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Non-Conventional Treatments of Imidacloprid, Ascorbic acid, and Salicylic Acid against the Cotton Aphid *Aphis gossypii (*Glover)

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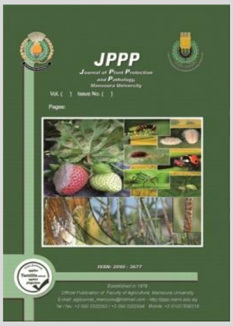

ABSTRACT

The present study was conducted to investigate the effects of selected inducers, including salicylic acid (SA), ascorbic acid (ASA), and the insecticide imidacloprid (IMI), on the cotton aphid, *Aphis gossypii*. All treatments demonstrated strong to moderate effects against aphids. Notably, (ASA) applied at concentrations of 2 and 4 mM significantly increased shoot and root lengths compared to both (IMI) and (SA) treatments. In the first season, based on the overall mean reduction in aphid populations, (IMI) showed the highest significant reduction 64.12% and 67.58% at application rates of 3.5 and 7 g/kg of seed, respectively. This was followed by IMI at 1.75 g/kg and SA at 2 mM, which resulted in reductions of 61.04% and 60.51%, respectively. The lowest reduction was observed with (ASA) at 1 mM, which achieved only 39.01%. Among all treatments, T18 (S&F with IMI at 7 g/kg + 0.75g/L) was the most effective, reducing cotton aphid populations by 64.12%, followed by T17 (S&F with IMI at 3.5 g/kg +0.375g/L) with 58.91%, and T11 (S&F with SA at 2 mM) achieving a 54.60% reduction. In contrast, seed treatment with ASA at 1 mM was the least effective, showing only a 27.32% reduction in aphid populations. Treatments with IMI, SA, and ASA significantly increased polyphenol oxidase activity and total protein levels compared to the control. Moreover, seed treatment with ASA at 4 mM resulted in a highly significant increase in chlorophyll content specifically, chlorophyll A, chlorophyll B, and total chlorophyll content increased by 20.51%, 41.42%, and 29.79%, respectively.

Keywords: Cotton, A. *gossypii*, salicylic acid, imidacloprid. Induced resistance,

INTRODUCTION

Cotton (*Gossypium barbadense* L.) is regarded as one of the most important economic crops in both Egypt and worldwide. However, cotton plants are vulnerable to infestation by numerous pests throughout their growth cycle, from planting to harvest (Burrows *et al*., 1982). Insects attack the crop from the seedling stage through to fruit development. The widespread and increasing use of chemical pesticides has led to growing challenges for farmers, particularly with managing sucking insect pests.

Aphids (Aphididae, Hemiptera) are major crop pests worldwide, causing significant economic damage by feeding directly on plants, excreting honeydew, and transmitting viruses (Dedryver *et al.,* 2010). The economic impact of aphids is substantial, with annual losses reaching millions of dollars globally (Farhan *et al.,* 2024). Depending on environmental conditions, aphids can reproduce both sexually and asexually (Le Trionnaire *et al*., 2008). The cotton aphid *A. gossypii* is considered one of the most important insect pests affecting cotton crops, leading to considerable yield reductions due to its rapid reproduction rate (Siviter & Muth, 2020). Moreover, the widespread use of pesticides to control aphid populations has resulted in environmental pollution and adverse health effects in humans (El-Ballal *et al*., 2019; Niu *et al*., 2020).

Therefore, it is essential to identify safe and effective tools for controlling insect pests. Over the past 20 years, systemic acquired resistance (SAR) has emerged as a promising form of induced resistance in plants. SAR is triggered by exposure to elicitors, which may include virulent, avirulent, or non-pathogenic microbes, as well as chemical compounds such as jasmonic acid (JA) and salicylic acid (SA) (Gozzo and Faoro, 2013). For example, treatment of tobacco plants with SA has been shown to induce the accumulation of pathogenesis-related (PR) proteins, which are strongly associated with enhanced plant resistance (White, 1979; Ward *et al.*, 1991).

Pre-soaking seeds in growth regulators before planting has also been found beneficial, particularly in mitigating the adverse effects of salinity on plant growth and physiological/biochemical responses (Ashraf & Rauf, 2001; Afzal *et al.*, 2005). Furthermore, such methods require significantly smaller quantities of active ingredients compared to conventional pest control applications. This not only reduces the exposure of agricultural workers and the environment to insecticides but also lessens harm to beneficial natural enemies (Nault *et al*., 2004; Younis *et al*., 2007).

Additionally, these treatments have been shown to activate defense-related genes, leading to the synthesis and accumulation of antioxidant enzymes, steroidal glycoalkaloids, and volatile organic compounds (VOCs). VOCs play a crucial role in strengthening indirect plant defenses by attracting natural enemies of herbivorous insects (Ryan, 2000; Chehab *et al.*, 2008). Moreover, the exogenous application of SA has been reported to reduce caterpillar feeding and delay the development of insect resistance (Mishra *et al*., 2024).

Imidacloprid, the first neonicotinoid insecticide approved by the United States Environmental Protection Agency (US EPA), belongs to the chloronicotinyl class

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and acts as a nicotinic analogue. It functions as a neurotoxin by binding to the nicotinic acetylcholine receptors (nAChRs) of insects, causing hyper-excitation that ultimately leads to death (Matsuda *et al.*, 2001). Due to its systemic properties, imidacloprid is widely used against soil-dwelling pests and is applied through seed treatments as well as foliar sprays (Magalhaes *et al.*, 2009; Lanka *et al.,* 2013). Its popularity has grown because of its selective toxicity to insects and relative safety for humans, accounting for 41.5% of global neonicotinoid usage (Liu *et al.*, 2022). As a broad-spectrum, systemic insecticide, imidacloprid acts both through contact and ingestion, and is effective against a variety of pests, including aphids, thrips, whiteflies, and termites (Zhang *et al*, 2011).

This study aims to enhance the resistance of cotton plants to *Aphis gossypii* Glover and reduce the reliance on insecticides by applying chemical inducers prior to insecticide treatments.

MATERIALS AND METHODS

Used Chemicals

a.Insecticides

- **Neonicotinoid – Imidacloprid**: Two formulations of imidacloprid were used: Gaucho 70% WS, obtained from Bayer Crop Science, Germany, and applied as a seed treatment at a rate of 7 g/kg of seeds; and Best 25% WP, obtained from El-Helb Company, Egypt, and applied as a foliar spray at a rate of 75 g per 100 L of water.

b. Plant defense inducers

- **Salicylic Acid (SA)**: Obtained from Piochem for Laboratory Chemicals Company.
- **Ascorbic Acid (ASA)**: Also obtained from Piochem for Laboratory Chemicals Company.

Seed Treatment Procedure

Delinted cotton seeds (*Gossypium barbadense* L., variety Giza 94) were immersed in various treatment solutions. Imidacloprid 70% WS was applied at three different rates: 1.75, 3.5, and 7 g/kg of seeds. Additionally, salicylic acid (SA) and ascorbic acid (ASA) were each applied at concentrations of 1, 2, and 4 mM. Seeds were soaked in these solutions for 6 hours, then transferred to white paper and allowed to dry. The treated seeds were subsequently used in the following experiments.

Laboratory Experiments

The experiments were conducted in the laboratory of the Plant Protection Research Institute, El-Mansoura, under controlled conditions (25 ± 2 °C, with a 16/8-hour light/dark photoperiod).

Effect of Imidacloprid (IMI), Salicylic Acid (SA), and Ascorbic Acid (ASA) on Cotton Seed Germination and Seedling Traits

The effects of imidacloprid at three rates (1.75, 3.5, and 7 g/kg seed), as well as salicylic acid (SA) and ascorbic acid (ASA) at three concentrations (1, 2, and 4 mM), on seed germination and selected seedling traits were evaluated under laboratory conditions. Each treatment was replicated three times, using 10 seeds per replicate, in addition to an untreated control. Treated and control seeds were placed on moistened cotton in 9 cm diameter Petri dishes and incubated for five days under the controlled laboratory conditions described above. After incubation, seed germination percentage and various seedling traits—including shoot length, root length, emergence rate index, and vigor index—were recorded. according to the methods described by **Sun *et al,* (2010)** the emergence percentage was calculated using the formula: number of germinated seeds with in first 5 days / total number of seeds × 100. To measure the emergence rate, the petri dishes were daily visited and the emerged seedlings were recorded. The emergence rate index was calculated using the following equation

$$\text{Emergence rate index} = \sum_{1}^{i} \frac{n_i}{d_i}$$

where *ni* is the number of emerged seedlings on day *i*, and *di* is the number of days after sowing. Vigor index was calculated by multiplying the mean seedling length by emergence percentage divided by 100 (Sun *et al,* 2010).

Effect of tested agents as seed treatments on plant defense enzymes, total soluble protein, and chlorophyll content

To assess the activity of key plant defense enzymes—peroxidase, catalase, and polyphenol oxidase—as well as total soluble protein and chlorophyll content in cotton seedlings, samples were collected five days after seedling emergence. Cotton seedlings treated with imidacloprid, salicylic acid (SA), and ascorbic acid (ASA), along with untreated control, which harvested and immediately frozen in liquid nitrogen for biochemical analysis.

For enzyme and protein assays, 0.5 g of seedling leaves were homogenized in 3 mL of 50 mM TRIS buffer (pH 7.8) containing 1 mM EDTA–Na_2 and 7.5% polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 12,000 rpm for 20 minutes at 4°C, and enzyme activities were measured spectrophotometrically in the supernatant, following the method of Hafez (2010). Peroxidase activity was determined according to Hammerschmidt *et al.* (1982). Catalase activity was measured using the method of Aebi (1984). Polyphenol oxidase activity was assessed following Malik and Singh (1980). Total soluble protein content was estimated using the method of Koller (1984). For chlorophyll content determination, 1 g of fresh leaves was extracted with 5 mL of dimethylformamide and kept overnight at 5°C. Chlorophyll concentrations were measured at 663 nm and 647 nm, following the procedure of Moran and Porath (1982). All spectrophotometric measurements were carried out at 25°C using a UV-160A spectrophotometer (Shimadzu, Japan). Each enzyme assay was performed in triplicate.

Field Experiments

The field experiments were conducted in the Aga region, El-Mansoura Governorate, during two consecutive cotton growing seasons (2022 and 2023), using the cotton variety Giza 94.

Evaluation of Cotton Seed Treatments with Imidacloprid, Salicylic Acid (SA), and Ascorbic Acid (ASA) Against the Cotton Aphid (*Aphis gossypii* Glov.)

A total field area of approximately 4,000 m² was divided into individual plots of 42 m² each, with buffer zones between plots to prevent cross-contamination. The cotton seeds (Giza 94) were obtained from the Cotton Research Institute, Sakha, Kafr El-Sheikh. Prior to sowing, seeds were treated with imidacloprid at three rates (1.75, 3.5, and 7 g/kg seed), and with salicylic acid (SA) and ascorbic acid (ASA) at three concentrations (1, 2, and 4 mM), as described in the laboratory experiments. Untreated seeds served as the control.

The experiment was arranged in a completely randomized block design (CRBD) with four replicates for each treatment. Sowing was carried out during the last week of April and the first week of May for the 2022 and 2023 growing seasons, respectively.

Foliar Application of Treatments

In addition to seed treatments, foliar applications were conducted using the insecticide Best 25% WP at three concentrations—0.25(0.18g/L), 0.5(0.37g/L, and the recommended field rate(0.75g/L)—along with salicylic acid (SA) and ascorbic acid (ASA) at 1, 2, and 4 mM. Spraying was carried out 30th day after sowing, coinciding with a noticeable increase in aphid (*Aphis gossypii* Glov.) populations.

The treatments were applied to previously seed-treated plots, plots receiving only seed treatment, plots receiving only foliar spray, and control plots (untreated). Applications were performed using a knapsack sprayer (model CP3) equipped with a single nozzle, delivering a spray volume of 200 L water per feddan.

For aphid population monitoring, 25 leaves were randomly selected from each plot at 40, 43, 46, 49, and 52 days after spraying. Aphid counts were recorded directly in the field.

The reduction percentage of infestation by aphid were calculated conferring to Abbott's formula (1925). %Reduction number in control –number in treatment / number in control * 100

Statistical Analysis

All data were expressed as means ± standard error (SE). Statistical significance was assessed using one-way and two-way analysis of variance (ANOVA) with cosStat software. Duncan's Multiple Range Test (DMRT) was used to determine significant differences between means. Differences were considered statistically significant at $p < 0.05$.

Results and discussion

Effect of cotton seed treatments with imidacloprid, salicylic acid (SA) and ascorbic acid (ASA) on germination and certain seedling traits:

Seed priming is one of the effective methods for enhancement seed growth (Mc Donald, 2000). It is a form of seed preparation in which seeds are pre-socked before planting by different chemicals to enhance the germination.

Effect of cotton seed treatment with imidacloprid at rates of 1.75, 3.5 and 7g/kg seed, also, salicylic acid (SA) and ascorbic acid (ASA) at concentration (1, 2 and 4 mM) on seed germination in addition to certain seedling traits was evaluated under laboratory conditions and the obtained results were presented in Table (1) and Figure (1). It was apparent that the use of ascorbic acid treatment (2 and 4mM) increased significantly the shoot and root length more than both imidacloprid (IMI) and salicylic acid (SA) one.

Table 1. Effect of treated cotton seeds with imidacloprid, salicylic acid (SA) and ascorbic acid (ASA) on emergence and certain seedling growth traits under laboratory conditions

Tr.	Coc.	Shoot length	Root length	% Emergence	ERI	Vigor index
	1mM	2.92±0.04 de(-20.68%)	1.45±0.12 cde(-8.80%)	50.00±5.77 def	0.64±0.07d	2.19±0.27 fg
SA	2mM	2.61±0.11 e(-29.26%)	1.42±0.08 de(-11.90%)	43.33±3.33 ef	1.11±0.12cd	1.73±0.05 fg
	4mM	2.48±0.11 e(-32.79%)	1.27±0.09 e(-32.00%)	40.00±5.77 f	0.92±0.21cd	1.48±0.15 g
	1mM	3.42±0.23 c(-7.31%)	1.94±0.12 bc(+22.01%)	76.67±8.81 abc	1.97±0.43b	4.05±0.22 c
ASA	2mM	3.98±0.17b(+7.85%)	2.35±0.27ab(+47.70%)	86.67±8.81 ab	3.33±0.22a	5.41±0.22 b
	4mM	4.56±0.27a(+23.57%)	2.63±0.25a(+65.40%)	93.33±3.33 a	3.67±0.08a	6.67±0.28 a
	1.75 gm/kg	3.71±0.10 bc(+2 .00%)	1.71±0.05 cde(+7.54%)	70.00±5.77 bcd	1.32±0.18c	3.79±0.28 cd
IMI	3.5 gm/kg	3.28±0.12 cd(-41.00%)	1.63±0.15 cde(+2.51%)	63.33±8.81 cde	1.37±0.14c	3.10±0.41 de
	7.00 gm/kg	2.63±0.08 e(-28.72%)	1.85±0.17 cd(+16.35%)	56.67±8.81 cdef	0.97±0.05cd	2.54±0.38 ef
Control	_____	3.69±0.09 bc	1.59±0.05 cde	73.33±8.81 abc	1.13±0.06cd	3.87±0.42 cd
L.S.D		0.435	0.462	21.09	0.567	0.862

Note: Tr. = treatment, ERI = Emergence rate index (seedlings per day), and means within a column denoted by the same letter(s) are not significantly different at 5% level according to DMRT (1955). Values in parenthesis refer to percentage of change with respect to control.

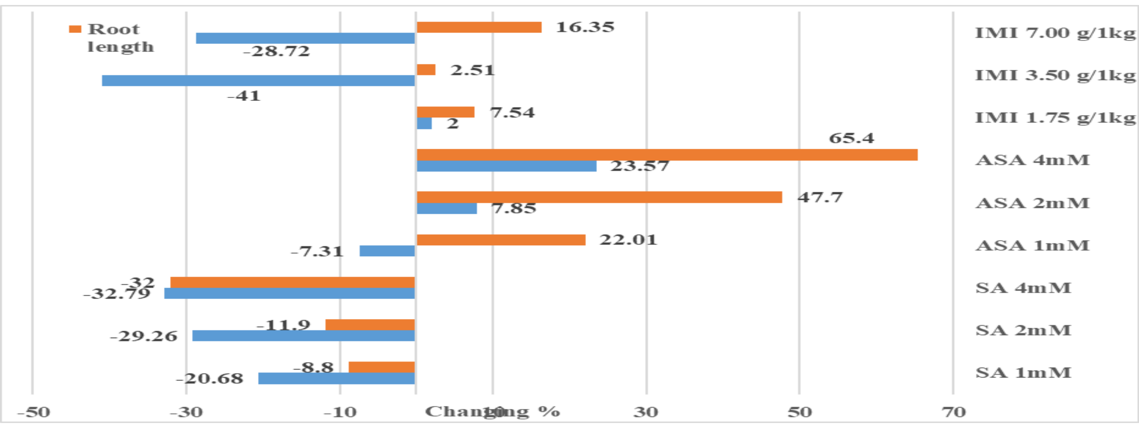

Fig. 1. Changing percentages of cotton shoot and root lengths affected by treated cotton seeds with imidacloprid, salicylic acid (SA) and ascorbic acid (ASA).

Also, the shoot and root length were increased with an increase of applied rate of ascorbic acid (ASA). The treated seeds with (ASA) at rate of 4 m molar recorded the highest percentage of shoot and root length by 23.57 and 65.40%, respectively. While the lowest shoot and root lengths were 2.48 and 1.27cm, respectively, at rate 4mM for salicylic acid.

Also, the treated seeds with ascorbic acid (ASA) at 4mM had a significant increase in % emergence, emergence rate index and vigor index by 93.33, 3.67 and 6.67% , respectively, compared with other treatments.

However, it has known for long that pretreatment of seeds with oxidants such as ascorbic acid can be scavenged the reactive oxygen species which are very harmful on the plant growth. It is a product of D-glucose metabolism which affects some nutritional cycle activities in higher plants and plays an important role in the electron transport system (El-Kobisy *et al*., 2005). Several studies have shown that ascorbic acid plays an important role in improving plant tolerance to abiotic stress (Shalata and Neumann, 2001; Al-Hakimi and Hamada, 2001; Athar *et al*., 2008).

The obtained results agreed with the findings of Sajjanar (2018) who reported that cotton seed treatment with imidacloprid at 3.5 g/kg seed recorded significantly greater germination percentage compared to control.

Also, Brar *et al.* (1983) found that spraying 100 ppm ascorbic acid at the flowering stage gave higher seed cotton yield. The obtained results confirmed with the findings of Jadhav and Bhamburdekar (2011) and Kaydan *et al.* (2007) who reported that pre-treatment of seeds with SA increased germination and emergence of seeds. Also, Ibrahim (2015) showed that abiotic SA acid played important role in enhancing seed vigor such as mean germination percentage (MPG) and speed germination (SG) compared to control.

Effect of the tested agents on some plant defense enzymes activities in cotton plants.

However, induced resistance in plants against insects can help control insects with low environmental impact. So, the change in antioxidant enzymes such as peroxidase, catalase and polyphenol oxidase which play a role in plant resistance as well as total protein content in cotton plants was estimated at seedling stage after treated seed with the tested agents. The obtained results were depicted in Table (2) and figure (2). It was observed that the seeds treated with imidacloprid decreased significantly the peroxidase activity by 43.64, 45.79 and 47.77% at low, moderate and high rate, respectively as compared to control. In constant, salicylic acid (SA) and ascorbic acid (ASA) treatments produced an increase in the enzyme activity.

Among treatments, salicylic acid (SA at 2mM) resulted in a significant increase in peroxidase by 13.47 compared to control. As for the effect of imidacloprid, salicylic acid and ascorbic acid treatments on catalase activity, it was showed that all treatments decreased significantly the activity of this enzyme compared with (SA at 2 mM)which was the least significant on the activity of it by 59.00 among treatments. It was observed that imidacloprid (IMI) , salicylic acid (SA) and ascorbic acid (ASA) treatments (at all rates) induced significantly higher increase in poly phenol oxidase activity, chlorophyll content and total protein compared to control (Table 2 and Fig., 2).

Chlorophyll (Leaf greenness)

Plant leaf colors are one of the most essential factors affecting attracting and preventing insects. Each insect has a proper range of wavelengths to which it is attracted. As chlorophyll (Leaf greenness) measurements were estimated. The results presented in Table (2) revealed that, all treatment increased significantly chlorophyll contents. As seed treatment with ASA at 4mM gave highest significance of chlorophyll contents (chlorophyll A, chlorophyll B and total chlorophyll by 20.51, 41.42 and 29.79, respectively) compared with other treatments.

Pretreating plants with various chemical inducers can enhance their resistance, offering protection against insect attacks. When faced with herbivore damage, plants activate a range of inducible defense mechanisms (Karabn and baldwin, 2007). In this study, seed application of IMI, tested agents to cotton plants significantly increased crop productivity and markedly reduced cotton aphid infestation compared to untreated controls.

The application of tested agents likely stimulated the production of plant defense-related enzymes—peroxidase (POX), polyphenol oxidase (PPO), and catalase (CAT)—which could explain the observed decline in whitefly populations. Previous research has shown that treatments with certain hormones, especially salicylic acid (SA), jasmonates, and ethylene, can reprogram host metabolism, influence gene expression, and enhance plant defense responses (Bari and jones,2009). This study also examined antioxidant enzymes involved in plant defense mechanisms against pests. Elevated activity levels of POX, PPO, and CAT were identified as key defense responses triggered by pest recognition (Van Loon *et al*.,2006). These findings align with the results of (Khan *et al*., 2015), who demonstrated that biological inducers trigger a complex resistance involving various enzymes and compounds, with POX and chitinase (CHI) playing a central role (Bargaus-Lars *et al*., 2007). High POX and PPO activity is among the most effective defense mechanisms following pathogen recognition (Karabn and baldwin, 2007). Guerra *et al.* (2013) and Liang *et al.* (2005). Also observed that increased POX and PPO activity promoted the accumulation of phenolic compounds, thereby enhancing resistance in cotton and cucumber to pests and diseases. Specifically, PPO activity facilitates phenol accumulation, which may impair the growth of aphids (Jafarbeigi *et al*.,2020). PPO catalyzes the oxidation of monophenols or o-diphenols into o-quinones in the presence of oxygen, a process that can reduce plant digestibility for insects.

Therefore, the reduction in the cotton aphid population observed in this study is likely due to the antifeedant properties of these defense-related enzymes and the adverse effects of phenolic compounds on insect digestion (Pérez-Hedo and Urbaneja, 2015)

The obtained results agreed with the findings of Chauhan *et al.,* (2013) who found that imidacloprid treatment of potato at field increased the total protein content. (Foyer *et al.,* 1997) reported that the level of antioxidants and the activities of antioxidants enzymes such as CAT and POX are generally increased in plants under stress condition and in several cases their activities correlate with enhanced tolerance. Ghazanfar *et al.* (2020) found that potato plants treated with SA had higher peroxidase and polyphenol oxidase activity than water treated plants. Enzymes produced as results of treatment with elicitors are responsible for protection against sucking insect pests. Locateli *et al*. (2019) showed that the application of elicitors as Salicylic Acid (SA -2mM) increased the path of the phenylpropanoids with the activation of development of phenolic compounds enzymes such as chitinase and peroxidases. in contrst, Kaur and Sohal (2015) found more significant increase in peroxidase activity on 8th day after treatment with imidacloprid in cotton hybrids

(RCH- 134, JKCH-1947 and NCEH-6R) seedlings as compared to their respective control.

From the obtained results during this work, it can be concluded that, improved seed germination after cotton seed treatment with imidacloprid , salicylic acid (SA) and ascorbic acid (ASA) may be due attributed to the enhanced activity of polyphenol oxidase enzymes and total protein as mentioned by Ghazanfar *et al.* (2020) and Chauhan *et al.,* (2013).

Results are also on line with outcomes of El-Shafey, (2017) who showed that foliar spray of salicylic or ascorbic acid significantly increased chlorophyll contents on soybean plants in both seasons 2012 and 2013. In this study, chlorophyll content was measured to evaluate the effects of the tested agents on the performance of tomato plants. As a vital natural pigment, chlorophyll absorbs light energy necessary for photosynthesis, and its levels serve as an important indicator of how plants respond to environmental stress (Golkar *et al.,* 2009).

Table 2. effect of the tested agents on some cotton plant parameters

Treatment	Peroxidase (µ Mole/mgprotein)	Catalase (µ Mole/protein)	polyphenol oxidase (µ Mole/mg protein)	total soluble protein mg/g fresh weight	Chlorophyll a(mg g-1)	Chlorophyll b(mg g-1)	Total Chlorophyll
SA 1	0.0125 b (7.45)±0.000231	12.667 d (-45.71)±2.333	0.0835 b (14.48) ±0.000348	5.171 b (14.026)±0.040	0.468 e (8.67)±0.00404	0.386 (12.42)±0.00405	0.854 e (10.331)±0.00809
SA 2	0.0132 a (13.47) ±0.000265	9.567 e (-59.00)±0.480	0.0867 a (18.82) ±0.000529	5.336 a (17.662)±0.104	0.477 c (10.68)±0.00433	0.407 d (18.43)±0.00832	0.884 d (14.120)±0.0126
SA 4	0.0123 b (5.44±0.00026	15.667 bc (-32.86)±1.763	0.0818 c (12.11) ±0.000321	5.032 c (10.966)±0.0203	0.465 f (7.97)±0.00378	0.375 (9.21)±0.00497	0.840 f (8.523)±0.00876
ASA 1	0.0122 b (4.58)±0.000202	16.567 bc (-29.00)±1.223	0.0765 f (4.89) ±0.000440	4.820 e (6.299) ±0.0382	0.472 d (9.67)±0.00375	0.444 c (29.29)±0.00705	0.917 c (18.381)±0.0107
ASA 2	0.0123 b (6.02)±0.00026	18.133 b (-22.29)±1.618	0.0773 e (5.94) ±0.000462	4.900 de (8.052)±0.0409	0.491 b (14.01)±0.00461	0.461 b (34.24)±0.00779	0.952 b (22.988)±0.0109
ASA 4	0.0126 b (8.31)±0.000208	18.300 b (-21.57)±1.026	0.0789 d (8.13) ±0.000346	5.003 c (10.325)±0.0409	0.519 a (20.51)±0.00461	0.486 a (41.42)±0.00378	1.005 a (29.789)±0.00838
IMI 1.75 g/kg	0.0066 d (-43.64)±0.0000384	12.767 d (-45.29)±0.375	0.0759 g (3.97) ±0.000318	5.141 b (13.377)±0.0486	0.465 f (8.05)±0.00480	0.387 e (12.51)±0.00296	0.852 e (10.030)±0.00776
IMI 3.50 g/kg	0.0063 d (-45.79) ±0.000038	14.167 cd (-39.29)±0.405	0.0747 h (2.42) ±0.000273	4.956 cd (9.286)±0.0537	0.455 g (5.57) ±±0.00433	0.381 ef (10.77)±0.00260	0.835 f (7.878)±0.00693
IMI 7 g/kg	0.0061 d (-47.77) ±0.00007	16.333 bc (-30.00)±0.375	0.0741 i (1.51) ±0.000841	4.809 e (6.039)±0.0311	0.451 h (4.64)±0.00318	0.372 f (8.34)±0.00441	0.823 g (6.285)±0.00757
CONT	0.0116 c±0.000067	23.333 a±2.603	0.0730 j±0.000581	4.535 f±0.0898	0.431 i±0.00433	0.344 g±0.00405	0.774 h±0.008373
L.SD	0.0006	2.548	0.0005	0.0932	0.0019	0.0087	0.0090

Note: Means within a column denoted by the same letter (s) are not significantly different at 5% level according to DMRT (1955).

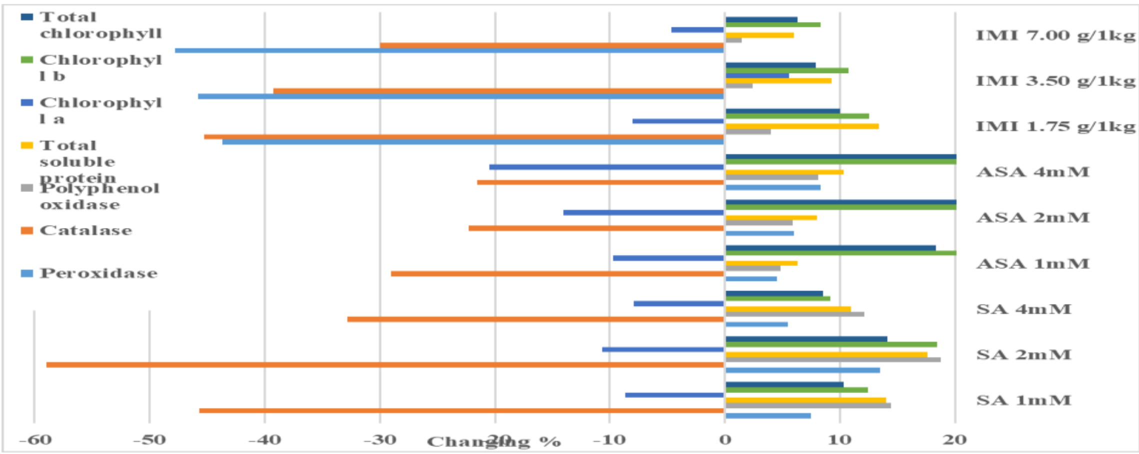

Fig. 2. Changing percentages of certain enzymes, soluble protein and chlorophyll in cotton seedlings after seed treatment with imidacloprid (IMI), salicylic acid (SA) and ascorbic acid (ASA).

The efficiency of Imidacloprid(IMI) , salicylic acid (SA) and ascorbic acid (ASA)as seed treatment was evaluated against the cotton aphid, *Aphis gossypii* Glov. during 2022 and 2023 cotton seasons

The results in Table (3) clear the reduction in the aphid population following imidacloprid, salicylic acid (SA) and ascorbic acid (ASA) as seed treatment throughout the experimental period during the two study seasons. In general, imidacloprid seed treatment exhibited higher reduction in aphid population than both salicylic acid (SA) and ascorbic acid (ASA) one.

In the first season, with regard to general mean of reduction , imidacloprid treatment recorded higher significant reduction in the population by 64.12 and 67.58 % at rate of

3.5 and 7 g/kg seed, respectively, followed by IMI at rate (1.75 g/kg) and SA at rate of 2 mM by 61.04 and 60.51% , respectively, while ascorbic acid (ASA)treatment induced the lowest reduction by 39.01 at rate of 1 m molar.

Based on the average of reduction in population of 2023 study season, imidacloprid treatment at rate of 7 g/kg seed was significantly superior in reducing the population as, the reduction percentage was 62.90 %. On the other hand, ascorbic acid (ASA) treatment caused low effect on the population by 30.88 % reduction in population at the low rate (1mM).

Finally, it can be concluded that imidacloprid, salicylic acid (SA) and ascorbic acid (ASA) had proved better performance compared to control. However, imidacloprid is aneonicotinoid compound belonging to the chloronicotinyl insecticide which has gut and contact activities against insects (Maienfisch *et al*., 2001). After seed treatment, imidacloprid shows systemic and residual toxicity in several crop plants and interferes with the transmission of stimuli or impulses in the nervous system on insect herbivores, and gives excellent control against a broad range of important sucking insect pests (Zhang *et al*., 2011). Also, (Shatpathy *et al,* 2018) reported that Salicylic acid (SA) also plays a major role in regulation of many physiological processes e.g. growth, development, ion absorption and germination of plants. Ghazanfar *et al*., (2020) studied Pre-treatment of plants with chemical elicitor salicylic acid (SA) which can induce systemic resistance against insect herbivores.

The obtained results agreed with those of Sajjanar (2018) who found that the cotton seeds treated with imidacloprid 70% WS (at 3.5 g/kg seed) recorded significantly lower aphid population followed by carbosulfan 25 %DS (at 30g /kg seed) and the highest aphid population was recorded with control. Also, Hossain *et al*., (2013) found that imidacloprid 70% WS (at rate of 1.5 and 2.5 g/kg seed) significantly reduced aphid population on cotton compared to untreated control or foliar spray of monocrotophos. Liang *et al.,* (2015) evaluated seed treatments with the neonicotinoid insecticides imidacloprid and thiamethoxam against aphid on oilseed rape, *Brassica napus*. They recorded that all of the plants treated with neonicotinoids were shown to have significant anti-aphid characteristics that persisted until the end of the trial. Atia and Alyousf (2021), who recorded decrease in the population density of *B. tabaci* adults on tomato plants due to the use of spraying salicylic acid (SA).

Table 3. Reduction percentage of *A. gossypii* following seed treatment with Imidacloprid, salicylic acid (SA) and ascorbic acid (ASA) during 2022 and 2023 cotton seasons.

Tr.	U.R	Reduction percentage of *A. gossypii* at:							
		Season of 2022				Season of 2023			
		2WAS	3WAS	4WAS	Mean	2WAS	3WAS	4WAS	Mean
SA	1mM	51.65± 3.01	50.53± 3.36	51.26± 4.85	51.15± 2.92 bc	46.24± 3.74	38.69± 2.69	35.14± 1.42	40.02± 1.79 e
	2 mM	61.75± 1.23	60.73± 2.88	59.07± 2.98	60.51± 2.03 ab	53.95± 2.31	52.19± 1.31	49.74± 0.61	51.96± 0.21bc
	4 mM	46.17± 2.51	47.77± 4.06	46.26± 4.26	46.73± 3.29 cd	41.93± 4.28	38.02± 2.37	33.22± 1.64	37.72± 1.74 ef
ASA	1 mM	41.42± 2.95	40.65± 6.17	34.96± 6.24	39.01± 4.57 d	32.37± 5.02	33.67± 4.17	26.60± 4.91	30.88± 2.57 g
	2 mM	43.60± 4.25	44.63± 5.75	48.35± 4.19	45.53± 4.46 cd	34.09± 3.56	36.63± 1.68	25.43± 2.50	32.05± 0.96 fg
	4 mM	53.53± 2.97	53.09± 5.46	52.05± 5.34	52.89± 4.42 bc	45.41± 3.55	40.24± 2.98	37.60± 1.88	41.08± 1.57 de
IMI1	1.75	62.87± 0.46	60.99± 2.17	59.26± 1.84	61.04± 1.41 ab	48.80± 2.58	44.84± 4.43	45.25± 2.74	46.30± 3.32 cd
	3.5	67.23± 1.46	63.72± 1.85	61.41± 2.17	64.12± 1.72 a	58.27± 1.80	51.61± 3.19	50.31± 2.21	53.40± 2.22 b
	7	71.32± 1.61	67.25± 2.04	64.17± 2.29	67.58± 1.96 a	67.54± 1.63	62.61± 2.90	58.54± 2.06	62.90± 2.08 a
L.S.D					9.405				5.815

Note: Tr =treatment, U.R= using rate, WAS = weeks after sowing, and Means in a column denoted by the same letter (s) are not significantly different at 5 % level according to DMRT (1955)

Effect of different treatments with imidacloprid , salicylic acid (SA) and ascorbic acid (ASA) against the aphid cotton, *Aphis gossypii* Glov.:

Effect of different treatments (seed treatment, seed treatment& foliar spray and foliar spray) with imidacloprid (IMI) , salicylic acid (SA) and ascorbic acid (ASA) against the cotton aphid, *Aphis gossypii* Glov. on cotton plants was evaluated during two successive cotton seasons; 2022 and 2023. The tested inducers were sprayed at 30 - day -old plants at the increase in aphid numbers. The data obtained in season of 2022 were summarized in Table (4). It was apparent that T18 treatment (S&F with IMI, 7g/kg seeds and 0.75g/L) was significantly more effective in the reduction of cotton aphids by 64.12% followed by T17 (S&F with IMI,3.5g/kg seeds and 0.375g/L)with 58.91% followed by T11(S&F with SA, 2mM) with 54.60 followed by T10(S&F with SA at 1mM and T 16 S&F (IMI) at 1.75g/kg and 0.187g/L)with 49.78% and 49.90% . While, seed treatment with ascorbic acid (ASA) at 1 m M was the least effective in reducing the population by 18.37% reduction.

As for the second season, the same trend of results was observed as shown in season of 2023 Table (5). Based on general mean, the reduction in population was significantly increased by 63.68 for T18 treatment (S & F with IMI at rate of 7g/kg seeds and 0.75g/L) followed by T11, T16 and T17 treatments (S&F with SA at 2Mm and IMI at its to smaller rates) with 60.41 , 57.78 and 57.56 % respectively. It was observed that, only seed treatment (S) with ascorbic acid (ASA, 1mM) caused a decrease in the reduction of aphid by 18.03.

Imidacloprid (IMI), salicylic acid (SA) and ascorbic acid (ASA) are used in the present study to lower the amounts of insecticides to their recommended dose. Salicylic acid is a potent plant hormone (Hayat *et al.,* 2010) and has a significant role for alleviating the devastating effects generated by various biotic and abiotic stresses on plants (Catinot *et al.,* 2008). Salicylic acid at a concentration of 1 mM acted synergis- tically with nitric oxide and hydrogen peroxide for redu- cing the deleterious effects of biotic stress caused by Podosphaera xanthii in squash plants through inducing their resistance to this pathogen (Maswada *et al.,* 2014). The obtained results agreed with those of Elhamahmy *et al*. (2016) who found that SA at (50 ppm) as foliar application was a successful elicitor for reducing cabbage aphid populations.

Also, Thakur *et al.* (2016) reported that spraying SA at 0.5 and 1 mM as foliar spray on Brassica cultivars resulted in a notable decrease in the population of the mustard aphids, *Lipaphis erysimi* Kalt. by 83.00 and 54.67%, respectively. El- Sherbeni et al., (2019) reported that adding SA to pesticides to control whitefly insects on cotton plants improved the methods and decreased the use of pesticides by 25% due to enhanced phenolic chemical contents in the leaves.

Table 4. Effect of imidacloprid (IMI), salicylic acid (SA) and ascorbic acid (ASA) as seed treatment and foliar spray against *A. gossypii* on cotton during season of 2022.

No.	Treatment		Reduction percentages of aphid population after treatments					
	S	F	40 days	43 days	46 days	49 days	52 days	Average
T1	SA 1	-----	27.74±1.01	29.13±0.73	29.45±0.69	26.98±1.40	23.30±1.96	27.32±0.89 m
T2	SA 2	-----	43.84±0.19	43.18±1.92	42.80±1.27	30.83±1.25	19.81±1.59	36.09±1.04 ijk
T3	SA 4	-----	26.92±1.50	28.95±1.58	28.46±2.17	24.30±2.00	21.24±2.11	25.98±1.74 m
T4	ASA 1	-----	20.53±0.19	21.81±1.55	20.89±2.25	15.41±1.12	13.23±1.28	18.37±1.17 n
T5	ASA 2	-----	25.38±1.08	25.51±1.23	24.43±1.02	13.57±4.11	10.86±4.95	19.95±2.36 n
T6	ASA 4	-----	21.11±0.18	23.58±0.93	23.74±1.514	21.23±1.517	19.89±2.34	21.91±0.93 n
T7	IMI 1.75	-----	38.05±0.42	39.63±0.78	38.34±1.05	29.02±1.84	19.86±4.38	32.98±0.97 ki
T8	IMI 3.50	-----	40.55±0.60	41.14±1.83	40.44±1.96	32.82±3.81	29.31±5.80	36.85±2.45 ijk
T9	IMI 7	-----	43.69±1.57	42.84±1.29	43.04±1.22	42.10±1.64	42.20±2.49	42.77±1.19 fgh
T10	SA 1	SA 1	54.55±0.90	51.78±0.88	49.12±1.06±	47.46±1.59	45.99±1.81	49.78±1.19 d
T11	SA 2	SA 2	57.57±0.50	54.861.23	52.02±1.38	53.95±2.05	54.582.25	54.60±1.42 c
T12	SA 4	SA 4	45.48±0.56	43.78±1.20	43.70±1.95	42.81±3.16	42.22±3.60	43.60±1.81 fg
T13	ASA 1	ASA 1	43.19±0.53	41.43±0.43	39.12±0.52	37.53±0.85	36.14±1.32	39.48±0.53 ghi
T14	ASA 2	ASA 2	47.01±0.46	44.04±0.96	41.57±1.77	40.13±2.52	37.84±4.09	42.12±1.83 fgh
T15	ASA 4	ASA 4	49.82±0.75	47.37±1.46	44.73±1.79	44.61±2.13	44.48±2.59	46.20±1.63 def
T16	IMI 1.75	IMI 0.187	53.56±0.80	51.56±0.97	48.46±1.37	47.99±1.10	47.95±1.61	49.90±1.12 d
T17	IMI 3.50	IMI 0.375	64.63±1.05	58.99±1.90	56.85±1.83	57.27±2.26	56.80±2.86	58.91±1.93 b
T18	IMI 7	IMI 0.75	66.92±1.35	65.22±1.57	62.70±1.44	63.05±1.29	62.71±1.54	64.12±1.39 a
T19	-----	SA 1	33.66±0.77	34.11±0.87	42.39±1.46	41.06±1.49	30.73±1.58	36.39±0.87 ijk
T20	-----	SA 2	41.56±3.97	40.35±3.80	40.92±3.84	38.61±4.29	36.96±4.38	39.68±3.87 ghi
T21	-----	SA 4	35.11±1.02	35.67±1.51	35.01±1.95	33.47±2.70	32.45±2.48	34.34±1.86 jk
T22	-----	ASA 1	31.29±0.83	32.16±0.31	32.73±0.83	27.71±0.94	25.54±1.24	29.88±0.73 im
T23	-----	ASA 2	35.67±0.92	36.73±0.72	36.00±1.13	33.00±2.25	31.34±2.43	34.55±1.40 jk
T24	-----	ASA 4	39.21±0.60	38.41±0.53	38.34±1.05	37.66±0.93	37.38±2.19	38.20±0.79 hij
T25	-----	IMI 0.187	41.96±0.77	41.71±0.70	39.78±1.13	37.36±1.22	36.15±0.96	39.39±0.92 ghi
T26	-----	IMI 0.375	46.85±0.64	45.68±0.60	44.72±0.69	43.68±1.19	42.80±1.88	44.75±0.66 ef
T27		IMI 0.75	51.32±0.87	49.82±0.75	47.58±1.02	46.85±1.94	46.56±2.53	48.42±1.29 de
L.S.D								4.065

Note: S = Seed treatment, F = Foliar spray at 30th day , and means within a column denoted by the same letter (s) are not significantly different at 5% level according to DMRT (1955).

Table 5. Effect of imidacloprid(IMI) , salicylic acid (SA) and ascorbic acid(ASA)as seed treatment and foliar spray against the cotton aphid, *Aphis gossypii* Glov. on cotton during season of2023

No.	Treatment		Reduction percentages of aphid population after treatments					
	S	F	40 days	43 days	46 days	49 days	52 days	Average
T1	SA 1	-----	28.49±0.77	27.74±0.49	27.72±0.44	15.11±0.15	13.99±1.41	22.61± 0.29 j
T2	SA 2	-----	39.17±0.44	37.94±0.63	37.11±0.81	28.47±3.28	26.74±4.00	33.89±1.62 gh
T3	SA 4	-----	23.02±0.67	23.30±0.54	23.99±0.83	13.60±1.88	12.44±1.66	19.27±0.87 jk
T4	ASA 1	-----	20.63±0.43	19.85±0.53	19.39±0.71	15.12±0.73	15.13±0.99	18.03±0.56 k
T5	ASA 2	-----	26.42±1.30	26.67±1.57	26.51±1.38	17.49±2.02	16.09±1.88	22.64±1.33 j
T6	ASA 4	-----	31.47±0.95	31.45±0.55	31.68±0.79	19.47±0.98	19.69±1.80	26.75±0.86 i
T7	IMI 1.75	-----	34.87±1.48	34.61±1.44	33.94±1.60	22.28±1.12	25.35±1.58	30.21±1.40hi
T8	IMI 3.50	-----	39.32±1.40	38.81±1.66	38.35±1.78	26.46±1.785	26.70±2.92	33.93±1.83 gh
T9	IMI 7.00	-----	46.48±0.99	43.89±0.86	41.81±1.04	37.48±3.64	38.77±4.29	41.69±1.94 f
T10	SA 1	SA 1	52.67±0.65	50.40±0.84	47.74±0.82	46.38±0.47	48.74±0.80	49.19±0.68 cd
T11	SA 2	SA 2	63.80±0.31	61.58±0.44	59.15±0.66	58.37±1.62	59.14±2.07	60.41±0.80 ab
T12	SA 4	SA 4	49.92±0.57	49.23±0.50	47.74±0.82	44.37±1.98	42.95±3.89	46.84±1.62 de
T13	ASA 1	ASA 1	42.36±0.76	40.74±0.79	39.79±0.52	40.48±0.27	41.93±0.08	41.06±0.32f
T14	ASA 2	ASA 2	47.93±0.51	47.36±0.44	45.53±0.60	43.39±1.32	44.77±1.10	45.80±0.56 de
T15	ASA 4	ASA 4	55.65±1.20	54.37±0.74	53.46±0.89	50.93±1.40	47.47±3.26	52.38±0.37c
T16	IMI 1.75	IMI 0.187	59.74±1.85	57.76±1.97	56.44±2.43	56.85±2.79	58.11±3.09	57.78±2.41 b
T17	IMI 3.50	IMI 0.375	60.65±1.39	58.63±1.73	56.15±2.03	55.82±2.29	56.53±2.96	57.56±2.02b
T18	IMI 7.00	IMI 0.75	66.40±1.58	63.75±1.30	61.45±1.84	62.17±2.39	64.62±3.20	63.68±2.02 a
T19	-----	SA 1	44.00±0.85	42.71±1.28	41.61±1.13	31.00±0.67	20.32±0.94	35.93±0.89g
T20	-----	SA 2	47.24±1.23	49.37±3.55	48.34±3.88	37.05±4.28	28.04±4.80	42.01±3.08 f
T21	-----	SA 4	41.02±0.73	40.16±0.79	39.65±1.25	28.13±1.15	18.05±1.09	33.40±0.97gh
T22	-----	ASA 1	37.99±1.18	38.04±0.87	38.08±1.03	27.10±0.92	16.67±0.47	31.58±0.81 h
T23	-----	ASA 2	34.39±1.029	34.18±1.02	33.38±0.54	21.84±1.20	10.77±1.74	26.91±1.05 i
T24	-----	ASA 4	42.97±0.92	42.71±0.71	42.47±0.93	30.51±0.37	20.60±0.88	35.85±0.56 g
T25	-----	IMI 0.187	47.41±0.84	46.87±0.83	46.40±0.93	36.58±0.60	27.84±0.73	41.02±0.71 f
T26	-----	IMI 0.375	50.84±0.47	49.61±0.42	48.78±0.28	38.23±0.78	28.58±0.82	43.21±0.48 ef
T27		IMI 0.75	53.91±0.69	53.45±0.85	52.61±1.24	42.03±0.96	32.58±0.46	46.92±0.77de
L.S.D								3.542

Note: S = Seed treatment, F = Foliar spray at 30th day, and means within a column denoted by the same letter (s) are not significantly different at 5% level according to DMRT (1955).

CONCLUSION

To sum up this study aim to the teteted agents may be play a vital role increasing the acquired systemic resistance of cotton plants. and might be a potent alternative to conventional insecticides and compatible with an integrated pest management program.

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المعاملات غير التقليديه ايميداكلوبريد، أسكوربيك أسيد، ساليساليك أسيد ضد حشرة من القطن.

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الملخص

أجريت هذه الدراسه بهدف أختبار تأثير المستحثات المختاره والتى تشمل كل من حمض الساليساليك وحمض الاسكوربيك وكذلك مبيد الايميداكلوبريد ضد حشرة من القطن. أظهرت جميع المعاملات تأثيرات من قويه الى معتدله .ومن الجدير بالذكر ان حامض الأسكوربيك المطبق بتركزات ٢و٤ مليمولر أظهرت زيادة معنويه فى مساحة الساق والجذر مقارنة بكل من حامض الساليساليك ومبيد الايميداكلوبريد .فى الموسم الاول بناءا على انخفاض متوسط تعداد حشرة من القطن اظهر مبيد الايميداكلوبريد انخفاض معنوى كبير فى التعداد حيث كانت نسبة الانخفاض ٦٤,١٢ و ٦٧,٥٨ % لكل من معدلات التطبيق ٣,٥ و٧ جم /كجم من البذور, على التوالى. يلى ذلك معدل تطبيق ٢مليملر من حامض الساليساليك ومبيد ايميداكلوبريد على ١,٧٥ جم/كجم مما ادى لنسبة خفض ٦١,٠٤٪ و ٦٠,٥١ %. لوحظ ادنى تخفيض مع حمض الاسكوربيك بتطبيق ١ مليمولر والذى حقق ٣٩,٠١٪ فقط من بين جميع المعاملات. ,T18(S&F) مع الايميداكلوبريد بتركيز ٧ جم /كجم بذور+٠,٧٥ جم / لتر كان الاكثر فعالية بنسبة خفض ف تعداد من القطن ٦٤,١٢٪ تليها T17 (S&F)ع ٣,٥ جم/كجم بذره +٠,٣٧٥جم /لتر كان٥٨,٩١٪ و T11 (S&f)مع ٢ مليمولر من حمض الساليساليك حقق ٥٤,٦٠ % وكان ١ مليملر من حمض الساليساليك هو الاقل فعاليه وعرض انخفاضا بنسبة ٢٧,٣٢٪. معاملات كل من حامضى الاسكوربيك والساليساليك وكذلك الايميداكلوبرسد أدت الى زياده كبيره فى ف نشاط انزيم البوليفينول اوكسيداز والبروتين الكلى مقارنة بالكنترول ,علاوة على ذلك أدى معاملة البذور ب ٤مليمولر من حمض الاسكوربيك الى زياده معنويه ف محتوى الكلوروفيل على وجه التحديد وذاد اجمالى محتوى الكلوروفيل بنسبة ٢٠,٥١٪و ٤١,٤٢٪,٢٩,٧٩٪ على التوالى.