentage (C.M.%) 60.0% followed by Bt formula (Agrien) with 54.67% with no significant differences. On the contrary, the Bt + K_2SO_4 0.1% treatment gave the lowest (C.M.%) 13.3%. Regarding the effect of the tested compounds on the activities of certain enzymes, its noticed that; the activity of Acetylcholinesterase enzyme (AchE) increased significantly in the 2nd instar larvae under four treatments; Bt+ZnS 0.25%, Bt+KCl 0.25%, Bt+CaO 0.25%, and Bt+K_2SO_4 0.25% compared to the control. The activity of (AchE) decreased under the Agrien (92.67 ug AchBr/min/g.b.wt) formula treatment compared to the control (103.3 ug AchBr/min/g.b.wt) with no significant differences. On the other hand, three of the tested compounds significantly inhibited the activity of Acid phosphatase (ACP) compared with the control, Agrien gave the lowest activity (430.67Ux103 /g.b.wt) followed by Bt+KCl 0.25%and Bt+K_2SO_4 0.25% (675.67 and 754.33 Ux103 /g.b.wt), respectively as compared with the control (810.3 Ux103 /g.b.wt). All treatments resulted in the inhibition of Protease enzyme activity. There were significant differences between the control and all other treatments. Finally, the activity of the Amylase enzyme increased under two treatments, Bt+$NaHCO_3$ 0.25% (153.33 µg glucose /g.b.wt.) and Bt+K_2SO_4 0.25% (115.67 µg glucose /g.b.wt.).

INTRODUCTION

Fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is a significant pest with a very broad host range of plants, where it has been observed on more than 353 plant crops, such as maize, sweet corn, sorghum and rice (Casmuz et al., 2010 and Montezano *et al.*, 2018). In addition, *S. frugiperda* can infest and damage vegetable crops

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including capsicum, chilli, tomato, pumpkins, cucumber, beans, eggplant and other vegetables (Acharya *et al.*, 2020). The first outbreak of Fall Armyworm (FAW) in Africa was reported in West Africa in late 2016 and Sisay *et al.* 2019). In Egypt, FAW is considered one of the most serious pests, and according to the Agricultural Pesticide Committee (APC), Ministry of Agriculture the FAW was recorded on maize fields in a village of Kom-Ombo city, Aswan Governorate, Upper Egypt in May 2019 (Dahi *et al.,* 2020). Subsequently, it invaded Qena, Sohag, Luxor and Assuit Governorates causing damage to maize fields (Hend *et al.,* 2022). In general, the fall armyworm is a major limiting factor affecting crop and vegetable production, not only in Egypt but also in many other countries. The current use of synthetic insecticides is not effective against fall armyworms; this leads to the use of high doses with repeated applications. The extensive use of insecticides has led to the development of insect resistance, with subsequent pest population outbreaks, a negative impact on the environment, and a great threat to human health (Bakr and Abd El-Bar 2017; Sisay *et al.,* 2019). The best alternatives to the use of chemical pesticides are biopesticides, which are based on microorganisms and are beneficial for agriculture and less harmful to the environment and health (Oliveira *et al.,* 2018). Among the group of bacteria used for biocontrol means, *Bacillus thuringiensis* has been successfully used for many years as both a bioinsecticide (Fang *et al.,* 2009; Raymond *et al.,* 2010; Oliveira *et al.,* 2018 and GC *et al.,* 2020).

Therefore, the main objective of the current study is to assess the impact of some chemical salts additives on *B. thuringiensis* (Agrien) to increase its ability to control the fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae).

MATERIALS AND METHODS

Field and Semi-Field Experiments:

In order to achieve the objective of this study one Kirat (175 m^2) was planted with cabbage (*Brassica oleracea*) seedlings at Qaha research station, Qalubya Governorate in October 2022. To assess the efficacy of Agrien as a Bt. Compound and some chemical additives (inorganic salts) against the fall armyworm larvae were applied as flow: Agrien was sprayed at the recommended rate of 250 gm/ fed and serial dilutions of the selected samples were incorporated with certain concentrations of additive and then were used to treat the cabbage leaves before feeding to the larvae at three concentrations; 0.1%, 0.25% and 0.5% with the following salts Zinc sulfide (ZnS), Potassium chloride (KCl), Calcium oxide (CaO), Sodium bicarbonate ($NaHCO_3$) and Potassium Sulfate (K_2SO_4). Mixtures were prepared by adding 1.25 gm from Agrien to each concentration of salts. The treatments were sprayed on cabbage plants using a solo motor under field dilution rate (250 L water / fed.).

Rearing of Fall Armyworm; *Spodoptera frugiperda*:

A stock culture of *Spodoptera frugiperda* larvae was provided from the cotton leaf worm Department, Plant Protection Research Institute; Agricultural Research Centre (ARC), Giza, Egypt. The *S. frugiperda* larvae were maintained under controlled conditions; 65 ± 5% relative humidity (RH) and 27±1 °C temperature; (Dahi *et al.*, 2020). The larvae were reared on cabbage leaves as natural food until entered the pupae stage. The pupae were observed daily until the adult moths emerged. Moths were transferred to a plastic container (20 lit. capacity with 27 cm height and 24 cm diameter), covered with muslin cloth. The plastic containers were supplied with a cotton plug soaked in 10% honey solution as a sugar source for moths feeding. Another piece of muslin cloth was hung inside the cage for oviposition (Sharanabasappa *et al.*, 2018; Haq *et al.,* 2022). Eggs of *S. frugiperda* were collected from insect colonies and kept in an incubator at 65 % R. H and. 25 °C for hatching. Newly emerging larvae were transferred into Petri dishes and were supplied with discs of cabbage leaves to perform the experiments.

Bioinsecticide:

Agrein 6.5% WP. Bt. compound produced by the agricultural genetic Engineering Research Institute, ARC, Giza, Egypt. It contains *Bacillus thuringiensis* aegypti that distributes a different profile with various combinations of genes from groups; cry1, cry2, cry8, and cry9.

Inorganic Salts:

Zinc sulfide (ZnS), Potassium chloride (KCl), Calcium oxide (CaO), Sodium bicarbonate ($NaHCO_3$) and Potassium sulfate (K_2SO_4). The additives used were obtained from local companies El-Nasr Pharmaceutical Chemical Company and EL-Gomhouria Chemical Company, Cairo, Egypt.

Bioassay with *B. thuringiensis* and Chemical Additives:

Bacillus thuringiensis formula (Agrien), as well as three concentrations for the five inorganic salts mentioned above mixed with Agrien, and control, were assayed against the 2^{nd} instar larvae of *S. frugiperda*. The 2^{nd} instar larvae were selected for the present study. Such cabbage leaves were randomized collected from each treatment after application with the materials under study and transferred to the laboratory and provided to the starved 2nd instar of *S. frugiperda* to feed on. The treated leaves were replaced with untreated ones after 24 hours. The assay was replicated five times for each concentration; twenty-five 2^{nd} instar larvae were used in each treatment. The mortality was recorded 3, 5, and 7 days after feeding and the corrected mortality percentage was calculated.

Biochemical Studies:

Biochemical studies were conducted in the Department of Insect Physiological, Plant Protection Research Institute, Agricultural Research Centre to explain the effect of tested *Bt* formulation alone and its combination with chemical additives on some larvae enzymes.: Protease, Amylase, Acetylcholinesterase (AChE), and Acid phosphatase (ACP).

Enzyme activities in second-instar larvae were determined. The larvae were fed on either cabbage leaves that were first treated with Agrine or cabbage leaves treated with Agrine combined with 0.25% of Zinc Sulfide (ZnS), Potassium Chloride (KCl), Calcium Oxide (CaO), Sodium Bicarbonate (NaHCO3), or Potassium Sulfate (K_2SO_4).

Enzymes Determination: Preparation of Larval Enzymes Solution:

The samples of larvae used in enzyme assays were obtained from those subjected to the experimental biopesticide. The larval enzyme solution was prepared according to the method described by Ishaaya *et al.* (1971). The enzyme solutions were obtained by homogenizing 10 second-instar larvae, representing ca. 2 g. larval weight, in 20 ml distilled water, using a chilled glass Teflon grinder. The homogenate was centrifuged at 8000 r.p.m. for 15 min at 5°C, the deposits were discarded and the supernatants were kept in a deep freezer till use.

Determination of Enzymes Activities:

The determinations of Amylase activity were based on the digestion of starch respectively, by spectrophotometric methods Ishaaya *et al.* (1971). The determination of Protease activity: The proteolytic activity was determined by the casein digestion method described by Ishaaya *et al.* (1971).The determination of Acid phosphatase activity: Acid phosphatase activity was measured according to the method of Laufer and Schin (1971).The determination of Acetylcholinesterase (AChE) activity: was measured according to the method described by Simpson *et al.* (1964).

Statistical Analyses:

Data were statistically analyzed. Mortality data were corrected according to Abbott`s formula (Abbott, 1925). For testing the homogeneity of the control agent Chi-square analysis "$\chi 2$" method was used (Snedcor and Cochran 1982). Data analysis has been employed in this study SPSS Ver. 23 was used to compute ANOVA (P<0.05).

RESULTS AND DISCUSSION

The Efficiency of Certain Control Treatments against the 2nd Instar Larvae of *Spodoptera frugiperda*:

The efficiency of mixing *Bacillus thurigensis* (Agrien) with five inorganic salts in three different concentrations and (Agrien) alone were tested against 2nd instar larvae of *Spodoptera frugiperda* in a laboratory to explore their potential to control the pest. Data represented in Table (1) revealed that the Bt+KCl 0.5% recorded the highest reduction percentage 60.0% flowed by Bt (Agrien) with 54.67%. On the contrary, the Bt + K_2SO_4 0.1% treatment recorded the lowest reduction percentage13.3%. According to the chi-square test; there are significant differences between the 16 treatments whereas χ^2 = 12.59 and χ^2 = 15.51 sig. at 0.05. These treatments could be divided into three groups based on their reduction percentage, 1st group (a) contains on Bt+KCl (0.5%), Agrien, Bt.+KCl (0.25%), Bt.+ZnS (0.5%), Bt.+ ZnS (0.25%) and Bt.+ CaO (0.5%) respectively.

Table 1: Efficacy of some treatments on the percentage of reduction of the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) 2nd instars larvae.

Treatments	Mean number of larvae/ Replecat							
	Post-treatment observations							
	3days		5days		7days		%Total Corrected mortality	
	Mean No.	% Corrected mortality	Mean No.	% Corrected mortality.	Mean No.	% Corrected mortality	Mean No.	%CM.
Bt+ (KCl 0.5)	2.8	44	1.6	68	1.6	68	2.00	60.0 a
(Bt) Agrien 6.5% WP	2.8	44	2.2	56	1.8	64	2.27	54.67 a
Bt+ (KCl 0.25)	3.6	28	2.6	48	1.8	64	2.67	46.7 a
Bt + (ZnS 0.5)	3.8	24	2.2	56	2.0	60	2.67	46.7 a
Bt + (ZnS 0.25)	3.8	24	3.0	40	1.8	64	2.87	42.7 a
Bt+ (CaO 0.5)	3.6	28	3.0	40	2.2	56	2.93	41.3 a
Bt+ ($NaHCO_3$ 0.25)	4.2	16	3.0	33	2.2	56	3.13	34.9 b
Bt+(KCl 0.1)	3.6	28	3.2	36	3.2	36	3.33	33.3 b
Bt+ ($NaHCO_3$ 0.5)	3.6	28	3.6	28	3.0	40	3.40	32.0 b
Bt + (CaO 0.25)	4.0	20	3.8	24	3.2	36	3.67	26.7 b
Bt + (ZnS 0.1)	4.2	16	3.8	24	3.2	36	3.73	25.3 b
Bt + ($K2SO_4$ 0.5)	4.4	12	4.0	20	3.2	36	3.87	22.7 b
Bt+ ($NaHCO_3$0.1)	4.4	12	4.0	20	3.4	32	3.93	21.3 b
Bt+ (CaO 0.1)	4.6	8	4.2	16	3.4	32	4.07	18.7 b
Bt+ ($K2SO_4$ 0.25)	4.4	12	4.0	20	4.0	20	4.13	17.33 c
Bt + ($K2SO_4$ 0.1)	4.8	4	4.2	16	4.0	20	4.33	13.3 c
control	5	------	5	-----	5	----	5	-----

a, b, c mean there is significant difference using chi square (X^2) test at P< 0.05.
X^2 = 12.59 sig. at 0. 05 .
X^2 = 15.51 sig. at 0. 05.

The second group (b) contains Bt + $NaHCO_3$ (0.25%), Bt+KCl (0.1%), Bt + CaO (0.25%), Bt + CaO (0.25%), Bt + ZnS (0.1%), Bt + K_2SO_4 (0.5%), Bt + $NaHCO_3$ (0.1%) and Bt + CaO (0.1%); while the 3rd group contains on two treatments only Bt + K_2SO_4 (0.25%) and Bt + $K2SO_4$ (0.1%). Salama, *et al.* (1989) and Hefez *et al.* (1998) said that inorganic salts such as CaO, $CaCO_3$, $ZnSo_4$ and $K2CO_3$ could potentiate the activity of Bt. formulation (Agerin) against the corn borers, *Chilo agamemnon* and *Ostrinia nubilalis*. In addition, (Girgis 2007) found that the addition of K_2Co_3 and $CaCO_3$ increased the effectiveness of some biopesticides against *Phthorimaea operculella* under greenhouse and laboratory conditions. Zhang, *et al.* (2013) pointed out that many inorganic salts can increase the activity of *Bacillus thuringiensis* against *P.* xylostella larvae at different levels when combined with them, as he found calcium salts (calcium hydroxide, calcium chloride, calcium carbonate and calcium sulfate) have the preference increasing the activity of *B. thuringiensis*, followed by some other salts such as Sodium carbonate, potassium hydroxide and sodium acetate. Priyanka, *et al*. (2021) indicated that treatments of *B. thuringiensis* in addition to inorganic salts were significantly effective against fall armyworm larvae compared with *B. thuringiensis* alone.

Effect of the Tested Compounds on The Activities of Some Enzymes:

Effect on Acetylcholinesterase Enzymes (AchE):

Acetylcholinesterase (AchE) is considered a key enzyme in the nervous system of insects and is responsible for the hydrolysis of the neurotransmitter acetylcholine for ending the neurotransmission process. Thus, if this hydrolysis does not take place as a result of degradation or inhibition in the expression of AchE, build-up of acetylcholine occurs and leads to repeated firing of neurons and ultimately death of the insect (McCaffery, 1999; Gunning and Moores, 2001).

Data in Table (2) pointed that the activity of acetylcholinesterase increased significantly in the larvae of *Spodoptera frugiperda* under four treatments; Bt+ZnS 0.25%, Bt+KCl 0.25%, Bt+CaO 0.25%, and Bt+K_2SO_4 0.25% compared to the control. On the other hand, there were no significant differences between the control and the other two treatments (Agrien and Bt+$NaHCO_3$ 0.25%). The activity of (AChE) was significantly higher in Bt+ $K2SO_4$ 0.25% treatment as compared to the control and Agrien.

The activity of (AchE) correlated inversely and significantly with the total corrected mortality percentage (% CM) whereas r= 0.648 (Fig. 1). It is noticed that (% CM) was recorded at 17.33% when the concentration of (AchE) was 174.33 ug AchBr/min/g.b.wt and (% CM) was 54.67 when (AchE) recorded at 92.67 ug AchBr/min/g.b.wt. Gao (1992) said that increased expression levels of (AChE) in response to pesticide exposure showed insect tolerance and increased (AChE) activity, which seems to be a dominant mechanism conferring resistance in lepidopteran pests. Pesticides mostly, exert toxicity by inhibiting enzyme (AChE) (Bolton and Lim 1991; Muthusamy *et al.* 2011) which degrades acetylcholine (AChE), an essential neurotransmitter in the central nervous system (CNS) of insects, rodents, and humans (Jones 2005).

Table 2: Effect of certain treatments on some enzyme activities of fall armyworm, *Spodoptera frugiperda* (J.E. Smith)**.**

Treat. / Enz.	Control	Agrien	Bt+ZnS 0.25	Bt+KCl 0.25	Bt+CaO 0.25	Bt+$NaHCO_3$ 0.25	Bt+K_2SO_4 0.25	F value	P≤0.05
AchE (ug AchBr/min/g.b.wt)	103.3a	92.67 a	135.00b	146.67b	136.00b	100.33 a	174.33 c	63.98	0.001
Acid Phosphatase ((mU/g.b.wt)	810.3e	430.67b	790.33e	675.67 c	794.00e	1056.00a	754.33 d	831.35	0.001
Protease (ug alanine /min/g.b.wt)	188.88d	37.67a	36.33 a	44.00 a	56.67b	102.00c	56.33 b	218.86	0.003
Amylase (g glucose / min /g body weight)	109.17c	60.97a	80.03b	96.67 b	104.90 c	153.33e	115.67c	23.08	0.001

Means followed by different letters are significantly different according to Duncan's multiple range comparisons (DMRTs). Means followed by the same letter are not significantly different.

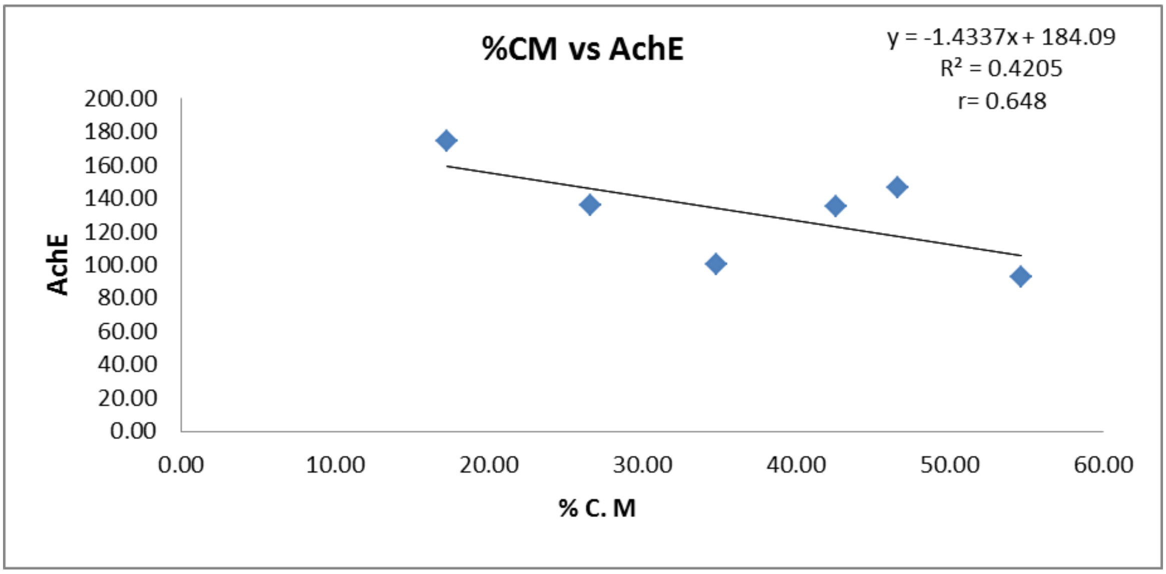

Fig. 1: The correlation between acetylcholinesterase enzyme activities (AchE) and corrected mortality percentage (%C.M.).

Effect on Acid Phosphatase (ACP) Enzymes:

Three of the tested compounds significantly inhibited the activity of Acid phosphatase enzyme (ACP) compared with the control, Agrien gave the lowest decrease (430.67 mU/g.b.wt) followed by Bt+KCl 0.25% and Bt+K_2SO_4 0.25% (675.67 and 754.33 mU/g.b.wt), respectively as compared with the control (810.3 mU/g.b.wt). On the contrary, the Bt+$NaHCO_3$ 0.25% increased phosphatase activity (1056.00 mU /g.b.wt). Figure (2) showed that the corrected mortality percentage correlated reversely and significantly with the activity of acid phosphatase enzyme where r=0.529.

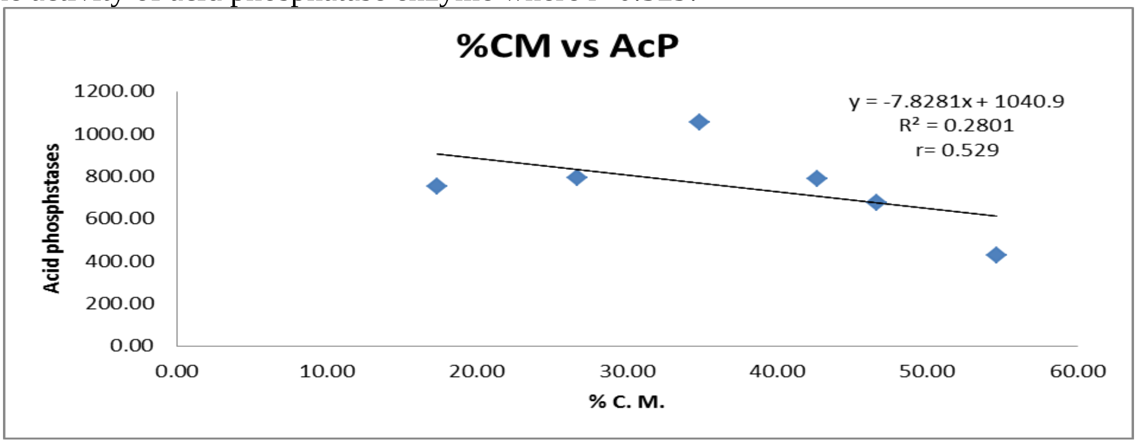

Fig. 2: The correlation between Acid Phosphatases enzyme activities ((AcP) and corrected mortality percentage (%C.M.).

The activity of phosphatase enzymes indicates the efficiency of digestion and absorption of nutrients in the stomach and their transfer to fat bodies. The decrease in the activity of this enzyme group can indicate a lack of digestive function and decreased metabolism due to a reduction in releasing phosphate groups for energy manufacture (SenthilNathan 2006; Younes *et al.*, 2011; Selin-Rani *et al.*, 2016).

Kamel *et al.* (2010) revealed that the ACPE activities decreased significantly in larvae of cotton leafworm *Spodoptera littoralis* after 48 hours of Agrien treatment compared to the control. Changes in ACPE activities after treatment with Bt indicate that changing the physiological balance of the midgut might affect these enzymes.

Effect on Protease Enzymes:

All treatments resulted in the inhibition of protease enzyme activity compared to the control. There were significant differences between the control and all other treatments. On the other hand, there were no significant differences between Agrien, Bt+ZnS 0.25% and Bt+KCl 0.25% (Table2). This result inagreement with Hassan *et al* (2015) who found that addition of Sodium bicarbonate (NaHCO3), and calcium carbonate (CaCO3) to *Bacillus thuringiensis* commercial formula induced a significant increase in the proteolytic activity in the second instar larvae of *Spodoptera littoralis* (Boisd.) compared to the control.

Protease enzyme activities showed inverse non-significant correlation with the total corrected mortality percentage (% CM) whereas r=0.40 (Fig. 3**)**. Salama, *et al.* (1989) said that inorganic salts demonstrated a considerable potentiation of the endotoxin action against the greasy cutworm *Agrotis ypsilon*. The impact of these salts has on the proteolytic enzymes like protease found in the midgut of insects may be connected to the salt's mood of action. Additionally, the efficacy of Bt was significantly increased by calcium salts such calcium carbonate and calcium oxide.

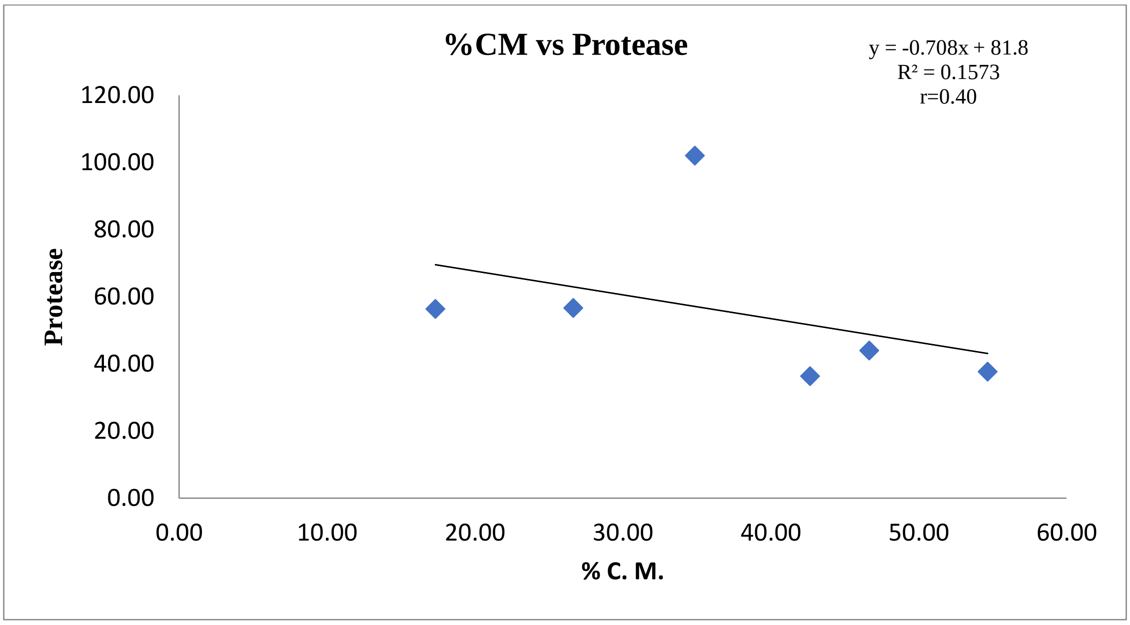

Fig. 3: The correlation between Protease enzyme activities and corrected mortality percentage (%C.M.).

Effect on Amylase Enzymes:

Data illustrated in Table (2**)** revealed the Agrien treatment resulted in the highest decrease in the activity of the Amylase enzyme (60.97 µg glucose /g.b.wt.) compared to the control (109.17 µg glucose /g.b.wt.) followed by Bt+ZnS 0.25%, and Bt+KCl 0.25% (80.03 and 96.67 µg glucose /g.b.wt.), respectively. Also, there were significant differences between the control and the three treatments mentioned above. Conversely, the activity of the Amylase enzyme increased under two treatments, Bt+$NaHCO_3$ 0.25% (153.33 µg glucose /g.b.wt.) and Bt+K_2SO_4 0.25% and (115.67 µg glucose /g.b.wt.) compared with the control (109.17 µg glucose /g.b.wt.).

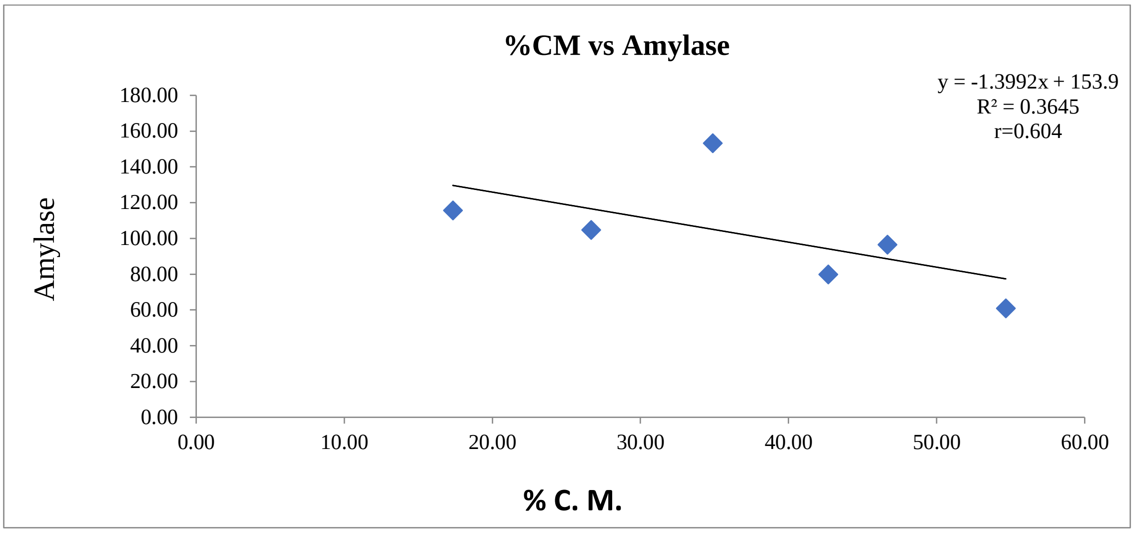

Fig. 4: The correlation between Amylase enzyme activities and corrected mortality percentage (%C.M.).

Figure (4) shows the simple correlation coefficients between the activity of the Amylase enzyme and corrected mortality percentage and the relations between them were inverse. Moreover, the corrected mortality percentage has a positive significant correlation coefficient, r=0.604 with Amylase enzyme activity where the high reduction percentage was 54.67% when the amylase activity was low (60.97 µg glucose /g.b.wt.) under Agrien treatment while Bt+ KCl 0.25% at 54.67% and 46.7%, respectively while the low one was recorded under Bt+K2SO4 0.25% treatment at 17.33% when the amylase activity was high (153.33 µg glucose /g.b.wt.).

Hammati *et al.* (2022) found that both proteases and amylases were most active in the larval midgut extract under alkaline conditions of pH 11 and 10, respectively. Yezdani *et al.* (2010) said that several chemicals compounds; (NaCl, KCl, $MgCl_2$ and $CaCl_2$) had variance effects on the enzyme activity in the midgut of *Glyphodes pyloalis* Walker (Lepidoptera: Pyralidae). Whereas, the highest amylase activity was recorded in the case of KCl at 20 mmol/L. Ca^{2+} ions increase the activity of *α*-amylase in *G. pyloalis* since *α*-amylase is a metalloproteinase and require calcium for maximal activity (De Sales *et al.*, 2008).

CONCLUSION:

The chemical additives with *Bacillus thuringiensis* (Agrien) caused a disturbance in the activities of enzymes such as Amylase, Protease, Acid Phosphatase (ACP), and Acetylcholinesterase (AchE) which play essential roles in the insect body. This indicates that many physiological functions, in the insect body, have been disrupted. Disturbance of the enzyme activities can be considered sub-lethal effects of the tested compounds but could finally lead to death.

REFERENCES

Abbott, W. S. (1925): A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*,18: 295-297.

Acharya, S., Kaphle, S., Upadhayay, J., Pokhrel, A., and Paudel, S. (2020): Damaging Nature of Fall Armyworm and Its Management Practices in Maize: A Review. *Tropical Agrobiodiversity*, 1: 82-85.

Bakr R. F. A. and Abd El-Bar, M. M. (2017): Effect of Lufenuron and Oriza sativa Bran

Extract on Fraction Protein and Acid Phosphatase Pattern in Haemolymph of Schistocerca gregaria. *Egyptian Academic Journal of Biological Sciences (A. Entomology)*, *10* (5): 21-33.

Bolton, T. B., and Lim, S. P. (1991): Action of acetylcholine on smooth muscle. *Zeitschrift fur Kardiologie*, *80*: 73-77.

Casmuz, A., Juárez, M. L., Socías, M. G., Murúa, M. G., Prieto, S., Medina, S., Willink, E., and Gastaminza, G. (2010): Revisión de los hospederos del gusano cogollero del maíz, *Spodoptera frugiperda* (Lepidoptera:Noctuidae). Revista de la Sociedad Entomológica. *Argentina*, 69: 209-231

Dahi, H. F., Salem, S. A., Gamil, W. E. and Mohamed, H. O. (2020): Heat requirements for the fall armyworm *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) as a new invasive pest in Egypt. *Egyptian Academic Journal of Biological Sciences (A. Entomology)*, 13 (4): 73-85.

De Sales, M. P., Alcazar, A., Lima, L. M., Amorim, T. M., Pitanga, J. C. M., Pereira, R. A., and Uchoa, A. F. (2008): Major digestive carbohydrase during larval development of meal moth, Plodia interpunctella (Lepidoptera: Pyralidae). *Protein and Peptide Letters*, *15*(9): 1022-1026.

Fang, Z. F.; Liu, Z. L.; Dai, J. J.; Qian, H. Y.; Qi, Z. L.; Ma, L. B.; Peng, J., (2009): Effects of enzyme addition on the nutritive value of broiler diets containing hulled or dehulled Chinese double-low rapeseed meals. *Journal of Animal Physiology and Animal Nutrition*, 93 (4): 467-476

Gao, X. W. (1992): Effects of host plant on Carboxylesterase activity in cotton aphid *Aphis gossypii* Glov. *Acta Entomologica Sinica*, 3:267-272.

GC, Y., Ghimire, K., and GC, A. (2020): Assessment of Bio-Pesticides against Fall Armyworm Management in Nepal. Advances in Agriculture. *Horticulture and Entomology, AAHE-119*

Girgis, N. R. (2007): Enhancement of the efficiency of some biopesticides against *Phthorimaea opreculella* by adjuvants under laboratory and greenhouse conditions. *Egyptian Journal of Agricultural Research*, 85(4):1327-1334.

Gomez, K.A. and A.A. Gomez, (1984): Statistical procedures of Agricultural research 2 ed. John Willy land Sanes. New York, U.S.A., pp: 680.

Gunning, R. V., and Moores, G. D. (2001): Insensitive acetylcholinesterase as sites for resistance to organophosphates and carbamates in insects: insensitive acetylcholinesterase confers resistance in Lepidoptera. *Biochemical Sites of Insecticide Action and Resistance*, 221-238.

Haq, I. UI., Zhang, K., Ali, S., Majid, M., Ashraf, H.J., Khurshid, A., Inayat, R., Li, C., Gou, Y., Al-Ghamdi, A.A. and Elshikh, M.S. (2022): Effectiveness of silicon on immature stages of the fall armyworm [*Spodoptera frugiperda* (JE Smith)]. *Journal of King Saud University-Science*, *34*(6), p.102152.

Hassan, H. A. A., El-Bar, A., and Marah, M. (2015): Strategies to Enhance the Toxicity of Bacillus thuringiensis Against Spodoptera littoralis (Boisd.). *Egyptian Academic Journal of Biological Sciences. A, Entomology*, *8* (2): 139-149.

Hemmati, S. A., Shishehbor, P., & Stelinski, L. L. (2022): Life table parameters and digestive enzyme activity of *Spodoptera littoralis* (Boisd)(Lepidoptera: Noctuidae) on selected legume cultivars. *Insects*, 13 (7): 661.

Hend, M. O., El-Heneidy, A. H., Dahi, H. F., and Awad, A. A. (2022): First Record of the Fall Armyworm, Spodoptera frugiperda (JE Smith) (Lepidoptera: Noctuidae) on Sorghum Plants, A new invasive pest in Upper Egypt. *Egyptian Academic Journal of Biological Sciences (A. Entomology)*, 15(1): 15-23.

Hirvonen, M.; Paljarvi, R.; and Savolainen, K. M. (1993): Malaoxoninduced neurotoxicity in old rats: alterations in cerebral inositol lipid signalling, brain tissue calcium levels and early neuronal injury.*Toxicology*, 79: 157-167.

Ishaaya, I.; I. Moore and D. Joseph (1971): Protease and amylase activity in larvae of the Egyptian cotton leafworm, *Spodoptera littoralis*. Journal of Insect Physiology. 17: 945-953

Jones, B. E. (2005). From waking to sleeping: neuronal and chemical substrates. *Trends in pharmacological sciences*, 26(11):578-586.

Kamel, A.S., Abd-EL Aziz, M.F. and EL-Barky, N.M., (2010). Biochemical effects of three commercial formulations of Bacillus thuringiensis (Agerin, Dipel 2X and Dipel DF) on *Spodoptera littoralis* larvae. *Egyptian Academic Journal of Biological Sciences. A, Entomology,* 3(1): 21-29.

Laufer, H. and Schin, K. S. (1971): Quantitative studies of hydrolytic enzymes activity in the salivary gland of *Chironomus tentans* (Diptera: Chironomidae) during metamorphosis. *The Canadian Entomologist,* 103: 454-457.

Malik, J. K.; and summer, K. M. (1982): Toxicity and metabolism of Malathion and its impurities in isolated rat hepatocytes: role of glutathione. *Toxicology and Applied Pharmacology,* 66: 69-76.

McCaffery, A. R. (1999): Resistance to insecticides in *heliothine* Lepidoptera: a global view In: Insecticide Resistance: From Mechanisms to Management*;* (Eds. I. Denholm A., Pickett and AL., Devonshire), *CABI* Publishing London, pp. 59-74.

Montezano, D. G., Specht, A., Sosa-Gomez, D. R., Roque-Specht, V. F., de Paula-Moraes, S. V., Peterson, J. A., and Hunt, T. E. (2019): Developmental parameters of Spodoptera frugiperda (Lepidoptera: Noctuidae) immature stages under controlled and standardized conditions. *Journal of Agricultural Science,* 11: 76–89.

Murúa, M. G., Nagoshi, R. N., Santos, D. A., Hay-Roe, M., Meagher, R. L., and Vilardi, J. C. (2015): Demonstration using field collections that Argentina fall armyworm populations exhibit strain-specific hostplant preferences. *Journal of Economic Entomology,* 108, 2305-15. https://doi.org/10.1093/jee/tov203

Muthusamy, Shivakumar, Karthi and Ramkumar (2011): Pesticide detoxifying mechanism in field population of Spodoptera *litura* (Lepidoptera: noctuidae) from South India. *Egyptian Academic Journal of Biological Sciences, F. Toxicology and Pest Control,* 3 (1): 51- 57.

Oliveira, M. A., Iost Filho, F. H., and Thuler, R. T. (2018): Efficiency of *Bacillus thuringiensis* in controlling the corn fall armyworm in lab conditions. *Revista Inova Ciência and Tecnologia/Innovative Science and Technology Journal,* 14-19.

Priyanka, M., Yasodha, P., Justin, C. G. L., Ejilane, J., and Rajanbabu, V. (2021): Biorational management of maize fall armyworm, *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) using Bacillus thuringiensis (Berliner) enriched with chemical additives. *Journal of Applied and Natural Science,* 13(4): 1231-1237.

Salama, H. S., M. S. Foda and A. Sharaby (1989): Potentiation of *Bacillus thuringiensis* endotoxin against the greasy cutworm, *Agrotis ypsilon*. *Journal of Applied Entomology,*108: 372-380.

Selin-Rani S et al (2016): Toxicity of Alangium salvifolium Wang chemical constituents against the tobacco cutworm Spodoptera litura Fab. *Pesticide Biochemistry and Physiolog,*126: 92–101.

Senthil-Nathan S (2006): Effects of Melia azedarach on nutritional physiology and enzyme activities of the rice leaffolder Cnaphalocrocis medinalis (Gnenee) (Lepidoptera: Pyralidae). *Pesticide Biochemistry and Physiolog,* 84:98–108.

Sharanabasappa, c.m. Kalleshwaraswamy, Maruthi M.S, and Pavithra H. B. (2018): Biology of invasive fall army worm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera:

Noctuidae) on maize. *Indian Journal of Entomology*, 80 (3): 540-543.

Simpson, D.R.; D.L. Bull and D.A. Linquist (1964): Asemimicro technique for estimation of cholinesterase activity in boll weevils. *Annals of the Entomological Society of America*, 57:367-371.

Sisay, B., Tefera, T., Wakgari, M., Ayalew, G., and Mendesil, E. (2019): The efficacy of selected synthetic insecticides and botanicals against fall armyworm, *Spodoptera frugiperda*, in maize. *Insects*, 10(2): 45.

Snedecor G.W. and Cochran W.G. (1982): Statistical method, 7th edition the IOWA state University press, Ames, Iowa, U.S.A.

SPSS (2004): Statistical package for Social Sciences. SPSS Inc. Version 13.0 for Windows Statistical package for Social Sciences. SPSS Inc. Version 23.0 for Windows IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.

Younes MWF (2011): Effect of seven plant oils on some biochemical parameters in khapra beetle *Trogoderma Granarium* Everts (Coleoptera: Dermestidae). Egyptian Journal of Experimental Biology, 7:53–61.

Zhang, L., Qiu, S., Huang, T., Huang, Z., Xu, L., Wu, C., and Guan, X. (2013): Effect of chemical additives on *Bacillus thuringiensis* (Bacillales: Bacillaceae) against *Plutella xylostella* (Lepidoptera: Pyralidae). *Journal of Economic Entomology*, *106*(3):1075-1080.

ARABIC SUMMARY

تأثير الأملاح المختلفة على فعالية بكتيريا *Bacillus thuringiensis* ضد الطور اليرقي لدودة الحشد الخريفية *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae)

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يهدف هذا البحث إلى دراسة فاعلية بعض الإضافات الكيماوية مع المركبات التجارية لبكتيريا *Bacillus thuringiensis* مثل (Agrien) لزيادة قدرتها على السيطرة على دودة الحشد الخريفية *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). ومن ثم ، أجريت تجربة نصف حقلية وذلك للتحقق من تأثير خمسة من الأملاح غير العضوية وهي: كبريتيد الزنك (ZnS) ، وكلوريد البوتاسيوم (KCl) ، وأكسيد الكالسيوم (CaO) ، وبيكربونات الصوديوم ($NaHCO_3$) وكبريتات البوتاسيوم (K_2SO_4) على فاعلية مستحضر *B. thuringiensis* (Bt) ضد يرقات *S. frugiperda*. تم تقييم ثلاثة تركيزات من كل ملح (0,1 ، 0,25 و 0,5٪) ممزوجة بالجرعة الموصى بها من Agrien ضد يرقات الطور الثاني من دودة الحشد الخريفية. أشارت النتائج المتحصل عليها إلى أن (Bt + % 0.5 KCl) سجلت أعلى نسبة تخفيض (60,0٪) يليها مركب Agrien بنسبة (54,67٪) مع عدم وجود فروق معنوية. على العكس من ذلك ، أعطت معاملة (Bt + K_2SO_4 0.1%) أقل نسبة موت 13.3٪. فيما يتعلق بتأثير المركبات المختبرة على نشاط بعض الإنزيمات ، فقد لوحظ أن ؛ زاد نشاط إنزيم Acetylcholinesterase (AchE) بشكل ملحوظ في يرقات الطور الثاني تحت أربعة معاملات Bt + ZnS 0.25٪ ، Bt + KCl 0.25٪ ، Bt + CaO 0.25٪ ، Bt + K_2SO_4 0.25٪ مقارنة بالكنترول. انخفض نشاط (AchE) تحت تأثير Agrien (92.67 ميكروغرام AchBr / min / g.b.wt) مقارنة بالكنترول (103,3 ميكروغرام AchBr / min / g.b.wt) مع عدم وجود فروق معنوية. من ناحية أخرى ، أدت ثلاثة من المركبات المختبرة إلى تثبيط نشاط حمض الفوسفاتيز (ACP) بشكل كبير مقارنة مع الكنترول ، حيث أعطى Agrien أقل نشاط (430,67 Ux103 /g.b.wt) يليه Bt + KCl 0.25٪ و Bt + K_2SO_4 0.25٪ (675.67 و 754,33 Ux103 /g.b.wt) ، على التوالي بالمقارنة مع عنصر التحكم (810,3 Ux103 /g.b.wt). أدت جميع المعالجات إلى تثبيط نشاط إنزيم البروتياز. كانت هناك فروق معنوية بين الكنترول وبين باقي المعاملات الأخرى. أخيرًا ، زاد نشاط إنزيم الأميليز تحت معاملتين وهما، Bt+$NaHCO_3$ 0.25٪ و Bt+ K_2SO_4 0.25٪ (153,33 و 115,67 µg glucose /g.b.wt.) على التوالي.