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The Leverage of Foliar Nutrients Combined with Insecticides on The DNA Structure, Protease Enzyme of The Cotton Bollworm, *Earias insulana* (Lepidoptera: Nolidae)

Hassan Mesbah¹, Ahamed Abdel- Mageed¹, Dina ELkobrosy², Aly El-Naggar ³, Hend Abouzaid³ and Hisham M. EL-bassouiny^{*3}

1-Department of Plants Protection, Faculty of Agriculture (Saba-Bacha), Alexandria University.

2-City Sci. Res. & Technol. Applicant. Plant Protect & Biomol. Diag. Dep., Arid Land Cultivate Res. Inst. Alexandria

3-Plant Protection Research Institute, Agriculture Research Center, Giza, Egypt *E-mail: elbassouiny.ppi@arc.sci.eg

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ABSTRACT

In these difficult times, growers must pay special attention to the efficiency of their agricultural operation. Anywhere many growers reduce their staff significantly. So, it's important to utilize the workforce as efficiently as possible in order to stay in business. One source of efficiency is the proper use of tank mixes in the spray program. Tank mix is a combination of more than one crop protection pesticide and/or fertilizer in the spray at the same time. This article will focus on the efficiency of some mixing fertilizer foliar nutrients (Novatrin®, Super Mix® and Potasin-F®) combined with biorationanl insecticide (Radiant®) on Some biochemical processes of spiny bollworm Earias insulana (Boisd.) Use these compounds against E. insulana during the two following seasons in 2018 and 2019 at the experimental farms of the faculties of Agriculture, Saba Bacha- Alexandria-Egypt. The achieved results specified that the highest efficacy was gained by Novatrin/ Radiant®, which decreased the incidence of E. insulana (0.84 larvae /10 green bolls) in the season of 2017. Furthermore, the foliar treatments of Novatrin/ Super Mix/Radiant® and Super Mix/ Potasin-F/ Radiant[®] gave the highest decrease in the level of the *E. insulana* infestation (0.53 and 0.65 larvae /10 green bolls, respectively) in the season of 2019. The consequent treatment of Novatrin/ Radiant® had severity affected on DNA structure and protease enzyme activity in the cotton season of 2017, While, in the cotton season of 2019, all of the performed treatments, to a more or a less, extent unprofitably affected the DNA and Protease enzyme activity but the treatment of Novatrin/ Super Mix/ Potasin-F had more effect on DNA structure and protease enzyme activity.

INTRODUCTION

Cotton is still a major fiber crop of global significance, cultivated in more than seventy countries in the world. Cotton crop is playing an important role in economic, political and social businesses of the world, in terms of both employment and foreign exchange, hence it is popularly known as "White Gold" and "Friendly Fiber". Though it's the most important cash crop in Egypt, and it performs a principal role in the industrial and

Citation: *Egypt. Acad. J. Biolog. Sci.* (F. Toxicology& Pest control) *Vol.14(2) pp 207-217 (2022)* DOI: 10.21608/EAJBSF.2022.276974 agricultural economy of the country. Cotton suffers from numerous injurious species of most serious insects such as bollworms, sucking pests, and leaf worms. Cotton bollworms are considered a problem in growing cotton, because of feeding preferentially on the fruiting structures, which are normally shed after injury (Hearn and Fitt, 1992); they are namely known as; Spotted bollworms, *Earias insulana* (Boisduval), Pink bollworm, *Pectinophora gossypiella* (Saunders); *Earias vittella* (Fabricius) and American bollworm *Helicoverpa armigera* (Hubner) (Akhtar and Farooq, 2019). These bollworms are said to be notorious insect pests, which can be the reason for up to 50 % loss (Dhawan *et al.*, 1998). Bollworms pertaining to OR. Lepidoptera is called lepidopteran species; attacks different plant's fruiting bodies including cotton.

Controls of these insect pests have depended exclusively on conventional. Insecticides though, the Excessive indiscriminate use of organophosphates, carbamates and synthetic pyrethroids has shaped a number of problems such as resistance and pest resurgence (Bajya *et al.*, 2010) and earnestly influenced the population consisting of the natural enemies (Yang *et al.*, 2002 and Younis *et al.*, 2007). A new approach in insect pest control is the use of substances that affect insect growth and development because the reduction of pesticide uses, not only arouse increased economic profit but also contribute to a cleaner environment and improve the health status of farmers (Hossain *et al.*, 2004; Kouser and Qaim 2011; Abdullah *et al.*, 2015).

This goal leads to the search for some alternative methods; the use of foliar fertilization to relieve physiological stress has great potential (Gray and Akin, 1984). The role of bio-fertilizers in agriculture assumes special significance, particularly in the present context of the increased cost of chemical fertilizers and their hazardous effects on soil health. (Kumar *et al.*, 2017). Therefore, foliar nutrient sprays (fertilization) are good instruments to produce a profitable cotton crop to outdo the possible influence and problems resulting from insect and disease damage. also, foliar spray enables mend deficiencies of certain required nutrients in large amounts (macro-elements) and required in trace amounts (micro-elements) (Treshow, 1970; El-Naggar, 1998; Mesbah *et al.*, 2000; El-Naggar, 2009). Recently, great attention has been paid to by several Egyptian and foreign investigators. They proved that the productivity of cotton increased by applying the technique of foliar feeding (El-Menshawi, 2003; Pettigrew *et al.*, 2005; Zakaria *et al.*, 2011).

Therefore, in contribution to the former carried out investigations, the present study aims to evaluate newly adopted trials included, including the bio-fertilizer Microbin, many macros and micro foliar nutrients and alternative chemical compounds, such as standard insecticides (Lambda-cyhalothrin, chloropyrifos) and bio-rational insecticide (Radiant[®]) are known to be effective against bollworms (El-Bassouiny *et al.*, 2015) were evaluated for controlling the incidence of spiny bollworms on the cotton plant; besides, estimating their side effects on the protease enzyme and DNA structure of the inspected larvae of spiny cotton bollworms.

MATERIALS AND METHODS

The experiment of the investigation was conducted along two consequent cotton seasons, namely 2018 and 2019, at two experimental farms in Alexandria Government, the faculty of Agriculture, (Saba Bacha) and the faculty of Agriculture (El-chatby), respectively in Egypt. Whereas, cotton variety "Giza 86" was cultivated on the 20th of April and 1st of May, in both seasons respect. The experimental area was $\frac{1}{2}$ faddan (2100 m²) divided into plots each of 0.01 feddan (42 m²). In both seasons of 2018 and 2019 the randomized

complete block design was utilized in the trial program with three and four replicates for each treatment, as well as, the untreated check, in respect. Each plot was separated from the adjacent one by a half-meter belt to minimize the interference of spray drift from one treatment to another.

In the cotton seasons of 2017 and 2019, pre-planting treated the cotton seeds with bio-fertilizer "Microbin[®]". The trial area was divided into 6 and 7 treatments in addition to an untreated check. Treatments included: 1) foliar fertilizers: "Super Mix, novatrin and potasin -F; 2) synthetic insecticide lambda-cyhalothrin (Katron[®] 5%EC) and chlorpyrifos (Pestban[®] 48%EC), 3) A bio-pesticide "Radiant[®]" which was individually sprayed, three following periods applied in treatments at different period, i.e., (beginning of flowering, fifty percentage of flowering and of the beginning of fruiting).

Assessments were executed for determining the efficiency of the applied treatments to confirm the mixtures against the spiny bollworms *E. insulana*. Also, the side effect of performed treatments was determined on the protease enzyme and DNA structure of occurring spiny bollworm larvae on the treated cotton plants.

Estimation of Bollworms Infestation:

ten green bolls/plot had taken at random as a weekly sample, the inspected bolls for each treatment were 30 and 40 green bolls in both seasons, respectively. In each taken sample, the bolls were inspected externally before dissection and internally examined. Infestation records were based on the existence of injury symptoms regardless of the presence of the larvae. The level of infestation by bollworms was weekly estimated along ten or/ and nine weeks during 2017 and 2019 seasons, respectively.

Extraction, Isolation and Purification of Lepidopteran Spiny Cotton Bollworm Larval Genomic DNA:

The Random Amplified Polymorphic DNA (RAPD) – PCR (Polymerase Chain Reaction) analysis was carried out in the faculty of Agriculture, (Saba Bacha) and city of scientific research and technological applications, District New Borg EL-Arab city, Alexandria Government, Egypt; in the growing seasons of 2017 and 2019, respectively. Genomic DNA was isolated from cotton spiny bollworms larvae which were collected from the field and kept at -20°C. The method of determination is described by Murray and Thampson (1980).

Quantification of Protease Enzyme Expression Using Real-time PCR:

E. insulana larvae collected lively of the almost same age and weight from each plot and transferred to the lab. Comparative quantification analysis was done using Rotor-Gene-6000 Series Software based on the adopted equation by Rasmussen (2001).

Tested Compounds:

1-Foliar Fertilizers; manufactured by the General Organization Agricultural Economic Fund (G.O.A.E.F) (Egyptian Ministry of Agric.):

- **a. Potasin-F:** The ingredients are 30% potassium oxide, 10% fivith potassium oxide, NPK (0: 10: 30).
- b. Novatrin:Contains: K 5%; N 5%; P 5%; Fe 1.5%; Zn 1.5%; Mn 1%; Mo 0.01%; B 0.5%
- **c. Super Mix:**It contains micro and rare elements, a group of amino acids, magnesium and natural growth stimulants.

2-Chlorpyrifos (Pestban[®] 48%EC): PP321; ICIA0321 (both ICI) (Agrochem for Fertilizers & Chemicals, 473 El-Horreya Street, Bolkeley, Alexandria, Egypt).

3-Lambda Cyhalothrin (KATRON[®] 5%EC): PP321; ICIA0321 (both ICI) (Agrochem for Fertilizers & Chemicals, 473 El-Horreya Street, Bolkeley, Alexandria, Egypt).

4-Spinetoram (Radiant® 12 SC): XDE-105; DE-105 (both Dow), Dow, Cairo, Heliopolis 11771, Egypt.

The application of foliar's sequences compared with chlorpyrifos and lambdacyhalothrin as standard insecticides are obtainable in Table (1).

Seasons 2018		Seasons 2019			
Treatments	Rate of application	Treatments	Rate of application		
Novitrin	3.5ml/L.	Novitrin / Super Mix/ Radiant®	3.5ml/L./6.5ml/L/0.4ml/L		
Novitrin / Radiant®	3.5ml /L./0.4ml/L.	Novitrin / Super Mix/ Potasin-F	3.5ml/L./ 6.5ml/L/5ml/L		
Novitrin / SuperMix	3.5ml/L./6.5ml/L.	Novitrin / Potasin-F / Radiant®	3.5ml/L./ 5ml/L/0.4ml/L		
Super Mix / Radiant	6.5ml/L./ 0.4ml/L	Novitrin / Potasin-F / Chloropyrifos	3.5ml/L./ 5ml/L/5ml/L		
Super Mix	6.5ml/L	Super Mix/ Potasin-F/ Radiant®	6.5ml/L./ 5ml/L/0.4ml/L		
Lambda cyhalothrin 1.9m/L.		Super Mix/ Potasin-F/ lambda cyhalothrin	6.5ml/L./ 5ml/L/1.9ml/L		
		Chloropyrifos	5ml/L.		

Table 1: Foliar compound and their rates during the growing seasons of 2018 and 2019.

Statically Analysis:

The data were exposed to the analysis of variance test (ANOVA), with mean separation at 5% levels of significance, Computer program COSTAT and Duncan's Multiple was used to compare the averages according to the method of Snedecor and Cochran (1967).

RESULTS

1-The Combined Effect of Bio-Fertilizers, Foliar Sprays and Certain Insecticides on The Incidence of *E. insulana* Infestation Throughout Two Growing Seasons (2017 and 2019):

A- Season 2017:

The exhibited results in Table 2 show the revealed effects of the achieved foliar treatments on the mean numbers of inspected spiny bollworm larvae /10 green bolls of the bio-fertilized cotton plants. From the table, it could be noticed that each of the subsequently performed sprays of Novatrin / Radiant[®] was highly efficient and recorded the utmost lower level of infestation amounting to 0.84 larvae /10 bolls, while, the other applied foliar treatments, gave a more or a less increased infestation level calibrated from 1.24 larvae / 10 bolls for the treatment of Novatrin / Super Mix to 1.45 larvae / 10 bolls for the treatment of Novatrin. Remarkably, the measured level of the spiny bollworm infestation in untreated check was higher than that calculated for each of the aforementioned treatments and comprised 1.75 larvae / 10 bolls.

vae /10 bol	ls during the growing se	eason of 2018
	Treatments	Mean Numbers of larva/10 bolls
	Novatrin	1.45 ^b
	Novatrin / Radiant®	0.84°
	Novatrin / Super Mix	1.24 ^b
	Super Mix / Radiant®	1.27 ^b

1.24^b

1.42^b

1.75ª

0.29

Table 2: The influence of tested foliar treatments on the mean numbers of *Earias insulana*larvae /10 bolls during the growing season of 2018

Super Mix

LSD.05

P value

Untreated Check

Lambda Cyhalothrin 5%

Means followed by the same latter are not significantly different at the 5 % level

B- Season 2019:

The demonstrated data in Table 3 elucidate the response of bio-fertilized cotton plants to the detected effect of each performed foliar treatment on the calculated means of inspected spiny bollworm larvae / 10 bolls. From the table, it could be revealed that each of the subsequently applied sprayings of Novatrin / Super Mix/ Radiant[®] gave a highly efficient control of the spiny bollworm (0.53 larvae / 10 bolls), followed by Super Mix/ Potasin-F/ Radiant[®] (0.65 larvae / 10 bolls). Consequently, for the other foliar applied treatments, the rate of the determined spiny bollworm infestation amounted to (0.71 larvae / 10 bolls) in the case of Novatrin / Potasin-F / Radiant[®] treatment, (0.78 larvae)-Novatrin / Potasin-F / Chloropyrifos, (1.03 larvae)-Chloropyrifos, (1.06 larvae)-Super Mix/ Potasin-F/ Lambda Cyhalothrin 5% and (1.09 larvae)-Novatrin / Super Mix/ Potasin-F. Comparatively the untreated check gave a high level of spiny bollworm infestation amounting to 1.71 larvae / 10 bolls.

Table 3: The influence of tested foliar treatments on the mean numbers of Earias insulana larvae /10 bolls during the growing season of 2019.

Treatments	Mean Numbers of larvae / 10 bolls		
Novatrin / Super Mix/ Radiant®	0.53 ^d		
Novatrin / Super Mix/ Potasin-F	1.09 ^b		
Novatrin / Potasin-F / Radiant®	0.71 ^{bcd}		
Novatrin / Potasin-F / Chloropyrifos	0.78 ^{bcd}		
Super Mix/ Potasin-F/ Radiant®	0.65 ^{cd}		
Super Mix/ Potasin-F/ Lambda Cyhalothrin 5%	1.06 ^{bc}		
Chloropyrifos	1.03 ^{bc}		
Untreated Check	1.71ª		
LSD.05	0.37		
P value	0.0001		

Means followed by the same latter are not significantly different at the 5 % level

2-The Efficiency of Foliar Application on The Larval Genomic DNA of Spiny Cotton Bollworm Throughout Two Growing Seasons (2017 and 2019).

A. Season 2018:

The illustrated results in Figure (1) considered a confirmative tool for the evaluation of genomic template stability (a qualitative measure reflecting changes in RAPD profiles, which is affected by certain performed foliar compounds of plant nutrients, alternative chemical and bio- compounds. Exposure to these treatments led to an increase or a decrease in the number of amplified polymorphic DNA and increased gene expression. Exposure to such chemicals undoubtedly contributed to the formation of DNA damage in the genomic pool of the lepidopteran bollworm larvae. However, the level of alteration was clearly dependent on the sequent effect (before and after) on the post-treated larvae.

From Figure (1), it could be revealed that each of the performed sprayings of Novitrin / Radiant[®] (2) and Super Mix / Radiant[®] (4) induced DNA damage of the spiny bollworm larvae, followed by the foliar treatment of Super Mix (5). Vice versa, there were no differences between Novitrin (1), Novitrin / Super Mix (3), Lambda Cyhalothrin 5% (6) like the Untreated Check(7), was less effective on the larvae DNA of spiny bollworms.

B. Season 2019:

The illustrated RAPD-PCR profiles in Figure 2, elucidate that exposure to the performed sprayings of foliar nutrients, alternative and biorationanl insecticides gave, a more or less, higher levels of DNA alteration, which are indicated by the appearance and /or the disappearance of DNA bands, compared with those detected in the untreated control. These DNA alterations to more extent are related to the differences in the determined rated mortality of spiny bollworm (*Earias insulana*) induced by each of these consequently tested compounds.



Fig.1: RAPD -PCR profiles of larval genomic DNA bands of spiny cotton Bollworm after tested foliar treatments, throughout the growing season of 2018. Whereas: (M) DNA marker; (B) Blank; (1) Novitrin; (2) Novitrin / Radiant[®]; (3) Novitrin / Super Mix; (4) Super Mix / Radiant[®]; (5) Super Mix; (6) Lambda Cyhalothrin 5%; (7) Untreated Check.



Fig. 2: RAPD-PCR profiles using primer (RAPD15), of genomic DNA bands of spiny cotton Bollworm larvae after foliar treatments throughout the growing season of 2019. Whereas: (M) DNA marker; (C) Untreated Check; (1) Novitrin / Super Mix/ Radiant[®]; ⁽²⁾ Novitrin / Super Mix/ Potasin-F; (3) Novitrin / Potasin-F / Radiant[®]; (4) Novitrin / Potasin-F / Chloropyrifos; (5) Super Mix/ Potasin-F/ Radiant[®]; (6) Super Mix/ Potasin-F/ Lambda Cyhalothrin 5%; (7) Chloropyrifos.

From the figure it could be noticed that each of the performed foliar sprays of Novitrin / Super Mix/ Potasin-F (2) and Super Mix/ Potasin-F/ Lambda Cyhalothrin5% (6) were highly efficient and gave the utmost lower level of the detected spiny bollworm DNA, followed by Novitrin / Potasin-F / Chloropyrifos (4), Novitrin / Super Mix/ Radiant[®] (1), *Novitrin* / Potasin-F / Radiant^(®) (3) Chloropyrifos (7) and Super Mix/ Potasin-F/ Radiant^(®) (5), respectively, whereas, the measured level of DNA band, in the untreated spiny bollworm larvae (C) was comparatively higher than that detected for each of these tested foliar sprays

in the above-mentioned treatments; therefore, it could be suggested that these sequent applied foliar treatments were more or less effective on the synthesis of larval DNA. On the other hand, slight changes occurred in the measured DNA patterns in the other applied foliar treatments Figure (2).

3-The Efficiency of Foliar Application on The Activity of Larval Protease Enzyme Throughout the Two Growing Seasons (2018 and 2019): A-Season 2019:

Table (4) shows to the tested foliar fertilizers and all alternative and biorationanl insecticides have severe effects on the activity of larval protease of the spiny bollworm, *E. insulana* in comparison to the untreated check. In this respect, the foliar treatments of Novatrin/ Super Mix and Novatrin/Radiant[®] decreased the expression level of enzyme activity up to the lowest value of 1.6E-05 and 7.67E-05-fold, in respect, followed by the treatments of Novatrin and Super Mix /Radiant[®] (0.00021and 0.000222-fold, respectively), compared to the untreated check which gave a value amounted to 1.00 (fold). While, the treatments of Lambda Cyhalothrin 5% and SuperMix gave more increased values of the expression level of the enzyme activity, which amounted to 0.000375 and 0.000488, respectively, (Table,4), but less than the untreated check.

Table 4	: Quantitative	SYBR Gree	n RT-PCR	expression	of protease	enzyme	from	cotton
	bollworm und	ler different	reatment du	uring season	n of 2018.			

Treatments	Ratio of expression (fold)		
Novatrin	0.00021		
Novatrin / Radiant®	7.67E-05		
Novatrin / Super Mix	1.6E-05		
Super Mix / Radiant®	0.000222		
Super Mix	0.000488		
Lambda Cyhalothrin 5%	0.000375		
Untreated Check	1.00		

B. Season 2019:

The exhibited results in Table 5 exhibit the determined effects of performed foliar treatments on the estimated values of protease enzyme activity, of inspected spiny bollworms. It could be noticed that the treatments of Novatrin / Potasin-F / Radiant[®] and Novatrin / Super Mix/ Potasin-F gave a greatly decreased expression level of the enzyme activity which amounted to (0.9E-05 - 6.87E-05fold, with respect), followed by the treatments of Chloropyrifos and Novatrin / Super Mix/ Radiant[®](0.000118 and 0.00018 fold, respectively). Versus the other treatments of Novatrin / Potasin-F /Chloropyrifos and Super Mix/ Potasin-F/ Lambda Cyhalothrin 5% which gave slightly increased values of protease activity (0.000188-0.000263 fold, in respect); Super Mix/ Potasin-F/ Radiant[®] which gave a more increased value (0.000508), in comparison to the untreated check 1.00 fold.

Table 5:	Quantitative	SYBR Gree	n RT-PCR	expression	of protease	enzyme	from	cotton
	bollworm und	ler different t	reatment du	uring season	n of 2019.			

Treatments	Ratio of expression (fold)
Novatrin / Super Mix/ Radiant®	0.00018
Novatrin / Super Mix/ Potasin-F	6.87E-05
Novatrin / Potasin-F / Radiant®	0.9E-05
Novatrin / Potasin-F /Chloropyrifos	0.000188
Super Mix/ Potasin-F/ Radiant®	0.000508
Super Mix/ Potasin-F/ Lambda Cyhalothrin 5%	0.000263
Chloropyrifos	0.000118
Untreated Check	1.00

DISCUSSION

Results proved the high efficiency of the evaluated foliar fertilizers and/ or biofertilizer as efficient control agents against the bollworms which are in agreement with those recorded by El-Naggar *et al.* (2008) who found that the applied tri and/or bi - sequent sprays of Baythroid[®] with Greenzit N.P.K and Neem oil with Greenzit S.P₁₀₀ efficiently decreased the rate of spiny bollworm infestation. Also, Gogi *et al.* (2012) observed that nutrient management improves plant health, which enables the plant to tolerate the incidence of herbivores sucking as well as chewing insect pests. Torres-Olivar *et al.* (2014) elucidated that fluctuation in the nitrate (NO₃⁻) application can increase the susceptibility of plants to insect pests as its excessive use decreases lignin concentration, which is a substance used by plants as a physical defense against various insect pests. In addition, Kiran *et al.* (2018) found that the need for more healthful foods is stimulating the development of new techniques to increase plant resistance to phytophagous insects.

The end result also revealed that the application of foliar fertilizers with bioinsecticides was highly effective on the incidence of the bollworms. In this concern, El-Naggar (2009) stated that the foliar treatment with Easterna Aminofert / Greenzit S.P₁₀₀ / Spinosad decreased the incidence of spiny bollworm infestation in cotton season 2006. The foliar treatments of Easterna. / Greenzit S.P₁₀₀ with Spinosad or/and Chlopyrifos were also highly efficient in reducing the level of spiny bollworm infestation.

Herein the changes of DNA polymorphisms detected by RAPD analysis could be used to investigate pest toxicology and as a useful bio-marker assay for the detection of genotoxic effects of plant nutrients and alternative chemical and/or bio-compounds on *E. insulana* larvae. These results are in agreement with those attained in the previous studies, of Saxena *et al.* (2004) who stated that the decrease in DNA bands could be either due to the blocking of such protein synthesis, suppressing DNA synthesis, or blocking in certain proteins, thus preventing the cells from entering mitosis leading to the entanglement of chromatin threads. Also, Maluszynska (2005) found that genotoxic agents cause DNA damage which is either repaired or otherwise leads to iterations of the DNA. Chromosome aberrations are the consequences of a DNA double-strand break that was unrepaired or repaired improperly.

Packiam *et al.* (2015) documented a significant increase in tail length, percent tail DNA and alive tail moment in the treatment of *H. armigera* larvae with a phytopesticide. Plant phenolics have been reported to cause oxidative stress in lepidopteran insects. Also, Wang *et al.* (2018) determined that malathion exposure stimulates DNA damage as evidenced by increased expression of HR (homologous recombination) - and NHEJ (non-homologous end joining)-related genes, which usually participate in the DNA repair process, through HR/NHEJ related proteins.

The above-cited results show that the application of foliar nutrients, insecticides and/ or bio-insecticides, to a more or a less, extent affected the Protease enzyme activity and can result in a break of the balance among the proteases, causing a disorder in the digestive system, which would furtherly affect the growth and reproduction of the insect. That was ascertained by the arrived findings by many workers, i.e., Amorim *et al* (2008) who found that the examined protease activity in *Glyphodes pyloalis* (Lepidoptera: Pyralidae) was inhibited by iodoacetamide, a cysteine protease inhibitor, TPCK, a chymotrypsin inhibitor, and EDTA, a metalloprotease inhibitor, at 47%, 8%, and 28%, respectively.

Also, EI-Naggar and Tawfeek (2012) determined that the treatments sequence of EAP/ Radiant[®] and Methoxyfenozide[®] recorded the high activity of *Earias insulana* larvae Protease enzyme, on the other hand, Radiant[®]/ Methoxyfenozide[®]/ Chlorpyrifos shows the lowest activity. In addition, Mahdavi *et al.*(2013) studied the effect of several metal ions on the activity of proteases and showed that NaCl, CaCl₂, CoCl₂ (5 and 10 mM), and MnCl₂(5mM) reduced the protease activity. Also, Zhao (2019) observed that protease inhibitors had varying degrees of inhibitory effect on protease activities, consequently, extending the growth and development periods of *P. xylostella*.

(Hend, 2020; Mesbah *et. al.* 2020) indicated that the treatments of Novitrin/Radiant[®], Novitrin/Super Mix/ Radiant[®], Novitrin/Potasin-F/ Radiant[®] and Novitrin/Super Mix/ Potasin-F increased the cotton plant tolerance to infestation incidence of cotton bollworms, the highest productivity, a lower percentage of losses and improved fiber properties; besides, it reduced the activity of the protease enzyme, drastically affected the structure of DNA, compared with the untreated check; and gave high economic benefit.

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