

## EFFECT OF FOLIAR APPLICATIONS OF GIBBERELLIC ACID, INDOLE ACETIC ACID AND SALICYLIC ACID ON FABA BEAN GROWTH, YIELD AND CHOCOLATE SPOT DISEASE SEVERITY

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**ABSTRACT:** Field experiment was conducted at the two successive seasons 2011/2012 and 2012/2013 to study the effect of salicylic acid (SA), gibberellic acid (GA<sub>3</sub>) and indole acetic acid (IAA) singly (SA) or in combinations at the concentrations of 10<sup>-4</sup>, 10<sup>-5</sup> and 10<sup>-6</sup> M on faba bean, vegetative growth, yield components and some biochemical constituents in correlation with chocolate spot disease incidence. Foliar applications of GA<sub>3</sub> and SA+IAA at 10<sup>-5</sup> M had significant effect in increasing plant height, fresh and dry weight of shoots in both seasons as compared with those of control. Moreover GA<sub>3</sub>, IAA and SA+IAA at 10<sup>-5</sup> M recorded the maximum responses in most parameters of both plant growth and yield components, i.e. plant height, number of branches/plant, number of pods/plant, number of seeds/plant, weight of seeds/plant 100-seed/weight and seed yield/fed. Generally, the contents of chlorophyll a, chlorophyll b and total chlorophyll, peroxidase activity and total phenol content were significantly the highest in response to the applications of SA, GA<sub>3</sub> and IAA singly or in combinations at 10<sup>-4</sup> and 10<sup>-5</sup> M, compared to nontreated control. Crude protein and total carbohydrates were increased significantly at 10<sup>-4</sup> or 10<sup>-5</sup> M. Application of IAA and SA+GA<sub>3</sub> at 10<sup>-5</sup> M resulted the highest values of protein with the average of 26.38 and 25.88% and carbohydrate with the average of 56.85 and 58.32%, respectively. All treatments at the rate of 10<sup>-4</sup> and 10<sup>-5</sup> M significantly decreased chocolate spot disease severity and increased the yield components of faba bean compared with the untreated control plants. The maximum reduction of disease severity was obtained with Kocide 101 and SA+GA<sub>3</sub> followed by SA+IAA at 10<sup>-5</sup> M with the average of 76.09, 71.74 and 64.13%, respectively in the first season. On the other hand, the same results were obtained with SA+IAA and SA at rate 10<sup>-5</sup> M with an average of 18.0% in the second season. So it could be concluded that the used chemical compounds could resist the detrimental effects of *Botrytis fabae* on the plant growth and improve yield production.

**Key words:** Growth regulators, salicylic acid, faba bean, chocolate spot, peroxidase, total phenol.

### INTRODUCTION

Faba bean (*Vicia faba* L.) is one of the earliest domesticated legumes which represents a very important source of protein for the human diet, especially for poor section of the population in Egypt and/or for animal feed in several countries. However, this crop is subjected to many abiotic and biotic stresses that seriously compromise the final yield (Tivoli *et al.*, 1988 and Abdalla Abdalla and Darwish, 2002). Among the menacing stresses, chocolate spot disease caused by *Botrytis fabae* Sard. is one of the most economically important diseases that damages the plant foliage, limit photosynthesis activity, and reduces

faba bean production globally (Torres *et al.*, 2004). For example, The subsequent loss was estimated to be more than 55% for susceptible cultivars (El-Bramawy and Abdul Wahid, 2007) and in other cases, losses were found to score approximately 100%, especially when favorable conditions prevail (Yi, 1986). Therefore, it is necessary to protect faba bean from this disease to cover the gap between production and consumption of this crop in Egypt.

At last few years, many great efforts were carried out to save the environment from pollution. Application of pesticides is considered one of the most famous

environmental pollutants. Ryals and Ward (1994) mentioned that all plants have the ability to defend themselves against pathogenic infection through a wide variety of mechanisms that can be local or systemic, constitutive or inducible. Moreover, plant diseases are a major problem for agriculture worldwide, understanding the mechanisms employed by plants to defend themselves against pathogens may lead to novel strategies to enhance disease resistance in crop plants (Pozo *et al.*, 2005).

The systemic acquired resistance (SAR) is a pathogen inducible defense mechanism that dependent on salicylic acid (SA) and is associated with a system expression of a subset of defense gene, e.g. the acidic proteins (Ward *et al.*, 1991). Oostendorp *et al.* (2001) revealed that the best-characterized signal pathway for systemically induced resistance is SAR that is activated by localized infections with necrotizing pathogen. It is characterized by protection against a broad range of pathogens through formation of set of induced proteins and its dependence of salicylic acid. Salicylic acid (SA) was reported as plant resistance inducer in many plants represents an interesting new opportunity in controlling fungal and bacterial diseases within environmental friendly integrated crop protection system through enhancing of plant to pathogen ( Hassan *et al* 2006, Rajkumar *et al.*, 2008; Wang *et al.*, 2010 and Yehia, *et al.*, 2011). Salicylic acid showed synergetic effect with auxin and gibberellins (Sanaa *et al.*, 2006). Moreover, in a number of species SA promoted flowering in combination with other plant growth regulators such as kinetin, indole acetic acid and gibberellins (Shehata, *et al.*, 2000).

The regulation of plant growth and biosynthesis of important economic constituents could be achieved through plant growth regulators. Applying growth regulators may modify morphological and physiological characteristic of plant and may induce better adaptation of plants to the environment, which could improve the growth and yield. The use of plant growth

regulators are directed, in general, to improve the yield quality and/or quantity of many crop (Rabie 1996). Gibberellic acid (GA<sub>3</sub>) and benzyladenine (BA) are the most important natural growth regulators in use. They are used to induce great changes in the growth regulators characters, chemical composition and yield criteria of *Hibiscus sabdariffa* L. In this concern, Nowak *et al.* (1997), Ramadan (1998) and Ibrahim *et al.* (2007) reported that GA<sub>3</sub> and (BA) treatments increased *Vicia faba* yield components. Foliar application of IAA also increased plant height, number of leaves /plant, fruit size with consequent enhancement in seed yield in different crop like groundnut (Lee, 1990), and cowpea ( Khalil and Mandurah, 1989). Gaber *et al.* (2000) reported that foliar spray of faba bean with GA<sub>3</sub> increased the polysaccharides which form the predominant fraction in the carbohydrate pool in the yielded seeds and thereby increased significantly the total carbohydrates.

Growth regulators (GR<sub>S</sub>) are generally used in commercial farming to direct the plant growth in particular dimension. Plant pathogens also disturb the balance of these growth vitals and cause abnormal growth. Although GR<sub>S</sub>, e.g. prohexandione-Ca are inactive with respect to pesticides (Rademacher and Bucci, 2002), it had been reported that plants do not only show better growth under the action of growth regulators but also show reduced occurrence of the disease (Costa *et al.*, 2001; Maxson and Jones, 2002 and Roemmelt *et al.*, 2003).

Hopkins (1985) mentioned that foliar application of indole acetic acid (IAA) at 200 mg/ml and kinetin at 1.000 mg/ml on *vitid rotundifolia* (Carlos) showed moderately resistant to pierces 's disease (PD), prevented the development of PD symptoms in inoculated plants in the greenhouse. These treatments also prevented accumulation of PD bacteria in leaves of the plants. El- korashy (1998) found that spraying growth regulators (GA<sub>3</sub>), naphthalene actic acid (NAA) and indole acetic acid (IAA) reduced the incidence of soil borne diseases, pod rot and increased peanut yield. Indol acetic acid (IAA) showed

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best prevention in case of pre and post emergence damping-off (Shafique *et al.*, 2011), followed by Indole butyric acid (IBA). Bazzi *et al.* (2003a and 2003b) investigated bacterial wilts and downy mildews of different crop plants and reported that application of GR<sub>S</sub> is also a beneficially preventive measure against them. Halbwirth *et al.*, (2003) concluded that use of growth regulators might be a considerable alternative of antibiotics against bacterial blights. El-Desouky (2006) reported that treating cucumber plants with plant growth regulators Ethephon, cycocyl, terpal and morphactin reduced disease severity of powdery and downy mildew and consequently increased protection of different cucumber cultivars under both greenhouse and field conditions.

The objective of this research was to determine the efficacy of the commercial inducer resistance of salicylic acid (SA) and plant growth regulators gibberellic acid (GA<sub>3</sub>) and indole acetic acid (IAA) singly or in combinations with (SA) to reduced the faba bean chocolate spot disease under field conditions and evaluating the enhancement of growth and seed yield plants. In addition, estimation of some associated biochemical changes in treated plants were studied.

### **MATERIALS AND METHODS**

Field experiments were carried out at Etay- El-Baroud Agric. Res. Station during the seasons 2011-2012 and 2012-2013 in randomized complete blocks design with three replicates. Experimental plot (5.4m<sup>2</sup>) had two ridges, 60 cm in between, and 3 meters long. Planting took place at the recommended in two sides/ridge with 2 seeds per hill; 20 cm apart which had been sown on 13 November of both seasons. Faba bean cultivar Giza 2 was used in this investigation, which obtained from Legumes Research Section, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt. All agricultural practices were carried out according to the recommendations of Ministry of Agriculture, Egypt. In both seasons, foliar application of the growth regulators used were conducted, *i.e.*,

gibberellic acid (GA<sub>3</sub>) and indole acetic acid (IAA) besides the growth inducer of salicylic acid (SA), supplied from Sigma Chemical Company, and used at 10<sup>-4</sup>, 10<sup>-5</sup> and 10<sup>-6</sup> M concentrations and tap water as negative control. They were freshly prepared solutions, which sprayed after 30, 45 and 60 days after sowing. The surfactant super film 0.3%, was added to the prepared solutions before using and kocide 101 at 2.5 g/L as fungicide against chocolate spot disease and used as positive control.

The tested treatments were:

- 1- 10<sup>-4</sup>, 10<sup>-5</sup> and 10<sup>-6</sup> M (SA)
- 2- 10<sup>-4</sup>, 10<sup>-5</sup> and 10<sup>-6</sup> M (GA<sub>3</sub>)
- 3- 10<sup>-4</sup>, 10<sup>-5</sup> and 10<sup>-6</sup> M (IAA)
- 4- 10<sup>-4</sup> M (SA + GA<sub>3</sub>)
- 5- 10<sup>-5</sup> M (SA + GA<sub>3</sub>)
- 6- 10<sup>-6</sup> M (SA + GA<sub>3</sub>)
- 7- 10<sup>-4</sup> M (SA + IAA)
- 8 - 10<sup>-5</sup> M (SA + IAA)
- 9 - 10<sup>-6</sup> M (SA + IAA)
- 10 - Tap water as negative control.
- 11- kocide 101 at 2.5g/L positive control.

### **Growth parameters:**

Five plants were randomly chosen from the center row of each plot at 90 days after sowing in both seasons to estimate plant height (cm), fresh and dry weight of shoots/plant (g).

### **Yield and its components:**

After physiological maturity about 170 days after sowing, five plants from each plot were harvested at random to determine plant height (cm); number of pods/plant; number of seeds/plant; weight of seeds/plant, number of branches/plant and weight of 100-seeds (g).

### **Disease assessment:**

The disease severity of chocolate spot was estimated after 55 and 75 days from sowing under natural infection conditions. Twenty plants were selected randomly from each plot using a 0-9 scale, where 0, 1, 2, 3, 4, 5, 6, 7 and 8 represent no visible infection (0) or disease covering less than 10%, 20%, 30%, 40%, 50%, 60% 70% or 80% of foliar tissue, respectively and 9 represents disease covering more than 80% of the foliar

tissue. Disease severity (DS) values were calculated using the following formula, according to Wheeler (1969) and ICARDA, (1986):

$$DS(\%) = \frac{\text{isease grade} \times \text{number of plant in each grade}}{\text{(total number of plants} \times \text{highest disease grade)}}$$

### **Chemical analysis:**

#### **Estimation of photosynthetic pigments:**

Chlorophyll a, b and chlorophyll a+b were determined quantitatively using N, N-dimethylformamide (DMF) as described by (Mortan and Porath 1980). Leaves were selected from different positions on the stem. Fresh weight samples were taken after 90 days after sowing and immersed directly in pure DMF {2-5% w/v}, then kept in the dark for 1-2 days at 4°C. The extracts were centrifuged for 15 minutes at 3000 rpm, and then the supernatant was diluted to the appropriate volume. Thereafter, the extinction of the extracts were measured photometrically (Uvnis Spectrophotometer, Lambda 2, Perkin-Elmer and Co. GmbH, 8027, F.R.G.) against a blank of pure DMF at 646.8, 663.8 and 750 nm.

The following equations were applied for determining the Chl content (mg/ml) of the leaf sample:

$$\text{Chl a} = 12.00 (E_{663.8} - E_{750}) - 3.11 (E_{646.8} - E_{750})$$

$$\text{Chl b} = 20.78 (E_{646.8} - E_{750}) - 4.88 (E_{663.8} - E_{750})$$

$$\text{Chl a+b} = 17.67 (E_{646.8} - E_{750}) + 7.12 (E_{663.8} - E_{750})$$

The Chl content was then expressed as mg/g f. wt.

#### **Determination of peroxidase and total phenol content:**

The activity of peroxidase enzyme was determined by employing the method of Thimmaiah (1999), after 90 days from sowing, 3 g fresh leaves were ground in a precooled mortar and pestle containing 9 ml of 0.1 M phosphate buffer (pH 7.1). The extract was centrifuged at 3000 rpm at 6°C for 20 min. Peroxidase activity was expressed as changes in absorbance min<sup>-1</sup> at 425 nm.

Total soluble phenols in fresh leaves (90 days age) were determined using the

colorimetric method described by Folin and Ciocalteu (AOAC 1985).

#### **Determination of carbohydrate and total protein:**

Total carbohydrate was determined in the dried seeds using phenol sulphuric method (Dubois *et al.*, 1956). Total nitrogen percentage was determined by micro-kjeldal method as described by A.O.A.C. (1975), and the percentage of protein was calculated by multiplying total N by factor 6.25.

#### **Statistical analyses:**

Data obtained were subjected to the statistical analysis according to the standard methods recommended by (Gomez and Gomez, 1984) using the computer program (costate). Means were compared using L.S.D. at the level 5% of probability.

### **RESULTS AND DISCUSSION**

Data present in Table (1) show the effect of foliar application of SA, GA<sub>3</sub> and IAA at 10<sup>-4</sup>, 10<sup>-5</sup> and 10<sup>-6</sup>M singly or in combinations on plant height, fresh and dry weights of faba bean shoots after 90 days after sowing. Generally, application of the tested compounds led to significant differences between these treatments in both seasons. In the first season, the highest values of plant height were obtained from the application of SA+IAA at 10<sup>-5</sup> M with an average of 89.40 cm compared to control followed by GA<sub>3</sub>, SA+GA<sub>3</sub> and SA at the rate of 10<sup>-5</sup> with average of 87.40, 87.40 and 84.60 cm, respectively. However, kocide 101 had similar value obtained from the application of SA at 10<sup>-4</sup> and 10<sup>-6</sup> M with an average of 78.60 cm for them. In the second season, GA<sub>3</sub>, SA+IAA and IAA at 10<sup>-5</sup> M increased plant height and their averages were 89.80, 88.80 and 87.80 cm, respectively. Marschner (1986) indicated that application of GA<sub>3</sub> or IAA caused elongation in the primary plant cells in the young tissues and growth centers. In this respect, other investigators reported that faba bean plant height was increased due to foliar application of GA<sub>3</sub> or IAA (Shalaby and Ahmed 1994 and Abdel-Fattah 1997). Also,

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Bekheta (2004) reported that foliar application of GA<sub>3</sub>, on wheat plants increased plant height.

**Table (1). Effect of foliar spray of SA, GA<sub>3</sub>, IAA singly or in combinations on faba bean plant growth parameters after 90 days from sowing, under field conditions.**

Growth parameter Treatment	2011/2012			2012/2013		
	Plant height (cm)	Fresh weight (g)	Dry weight (g)	Plant height (cm)	Fresh weight (g)	Dry weight (g)
10 <sup>-4</sup> M SA	78.60	67.85	10.38	76.00	77.72	9.61
10 <sup>-5</sup> M SA	84.60	76.52	11.59	78.20	81.12	11.37
10 <sup>-6</sup> M SA	78.60	59.87	9.16	69.40	70.23	8.81
10 <sup>-4</sup> M GA <sub>3</sub>	82.20	78.88	12.79	84.80	78.45	11.05
10 <sup>-5</sup> M GA <sub>3</sub>	87.40	91.73	16.47	89.80	92.24	12.99
10 <sup>-6</sup> M GA <sub>3</sub>	72.80	74.21	12.46	81.60	77.07	10.31
10 <sup>-4</sup> M IAA	81.00	80.84	12.87	84.20	84.58	10.48
10 <sup>-5</sup> M IAA	87.40	79.73	13.58	87.80	93.62	12.18
10 <sup>-6</sup> M IAA	79.40	71.33	10.90	75.60	76.35	10.27
10 <sup>-4</sup> M SA+GA <sub>3</sub>	80.40	69.04	11.17	77.60	70.15	9.71
10 <sup>-5</sup> M SA+GA <sub>3</sub>	87.40	68.82	9.91	81.00	71.34	10.34
10 <sup>-6</sup> M SA+GA <sub>3</sub>	82.00	58.40	9.18	75.20	62.08	9.19
10 <sup>-4</sup> M SA+IAA	79.40	87.25	13.15	76.20	81.77	8.46
10 <sup>-5</sup> M SA+IAA	89.40	80.85	13.60	88.80	81.49	11.16
10 <sup>-6</sup> M SA+IAA	77.60	63.86	10.14	76.20	69.57	8.46
Kocide 101	78.60	64.17	9.54	74.00	65.69	9.02
Control	75.80	61.13	9.36	72.20	64.35	8.03
LSD at 5%	8.62	16.06	2.95	7.40	14.48	2.49

Generally, fresh weight of shoots was increased significantly in plants sprayed with SA, GA<sub>3</sub> and IAA singly or in combinations of (SA+GA<sub>3</sub>) and (SA+IAA). In the first season, the best results were obtained with GA<sub>3</sub> at 10<sup>-5</sup> M and (SA+IAA) at 10<sup>-4</sup> M with the average of 91.73 and 87.25 g, respectively. On the contrary, the lowest values were obtained with (SA+GA<sub>3</sub>) and SA at 10<sup>-6</sup> M with the average of 58.40 and 59.87 g, respectively compared to all treatments. In the second season significant increases were clear in the fresh weight of plants sprayed with IAA and GA<sub>3</sub> at 10<sup>-5</sup> M with the average of 93.62 and 92.24 g., respectively, followed by IAA at 10<sup>-4</sup> M and SA+IAA at 10<sup>-4</sup> or 10<sup>-5</sup> M with the average of 84.58, 81.49 and 81.77 g, respectively.

Dry weight of faba bean shoots was significantly differed according to variable treatments. However, GA<sub>3</sub> at 10<sup>-5</sup> M resulted the highest shoot dry weight at both seasons (16.47 and 12.99 g), respectively. Also, the concentrations of 10<sup>-4</sup> M (SA+IAA), 10<sup>-5</sup> M (IAA) and 10<sup>-4</sup> M (SA+IAA) gave the average of 13.60, 13.58 and 13.15 g, respectively in the first season. However, in the second season the best values obtained with 10<sup>-5</sup> M (GA<sub>3</sub>) followed by 10<sup>-5</sup> M (IAA) and 10<sup>-5</sup> M (SA) with the average of 12.99, 12.18 and 11.37 g, respectively. Abdel-Latef (2003) and Abdel-Latef *et al.* (2009) reported that the increase in dry matter due to soaking in GA<sub>3</sub> and IAA solution might be attributed to rapid increase in cell division, cell enlargement and accumulation of building units that

accompanied by greater saccharides content than those of untreated plants

Data recorded in Table (2) clear that spraying faba bean plants with SA, GA<sub>3</sub> and IAA either singly or in combinations caused marked effect on plant height, number of branches/plant, number of pods/plant; number of seeds/plant; weight of seeds/plant, 100-seeds/weight and seed yield/fed. in comparison with the untreated control plants. The highest values of plant height were achieved when 10<sup>-4</sup> and 10<sup>-5</sup> M (IAA) and 10<sup>-4</sup> M (GA<sub>3</sub>) were applied, followed by SA+ GA<sub>3</sub> at 10<sup>-5</sup> M with the average of 112.4, 112.0, 108.6 and 108.0 cm, respectively. About the number of branches /plant also GA<sub>3</sub> at 10<sup>-4</sup> and 10<sup>-5</sup> M had the best values with the average of 6.8 and 6.4, respectively. Sarkar *et al.* (2006) found that GA<sub>3</sub> and IAA had regulatory effect to enhance plant height, number of branches, compared to other plant growth regulators and control. The highest number of pods/ plant was clear in case of 10<sup>-4</sup>M (GA<sub>3</sub>) and 10<sup>-5</sup> M (SA+GA<sub>3</sub>) with the average of 32.00 and 30.00, respectively. In the case of number of seeds/plant, 10<sup>-4</sup> M (GA<sub>3</sub>) and 10<sup>-5</sup> M (IAA) recorded the best values with the average of 87.00 and 85.80, respectively and the same trend was clear in case of seeds weight/plant with the average of 59.09 and 56.89 g, respectively. Both treatments of GA<sub>3</sub> and SA+ GA<sub>3</sub> at 10<sup>-5</sup> M increased 100-seed weight with the average of 76.81 and 76.18 g, respectively. Finally, from the same table it is clear that, seed yield/fed. was in harmony with the weight of seeds/plant especially in case of 10<sup>-4</sup> M (GA<sub>3</sub>), 10<sup>-5</sup> M (IAA), 10<sup>-4</sup> M (SA+GA<sub>3</sub>) and IAA at 10<sup>-4</sup>M with the average of 3.22, 3.14, 3.09 and 3.00 (ton), respectively. Ibrahim *et al.* (2007) illustrate that the increase in the yield could be reflection of bioregulators effect on growth and development, it might be due to a) marked increase in the number of branches/plant which gave a chance to the plant to carry more flowers, pods and hence more seeds b) marked increase in the phytosynthetic pigments content, which could lead to increase in phytosynthesis, resulting in greater transfer of assimilates to

seeds causing increase in their weight (Table,4).

Table (3) shows the results of the second growing season where significant increase in plant height was noticed when any of GA<sub>3</sub>, IAA and SA+GA<sub>3</sub> at 10<sup>-5</sup> M was applied compared to the untreated plants (control) with the average of 112.6, 111.6 and 110.0 cm, respectively. Gibberellic acid was also reported to increase plant height, number of leaves and total plant weight of *Hibiscus sabdariffa* L. (Mukhtar, 2008) .On the other hand, the differences in the number of branches/plant were not significant during second season. These results are supported by the findings of Ibrahim *et al.* (2007) who reported that the differences in the number of branches/plant due to application of benzyl adenine, ancymidol or IAA were not significant at all studied growth stages during two seasons. Number of pods/plant was increased significantly in plants sprayed with 10<sup>-5</sup> M (IAA and SA+ GA<sub>3</sub>) and 10<sup>-4</sup> M (SA+IAA) with the average of 32.80, 31.20 and 30.80 pod/plant, respectively. Significant increase in number of seeds/plant cleared with the application of 10<sup>-5</sup> and 10<sup>-4</sup> M (IAA), 10<sup>-5</sup> M (SA+ IAA) and 10<sup>-4</sup> M (GA<sub>3</sub>) with the average of 88.00, 87.40, 85.20 and 85.00 g, respectively. The highest 100-seeds weight was found in the cases of 10<sup>-5</sup>M (GA<sub>3</sub>), SA+ GA<sub>3</sub> at 10<sup>-4</sup>M and 10<sup>-5</sup> M (IAA) with the average of 70.43, 69.20 and 67.02 g., respectively. (Asharf *et al.*, 2006) concluded that IAA is successful in enhancing the plant growth and yield of barley cultivars. The highest values of yield/fed (ton) were obtained by using 10<sup>-6</sup> or 10<sup>-4</sup> M (IAA) and SA+IAA at 10<sup>-5</sup> M with the average of 3.12, 3.10 and 3.03 (ton), respectively. The increment of the above agronomic characters is in agreement with the findings of Mostafa *et al.* (2005) and Nair *et al.* (2002) who, found that the increase in seed yield of roselle plants by application of GA<sub>3</sub> and /or benzyladenine (BA) relative to untreated plants might be result a through breaking the apical dominate of rosella plants which led to increase both flowers and branches and consequently the number

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of fruits. The increase in seed weights might be ascribed to the promotive effect of GA<sub>3</sub> and BA in increasing the assimilates and their translocations from leaves to the fruits.

**Table (2). Effect of foliar spray of SA, GA<sub>3</sub> and IAA singly or in combinations on yield components of faba bean plants after 170 days from sowing grown under field conditions during first season.**

Yield component Treatment	Plant height (cm)	No. of branches /plant	No. of pods per plant	No. of seeds per plant	Weight of seeds/plant (g)	100-seeds weight (g)	Seed yield per fed. (ton)
10 <sup>-4</sup> M SA	89.80	5.8	21.00	70.00	43.45	66.53	2.95
10 <sup>-5</sup> M SA	96.40	6.0	29.20	79.60	46.74	68.42	2.86
10 <sup>-6</sup> M SA	89.80	5.2	21.00	62.20	37.49	64.30	2.16
10 <sup>-4</sup> M GA <sub>3</sub>	108.6	6.8	32.00	87.00	59.09	68.27	3.22
10 <sup>-5</sup> M GA <sub>3</sub>	106.8	6.4	26.80	83.80	54.72	76.81	3.09
10 <sup>-6</sup> M GA <sub>3</sub>	104.0	6.4	24.00	64.60	35.73	60.17	2.41
10 <sup>-4</sup> M IAA	112.4	6.4	29.80	79.00	51.47	63.56	3.00
10 <sup>-5</sup> M IAA	112.0	6.0	29.20	85.80	56.89	67.16	3.14
10 <sup>-6</sup> M IAA	104.6	5.4	24.80	77.80	48.86	60.20	2.91
10 <sup>-4</sup> M SA+ GA <sub>3</sub>	105.0	5.8	26.00	75.00	55.51	66.99	3.09
10 <sup>-5</sup> M SA+ GA <sub>3</sub>	108.0	6.4	30.00	84.40	49.57	76.18	2.95
10 <sup>-6</sup> M SA+ GA <sub>3</sub>	95.20	5.8	22.00	63.00	30.24	63.95	2.80
10 <sup>-4</sup> M SA+IAA	103.4	5.2	21.20	80.00	44.32	68.71	2.97
10 <sup>-5</sup> M SA+IAA	104.0	5.4	24.60	83.00	47.35	71.00	2.98
10 <sup>-6</sup> M SA+IAA	103.2	4.6	22.40	64.40	42.61	66.99	2.89
Kocide 101	94.80	6.2	26.00	73.80	53.66	65.13	2.95
Control	94.20	4.4	20.20	58.60	34.97	59.46	2.41
LSD at 5%	9.65	0.913	7.03	14.82	13.30	3.63	0.52

**Table (3). Effect of foliar spray of SA, GA<sub>3</sub> and IAA singly or in combinations on yield components of faba bean plants after 170 days from sowing grown under field conditions during second season.**

Yield component Treatment	Plant height (cm)	No. of branches /plant	No. of pods per plant	No. of seeds per plant	Weight of seeds/plant (g)	100-seeds weight (g)	Seed yield per fed. (ton)
10 <sup>-4</sup> M SA	97.00	5.0	28.20	74.60	48.49	62.14	2.96
10 <sup>-5</sup> M SA	97.00	5.2	28.20	80.80	51.52	65.46	2.87
10 <sup>-6</sup> M SA	89.00	4.8	23.20	66.60	41.06	62.53	2.14
10 <sup>-4</sup> M GA <sub>3</sub>	108.8	5.8	30.00	85.00	60.35	65.31	3.03
10 <sup>-5</sup> M GA <sub>3</sub>	112.6	5.4	29.40	80.00	57.82	70.43	3.00
10 <sup>-6</sup> M GA <sub>3</sub>	106.2	5.4	25.40	67.40	43.58	61.67	2.23
10 <sup>-4</sup> M IAA	108.4	5.6	29.60	87.40	62.04	62.62	3.10
10 <sup>-5</sup> M IAA	111.6	5.6	32.80	88.00	65.47	67.02	3.12
10 <sup>-6</sup> M IAA	105	5.2	26.00	66.60	50.55	59.68	2.59
10 <sup>-4</sup> M SA+ GA <sub>3</sub>	104	4.8	29.00	70.00	51.85	69.20	2.94
10 <sup>-5</sup> M SA+ GA <sub>3</sub>	110	5.2	31.20	81.00	47.31	67.00	3.02
10 <sup>-6</sup> M SA+ GA <sub>3</sub>	99.00	4.8	25.00	71.80	31.19	62.14	2.46
10 <sup>-4</sup> M SA+IAA	103	4.8	28.80	79.80	52.17	64.24	2.79
10 <sup>-5</sup> M SA+IAA	108.2	5.0	30.80	85.20	60.56	66.33	3.03
10 <sup>-6</sup> M SA+IAA	102	4.4	20.20	69.20	46.87	63.61	2.88

Kocide 101	95.2	5.2	27.20	72.80	56.23	62.21	2.74
Control	95.2	4.8	22.80	64.00	48.39	60.70	2.37
LSD at 5%	7.41	ns	5.98	14.58	8.38	4.96	0.56

**Table (4). Effect of foliar spray of SA, GA<sub>3</sub> and IAA singly or in combinations on chlorophyll a,b and total chlorophyll as mg/g leaves of faba bean during two seasons.**

Treatment	(2011/2012)			(2012/2013)		
	Chl. (a)	Chl. (b)	Chl. (a+b)	Chl. (a)	Chl. (b)	Chl. (a+b)
10 <sup>-4</sup> M	1.03	0.52	1.55	0.94	0.47	1.41
10 <sup>-5</sup> M SA	1.10	0.49	1.60	0.98	0.53	1.51
10 <sup>-6</sup> M	0.91	0.52	1.43	0.95	0.42	1.37
10 <sup>-4</sup> M	1.30	0.67	1.97	0.96	0.45	1.41
10 <sup>-5</sup> M GA <sub>3</sub>	1.22	0.59	1.81	0.98	0.49	1.47
10 <sup>-6</sup> M	1.05	0.44	1.49	0.95	0.43	1.38
10 <sup>-4</sup> M	0.98	0.51	1.49	0.98	0.45	1.39
10 <sup>-5</sup> M IAA	1.27	0.66	1.96	0.96	0.47	1.45
10 <sup>-6</sup> M	0.94	0.50	1.44	0.96	0.43	1.38
10 <sup>-4</sup> M	1.14	0.49	1.63	0.97	0.46	1.43
10 <sup>-5</sup> M SA+ GA <sub>3</sub>	1.25	0.60	1.85	0.95	0.51	1.46
10 <sup>-6</sup> M	1.06	0.50	1.56	0.96	0.44	1.40
10 <sup>-4</sup> M	0.96	0.59	1.55	0.95	0.40	1.35
10 <sup>-5</sup> M SA+IAA	1.09	0.55	1.64	0.98	0.49	1.47
10 <sup>-6</sup> M	0.99	0.53	1.52	0.94	0.34	1.28
Kocide 101	0.98	0.59	1.57	0.96	0.46	1.42
Control	0.95	0.50	1.45	0.95	0.43	1.38
LSD at 5%	0.11	0.04	0.14	ns	0.05	ns

Data recorded in Table (4) indicate that spraying faba bean plants in the first season (2011/2012) with SA, GA<sub>3</sub>, and IAA at 10<sup>-4</sup> or 10<sup>-5</sup> M singly or in combinations caused significant increase in photosynthetic pigments content (chlorophyll a, chlorophyll b and chl. a+b) in the leaves of faba bean plants collected after 90 days from sowing. The data revealed that foliar application of 10<sup>-4</sup> M (GA<sub>3</sub>), IAA at 10<sup>-5</sup> M and/or SA+ GA<sub>3</sub> at 10<sup>-4</sup> M were more effective on the content of total chlorophyll than other treatments. The present results are in agreement with the findings of Ibrahim *et al.* (2007) who reported that spraying faba bean plants with GA<sub>3</sub>, IAA and BA caused significant increase in the photosynthetic pigments

content (chlorophyll a, chlorophyll b and chl. a+b) at 90 days after sowing. In the second season, photosynthetic pigments content Chl. a and Chl. a+b were not significantly affected with the studied treatments. On the other hand, significant increase in Chl. b obtained with SA, SA+GA<sub>3</sub>, SA+IAA and GA<sub>3</sub> at 10<sup>-5</sup> M. The increase in chlorophyll biosynthesis and inhibition of chlorophyllase enzyme leading to higher accumulation of chlorophyll (Paricha *et al.*, 1977 and Sivakumar *et al.*, 2002).

Generally, foliar application of SA, GA<sub>3</sub> and IAA significantly increased both protein and total carbohydrate contents of faba bean except in the case of SA at 10<sup>-4</sup> and 10<sup>-6</sup> M and GA<sub>3</sub> at 10<sup>-6</sup> M. (Table, 5). Seeds



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produced from the plants sprayed with IAA at  $10^{-5}$  M and SA+ GA<sub>3</sub> at  $10^{-5}$  or  $10^{-4}$  M possessed the highest values of protein percentages with the average of 26.38, 25.88, 24.94 and 24.44, respectively. Total carbohydrate percentages were increased in the cases of  $10^{-5}$ ,  $10^{-4}$  M (SA+GA<sub>3</sub>),  $10^{-5}$  M (IAA) and  $10^{-5}$ ,  $10^{-4}$  M (SA+IAA) with the average of 58.32, 57.70, 56.85 56.25 and 55.24, respectively. Similar results were obtained by El-Etr, (2000) and Bardisi (2004) who found that application of kinetin on soybean and pea plants caused increase in seed protein and carbohydrate contents. Amin *et al.* (2006), also found that individual application of morphactin and indole-3-butric acid IBA at (25-100 mg) caused increase in carbohydrates and protein contents of maize grains as compared to their control. Similarly, Brnce (1990) and Chhun *et al.* (2004) showed that the highest values of carbohydrate and crude protein contents of *Oryza sativa* grains and soybean seeds were obtained.

Data present in Table (5) clear that faba bean foliar treatments with SA, GA<sub>3</sub> and IAA at  $10^{-4}$  or  $10^{-5}$  M singly or in combinations significantly increased the activity of peroxidase compared to the control treatment. In addition, foliar treatments of SA+GA<sub>3</sub>, SA+ IAA and SA at  $10^{-5}$  M recorded the highest increase in peroxidase activity compared to other treatments with the average of 0.785, 0.702, and 0.678 mg/g leaves, respectively. The results obtained in this study are confirmed with those mentioned by El-Fiki *et al.* (2007) in the case of sugar beet leaves infected by *Uromyces beta* and they point out the role played by catalase, peroxidase and polyphenoloxidase which are implicated in defensive mechanisms of host plant against the fungal pathogen; the results also are similar to the findings of Rodica (2012) who reported that peroxidase and polyphenoloxidase activities were found higher in the diseased leaves attacked by *Taphrina deformans* when compared to the enzymes activity of healthy leaves. This study suggests that the accumulation of reactive oxygen species in

peach tissues, increase activities of such enzymes being involved in host defence mechanisms. Also, foliar application with SA and SA+IAA at  $10^{-5}$  M resulted the highest increase in total phenol content (0.390 mg/g f.w.) respectively, followed by SA+GA<sub>3</sub> and SA at  $10^{-4}$  M with the average of 0.380 and 0.360 mg/g f.w., respectively. Gozzo (2004) reported that salicylic acid might interact with iron-based enzymes, either as a chelator of metal ion or through binding to related proteins. The plants forming phenolic free radicals, resulting from the interaction with catalase or peroxidase has proposed to be involved in the induction of systemic acquired resistance (SAR). These results are in agreement with the El-Nagar (1998) who indicated that the phenolic compounds, especially total phenols were more increased in the stem rust infected wheat plants pretreated with different concentrations of GA<sub>3</sub>, as compared with the infected-untreated plants. Also, Chowdhury (2003) found that auxin led to increase in total phenol, calcium content and activity of catechol oxidase, and mentioned that these materials protect plants against pathogen stress. Wasternack *et al.* (2006) concluded that phenomenon of SAR has been found to be mediated by salicylic acid following the observation that exogenous treatment with salicylic acid induced PR-protein synthesis and enhanced resistance to infections. The expression of defense related protein (PR) are peroxidases, polyphenol oxidase etc. which are plant specific and released by pathogen in response to elicitors.

The effect of different treatments on chocolate spot disease severity (DS) and consequently protection of faba bean (Giza 2 cv.) during the two seasons 2011/2012 and 2012/2013 were shown in Table (6). All the treatments, salicylic acid (SA), gibberellic acid (GA<sub>3</sub>) and indole acetic acid (IAA) at  $10^{-4}$   $10^{-5}$  and  $10^{-6}$  M either separately or in combinations significantly decreased the (DS) of chocolate spot during the two seasons under natural infection conditions. Kocide 101 had the best effect in

reducing the disease severity followed by  $10^{-5}$  M (SA+ GA<sub>3</sub>) with disease reduction averages of 76.09 and 71.74% then  $10^{-4}$ M (SA+GA<sub>3</sub>) and SA+IAA at  $10^{-5}$  M with the average of 67.39 and 64.13%, respectively. Saswati *et al.* (1988) found that, the pretreated rice plants with GA<sub>3</sub> exhibited induced resistance to rice sheath rot disease. The obtained results are in harmony with the finding of El-Fiki *et al.* (2007) who mentioned that spraying plants of sugar beets with any of IAA, NAA and GA<sub>3</sub> was significantly effective for suppressing natural rust infection in sugar beet during 2000 and 2001. They also reported that spraying with IAA was the most effective followed by NAA and GA<sub>3</sub> compared with control. In this respect, the highest reduction was recorded in the second season with Kocide 101 followed by  $10^{-5}$  M (SA and SA+IAA) with the averages of 71.43, 63.27 and 63.27% disease

reduction, respectively. Indol acetic acid (IAA) showed best prevention results in case of pre and post emergence damping-off (Ragab *et al.*, 2009), followed by Indole butric acid (IBA) which provided second best prevention under identical circumstances. Salicylic acid plays a control role in the activation of defense responses against biotrophic and semi-biotrophic pathogens as well as the establishment of systemic resistance (Vlot *et al.*, 2009 Peleg and Blumwald, 2011). The phytohormones affect all phases of the plant life cycle and their responses to environmental stresses, both biotic and abiotic. (Williams, 2010). These results are in agreement with the findings of El-Korashy (1998) who found that spraying growth regulators gibberellin (GA<sub>3</sub>), naphalene acetic acid (NAA) and indole acetic acid (IAA) on peanut plants reduced the incidence of soil borne disease, pod rot and increased as well; the yield.

**Table (5). Protein, total carbohydrate contents of produced seeds, peroxidase activity and total phenols content as mg/g leaves of faba bean plants sprayed with SA, GA<sub>3</sub> and IAA singly or in combinations (2011/2012).**

Treatment	Protein %	Total Carbohydrate %	Peroxidase activity(Po) A424/min	Total phenols mg/g/f.w.
$10^{-4}$ M	19.75	44.01	0.551	0.360
$10^{-5}$ M SA	24.19	51.45	0.678	0.390
$10^{-6}$ M	19.25	43.63	0.365	0.240
$10^{-4}$ M	22.50	48.91	0.434	0.340
$10^{-5}$ M GA <sub>3</sub>	23.66	50.57	0.599	0.350
$10^{-6}$ M	18.72	46.36	0.312	0.250
$10^{-4}$ M	24.44	49.74	0.443	0.330
$10^{-5}$ M IAA	26.38	56.85	0.613	0.330
$10^{-6}$ M	22.32	47.91	0.402	0.240
$10^{-4}$ M	24.94	57.70	0.653	0.300
$10^{-5}$ M SA+ GA <sub>3</sub>	25.88	58.32	0.785	0.380
$10^{-6}$ M	22.67	47.22	0.336	0.220
$10^{-4}$ M	21.51	55.24	0.634	0.350
$10^{-5}$ M SA+IAA	23.67	56.25	0.702	0.390
$10^{-6}$ M	21.11	48.39	0.415	0.210
Kocide 101	23.44	50.28	0.626	0.240
Control	23.44	46.43	0.413	0.210

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LSD at 5%	3.06	3.27	0.129	0.030
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**Table (6): Effect of foliar spray of SA, GA<sub>3</sub> and IAA singly or in combinations on chocolate spot disease severity (DS) of faba bean cv. Giza 2 under field conditions during two seasons.**

Treatment	Chocolate spot disease severity (DS)			
	2011/2012		2012/2013	
	DS	Reduction%	DS	Reduction%
10 <sup>-4</sup> M	18.50	59.78	19.00	61.22
10 <sup>-5</sup> M SA	16.75	63.59	18.00	63.27
10 <sup>-6</sup> M	32.94	28.39	35.25	28.06
10 <sup>-4</sup> M	19.00	58.70	22.50	54.08
10 <sup>-5</sup> M GA <sub>3</sub>	20.00	56.52	20.00	59.18
10 <sup>-6</sup> M	32.94	28.39	33.67	31.29
10 <sup>-4</sup> M	21.80	52.61	20.50	58.16
10 <sup>-5</sup> M IAA	23.80	48.26	25.22	48.53
10 <sup>-6</sup> M	34.00	26.09	37.99	22.47
10 <sup>-4</sup> M	15.00	67.39	24.00	51.02
10 <sup>-5</sup> M SA+ GA <sub>3</sub>	13.00	71.74	20.00	59.18
10 <sup>-6</sup> M	26.50	42.39	28.00	42.86
10 <sup>-4</sup> M	19.00	58.70	20.00	59.18
10 <sup>-5</sup> M SA+IAA	16.50	64.13	18.00	63.27
10 <sup>-6</sup> M	25.00	45.65	29.00	40.82
Kocide 101	11.00	76.09	14.00	71.43
Control	46.00	-----	49.00	-----
LSD at 5%	3.71		4.26	

Data in Tables (5 and 6) show that the highest peroxidase activity and total phenol contents in addition to the lowest chocolate spot disease severity were obtained at the concentration of 10<sup>-5</sup> M of either (SA+GA, SA+IAA and SA) treatments. Sarma *et al.* (2007) reported that chickpea foliar spray with zinc sulphate, oxalic acid, sodium malonate and sodium selenite reduced mortality of chickpea from *Sclerotinia sclerotiorum*. HPLC analysis of treated chickpea leaves revealed the activation synthesis of several phenolic compounds after application and a positive correlation was observed between induction of phenolic compounds and survival of the plants. Foliar spray with different concentrations especially (10<sup>-5</sup>M) conc. resulted in a significant increase in peroxidase and total phenols beside disease severity reduction

compared with the untreated control Tables (5 and 6). Figures (1 and 2) illustrate negative correlation relationship between disease severity and peroxidase activity and accumulation of total phenols. Tarrad *et al.* (1993) and Cherif, *et al.* (2007) reported that the increase in peroxidase activity enhanced lignification in response to chocolate spot disease, which may restrict fungal penetration and play an important role in induced resistance of plant to the pathogen. Another supportive suggestions was made by Nawar and Kuti (2003), who stated that an increase in peroxidase activity is considered to be a preliminary indicator for resistance of broad beans to chocolate spot disease. Segarra *et al.* (2006) conducted a study on induction of resistance under the activity of chemical inducers against root rot diseases and found induced systemic

resistance (ISR) rapidly developed in plants marking the resistance. They mentioned that chemicals play a role behind this resistance induction as accumulation of phytoalexins, lignifications of phenols and activation of

chitinase, polyphenoloxidase and peroxidase.

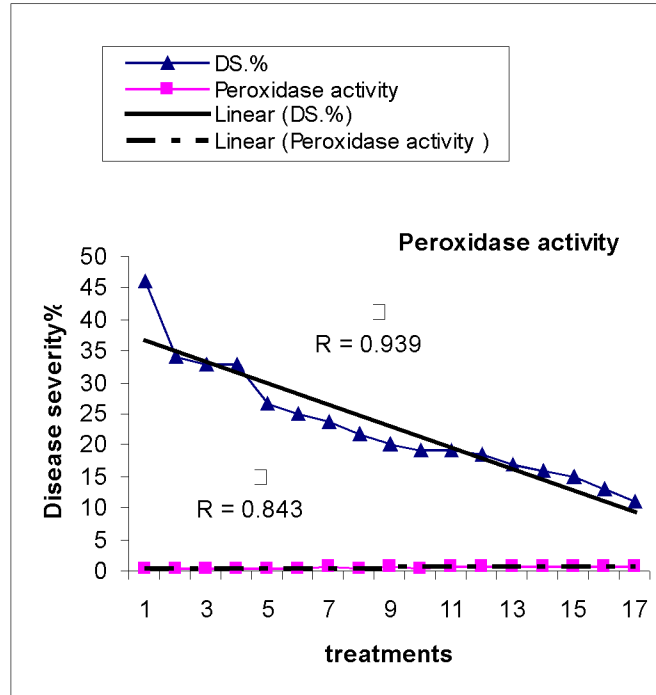
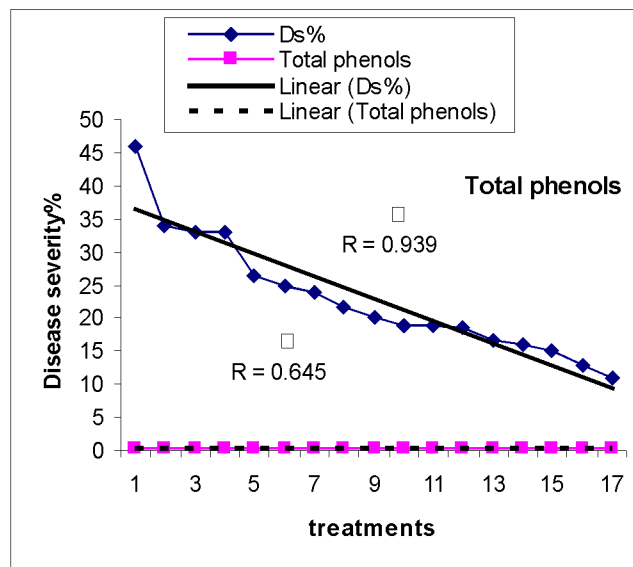


Fig (1): Effect of foliar spray of SA, GA<sub>3</sub> and IAA singly or in combinations on peroxidase activity and chocolate spot disease severity of faba bean cv. Giza 2 under field conditions and natural inoculation during first season.



**Fig (1): Effect of foliar spray of SA, GA<sub>3</sub> and IAA singly or in combinations on total phenols content and chocolate spot disease severity of faba bean cv. Giza 2 under field and natural inoculation conditions during first season.**

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## **تأثير المعاملة الورقية لحمض الجبريليك وإندول حمض الخليك وحمض الساليسليك على نمو ومحصول الفول البلدى وكذلك على شدة إصابة بالتبقع الشكولاتى**

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### **المخلص العربى**

تحت ظروف الحقل بمحطة البحوث الزراعية - إيتاى البارود- محافظة البحيرة لموسمين متتالين ٢٠١٢/٢٠١١ و ٢٠١٣/٢٠١٢ تم دراسة تأثير كل من حمض الساليسليك وحمض الجبريليك وإندول حمض الخليك ومخاليطهم عند تركيزات  $10^{-4}$ ،  $10^{-5}$ ،  $10^{-6}$  مولار على مقاومة مرض التبقع الشكولاتى فى الفول البلدى صنف جيزة ٢ وكذلك على بعض الصفات المحصولية والمحصول البذرى وبعض التغيرات البيوكيميائية (محتوى الأوراق من الكلوروفيل ا، ب والكلى وكذلك نشاط إنزيم البيروكسيداز والمحتوى الكلى للفينول). وجد أن رش حمض الجبريليك و حمض الساليسليك + إندول حمض الخليك عند تركيز  $10^{-5}$  مولار أدى إلى زيادة فى إرتفاع النبات والوزن الرطب والجاف للمجموع الخضرى مقارنة بالنباتات غير المعاملة. كما وجد زيادة فى بعض الصفات المحصولية وهى طول النبات، عدد الفروع/نبات، عددالقرون/نبات، عدد البذور/نبات، وزن ١٠٠ بذرة والمحصول البذرى نتيجة للمعاملات بحمض الجبريليك وإندول حمض الخليك وحمض الساليسليك + إندول حمض الخليك عند تركيز  $10^{-4}$  و  $10^{-5}$  مولار. بصفة عامة المحتوى الكلوروفيل أ و ب وكذلك الكلوروفيل الكلى أ+ب ونشاط إنزيم البيروكسيداز يزداد عند كل المعاملات ذات التركيز  $10^{-4}$  و  $10^{-5}$  مولار. وجد أن أعلى معدل زيادة فى محتوى البذور من البروتين والكربوهيدرات عند المعاملة بإندول حمض الخليك، حمض الساليسليك + حمض الجبريليك عند التركيز  $10^{-5}$  مولار بنسبة ٢٦,٣٨ ، ٢٥,٨٨ % للبروتين و ٥٨,٣٢ ، ٥٦,٨٥ % للكربوهيدرات على التوالى. وجد أن كل المعاملات عند التركيز  $10^{-4}$  و  $10^{-5}$  مولار تقلل من شدة الإصابة بالتبقع الشكولاتى مقارنة بالكنترول. وظهرت أعلى مقاومة لشدة الإصابة عند المعاملة بالمبيد كوسيد ١٠١ يليها حمض الساليسليك + حمض الجبريليك ، حمض الساليسليك + إندول حمض الخليك عند التركيز  $10^{-5}$  مولار بنسبة ١١,٠٠ ، ١٣,٠٠ ، ١٦,٥٠ % على الترتيب فى الموسم الأول أما فى الموسم التالى تساوى تأثير المعاملة وحمض الساليسليك + إندول حمض الخليك مع المعاملة بحمض الساليسليك عند التركيز  $10^{-5}$  مولار بنسبة ١٨,٠٠%. وبناء على ماسبق يمكن الإستنتاج أن المركبات الكيميائية المستخدمة يمكنها مقامة التأثير المدمر لفطر البوتريتس فابى على مستوى النمو والإنتاجية للفول البلدى.