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6. SUMMARY

The cotton is one of the major sources for the national economy in Egypt. The cotton leafworm *Spodoptera littoralis* (Boisduval) is considered to be one of the most destructive insect pests in the Egyptian cotton. The cotton leaf worm is a polyphagous insect and causes great damage to a wide variety of hosts including corn, clover, vegetables and fruit trees. The heavy use of conventional pesticides has led to several adverse consequences such as the appearance of resistant strains of this pest towards chemical insecticides, environmental pollution, along with disturbing the natural balance by killing the non-target and beneficial organisms. Therefore, it was a must to search for safer alternatives depending on living organisms, or their products. Biocides, including microbial pesticides and plant derived pesticides are efficient tools of biological control and a corner stone in the integrated pest management programs.

The present study aimed to evaluate the potency of some biocides or plant extracts, also the effectiveness of their combinations together or with the organophosphorus insecticide,

Lorsban (Chlorpyrifos) against a laboratory reared strain of the cotton leaf worm *Spodoptera littoralis* by dipping castor bean leaves in a serial concentrations of each tested compound and presented it for the 4th instar cotton leaf worm larvae for feeding on it.

This study focused on investigating the following points:

***First* : The toxic potential of two different formulations containing bacteria (*Bacillus thuringiensis* var. kurstaki) "Protecto" and "Agerin :** against the 4th instar cotton leaf worm larvae at different concentrations: This entomopathogenic bacteria is regarded as one of the most efficient microbial pest management because it is safe for human and the environment thus it consideres as key component of integrated pest management systems. In addition, *Bacillus thuringiensis* is compatible with other biocides which are entomopathogenic and with chemical insecticides and adjuvants.

***Second* : The toxic potential of two different formulations containing two types of entomopathogenic fungi:** Bio-Ranza (*Metarhizium anisopliae*) and Naturalis-L (*Beauveria bassiana*)

towards the 4th instar cotton leaf worm larvae at different concentrations, these fungi are a potentially useful control agent for suppressing the population levels of several economically important insect species and as environmentally friendly, but they are slower-acting, non-specific, additionally, the environmental factors have a major effect on their activity.

Third: **The toxic potential of a plant extract formulation from Neem tree :** (Achook) consists of "Azadirachtin" as triterpenoid compound which is a feeding deterrent, repellent and growth regulator. Besides it has no effect on the environment ecosystem, don't cause build up of resistance in target insects and is selectively harmless (non-toxic) to human and animals. This formulation is used against the 4th instar cotton leaf worm at different concentrations.

Fourth: **Comparing these five biopesticides with the reference conventional chemical insecticide Lorsban (Chlorpyrifos).**

The LC_{50} were recorded after 7 days of exposure for biocides showed significant difference as compared with the chemical insecticide "Chlorpyrifos" which recorded high significant effect after 1-3 days.

The values of LC_{50} were 3784.4 ppm for Protecto, 3945.2 ppm for Agerin, 4144.66 ppm for Bio-Ranza, 0.02 x 10^5 conidia/ml for Naturalis-L, 454.3 ppm for Achook after 7 days of treatment respectively. While for chlorpyrifos the LC_{50} was 12.73 ppm after 3 days of treatment. Thus, chlorpyrifos formulation exhibited higher toxicity level was followed by the plant extract Achook, then the bacterial formulations, while fungal formulations were the least toxic.

Fifth : **Joint effect study of combinations of these five biopesticides with the chemical insecticide chlorpyrifos** : which is used in controlling cotton leaf worm at the LC_{25} : LC_{25} ratio, the results after 3 days of feeding the 4th instar cotton leaf worm larvae on treated castor bean leaves indicated that the mixtures of chlorpyrifos with each of Agerin, Achook, Protecto and Naturalis-L produced a successive increase on larval mortality percentage which reached +60, +53. 4, +46.6 and + 26.6%, respectively over than the expected mortality (50%) thus showing significant potentiation effect while the binary mixture of Chlorpyrifos with Bio-Ranza revealed only additional effect (-14%).

Also, the biocide mixtures showed significant potentiation effect in the mixtures of each of Achook with Protecto, Agerin, Bio-Ranza

and Naturalis-L, and in the mixture of Naturalis-L with Bio-Ranza. While mixtures of Protecto with either Agerin or Naturalis-L showed additional effect. On the contrary, the binary mixture of Bio-Ranza with each of Protecto or Agerin and Agerin with Naturalis-L resulted in antagonism.

***Sixth* : The impact of subsequent treatment of the five biocides with chlorpyrifos at the LC_{25} levels after 48 hours on *Spodoptera littoralis* 4th instar larvae :** An important finding in the present study was that subsequent treatment of the 4th instar larvae of *Spodoptera littoralis* with LC_{25} of the biocides [two different formulations containing the pathogenic bacteria *(Bacillus thuringiensis)* "Protecto" and "Agerin"; two different formulations containing two types of the entomopathogenic fungi "Bio-Ranza" (*Metarhizium anisopliae)* and "Naturalis – L" (*B. bassiana)* and a plant extract formulation from Neem tree "Achook"] and then post treated with chlorpyrifos after 48 hours with LC_{25} levels resulted in 100% kill of the larvae under all treatments.

Seventh **: The biochemical interfere of biocides and Chlorpyrifos with some biological targets:** in the 4th instar cotton leafworm larvae after feeding the larvae on castor bean leaves treated with serial of concentrations for 48 hrs then feeding on untreated castor bean leaves until the 7th day for biocides treatments and after 48 hrs for Chlorpyrifos treatment. Alive larvae were taken for determining the effect of these toxicants on the specific enzyme activity of both alkaline and acid phosphatases, in addition to chitinase activity.

(A) Determination of alkaline phosphatase activity

1) Effect of Agerin (*Bacillus thuringiensis*)

The results indicated that there was a highly significant increase in the activity of alkaline phosphatase post treatment with all the tested concentrations of bacterial biocide compared to the control groups. This increase reached its maximum at the high concentration 10000 ppm (26.383 μ moles paranitrophenol (PNP). mg protein^{-1}. min^{-1}) almost triple the activity of alkaline-phosphatase in the untreated check, and which was then gradually declined in the activity as the concentration decreased until reached its minimum

value at concentration 1000 ppm recording 11.605 μ moles PNP. mg protein^{-1}. min^{-1} which was more closer to that recorded in the normal larvae (8.489 μ moles PNP. mg protein^{-1}. min^{-1}).

2) Effect of Naturalis-L (the fungus *Beauveria bassiana*)

The results showed that there was a significant increase in alkaline-phosphatase activity at the high concentration followed by a significant decline at the concentration 2.3 x 10^5 conidia/ml then a gradually significant decrease until reached to a level close to the amount of the enzyme in the normal larvae.

3) Effect of Achook (natural extract from Neem tree)

The results showed a high significant increase in the activity of the alkaline-phosphatase post treatment with all the tested concentrations of Achook (Azadirachtin) compared with the untreated check. This increase reached its maximum at the high concentration 1200 ppm recording 23.836 μ moles PNP. mg protein^{-1}. min^{-1} almost triple the activity of alkaline phosphatase in the untreated larvae. Also, there was no-significant increase in the enzyme activity when using low concentration 400 and 600 ppm where the percentage increase reached +5.08 and +16.05%

respectively, followed by a gradual high significant increase as the concentration of Achook increased where the percentage increase reached +20.58, +44.86 and +63.79% respectively at the concentrations 800, 1000 and 1200 ppm, respectively.

4) Effect of Lorsban (Chlorpyrifos)

The lowest level of alkaline-phosphatase activity was detected in the untreated larvae which was (8.489 $\pm$ 0.042) μ moles PNP. mg protein^{-1}. min^{-1} while the highest level in activity was recorded at the higher concentration 10000 ppm which was + 30.45% relative to the untreated check and the activity of the enzyme decreased gradually as the concentration of Chlorpyrifos decreased 1000, 100, 10 and 1 ppm where the reduction percentage recorded were +22.22, + 17.70, + 8.64 and + 3.70 respectively relative to the untreated check.

(B) Determination of acid phosphatase activity

1) Effect of Agerin (*Bacillus thuringiensis*)

The results showed a high significant decrease in acid phosphatase activity post treatment with all the tested

concentrations of the bacterial biocide compared with the untreated check. This decrease reached its maximum at the high concentration 10000 ppm which was 10.17 μ moles PNP. mg protein^{-1}. min^{-1} almost half the activity of acid-phosphatase in the untreated check. That means that the bacterial toxin caused a remarkable decrease in the enzyme activity. The percentage declined gradually by the concentration decrease until it reached its maximum value at concentration 1000 ppm recording 19.55 μ moles PNP. mg protein^{-1}. min^{-1} which was more closer to that recorded in the untreated control larvae (20.80 μ moles PNP. mg protein^{-1}. min^{-1}).

2) Effect of Naturalis-L (*Beauveria bassiana*)

The results indicated that the fungal biocide caused a highly significant decrease in acid-phosphatase activity especially at the higher concentration 2.3 x 10^6 conidia/ml where the reduction percentage were –67.62% followed by a significant rise at the concentrations (2.3 x 10^5, 2.3 x 10^4 and 2.3 x 10^3 conidia / ml) where this percentage reached - 48.49, - 33.72 and – 27.01% respectively relative to untreated check. On the other hand at the lower concentration 2.3x10^2 conidia / ml the enzyme activity was

increased where the inhibition percentage reached – 16.78% compared with untreated check.

3) Effect of Achook (natural extract from Neem tree)

The results showed a high significant decrease in the acid phosphatase at the high concentration 1200 ppm followed by a significant rise at 1000 ppm then a successive and significant increase to a level closer to the amount of the enzyme in the normal larvae which was (20.80 $\pm$ 0.04 μ moles PNP. mg protein^{-1}. min^{-1}) while the enzyme activity was (19.48 $\pm$ 0.115 μ moles PNP. mg protein^{-1}. min^{-1}) thus at lower concentration of Achook 400 ppm and the inhibition percentages were (-15.10, -23.66, - 45.97, - 55.03 and –64.43%) at the concentrations (400, 600, 800, 1000 and 1200 ppm) respectively.

4) Effect of Lorsban (Chlorpyrifos)

The results indicated that there were a highly significant decrease in the acid phosphatase activity post treatment with all the tested concentrations of Chlorpyrifos compared with the untreated check. The occurrence of highly inhibition was observed at the higher concentration 10000 ppm recording 9.73 μ moles PNP. mg

protein^{-1}. min^{-1} almost half the activity of acid phosphatase in the untreated check and the reduction percentages declined gradually by the concentration decrease until reached its minimum value at concentration 1ppm recording 20.37 μ moles PNP. mg protein^{-1}. min^{-1} which was more closer to that was recorded in the normal larvae (20.80 μ moles PNP. mg protein^{-1}. min^{-1}).

(C) Determination of Chitinase activity

1) Effect of Agerin (*Bacillus thuringiensis*)

The bacterial biocide induced a pronounced decrease in chitinase activity at all the five tested concentrions (10000, 8000, 7000, 5000 and 1000 ppm) which caused in general, reduction in enzyme activity of the treated larvae. Also, the results showed a significant reduction with the higher concentrations 10000 and 8000 ppm while at the concentrations 7000, 5000 and 1000 ppm the significant reduction was lower and the inhibition percentage reached –69.46, -61.42, -46.73, -36.05 and –21.01% respectively compared with the untreated check.

2) Effect of Naturalis-L (*Beauveria bassiana*)

The results showed that the inhibition effect of *Beauveria bassiana* on chitinase was much higher on the larvae treated with high concentrations than that treated with low concentrations. There was slight significant reduction in chitinase activity when using $2.3x10^3$ and 2.3×10^2 conidia/ml concentrations, where the reduction percentages were – 27.55 and – 13.78% respectively followed by a gradually highly significant decrease at the concentrations (2.3×10^4, 2.3×10^5 and 2.3×10^6 conidia / ml) where the reduction percentages reached (- 49.02, - 66.25 and – 76.23) respectively relative to untreated check.

3) Effect of Achook (natural extract from Neem tree)

The highest level of chitinase activity was detected in the untreated larvae which was (33.05 $\pm$ 0.049) λ_{416}. mg protein^{-1}. hr^{-1} while the lowest level in activity was recorded at the higher concentration (1200 ppm) which was – 83.35% relative to untreated groups. However, the activity increase thereafter gradually as the concentration of Achook decreased 1000, 800, 600 and 400 ppm where the reduction percentages recorded were – 78.53,-65.33, - 57.98 and – 44.89% respectively relative to untreated groups.

4) Effect of Lorsban (Chlorpyrifos)

The results showed that there were a highly significant decrease in chitinase activity post treatment with all the tested concentrations Chlorpyrifos compared with the untreated groups. This decrease reached its maximum at the high concentration (10000 ppm) which was (17.82) λ_{416}. mg protein^{-1}. hr^{-1} almost half the chitinase activity in the untreated groups, followed by a significant rise at the concentration (1000 ppm) then a successive and significant increase to a level close to the amount of the enzyme in the untreated larvae , where the reduction percentages were (-63.03, - 55.57, - 40.64, - 22.85 and – 9.64%) at the concentrations 10000, 1000, 100, 10 and 1 ppm respectively relative to untreated groups.

Eighth: Comparison between total proteases activity of treated cotton leaf worm with some biocides and Chlorpyrifos:

Total proteolytic activity of 2nd instar larval total homogenate of *S. littoralis* laboratory strain, was measured using azocasein as a substrate by testing three different levels (LC_{50}, LC_{25} and LC_5).

1) Effect of Agerin biocide (*Bacillus thuringiensis*) on total protease activity

The value of total protease activity (expressed as micro $O.D_{440}$ units / mg protein / min) ranged between 183.96, 167.88 and 148.52 at concentration levels of LC_{50}, LC_{25} and LC_5 respectively. Statistical analysis showed that there were significant differences. The percentage increase in activity compared with untreated larvae were 14.47 and 4.46% for LC_{50} and LC_{25} concentrations, respectively.

2) Effect of Naturalis-L biocide *(Beauveria bassiana)* on total protease activity

It was evident from the activity data of larvae treated with the LC_{50} concentration of the fungus biocide Naturalis-L possessed higher protease specific activity recording 181.94 micro $O.D_{440}$ units/ mg protein / min with 13.21% increase percentage compared with untreated larvae. While the larvae treated with LC_{25} concentration produced almost similar activity to that recorded for untreated larvae (159.49 micro $O.D_{440}$ units / mg protein / min) while the larvae treated with LC_5 concentration showed significant decrease in specific activity for this group of enzymes with reduction percentage in activity recording 11.51% compared with the control groups.

3) Effect of the natural product Achook (Azadirachtin) on total protease activity

The results showed a highly significant reduction in the enzyme specific activity post treatment with all the tested concentrations of Neem compared with the untreated check. This decrease reached its maximum at the level of LC_{50} concentration which was 72.36 micro $O.D_{440}$ units / mg protein / min followed by a significant rise at LC_{25} and LC_5 recording 106.8 and 129.47 micro $O.D_{440}$ units / mg protein / min respectively, where the reduction percentages were (-54.97, -33.54 and–19.44%) with LC_{50}, LC_{25} and LC_5 concentrations compared with the untreated check .

4) Effect of Lorsban (Chlorpyrifos) on total protease activity

The data showed that there were significant decrease in total protease activity at the high concentration level of the LC_{50} (98.3 micro $O.D_{440}$ units / mg protein / min), followed by a significant rise at the concentration LC_{25} (122.13 micro $O.D_{440}$ units / mg protein /min). While the value of total protease activity for LC_5 concentration reached (160.78 micro $O.D_{440}$ units / mg protein / min) which was more closer to that recorded at the untreated groups (160.71 micro O.D units / mg protein / min), where the reduction percentages were –38.83 and –24, 01% for LC_{50} and LC_{25} concentrations, respectively.