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Salicylic Acid Enhances the Activity of Some Insecticides Against Bollworms-Infested Cotton Plants

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ABSTRACT

Background: Cotton is one of the most important economic crops across the world and in Egypt. It was in the past, the first source of national income in Egypt. Cotton is affected by many pests, but the most economically harmful are cotton bollworms, which affect the yield at the fruiting stage. To reduce the economic cost of boll-worms controlling and reducing the environmental impact of insecticides using, A Field experiment was conducted, to study the effect of using and mixing salicylic acid (SA) with some recommended insecticides against boll-worms on cotton plants. **Results:** The most effective insecticide against Pink bollworms was profenfos (Prf). Salicylic acid had a slight effect on it. This effect increased with the increasing the SA conc. Adding SA to insecticides enhanced the effect of imidacloprid only. The most effective treatments were Prf+1mM SA. Concerning Spiny bollworm, all insecticide treatments and their combination with SA had highly effective against the pest, with no significant differences among them, SA treatments had a moderate effect against it. Regarding yield and the yield component, imidacloprid and cyhalothrin treatment had the highest No of open bolls/plants followed by profenfos treatment. Profenfos had increased the cotton bolls' weight, seed weight/boll, and yield/ hectare. Also, all insecticide treatments had increased the cotton yield/ hectare, and all treatments increased the crude oil percentage but there is no significant difference among them. **Conclusions:** Salicylic acid enhance the efficiency of selected insecticides against bollworms, and had a slight efficacy against these pests. The additions of SA enhanced the qualities of some productivity aspects but did not affect others. The total productivity (cotton yield/ hectare) has increased by 73.55: 85.67 %.

INTRODUCTION

Cotton is one of the most important fiber crops worldwide. Where it is cultivated in more than seventy countries. It is the raw material for several industries such as textile, ginning, food oil, soap, and many further industries. Cotton is one of the basic sources of national income from foreign currencies (Gillham *et al.*, 1996).

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Cotton plants are infected with several pests from planting until harvest time. including sap-sucking pests, cotton leaf worms, and cotton bollworms, which cause intense economic losses due to reduced yields, and low lint quality (Burrows *et al.*, 1982). The most serious pests are those that invade the fruiting portions, squares, flowers, and green bolls. Bollworms can cause cotton yield losses reached to 60% (Brettell 1986). Among bollworms, Pink bollworm, *Pectinophora gossyipella* (Saund.), and the spiny bollworm, *Earias insulana* (Boisd.) are the most significant pests of cotton in Egypt (Hussein *et al.*, 2002). Cotton plants greatly suffer from infestation with these pests during the late seasons (Mohamed *et al.*, 2013).

The use of insecticides is still the main process for controlling these pests. However, the repeated use of pesticides is associated with many problems, which are the appearance of resistant strains of pests to insecticides, in addition to environmental pollution, and health hazards to humans and farm animals (Nassef and Watson 1999).

Systemic acquired resistance (SAR) is a form of induced resistance that occurs after plants' exposure to elicitors, whether virulent, a virulent, or non-pathogenic microbes or even chemical compounds such as jasmonic acid (JA) or salicylic acid (SA) (Gozzo and Faoro 2013). Salicylic acid synthesis in many prokaryotic and eukaryotic organisms. A strong correlation was found between the presence of SA and SAR in numerous plant species (Klessig and Malamy 1994). The treatment of tobacco plants with SA led to the accumulation of resistance proteins (PR) which are strongly associated with plant resistance (White 1979; Ward *et al.*, 1991). Several studies have been done on the effect of SA on many plant diseases. But few had studied their effect on insect herbivores (Karban and Kuc 1999). Our present study aims, not only to study the effect of SA treatment against bollworms on cotton plants but also to study the effect of mixing SA with a reduced rate of the recommended insecticides used to control bollworms, to reduce the use of insecticides.

MATERIALS AND METHODS

Experimental trials were carried out at the farm of Itay El-Baroud Agric. Res. Station during two growing seasons (2010 and 2011), to evaluate the efficiency of three insecticides tank-mixed with salicylic acid against pink and spiny bollworms (*Pectinophora gossyipella* and *Earias insulana*). The insecticides formulation used were Actacrone 72% E.C (profenofos), Cyhalon 2.5% E.C (cyhalothrin) and Joun 20% WP (imidacloprid).

The experimental trials included 16 spray treatments included:

- Salicylic acid (SA) that used individually at three concentrations (0.5, 0.75, and 1.0 mM)
- The three insecticides (profenofos, cyhalothrin, and imidacloprid)) that used individually at 100% of the recommended rate (15, 37.5, and 75 ml/20L water)
- Nine combinations of treatments including the mixture of each insecticide at 75% of their recommended dose with SA at different concentrations.
- Control which sprayed with 20 ml ethyl alcohol/20 L water (the solvent used to dissolve the SA).

Each insecticide or/and salicylic acid is sprayed twice each season. The first spray was carried out on (August 20) and the second one was conducted after 15 days. The spray applying by a Knapsack Sprayer.

Soil texture was clay soil, with obvious uniforms with no remarkable texture deviances. The seeds of the Giza-88 variety were gotten from the Cotton Plant Research Institute, Giza, Egypt. The experiment design was a complete randomized block. With three replicates of each treatment. Experimental plots consist of 10 ridges (42 m^2). Three seeds were sowed per hill. Distances between hills were 25 cm, on one side of the ridge. After the plantation, one plant was preserved on the hill.

For assessing the infestation by the pink and spiny bollworms, hundred green bolls were collected after each spray for seasons 2010 and 2011 at random from both diagonals of the inner square area of each plot. According to the method of (Shaaban and Radwan 1974). The inspection started on August 20 and continued until the end of each season. Hundred full-sized middle-aged bolls as a total picked up weekly from each plot for estimating the number of infestations of both pink and spiny bollworm larvae. Samples of bolls were transferred to the laboratory and examined to fetch up the pink and spiny bollworm larvae. The numbers of pink bollworms (*P. gossyipella*) and spiny bollworm *E. insulana* larvae were assessed during the two cotton seasons.

The efficiency of pesticides is calculated according to the following equation:

$$\%\ \text{infestation reduction} = \frac{A-B}{A} \times 100$$

Where: A = Counts in control after spraying, B = Counts in treatments after spraying.

Growth and Yield Characters:

Ten plants were chosen at random from every treatment and marked by colored tape at the end of the growing season. The following data were recorded for all treatments:

1. An average number of 10 open bolls/ plant.
2. The average weight of 10 bolls/ plant.
3. Cotton yield/plot

Determination of Crude Oil Cotton Seeds:

Oil percentage in cottonseeds was determined according to the methods of A.O.A.C (1975), after certain modifications. Approximately 100 gm of cleaned cottonseeds were collected from each plot material and placed in a vial. Vials are placed in an oven at 85 - 90°C for two days until drying. Seeds were gridded in the mill and then enfold in (Whitman paper No. 1). Each sample was placed in the extracting unit of the Soxhlet apparatus. N-Hexane was used as a solvent for 6 hrs. After salvation, the samples (the oil content extracted by N-Hexane) were collected and the rotary apparatus was used for the evaporation of the solvent. Remained oil in a conical flask was weighed and calculated the weight of oil / 100 gm seeds.

Statistical Analysis:

All data were subjected to analyses of variance (ANOVA) using the Statistical Analysis System (SAS Institute 2011). Means separated by least significant difference (LSD) test at 5% probability level.

RESULTS

Effect of the Insecticides Used Alone and In Combination with Salicylic Acid Against Some Cotton Bollworm Larvae:

Pink Bollworm, *P. gossyipella* larvae:

All treatments reduced the average number of larvae compared with the control. The most effective insecticide against pink bollworms was profenfos (Prf). Salicylic acid (SA) had a slight effect on the pest. This effect increased with the increase of SA conc. Adding SA to insecticides enhanced the effect of imidacloprid (Imd). But reduced the effect of the other insecticides. The most effective treatments were Prf+1mM SA, profenofos alone, and Prf+0.75 mM SA in both seasons (Fig 1).

The Spiny Bollworm, *Earias insulana* (Boisd.):

All insecticide treatments and their combination with SA had highly effective against the spiny bollworm *E. insulana* (Boisd.). There are no significant differences among those treatments. SA treatments had a moderate effect against the pest.

In the case of the 2010 season, there are no significant differences between profenfos and cyhalothrin (Cyh) or their combination with SA treatments. Flowed by the imidacloprid and imidacloprid/SA combination treatments. The same trend was observed in the 2011 season. All insecticides and insecticides/SA combination treatments had a strong effect against the spiny bollworm. There are no significant differences detected among them.

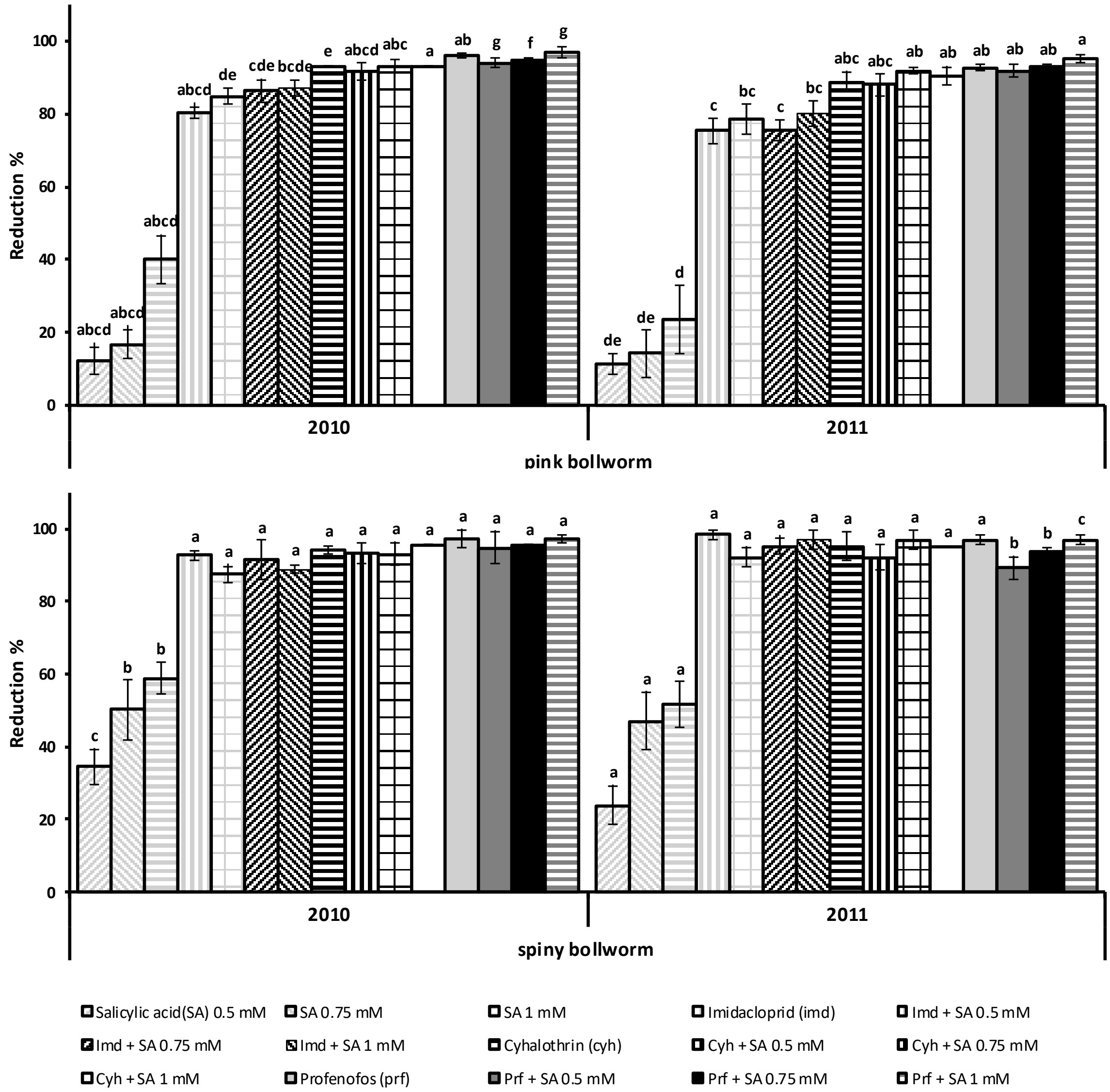

Fig 1: Efficiency of salicylic acid tank mixed with some insecticides against the Pink bollworm, *P. gossyipella*, and the Spiny bollworm, *Earias insulana* (Boisd.) in cotton plants.

Effect of the Insecticides Used Alone and In Combination with Salicylic Acid on Some Yield Characters:

The Number Of Open Bolls/Plant:

The average number of open bolls/plant was significantly influenced by spraying insecticides and salicylic acid compared with control (untreated check) in the two growing seasons (Fig 2). Regarding insecticides only, imidacloprid and cyhalothrin treatment had the highest No of open bolls/plant followed by profenfos treatment, in season 2010. While there are no significant differences among the insecticide treatments. SA treatments increase the No of open bolls/plant in both seasons (% control ranged between 112: 114 for the first season and 101: 117 for the second season). The effect had a positive correlation with the

SA rate of application. The maximum average number of open bolls/plant obtained from Imd + 0.75 mM SA followed by Imd +1 mM SA treatments. That, in the first season. While Imd + 0.75 mM SA had the maximum No of open bolls/ plant, no significant differences were observed among the rest of the insecticides and insecticides/SA combination. SA treatments were the least effective ones.

Average Bolls Weight:

Average bolls weight (g) was significantly influenced by spraying cotton plants with insecticides alone and in combination with salicylic acid in the two growing seasons (except for SA 0.5mM treatment in the second season, which had no significant difference between it and the control treatment). For the insecticides only, results showed that the highest average of bolls weight (g) was recorded in the profenfos treatments followed by imidacloprid and cyhalothrin, in season 2010. While in the 2011 season, no significant differences were observed among the insecticides. SA treatment had a slight effect on average bolls weight (g) in both seasons (% control ranged between 103:108 for the first season and 102:112 for the second season). With a positive dependent between increasing and application rats used. The highest bolls weight/boll recorded in Imd + 1 mM SA flowed by Cyh_l+SA1mM treatments, in the first season. While in the second season the highest boll weight was recorded in Imd + 1 mM SA flowed by Prf + 1 mM SA and Prf + 0.75 mM SA.

Seed Weight/boll:

Results shown in Figure (2**)** revealed the effect of the tested insecticides used alone and in combination with SA on seed weight/boll during the growing seasons of 2010 and 2011. data showed that. some treatments reduced the studied parameter. all imidacloprid/SA combination, cyhalothrin and Cyhl+SA 0.5 mM treatments (in season 2010) and Imd + 0.5 mM SA and Cyhl+ 0.5mM SA (in season 2011) had reduced the seed weight/boll significantly. while the rest treatments increased the studied parameter. for insecticide application only. Cyhalothrin reduced the seed weight/boll in both seasons. Profenfos increase the parameter in the 2011 season only. while imidacloprid increases the seed weight/boll significantly (% control 112.79 and 117.95 for the 2010 and 2011seasons, respectively). Salicylic acid treatments had a fluctuation effect on the seed weight/boll. the effect was not dependent on the SA application rats. the highest seed weight/boll recorded in Cyhl + 0.75mM SA, 0.75 mM SA, and Prf + 0.5 mM SA in both seasons.

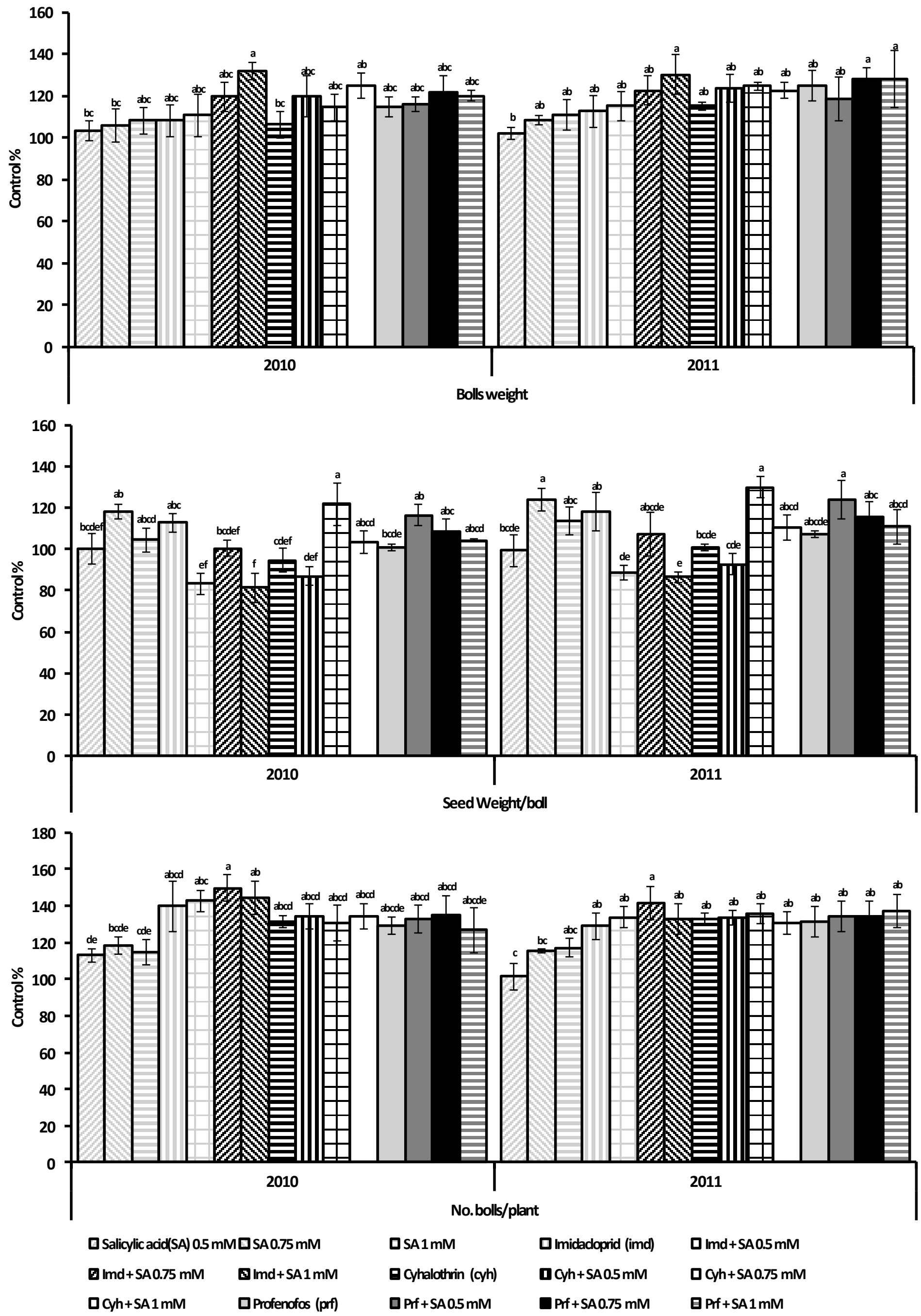

Fig.2: Effect of salicylic acid tank mixed with some insecticides on some cotton plant yield characteristics.

Yield/ Hectare:

The results illustrated in Figure (3) showed the effect of insecticides and salicylic acid at different rates on cotton yield/ hectare in growing seasons (2010 and 2011). All treatments significantly increased the cotton yield/hectare. Expect 0.5 mM SA treatment in the 2011 season, which had no significant difference between it and the control treatment. For insecticides only, all insecticides treatments had increased the cotton yield/ hectare, with no significant difference among them, in both seasons. But the highest cotton yield/ hectare was recorded in profenfos and cyhalothrin followed by imidacloprid. The highest cotton yield/ hectare recorded in Imd + 1 mM SA (%control 185.67) followed by Imd + 0.75 mM SA(%control 173.55). That was in season 2010. While, the highest cotton yield/ hectare observed in Prf+ 0.75 mM SA (% control 179.02) followed by Prf+1 mM SA, Imd + 0.75 mM SA and Imd+1 mM SA (% control values were 175.37, 174.17 and 173.90 respectively).

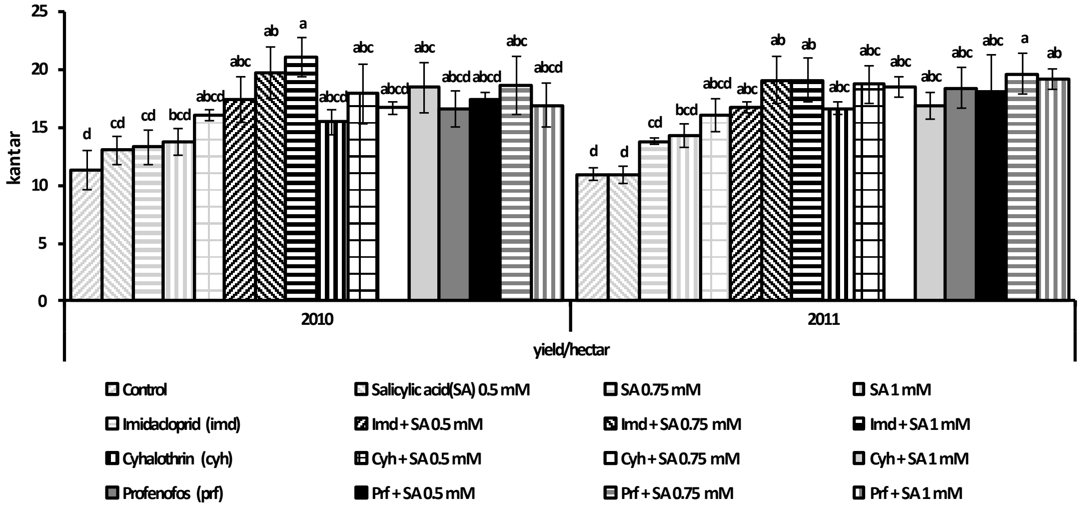

Fig. 3: Effect of salicylic acid tank mixed with some insecticides on cotton plant yield.

Crude Oil Content In Cotton Seeds:

The results shown in Figure (4) presented the effect of insecticides and salicylic acid at different rates on the percentage of crude oil in cotton seeds. All treatments increased the crude oil percentage but there is no significant difference among them.

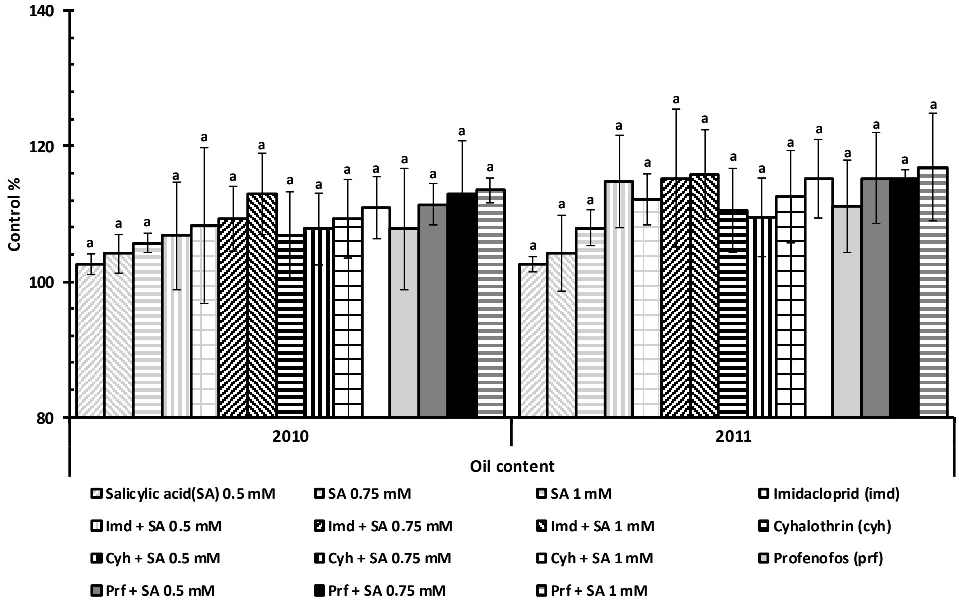

Fig.4: Effect of salicylic acid tank mixed with some insecticides on cottonseed oil content.

DISCUSSION

Effect of the Insecticides Used Alone and In Combination with Salicylic Acid Against Some Cotton Bollworm Larvae:

With insecticides only, we can conclude that all insecticides used had a potent effect against both bollworms. But, the most effective insecticide against pink bollworms (*Pectinophora gossyipella*) were profenfos and cyhalothrin. While there is no significant difference among the insecticides tested in controlling the spiny bollworms (*Earias* *insulana*). These results are going in line with those found by (Aslam *et al.* 2004; Younis *et al.* 2007; Al-Shannaf 2010; Al-Kazafy 2013; Elgohary 2014).

On the other hand, Salicylic acid had a slight to moderate effect against the two bollworms (pink and spiny bollworms respectively). This effect increased with the increase of SA conic. The insecticidal effect of Salicylic acid may be due to that SA and jasmonic acid (JA) act as signaling molecules in plants. Controlling the induced plant responses against infection with herbivorous and pathogen. Where it mediates the genes controlling plant resistance to insects (Arimura *et al.* 2005). There is a correlation between the level of salicylic acid and the attack of the herbivore on tomato plants (Peng *et al.* 2004). So, SA leads to many events that enhance plant resistance to insects. Exogenous applications of SA, either by spraying or even direct injection, reported originating a multitude of morphological and physiological effects on the plants (Pancheva *et al.* 1996). (Khoshkhoo *et al.*, 1993) found that treating the plants with salicylic acid increases the level of terpene aldehydes (e.g., gossypol). Which provides the plant's ability to resist insects and pathogens. (Levine *et al.*, 1994; Lamb and Dixon, 1997) found that, the first evidence of the SA pathway in tomato response to cotton bollworm feeding is the oxidative burst. Peng *et al.* 2004 found that the treatment of tomato seedlings with SA and Me-SA exogenously leads to an increase in the H_2O_2 concentration when these seedlings are subjected to grazing by cotton bollworm larvae. They suggested that the SA stimulated the production of H_2O_2. Also, salicylic acid obstructs the enzymatic breakdown of H_2O_2 by catalase and ascorbate peroxidase(Bi *et al.* 1997). H_2O_2 may play a role in plant defense against insect nourishing, as H_2O_2 can harm the digestive system of the insect, thereby suppressing their development. Feeding insects on wounded leaves cause oxidative damage to the midgut of the insect and hamper the absorption and transport systems of the herbivore insects (Felton and Summers 1993; Felton *et al.* 1994).
A volatile derivative of SA and Me-SA is also a major component of compounds emitted from plants when exposed to herbivore attack (Van Poecke *et al.* 2001). Because they are signal compounds, they may attract predatory mites (Dicke *et al.* 1993).

Adding SA to insecticides enhanced the effect of imidacloprid. But reduced the effect of the other insecticides. The most effective treatments against the two bollworms were Prf+ 1mM SA, Prf+ 0.75 mM SA, and Profenofos alone. Generally, all insecticides and insecticides/SA combination treatments had a strong effect against the two bollworms. The obtained results are going in line with those found by (Younis *et al.* 2007; Kumar Santhosh *et al.* 2012; Zidan *et al.* 2012).

This selective effect may be because the SA stimulates the production of ROS (oxidative burst) and inhibits the different H_2O_2 decomposition enzymes, leading to the rapid degradation of different pesticides, especially the systemic ones, but imidacloprid is oxidatively degrading in plants to 6-chloropyridinyl-3-carboxylic acid, which stimulates the induced resistance in plants (Ford *et al.* 2010).

Effect Of the Insecticides Used Alone and In Combination with Salicylic Acid on Some Yield Characters:

Regarding insecticides only: While, imidacloprid and cyhalothrin treatment had the highest No of open bolls/plant followed by profenfos treatment. Profenfos had increased the cotton bolls' weight, seed weight/boll, and yield/ hectare. Also, all insecticide treatments had increased the cotton yield/ hectare, with no significant difference among them. Also, all treatments increased the crude oil percentage but there is no significant difference among them. These results are in agreement with those reported by (Younis *et al.* 2007; Kumar Santhosh *et al.* 2012). They revealed that cotton cultivation produced significantly higher yields when plants were treated with pesticides.

Concerning SA, SA had a complex effect on yield and yield components. While, SA treatments increase the No of open bolls/ plant and had a slight effect on bolls' weight

(g) /boll, With a positive dependence on the SA rate of application. The SA treatments had a corrugated effect on the seed weight/boll, with no dependent on the SA application rates. This may be due to that salicylic acid (SA) prompts the plant to induce resistance. This leads to steering the plant's metabolic capability to resist pests, that may be absent. Which strains the plant and reduces its productivity (Akbar *et al.* 2012). As we have already explained, salicylic acid stimulates the production of ROS, which damages the plant itself to reduce the damage caused by pests. Therefore, the results of the treatment with SA are the result of the difference between the damage caused by the pests that have been avoided and the damage caused by the treatment with the SA.

Conclusion:

The addition of salicylic acid to insecticides used in the control of bollworms led to increasing the effectiveness of these insecticides. Also, salicylic acid has some efficacy against these pests. Also, these additions enhanced the qualities of some productivity aspects but did not affect others. However, total productivity (cotton yield/ hectare) has increased by73.55: 85.67 %.

Despite obtaining good control results for bollworms and increasing production at a rate greater than the increase resulting from the use of pesticides individually. However, the use of SA in pest control is hampered by many obstacles.

Firstly, the results of pest control using SA are varying from one plant species to another. That may be due to the complexity of the plant defense system with its various components (jasmonic, salicylic, ethylene) and their relationship with each other. Where the pathways of activating the plant, resistance cross the salicylic interfere with those of the jasmonic and vice versa. Also, The induced resistance within one species may vary depending on the genetic makeup of the plant variety and the availability of resistance protein genes or not. Also, vary from one pest to another. Where these pests evolved in the presence of these defense systems of the plant and have different capabilities to overcome those defenses.

Secondly, in our experiment, the pesticides were mixed with the salicylic acid during the field application only (tank mixed). Better to use by the farmers, is that the salicylic acid and different pesticides are mixed in one formulation. Here we have to answer some questions. Which, what is the effect of the addition of salicylic acid to the pesticide formulation immediately and after a period of storage, As well as their effect on the active ingredient of different pesticides? Does the SA enhance the degradation of the pesticide inside and outside the plant or vice versa? Does the presence of salicylic acid also affect the toxicity of these pesticides to mammals and non-target organisms and what is the affecting of these pesticides' mixtures with SA on the environment?

Therefore, our study recommends the use of salicylic acid in combination with pesticides to increase the effectiveness of the pest control process and reduce the economic cost and environmental impact of pesticides, but after conducting sufficient studies to answer the previous questions.

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