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Green Chemistry for Inducing Resistance Against Chocolate Spot Disease of Faba Bean

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ABSTRACT

Uses of a green chemistry in form of antioxidants and micronutrients for seed treatments or for foliar spray on faba bean plants was implemented as a delicate tactic for controlling chocolate spot disease cause by *Botrytis fabae*. The *in vitro* trials revealed that salicylic acid at 7 mM inhibited the growth of *B. fabae* followed by zinc at 8 g L^{-1} and iron at 4 g L^{-1}. The combinations of hydroquinone (HQ), salicylic acid (SA), zinc (Zn) and iron (Fe); (HQ+SA+Fe, HQ+SA+Zn and HQ+Zn+Fe) significantly reduced the growth of the fungus. Field experiment showed that all tested combinations in form of seed soaking or foliar spraying confirmed the *in vitro* results as they decreased the disease severity of chocolate spot. On the other hand, the physiological characters included the photosynthetic pigments (chlorophyll a, b and carotenoids), total phenols, proline content in the leaves, total protein in seeds, antioxidant activities in both leaves and seeds were also increased. So far, application of the above tactics may be recommended as delicate methods to increase the plant resistance against a number of plant pathogens including chocolate spot pathogen of faba bean (*B. fabae*). However, large scale application is needed to confirm these data.

Key words: Faba bean, green chemistry, inducing resistance, antioxidants, micronutrients, chocolate spot, *Botrytis fabae*

INTRODUCTION

Faba bean (*Vicia faba* L.) is a worldwide leguminous crop of a good source of protein. It contains minerals (iron, zinc, calcium) and vitamins (B1, B2, C). Also, it is used as a fodder and forage crop (Rubiales, 2010).

Faba bean is affected by several yield limiting factors including fungal diseases. Chocolate spot disease caused by *Botrytis fabae* Sardina is one of them. In the Nile Delta losses due to chocolate spots disease reaches 60-80% among susceptible cultivars and 34% among tolerant ones (Bouhassan *et al*., 2004; Sahile *et al*., 2008).

Application of green chemistry in agriculture is verified as a promising safe methods to control the diseases and produce seeds of high quality. These chemicals are environmentally sound more than synthetic fungicides; with lower economic costs.

A number of researchers applied these chemicals for controlling plant diseases and received promising data. Among these are salicylic, benzoic, citric and oxalic acids (Toal and Jones, 1999; Ziadi *et al*., 2001; Dmitriev *et al*., 2003; Achuo *et al*., 2004). One example is hydroquinone at a low

concentration as when soaking peanut seeds in 20 mM water solution of hydroquinone for 12 h. before sowing, a significant decrease in the incidence of soil-borne fungi and promotion in the growth parameters including yield and its components were obvious (Elwakil, 2003). Also, Hassan *et al*. (2006) showed a significant reduction in chocolate spot severity of faba bean upon the spray of citric, benzoic and salicylic acid on the plants.

Growing *B. fabae* under stress of Fe, Zn, Mn and Ca showed a significant decreased in its linear growth and sporulation while, foliar application of these micronutrients reduced chocolate spot syndrome on faba bean plant which was accompanied with an increase in the growth parameters, yield and its components of the plants (Abd El-Hai *et al*., 2007a).

El-Hendawy *et al*. (2010) showed that soaking seeds of faba bean in Di-potassium hydrogen phosphate (K_2HPO_4) along with calcium chloride ($CaCl_2$) at concentration 10 mM of each, moderately reduced the severity of the disease.

In this regard, El-Razek *et al*. (2013) revealed that spraying faba bean plants with Fe+Zn+Mn significantly decreased the incidence of Alternaria leaf spot, increased yield and yield components. Also, chlorophyll a, b, reducing sugars and the non-reducing sugars were significantly increased in all treatments when sprayed with these micronutrients compared with the non-treated ones (check).

Aldesuquy *et al*. (2014) revealed that exogenous application of shikimic and salicylic acid or their combination could counteract the adverse effects of *B. fabae* on osmotic pressure adjustment by inducing additional increase in proline, total soluble sugars, total soluble nitrogen and organic acids.

Since plants develop various mechanisms to overcome the deleterious effects of biotic or abiotic stresses including total phenols, enhancement the production of such compounds in the plants is a must (Jersch *et al*., 1989; MacRae and Towers, 1984; Scalbert, 1991). Also the amino acid proline proved to act as a potent scavenger for preventing the induction of programed cell death by Reactive Oxygen Species (ROS) (Ashry and Mohamed, 2011).

So far, the aim of this study was planned to evaluate the possible effect of antioxidants-micronutrients formulations as green chemicals for the control of chocolate spot caused by *Botrytis fabae* on faba bean plants under field conditions. Photosynthetic pigments, total phenols, total proline, total protein and antioxidant activity in both fresh leaves and dry seeds of faba bean when increased, this may be taken as persuasive evidence for the success of using the green chemicals for controlling the common diseases attacking plants.

MATERIALS AND METHODS

Trials were conducted in laboratories and the farm of Plant Pathology Department, Faculty of Agriculture, Mansoura University in the period from 2011-2014.

Isolation: Isolation of *Botrytis fabae* the causal agent of chocolate spot disease from faba bean seeds collected from different parts of Egypt was carried out according to the procedures described by the International Seed Testing Association (ISTA., 1996).

Pure isolates were identified in consultation with the description sheets of Mycological Institute, Kew, Surrey, England (CMI), Danish Government Institute of Seed Pathology (DGISP) publications, as well as publications of Ellis (1971) and Moubasher *et al*. (1977).

Pathogenicity: Pathogenicity test was carried out using healthy faba bean plants and the suspected aggressive isolates of *Botrytis fabae*. Plants were grown in net house under a winter

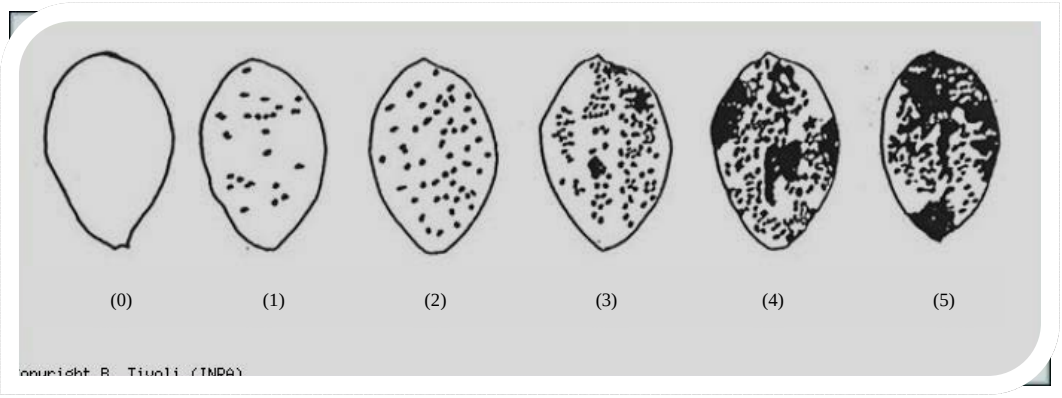

Fig. 1: Disease assessment recorded after 48 h of artificial inoculation following the disease index (0) Healthy plants, (1) Small spots, (2) Increasing spots number and spreading, (3) Coalesce spots together, (4) Half of the leaf is necrotic and (5) Leave died and fall

conditions. Giza 3 Mohassan seeds of faba bean were sown in plastic pots (20 cm in diameter) containing 5 kg clay-sandy soil (2:1, v/v) while five seeds were sown in each pot. After 45 days, plants were sprayed with the spore suspension of *B. fabae* and covered with polyethylene bags for 24 h to maintain humidity condition around the plants. Check plants were sprayed with tap water.

Disease assessment was recorded after 48 h of artificial inoculation following the disease index (Fig. 1) designed by ICARDA (2005).

While the disease severity was recorded using the formula adopted by Hanounik (1986):

$$\text{Disease severity index (\%)} = \frac{\sum(\text{NPC}\times\text{CR})}{\text{NIP}\times\text{MSC}}\times 100$$

where, NPC is no. of plants in each class rate, CR is class rate, NIP is no. of infected plants and MSC is maximum severity class rate.

Use of green chemicals i.e. antioxidants, micronutrients on *B. fabae*: Green chemicals including hydroquinone (HQ), salicylic acid (SA), chelated zinc (Zn), manganese (Mn) and iron (Fe) 13% (EDTA), as well as the formulated antioxidant GAWDA®(Patent No. 23798) consists of (tartaric acid 2 mM+hydroxyquinoline 1 mM+calcium chloride 6 mM+magnesium chloride 5 mM+calcium borate 5 mM) were used in this investigation to study their effect on *in vitro* growth of *B. fabae*.

Fungal growth: Four concentrations of these compounds, HQ (5, 10, 15 and 20 mM), SA (1, 3, 5 and 7 mM), Zn (2, 4, 6 and 8 g L^{-1}), Fe (1, 2, 3 and 4 g L^{-1}), Mn (1, 2, 3 and 4 g L^{-1}) and GAWDA®formulation (1, 2, 3 and 4 g L^{-1}) were incorporated in a PDA medium and poured in petri dishes. The plates were inoculated in the centers with 0.5 mm diameter discs of 5 days-old culture. For each treatment, three replicates were used. The cultures were incubated at 20±2°C. The linear growth of the fungus was measured after inoculation until the check covers the surface of the plate. The inhibition percentage was calculated using Topps and Wain (1957) as follows:

$$\text{Inhibition percentage} = \frac{\text{A-B}}{\text{A}}\times 100$$

where, A is mean of diameters in the control and B is mean of diameters in the treatment. The chemicals recorded significant inhibition percentage were selected to be used *in vivo*.

Mycelial dry weight: Erlenmeyer flasks each consists of one hundred ml of PD medium were autoclaved, then amended with the four concentrations of the tested chemicals. Each flask was inoculated with two discs of 0.6 mm diameter of the fungal culture, incubated at 20±2°C until check covers the surface of the flask. Three replicates were used from each concentration. At the end of incubation period, the mycelium was filtered and washed thoroughly in distilled water, then dried at 80°C for 48 h till constant weight (El-Morsy, 1993).

Field experiment: Field experiment was carried out to study the role of the tested antioxidants and micro-nutrients formulations as green chemistry on the disease severity of *B. fabae*. Growth parameters, yield and its components were also performed. Faba bean cultivar (Giza 3 Mohassan) was used in this investigation.

Split-plot design with three replicates was applied while the tested chemicals were the main plot factor and the application methods were the sub-plot factor. The plot area was 10.5 m^2 (3×3.5 m). The NPK fertilizers were applied following the recommendation of Egyptian Ministry of Agriculture, Egypt.

Application methods (soaking, spraying and soaking+spraying) were used as the sub-plot. Faba bean seeds were soaked in each treatment for 24 h and in water for the check. Each plot represents a treatment and consists of nine rows for the three application methods and three rows for each method. The concentrations of the tested chemicals were used as follow: HQ (15 mM), SA (3 mM), Zn-EDTA (8 g L^{-1}), Fe-EDTA (4 g L^{-1}) and GAWDA formulation (3 g L^{-1}) at these concentrations to form combinations shown in Fig. 2.

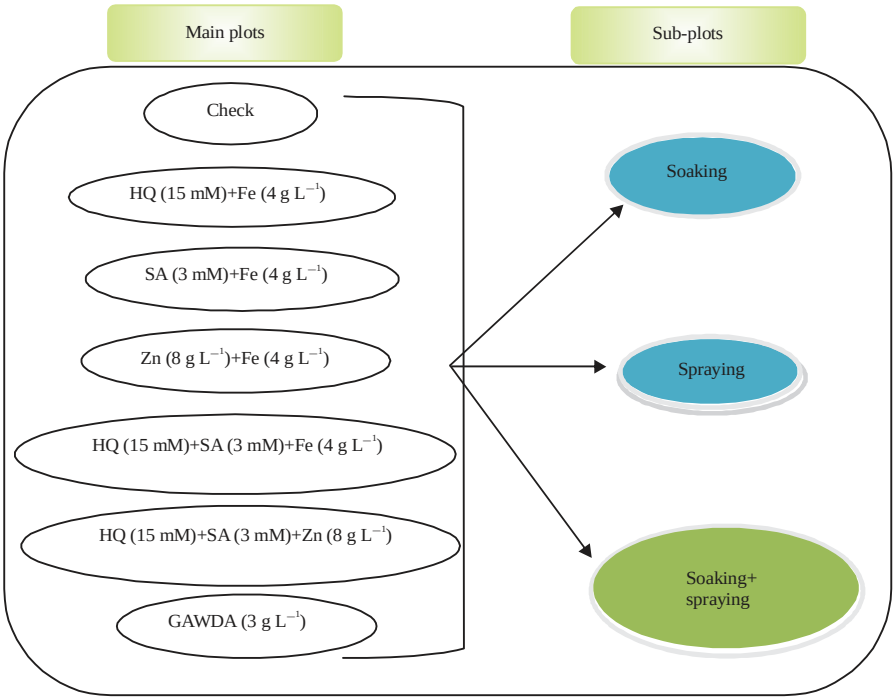

Fig. 2: Designed sketch for the application of the green chemicals combinations on faba bean plants under natural field conditions

Seeds were sown in hills spaced 15 cm on ridges. Thirty days later, the 1st spray of each treatment was carried out. After 15 days from the 1st spray, the 2nd spray was carried out followed by the 3rd one after another15 days. Disease assessment was recorded when the first disease symptoms were seen and after each spray. Samples of leaves were randomly collected for proline analysis and antioxidants activity. The samples were taken before and upon symptoms appeared. Estimation of photosynthetic pigments and total phenols in the leaf samples were carried out on 30 days old plants as well as after each time of spraying on 45, 60 and 75 days old plants.

In the mature plants, dry seeds of each treatment were used for estimating the total protein, antioxidant activity and total phenols.

Photosynthetic pigments: Determination of photosynthetic pigments (chlorophyll a, b and carotenoids) were carried out in samples of (0.05 g) of plant 3rd leaflet. Each sample was immersed in 10 mL methanol for 24 h and a trace of sodium carbonate was added then stored in dark (Robinson and Britz, 2000). The pigments were spectrophotometrically measured as described by Mackinney (1941).

Total phenols: Total phenols were determined using the Folin-Ciocalteau reagent (Singleton and Rossi Jr., 1965). The results were expressed as milligram catechol/100 g fresh weight material.

Proline content: Proline content in shoots was determined following the method of Bates *et al*. (1973). The proline content was expressed as follows:

$$\text{Proline } (\mu M)/\text{Fresh wt } (g) = \text{Proline } (\mu g)/mL \times \text{toluene } (mL) \times 5/115.5 \times \text{sample } (g)$$

where, 115.5 is molecular weight of proline.

Total protein: Protein content in the plant extract was determined spectrophotometrically following the method of Bradford (1976). The quantity of protein in each sample was estimated from the standard curve using Bovine Serum Albumin (BSA) solution as a standard protein.

Antioxidant activity: The effect of the extracts on diphenylpicrylhydrazyl (DPPH) radical was estimated using the method described by Kitts *et al*. (2000) and Liyana-Pathirana and Shahidi (2005).

Determination of metal elements

Zinc and iron: The concentration of these metals were estimated according to methods of AOAC (1990) using "Buck Scientific Accusys "214" Atomic Absorption Spectrophotometer, USA. The results were reported as milligram per killogram dry weight.

Statistical analysis: Data was statistically analyzed through CoStat 6.311 software of analysis of variance (Gomez and Gomez, 1984). The means were compared using Least Significant Difference (LSD) at $p \leq 0.05$ as outlined by Duncan (1955).

RESULTS

Isolation: Seven isolates of *Botrytis fabae* Sardina from different seed samples of faba bean plants were used in this investigation.

Pathogenicity tests: The disease severity of the *B. fabae* isolates showed that isolate No. 1 was the highest pathogenic one and presented disease severity and incidence of 56 and 100%, respectively. This isolate was selected for the further studies (Table 1).

In vitro studies

Effect of green chemicals on the growth of *B. fabae*: Table 2 presents the effect of four tested concentrations of each selected antioxidant and micronutrients on the linear growth and mycelial dry weight. An inverse relationship was found between the concentration and linear growth or mycelial dry weight of *B. fabae*. It was also found that SA at 7 mM is the most effective chemical in decreasing the linear growth of the fungus (0.60 cm) with inhibition percentage of 93.33%. The mycelial dry weight at 7 mM of SA and 4 g L^{-1} of Fe was inhibited as it record 0.047 and 0.048 g, respectively with inhibition percentage of 91.22% for both chemicals.

Table 1: Pathogenicity test of the isolated *Botrytis fabae* showing the disease severity and disease incidence

Isolates	Disease severity (%)	Disease incidence (%)
1	56.0^{a}*	100.00^{a}
2	36.0^{b}	100.00^{a}
3	20.0^{cd}	80.00^{b}
4	40.0^{b}	100.00^{a}
5	32.0^{bc}	60.00^{c}
6	36.0^{b}	100.00^{a}
7	08.0^{de}	40.00^{d}
Check	00.0^{e}	0.00^{e}

*Mean followed by different letter(s) in the same column are significantly different according to Duncan's multiple range tests at $p \leq 0.05$

Table 2: Effect of green chemicals on the growth of *B. fabae*

Treatments	Concentration	Linear growth	Inhibition (%)	Mycelial dry weight	Inhibition (%)
Hydroquinone	5 mM	6.97^{b}*	22.59^{i}	0.285^{e}	46.45^{g}
	10 mM	5.53^{c}	38.52^{h}	0.274^{e}	48.58^{g}
	15 mM	4.45^{de}	50.56^{fg}	0.175^{f-h}	67.16^{d-f}
	20 mM	4.40^{de}	51.11^{fg}	0.152^{h}	71.58^{d}
Salicylic acid	1 mM	8.67^{a}	03.71^{j}	0.362^{d}	32.12^{h}
	3 mM	1.00^{h-j}	88.89^{a-c}	0.415^{b}	22.07^{i}
	5 mM	0.63^{j}	92.96^{a}	0.084^{ij}	84.22^{bc}
	7 mM	0.60^{j}	93.33^{a}	0.047^{k}	91.22^{a}
Zn-EDTA	2 g L^{-1}	8.47^{a}	05.93^{j}	0.357^{d}	32.99^{h}
	4 g L^{-1}	4.93^{cd}	45.18^{gh}	0.299^{e}	44.00^{g}
	6 g L^{-1}	1.73^{gh}	80.74^{cd}	0.190^{fg}	64.30^{ef}
	8 g L^{-1}	0.93^{ij}	89.63^{ab}	0.099^{i}	81.46^{c}
Fe-EDTA	1 g L^{-1}	8.78^{a}	02.41^{j}	0.461^{b}	13.60^{j}
	2 g L^{-1}	4.13^{e}	54.07^{f}	0.268^{e}	49.65^{g}
	3 g L^{-1}	1.50^{g-i}	83.33^{b-d}	0.205^{f}	61.63^{f}
	4 g L^{-1}	1.10^{h-j}	87.78^{a-c}	0.048^{jk}	91.22^{a}
Mn-EDTA	1 g L^{-1}	9.00^{a}	00.00^{j}	0.500^{a}	06.20^{k}
	2 g L^{-1}	9.00^{a}	00.00^{j}	0.500^{a}	06.20^{k}
	3 g L^{-1}	9.00^{a}	00.00^{j}	0.500^{a}	06.20^{k}
	4 g L^{-1}	9.00^{a}	00.00^{j}	0.500^{a}	06.20^{k}
GAWDA®	1 g L^{-1}	8.38^{a}	06.85^{j}	0.161^{gh}	69.86^{c}
	2 g L^{-1}	5.60^{c}	37.78^{h}	0.074^{i-k}	86.16^{a-c}
	3 g L^{-1}	2.90^{f}	67.78^{e}	0.110^{i}	79.36^{c}
	4 g L^{-1}	2.18^{fg}	75.74^{de}	0.100^{i}	81.46^{c}
Check		9.00^{a}	00.00^{j}	0.533^{a}	00.00^{k}

*Means followed by different letter(s) in the column are significantly different according to Duncan's multiple range test at $p \leq 0.05$, HQ: Hydroquinone, SA: Salicylic acid, Zn: chelated zinc 13% (EDTA), Fe: chelated iron 13% (EDTA), Mn: chelated manganese 13% (EDTA), GAWDA®: A formulated antioxidants and mineral salts

Effect of the tested green chemicals on seed germination of faba bean: Soaking faba bean seeds in water solution of Zn and Fe at 8 and 4 g L^{-1} significantly reduced the percentage of un-germinated seeds, rotted or abnormal seedlings and enhanced the percentage of normal seedlings to record 100%. The check recorded 10.0, 3.33, 10.0 and 76.67%, respectively (Table 3).

Role of green chemicals combination on the growth of *B. fabae*: The combination of HQ+ SA+Fe at concentrations of 15 mM, 3 mM and 4 g L^{-1}, respectively inhibited the linear growth and mycelial dry weight of *B. fabae* while, the inhibition percentage reached 94.4 and 88.31%, respectively (Table 4).

Role of green chemicals combination on germination of faba bean seeds: Soaking seeds in water solution of HQ+SA+Fe combination at the same concentrations showed no symptoms (un-germinated seeds, rotted or abnormal seedlings). The normal seedlings increased up to 100% when compared with check which recorded 10.0, 10.0, 6.67 and 73.33%, respectively (Table 5).

Field experiments

Role of green chemicals on scaling up the growth of faba bean plants

Disease severity: Data in Table 6 showed that when all treatments previously used in the form of seed soaking were applied on 30 days old plants followed by three times of spraying (45, 60 and 75 days old plants), no chocolate spot symptoms on the tested plants were shown except GAWDA® formulation which showed the symptoms on 45 days old plants and record (1.33, 6.67) as disease severity and incidence, respectively.

Table 3: Effect of the tested green chemicals on germination of faba bean seeds

		Type of symptoms			
Treatments	Concentration	Un-germinated seeds	Rotted seedlings	Abnormal seedlings	Normal seedlings
Hydroquinone	5 mM	3.33^{de}*	13.33^{a}	10.00^{b-d}	73.33^{ef}
	10 mM	3.33^{de}	3.33^{bc}	20.00^{a}	73.33^{ef}
	15 mM	0.00^{e}	6.67^{a-c}	6.67^{c-e}	86.67^{b-d}
	20 mM	0.00^{e}	6.67^{a-c}	10.00^{b-d}	83.33^{b-e}
Salicylic acid	1 mM	3.33^{de}	3.33^{bc}	6.67^{c-e}	86.67^{b-d}
	3 mM	20.00^{c}	3.33^{bc}	6.67^{c-e}	70.00^{f}
	5 mM	66.67^{b}	6.67^{a-c}	10.00^{b-d}	16.67^{g}
	7 mM	86.67^{a}	3.33^{bc}	3.33^{de}	6.67^{g}
Zn-EDTA	2 g L^{-1}	0.00^{e}	13.33^{a}	13.33^{a-c}	73.33^{ef}
	4 g L^{-1}	0.00^{e}	6.67^{a-c}	13.33^{a-c}	80.00^{c-f}
	6 g L^{-1}	0.00^{e}	6.67^{a-c}	0.00^{e}	93.33^{ab}
	8 g L^{-1}	0.00^{e}	0.00^{c}	0.00^{e}	100.00^{a}
Fe-EDTA	1 g L^{-1}	0.00^{e}	10.00^{ab}	13.33^{a-c}	76.67^{d-f}
	2 g L^{-1}	0.00^{e}	3.33^{bc}	16.67^{ab}	80.00^{c-f}
	3 g L^{-1}	0.00^{e}	3.33^{bc}	6.67^{c-e}	90.00^{a-c}
	4 g L^{-1}	0.00^{e}	0.00^{c}	0.00^{e}	100.00^{a}
GAWDA®	1 g L^{-1}	6.67^{de}	6.67^{a-c}	10.00^{b-d}	76.67^{d-f}
	2 g L^{-1}	3.33^{de}	6.67^{a-c}	13.33^{a-c}	76.67^{d-f}
	3 g L^{-1}	0.00^{e}	3.33^{bc}	3.33^{de}	93.33^{ab}
	4 g L^{-1}	0.00^{e}	10.00^{ab}	3.33^{de}	86.67^{b-d}
Check		10.00^{d}	3.33^{bc}	10.00^{b-d}	76.67^{d-f}

*Means followed by different letter(s) in the column are significantly different according to Duncan's multiple range test at $p \leq 0.05$, HQ: Hydroquinone, SA: Salicylic acid, Zn: Chelated zinc 13% (EDTA), Fe: Chelated Iron 13% (EDTA), GAWDA®: A formulated antioxidants and mineral salts

Table 4: Role of green chemicals combination on the growth of *B. fabae*

Treatments	Linear growth	Inhibition (%)	Dry weight	Inhibition (%)
HQ+SA	3.15[d]*	65.00[c]	0.084[ef]	84.33[ab]
HQ+Zn	4.13[c]	54.07[d]	0.163[bc]	69.41[de]
HQ+Fe	5.08[b]	43.52[e]	0.186[b]	65.06[e]
SA+Zn	4.25[c]	52.78[d]	0.140[cd]	73.72[cd]
SA+Fe	0.70[f]	92.22[a]	0.100[d-f]	81.21[a-c]
Zn+Fe	1.58[e]	82.41[b]	0.078[ef]	85.28[ab]
HQ+SA+Zn	0.58[f]	93.52[a]	0.100[d-f]	81.15[a-c]
HQ+SA+Fe	0.50[f]	94.44[a]	0.062[f]	88.31[a]
HQ+Zn+Fe	0.60[f]	93.33[a]	0.064[f]	87.97[a]
SA+Zn+Fe	0.75[f]	91.67[a]	0.082[ef]	84.62[ab]
GAWDA®	2.90[d]	67.78[c]	0.110[de]	79.36[bc]
Check	9.00[a]	00.00[f]	0.533[a]	00.00[f]

*Means followed by different letter(s) in the column are significantly different according to Duncan's multiple range test at p≤0.05, HQ: Hydroquinone, SA: Salicylic Acid, Zn: Chelated zinc 13% (EDTA), Fe: Chelated Iron 13% (EDTA), GAWDA®: A formulated antioxidants and mineral salts

Table 5: Role of green chemicals combination on the germination of faba bean seeds

	Type of symptoms			
Treatments	Un-germinated seeds	Rotted seedlings	Abnormal seedlings	Normal seedlings
HQ+SA	6.67[ab]*	6.67[ab]	13.33[a]	73.33[d]
HQ+Zn	3.33[ab]	10.00[a]	10.00[ab]	76.67[cd]
HQ+Fe	6.67[ab]	10.00[a]	10.00[ab]	73.33[d]
SA+Zn	10.00[a]	6.67[ab]	10.00[ab]	73.33[d]
SA+Fe	3.33[ab]	0.00 c	6.67[a-c]	90.00[ab]
Zn+Fe	6.67[ab]	3.33[bc]	10.00[ab]	80.00[b-d]
HQ+SA+Zn	6.67[ab]	0.00[c]	6.67[a-c]	86.67[bc]
HQ+SA+Fe	0.00[b]	0.00[c]	0.00[c]	100.00[a]
HQ+Zn+Fe	6.67[ab]	3.33[bc]	3.33[bc]	86.67[bc]
SA+Zn+Fe	6.67[ab]	3.33[bc]	10.00[ab]	80.00[b-d]
GAWDA®	0.00[b]	10.00[a]	3.33[bc]	86.67[bc]
Check	10.00[a]	10.00[a]	6.67[a-c]	73.33[d]

*Means followed by different letter(s) in the column are significantly different according to Duncan's multiple range test at p≤0.05, HQ: Hydroquinone, SA: Salicylic Acid, Zn: chelated zinc 13% (EDTA), Fe: chelated Iron 13% (EDTA), GAWDA®: a formulated antioxidants and mineral salts

Photosynthetic pigments and total phenols in leaves of faba bean treated with green chemicals combinations

Chlorophyll a: The HQ+SA+Fe combination at the same concentrationsin form of seed soaking followed by three times of spraying and 15 days interval between each spray on 30 days old plants showed a significant increase in chlorophyll a content to record 1.502, 1.529, 2.094 and 1.807 mg g^{-1} fresh weight, respectively. The check recorded 1.064, 1.084, 1.083 and 1.142 mg g^{-1} fresh weight, respectively (Table 7).

Chlorophyll b: The HQ+SA+Fe combination at the same concentrationsin form of seed soaking followed by three time of spraying and 15 days interval between each spray on 30 days old plants also showed a significant increase in chlorophyll b content to record 0.850, 1.033, 1.321 and 0.975 mg g^{-1} fresh weight, respectively. The check recorded 0.375, 0.401, 0.408 and 0.403 mg g^{-1} fresh weight, respectively (Table 8).

Carotenoids: Seed soaking in HQ+SA+Fe combination at the same concentrations followed by three times and 15 days interval between each spray on 30 days old plants, increased the

Table 6: Role of green chemicals combination and the number of applications on the natural disease severity after three times of spraying

	Disease severities					
	After 1st spray (45 days old plants)		After 2nd spray (60 days old plants)		After 3rd spray (75 days old plants)	
Treatments	Ds 1	Di 1	Ds 2	Di 2	Ds 3	Di 3
HQ+Fe						
So	10.67^{c}	13.33^{b}	8.00^{cd}	10.00^{b}	6.00^{cd}	10.00^{b}
Sp	6.67^{ef}	10.00^{bc}	4.00^{f}	10.00^{b}	4.00^{d}	10.00^{b}
So+Sp	0.00^{g}	0.00^{e}	0.00^{g}	0.00^{c}	0.00^{e}	0.00^{d}
SA+Fe						
So	8.00^{de}	10.00^{bc}	6.00^{d-f}	10.00^{b}	4.00^{d}	10.00^{b}
Sp	5.33^{f}	9.00^{bc}	4.67^{ef}	10.00^{b}	4.00^{d}	10.00^{b}
So+Sp	0.00^{g}	0.00^{e}	0.00^{g}	0.00^{c}	0.00^{e}	0.00^{d}
Zn+Fe						
So	10.67^{c}	13.33^{b}	8.00^{cd}	10.00^{b}	6.00^{cd}	10.00^{b}
Sp	9.33^{cd}	10.00^{bc}	6.67^{de}	10.00^{b}	4.00^{d}	10.00^{b}
So+Sp	0.00^{g}	0.00^{e}	0.00^{g}	0.00^{c}	0.00^{e}	0.00^{d}
HQ+SA+Zn						
So	10.00^{cd}	10.00^{bc}	8.00^{cd}	10.00^{b}	5.33^{cd}	10.00^{b}
Sp	6.67^{ef}	10.00^{bc}	4.00^{f}	10.00^{b}	4.00^{d}	10.00^{b}
So+Sp	0.00^{g}	0.00^{e}	0.00^{g}	0.00^{c}	0.00^{e}	0.00^{d}
HQ+SA+Fe						
So	5.33^{f}	10.00^{bc}	4.67^{ef}	10.00^{b}	4.00^{d}	10.00^{b}
Sp	2.00^{g}	3.33^{de}	1.33^{g}	3.33^{c}	1.33^{e}	3.33^{c}
So+Sp	0.00^{g}	0.00^{e}	0.00^{g}	0.00^{c}	0.00^{e}	0.00^{d}
GAWDA®						
So	14.00^{b}	10.00^{bc}	10.00^{c}	13.33^{b}	6.67^{c}	10.00^{b}
Sp	8.00^{de}	10.00^{bc}	6.00^{d-f}	10.00^{b}	5.33^{cd}	10.00^{b}
So+Sp	1.33^{g}	6.67^{cd}	0.00^{g}	0.00^{c}	0.00^{e}	0.00^{d}
Check						
So	18.67^{a}*	40.00^{a}	28.67^{a}	76.67^{a}	38.00^{b}	100.00^{a}
Sp	14.67^{b}	40.00^{a}	23.33^{b}	73.33^{a}	36.67^{b}	100.00^{a}
So+Sp	18.00^{a}	36.67^{a}	27.33^{a}	76.67^{a}	46.67^{a}	100.00^{a}

*Means followed by different letter(s) in the column are significantly different according to Duncan's multiple range test at $p \leq 0.05$, So: Soaking, Sp: Spraying, So+Sp: Soaking+spraying, HQ: Hydroquinone, SA: Salicylic acid, Zn: Zinc, Fe: Iron, GAWDA®: A formulated antioxidants and mineral salts, Ds: Disease severity, Di: Disease incidence

carotenoids content in the plant leaves before and after spraying to record 0.382, 0.422, 0.459 and 0.490 mg g^{-1} fresh weight, respectively. The check recorded 0.208, 0.228, 0.292 and 0.291 mg g^{-1} fresh weight, respectively (Table 9).

Total phenols: The HQ+SA+Fe combination at the same concentrations applied as soaking+spraying significantly increased total phenols content in the plant leaves to record 396.78, 522.20, 620.20 and 1063.37 mg catechol/100 g fresh water, respectively. The check recorded 161.37, 178.12, 282.49 and 208.81 mg catechol/100 g fresh water, respectively (Table 10).

Changes in proline, total protein and total phenols content: As presented in Table 11 seed soaking in the combination of HQ+SA+Fe at the same concentrations followed by three times of spraying with the water solution of the same combination significantly increased the content of proline in leaf extract, total protein and total phenols in seed extract of faba bean grown under natural exposure to *B. fabae* in the field to record 0.206 μM g^{-1} tissue, 144.1 μg mL^{-1} and 154.47 mg catechol/100 g fresh water, respectively. The check recorded 0.035 μM g^{-1} tissue, 108.8 μg mL^{-1} and 88.75 mg catechol/100 g fresh water, respectively.

Table 7: Effect of green chemicals combinations and the number of applications on chlorophyll a content in plants grown under natural infection of field conditions

Treatments	Chlorophyll a (fresh weight) (mg g^{-1})			
	30 days old plants (before spray)	45 days old plants (After 1st spray)	60 days old plants (After 2nd spray)	75 days old plants (After 3rd spray)
HQ+Fe				
So	1.364^{ef}*	1.476^{c-e}	1.792^{fg}	1.460^{gh}
Sp	1.270^{h}	1.519^{b-d}	1.810^{e-g}	1.540^{e-g}
So+Sp	1.446^{cd}	1.564^{ab}	1.892^{c-f}	1.691^{bc}
SA+Fe				
So	1.392^{de}	1.452^{de}	1.891^{c-f}	1.561^{d-f}
Sp	1.332^{fg}	1.598^{a}	1.994^{a-c}	1.566^{d-f}
So+Sp	1.502^{b}	1.598^{a}	2.037^{ab}	1.691^{bc}
Zn+Fe				
So	1.338^{e-g}	1.444^{de}	1.791^{fg}	1.403^{hi}
Sp	1.308^{gh}	1.554^{ab}	1.795^{fg}	1.564^{d-f}
So+Sp	1.509^{b}	1.569^{ab}	1.925^{b-e}	1.763^{ab}
HQ+SA+Zn				
So	1.374^{ef}	1.530^{a-c}	1.733^{g}	1.449^{gh}
Sp	1.333^{fg}	1.533^{a-c}	1.822^{e-g}	1.635^{cd}
So+Sp	1.514^{b}	1.561^{ab}	2.015^{ab}	1.686^{bc}
HQ+SA+Fe				
So	1.505^{b}	1.529^{a-c}	1.960^{b-d}	1.506^{fg}
Sp	1.486^{bc}	1.541^{a-c}	2.011^{ab}	1.603^{c-e}
So+Sp	1.644^{a}	1.597^{a}	2.094^{a}	1.807^{a}
GAWDA®				
So	1.301^{gh}	1.440^{e}	1.742^{g}	1.352^{i}
Sp	1.206^{i}	1.445^{de}	1.823^{e-g}	1.634^{cd}
So+Sp	1.528^{b}	1.574^{ab}	1.864^{d-f}	1.658^{c}
Check				
So	1.029^{k}	1.064^{f}	1.045^{h}	1.091^{j}
Sp	1.063^{jk}	1.081^{f}	1.108^{h}	1.154^{j}
So+Sp	1.100^{j}	1.108^{f}	1.097^{h}	1.150^{j}

*Means followed by different letter(s) in the column are significantly different according to Duncan's multiple range test at $p \leq 0.05$, So: Soaking, Sp: Spraying, So+Sp: Soaking+spraying, HQ: Hydroquinone, SA: Salicylic acid, Zn: Chelated zinc 13% (EDTA), Fe: Chelated iron 13% (EDTA), GAWDA®: A formulated antioxidants and mineral salts

Antioxidant activity: The antioxidant activity of the ethanolic extracts prepared from the leaves and dry seeds of faba bean plants subjected to different treatments i.e., soaking, spraying and soaking+spraying with the green chemical combinations are shown in Table 11. Data recorded variable antioxidant activities among the tested extracts. The concentration of the extract needed to decrease the initial DPPH concentration by 50% (IC 50) is measured in comparison with that of ascorbic acid (Reference compound). The lower the IC 50, the higher the antioxidant power. The strongest activity was detected in the ethanol extracts of the seeds and leaves treated with GAWDA formulation at concentration of 3 g L^{-1}, IC50% recorded 1.84 and 0.046, respectively as well as in the combination of HQ+SA+Fe at the concentrations of 15 mM, 3 mM and 4 g L^{-1} IC50% recorded 1.90 and 0.048, respectively compared with other treatments and ascorbic acid that recorded 3.47.

Micronutrient content: The treatment of HQ+SA+Zn at the same concentrations in the form of seed soaking followed by three times of spraying increased Zn content in seeds to record 17.70 mg kg^{-1}. Also, seed soaking in the combination of HQ+SA+Fe showed a significant increase in Fe seed content to record 33.91 mg kg^{-1} comparing to the check which recorded 3.40 and 6.36 mg kg^{-1}, respectively (Table 11).

Table 8: Effect of green chemicals combinations and the number of applications on chlorophyll b content in plants grown under natural infection of field conditions

Treatments	Chlorophyll b (fresh weight) (mg g^{-1}) 30 days old plants (before spray)	45 days old plants (After 1st spray)	60 days old plants (After 2nd spray)	75 days old plants (After 3rd spray)
HQ+Fe				
So	0.624^{g}*	0.714^{h}	0.945^{g}	0.709^{h-j}
Sp	0.620^{g}	0.722^{gh}	1.081^{b-g}	0.814^{e-g}
So+Sp	0.728^{b-f}	0.843^{b-d}	1.130^{b-f}	0.858^{c-f}
SA+Fe				
So	0.787^{a-c}	0.801^{c-g}	1.045^{c-g}	0.789^{f-h}
Sp	0.661^{fg}	0.823^{c-e}	1.067^{b-g}	0.817^{e-g}
So+Sp	0.752^{b-e}	0.878^{bc}	1.205^{ab}	0.882^{b-e}
Zn+Fe				
So	0.706^{c-g}	0.728^{f-h}	0.992^{fg}	0.616^{k}
Sp	0.622^{g}	0.765^{d-h}	1.014^{d-g}	0.906^{a-d}
So+Sp	0.798^{ab}	0.804^{c-f}	1.158^{b-d}	0.940^{ab}
HQ+SA+Zn				
So	0.715^{b-f}	0.772^{d-h}	1.007^{e-g}	0.737^{g-i}
Sp	0.671^{e-g}	0.720^{h}	1.046^{c-g}	0.822^{ef}
So+Sp	0.773^{a-c}	0.908^{b}	1.143^{b-e}	0.920^{a-c}
HQ+SA+Fe				
So	0.757^{b-e}	0.803^{c-f}	1.066^{b-g}	0.682^{i-k}
Sp	0.686^{d-g}	0.746^{d-h}	1.161^{bc}	0.835^{d-f}
So+Sp	0.850^{a}	1.033^{a}	1.321^{a}	0.975^{a}
GAWDA®				
So	0.702^{c-g}	0.753^{e-h}	0.950^{g}	0.640^{ijk}
Sp	0.642^{fg}	0.773^{d-h}	1.049^{c-g}	0.878^{b-e}
So+Sp	0.767^{a-d}	0.871^{bc}	1.122^{b-f}	0.853^{c-f}
Check				
So	0.398^{h}	0.359^{i}	0.405^{h}	0.370^{l}
Sp	0.366^{h}	0.415^{i}	0.393^{h}	0.401^{l}
So+Sp	0.362^{h}	0.428^{i}	0.462^{h}	0.432^{l}

*Means followed by different letter(s) in the column are significantly different according to Duncan's multiple range test at $p \leq 0.05$, So: Soaking, Sp: Spraying, So+Sp: Soaking+spraying, HQ: Hydroquinone, SA: Salicylic Acid, Zn: chelated zinc 13% (EDTA), Fe: chelated iron 13% (EDTA), GAWDA®: A formulated antioxidants and mineral salts

DISCUSSION

Chocolate spot disease caused by *Botrytis fabae* Sardina is an important disease of faba bean (*Vicia faba* L.). The disease has been reported in many parts of the world (Deverall and Wood, 1961; Khalil and Harrison, 1981; Hanounik and Hawtin, 1982; Sundheim, 1973; Liang, 1989; Akem and Bellar, 1999; Bouhassan *et al*., 2004; Tivoli *et al*., 2006; Stoddard *et al*., 2010). It attacks the above ground parts of the plant and causes losses of seed yield and seed quality (Mohamed *et al*., 1981; Morsy, 1993). The economic loss in Egypt reach 50% especially in the Northern part of Nile Delta (Hogg, 1956; Mohamed *et al*., 1981; Harrison, 1988; Koike, 1998; Morsy, 2000).

Because of successive hazards of using pesticides on public health and environment, it was necessary to search for a new approach to increase the plant resistance and avoid the use of fungicides as we can as possible. Application of different chemicals with antioxidant properties which stimulate the inherent defense mechanism of the host plant, are relatively recent directions as friendly environmental method to manage the disease incidence. Induced resistance is a promising modern approach in the plant disease control tactics. It could be induced in plants by applying chemical elicitors (Reglinski *et al*., 2001). These inducers or the green chemicals are much more environmentally sound than synthetic fungicides; also they have lower costs when compared with the toxic fungicides.

Out of these green chemicals hydroquinone, salicylic acid, chelated zinc and iron were selected to be used in this study *in vitro* and *in vivo*. Hydroquinone, SA, Zn and Fe strongly affected the hyphal growth *in vitro* as the mycelial dry weight in liquid cultures was significantly reduced

Table 9: Effect of green chemicals combinations and the number of applications on the carotenoids content in plants grown under natural infection of field conditions

Treatments	Carotenoids (fresh weight) (mg g^{-1}) 30 days old plants (before spray)	45 days old plants (After 1st spray)	60 days old plants (After 2nd spray)	75 days old plants (After 3rd spray)
HQ+Fe				
So	0.266^{cd}	0.293^{gh}	0.416^{b-d}	0.310^{g-i}
Sp	0.266^{cd}	0.366^{b-d}	0.424^{a-d}	0.354^{b-e}
So+Sp	0.316^{b}	0.396^{ab}	0.430^{a-d}	0.358^{b-e}
SA+Fe				
So	0.310^{b}	0.353^{b-e}	0.433^{a-d}	0.334^{c-g}
Sp	0.262^{cd}	0.364^{b-d}	0.435^{a-c}	0.345^{b-f}
So+Sp	0.318^{b}	0.398^{ab}	0.441^{a-c}	0.373^{b}
Zn+Fe				
So	0.296^{bc}	0.317^{d-g}	0.398^{cd}	0.326^{e-h}
Sp	0.266^{cd}	0.373^{bc}	0.414^{b-d}	0.330^{d-g}
So+Sp	0.298^{bc}	0.396^{ab}	0.452^{ab}	0.363^{b-d}
HQ+SA+Zn				
So	0.310^{b}	0.342^{c-f}	0.429^{a-d}	0.305^{g-i}
Sp	0.246^{de}	0.377^{a-c}	0.426^{a-d}	0.360^{b-e}
So+Sp	0.322^{b}	0.391^{ab}	0.440^{a-c}	0.366^{bc}
HQ+SA+Fe				
So	0.294^{bc}	0.380^{a-c}	0.428^{a-d}	0.315^{f-i}
Sp	0.261^{cd}	0.386^{a-c}	0.444^{ab}	0.329^{d-g}
So+Sp	0.382^{a}	0.422^{a}	0.459^{a}	0.490^{a}
GAWDA®				
So	0.264^{cd}	0.297^{f-h}	0.391^{d}	0.333^{c-g}
Sp	0.243^{de}	0.315^{e-g}	0.429^{a-d}	0.338^{c-g}
So+Sp	0.334^{b}	0.391^{ab}	0.445^{ab}	0.350^{b-e}
Check				
So	0.215^{e}	0.196^{j}	0.286^{e}	0.290^{i}
Sp	0.205^{e}	0.235^{ij}	0.293^{e}	0.291^{i}
So+Sp	0.204^{e}	0.252^{hi}	0.297^{e}	0.292^{hi}

Means followed by different letter(s) in the column are significantly different according to Duncan's multiple range test at $p \leq 0.05$, So: Soaking, Sp: Spraying, So+Sp: Soaking+spraying, HQ: Hydroquinone, SA: Salicylic Acid, Zn: chelated zinc 13% (EDTA), Fe: Chelated iron 13% (EDTA), GAWDA®: A formulated antioxidants and mineral salts

(Table 4). These results were in agreement with the findings of Yao and Tian (2005), Wu *et al*. (2008) and Shabana *et al*. (2008). An inverse relationship was found between the concentration of both antioxidants, micronutrient and the linear growth and mycelial dry weight of *B. fabae* when they applied individually or in combinations as shown in Table 4. These results are in agreement with Abd El-Hai *et al*. (2007b), Wu *et al*. (2008), Shabana *et al*. (2008), Ali *et al*. (2009), Aldesuquy *et al*. (2014) and Seadh and El-Metwally (2015).

Results in the present study show that pre-treatment of faba bean seeds when soaked in water solution of these green chemicals (HQ, SA, Zn and Fe) as shown in Fig. 2 and followed by three successive spraying times with the same combination significantly reduced the disease severity and the incidence of *B. fabae* found under natural infection of field conditions (Table 6). These findings are in agreement with Elwakil (2003), who reported that soaking peanut seeds in 20 mM of water solution of hydroquinone of inhibits the common seed-borne fungi of peanut, as well as Hassan *et al*. (2006), who found that foliar application of salicylic, benzoic, citric and oxalic acids significantly reduced chocolate spot severity caused by *B. fabae* and/or *B. cinerea* compared with the check treatments. Abd El-Hai *et al*. (2007a) cleared that foliar application of Fe, Zn, Mn and Ca reduced chocolate spot disease of faba bean plants, El-Hendawy *et al*. (2010) demonstrate that KH_2PO_4, $CaCl_2$, ascorbic acid, salicylic acid and oxalic acid were varied in reducing chocolate spot disease severity of faba bean.

Table 10: Effect of green chemicals combinations and the number of applications on the total phenols content in plants grown under natural infection of field conditions

Treatments	Total phenols (mg catechol /100 g fresh weight)			
	30 days old plants (before spray)	45 days old plants (After 1st spray)	60 days old plants (After 2nd spray)	75 days old plants (After 3rd spray)
HQ+Fe				
So	293.72^{d-g}	309.48^{fg}	356.52^{j}	666.66^{kl}
Sp	278.83^{gh}	356.37^{e-g}	415.23^{i}	676.68^{j-l}
So+Sp	307.55^{c-f}	376.40^{d-f}	641.94^{a}	713.74^{i-k}
SA+Fe				
So	295.86^{d-g}	365.45^{e-g}	503.32^{c-g}	633.60^{lm}
Sp	286.63^{e-h}	354.90^{e-g}	526.63^{b-e}	872.03^{cd}
So+Sp	344.31^{b}	464.63^{a-c}	546.60^{bc}	944.16^{b}
Zn+Fe				
So	327.53^{bc}	350.71^{e-g}	460.38^{g-i}	738.79^{h-j}
Sp	262.33^{h}	378.08^{d-f}	492.03^{d-h}	805.91^{e-g}
So+Sp	310.31^{c-e}	411.27^{c-e}	524.76^{b-e}	868.02^{c-e}
HQ+SA+Zn				
So	267.54^{h}	295.73^{g}	479.88^{e-h}	793.89^{f-h}
Sp	268.60^{h}	370.73^{d-f}	532.24^{b-d}	829.95^{d-f}
So+Sp	311.88^{cd}	482.00^{ab}	534.14^{b-d}	914.11^{bc}
HQ+SA+Fe				
So	329.45^{bc}	386.23^{de}	514.28^{b-f}	857.00^{c-e}
Sp	299.38^{d-g}	435.84^{b-d}	559.31^{b}	889.06^{b-d}
So+Sp	396.78^{a}	522.20^{a}	620.20^{a}	1063.37^{a}
GAWDA®				
So	284.97^{f-h}	295.26^{h}	446.38^{hi}	587.52^{m}
Sp	267.08^{h}	345.75^{e-g}	456.80^{g-i}	678.68^{j-l}
So+Sp	293.92^{d-g}	399.98^{c-e}	472.03^{f-h}	755.82^{g-i}
Check				
So	156.52^{j}	211.14^{i}	213.91^{l}	216.67^{no}
Sp	154.07^{j}	153.23^{i}	278.49^{k}	169.48^{o}
So+Sp	173.54^{j}	170.01^{i}	355.07^{j}	240.30^{n}

Means followed by different letter(s) in the column are significantly different according to Duncan's multiple range test at $p \leq 0.05$, So: Soaking, Sp: Spraying, So+Sp: Soaking+spraying, HQ: Hydroquinone, SA: Salicylic Acid, Zn: chelated zinc 13% (EDTA), Fe: Chelated iron 13% (EDTA), GAWDA®: A formulated antioxidants and mineral salts

The photosynthetic pigments in plants treated with these antioxidants and micronutrients in all tested combinations including chlorophyll a, b and carotenoids significantly increased with variable percentages as shown in Table 7-9. These results are compatible with those of Rahhal (1993), who found that chlorophyll content was significantly increased when Zn and Mn at 8 g L^{-1} were used, El-Tantawy and Nawar (2013) cleared that Fe foliar application enhanced total chlorophyll and carotenoids. Gehad and Amina (2014) reported that the content of chlorophyll a, b and total chlorophyll significantly increased in faba bean plants in response to the application of salicylic acid.

A significant accumulation of total phenols in faba bean tissues as a result of *B. fabae* infection was increased in the plants treated with both antioxidants and micronutrients combinations. These findings are in agreement with Mahmoud *et al*. (2011) and El-Hendawy *et al*. (2010). However, Kruger *et al*. (2002) emphasized that phenols are the early stage of response in the plants to overcome the invasion of different pathogens.

It was also found in this research that proline content in faba bean plants was increased after *B. fabae* infection. In the same time, application of these green chemicals (HQ and SA) and (Zn and Fe) combinations accelerated the accumulation of proline in leaves tissue as shown in Table 11. These findings are confirmed by Ali *et al*. (2007), who noticed an increase in proline content in ginseng root treated with SA; Faheed *et al*. (2005) found that low concentration of salicylic acid stimulates the proline accumulation in tomato plants, as well as Aldesuquy *et al*. (2014) who found that application of shikimic and salicylic acid increases proline content in faba bean plants infected with *B. fabae*.

Table 11: Effect of green chemicals combinations and the number of applications on proline, total protein, total phenols and antioxidant activity in faba bean leaves and seeds under field conditions

Treatments	Proline (μM g^{-1} tissue)		Total protein (μg mL^{-1})	Total phenols (mg catechol/100 g fresh water)	Antioxidant activity IC 50%		Micronutrient content (mg kg^{-1} dry weight)	
	Before spray	After spray			Seeds	Leaves	Zn	Fe
HQ+Fe								
So	0.113^{cd}	0.134^{cd}	133.3^{fg}	118.81^{f}	4.78^{d}	0.120^{d}	4.70^{n}	11.87^{q}
Sp	0.097^{d}	0.115^{d}	131.5^{g}	142.52^{a-d}	3.76^{i}	0.094^{i}	7.07^{k}	12.14^{p}
So+sp	0.174^{a}	0.205^{a}	128.1^{hi}	141.38^{a-d}	2.93^{m}	0.073^{m}	12.02^{g}	28.76^{b}
SA+Fe								
So	0.150^{b}	0.177^{b}	133.7^{e-g}	126.22^{ef}	3.31^{k}	0.083^{k}	7.30^{j}	15.61^{m}
Sp	0.167^{ab}	0.197^{ab}	136.6^{de}	149.86^{ab}	3.56^{j}	0.089^{j}	10.40^{h}	22.33^{h}
So+sp	0.174^{a}	0.205^{a}	140.9^{bc}	147.39^{a-c}	2.68^{n}	0.067^{n}	13.11^{f}	27.39^{c}
Zn+Fe								
So	0.102^{d}	0.120^{d}	121.0^{k}	129.69^{d-f}	5.60^{a}	0.140^{a}	3.85^{p}	14.36^{o}
Sp	0.161^{ab}	0.190^{ab}	136.3^{d-f}	138.71^{b-e}	4.46^{e}	0.112^{e}	6.99^{l}	21.56^{i}
So+sp	0.174^{a}	0.205^{a}	143.4^{ab}	151.87^{ab}	2.71^{n}	0.068^{n}	13.87^{c}	22.56^{g}
HQ+SA+Zn								
So	0.124^{c}	0.146^{c}	133.2^{fg}	133.17^{c-f}	4.32^{f}	0.108^{f}	4.26^{o}	14.56^{n}
Sp	0.162^{ab}	0.191^{ab}	131.2^{gh}	146.86$_{a-c}$	4.01^{g}	0.101^{g}	8.66^{i}	22.62^{f}
So+sp	0.174^{a}	0.206^{a}	136.6^{de}	150.13^{ab}	3.83^{hi}	0.096^{hi}	17.70^{a}	25.43^{e}
HQ+SA+Fe								
So	0.173^{a}	0.205^{a}	139.3^{cd}	143.59^{a-d}	2.01^{op}	0.050^{op}	7.31^{j}	19.13^{k}
Sp	0.163^{ab}	0.193^{ab}	137.6^{d}	144.19^{a-d}	2.03^{o}	0.051^{o}	13.21^{e}	17.56^{l}
So+sp	0.174^{a}	0.206^{a}	144.1^{a}	154.47^{a}	1.90^{pq}	0.048^{pq}	16.32^{b}	33.91^{a}
GAWDA®								
So	0.111^{cd}	0.131^{cd}	124.6^{j}	133.97^{c-e}	3.40^{k}	0.085^{k}	3.53^{q}	4.16^{u}
Sp	0.113^{cd}	0.133^{cd}	126.8^{ij}	125.69^{ef}	3.07^{l}	0.077^{l}	5.97^{m}	21.39^{j}
So+sp	0.153^{b}	0.180^{b}	138.8^{cd}	142.58^{a-d}	1.84^{q}	0.046^{q}	13.44^{d}	25.91^{d}
Check								
So	0.022^{e}	0.026^{e}	103.6^{n}	88.75^{g}	3.91^{gh}	0.098^{gh}	3.45^{r}	6.58^{r}
Sp	0.038^{e}	0.045^{e}	109.1^{m}	88.75^{g}	5.35^{b}	0.134^{b}	3.25^{s}	6.00^{t}
So+sp	0.028^{e}	0.033^{e}	113.6^{l}	88.75^{g}	5.17^{c}	0.129^{e}	3.50^{q}	6.50^{s}

Means followed by different letter(s) in the column are significantly different according to Duncan's multiple range test at $p \leq 0.05$, So: Soaking, Sp: Spraying, So+Sp: Soaking+spraying, HQ: Hydroquinone, SA: Salicylic acid, Zn: Chelated zinc 13% (EDTA), Fe: Chelated iron 13% (EDTA), GAWDA®: A formulated antioxidants and mineral salts

In addition, a significant increase in total protein content in all faba bean seeds treated with the selected combination (HQ, SA, Zn and Fe). These findings are in agreement with Sarangthem and Singh (2003), who also found that the protein content, increased in *Phaseolus vulgaris* by foliar application of salicylic acid at 0.1%, Abd El-Monem *et al*. (2009), who found that foliar application of Zn at 100 ppm significantly increased protein content in faba bean seeds, as well as Gehad and Amina (2014), who found that foliar application of SA at concentration of 10^{-4} M significantly increased crude protein content in faba bean seeds.

The antioxidant activity found in the ethanolic extracts prepared from the leaves or dry seeds of faba bean plants that subjected to different treatments included soaking, spraying or soaking+spraying with antioxidants and micronutrients as well as combinations are reported in Table 11 and showed that there are variable antioxidant activities among the tested extracts.

The strongest antioxidant activity was detected in the ethanol extracts of the seeds or leaves treated with GAWDA formulation as well as the combination of HQ+SA+Fe. These findings are in agreement with a number of studies which showed that the contents of total phenols were positively correlated to the antioxidant activity in plant extracts (Osman *et al*., 2009; Doss *et al*, 2010; Isabelle *et al*., 2010; Hossain and Rahman, 2011).

The application of green chemicals by seed soaking, foliar spraying or interaction between them significantly increased Zn and Fe concentrations in seeds as shown in Table 11. These findings are in harmony with Abou El-Yazied (2011), who indicated that foliar application of SA and/or chelated zinc increased Zn concentrations in sweet pepper leaves, as well as Elham *et al*. (2014) found that foliar application of chelated iron increased iron concentration in spotted bean leaves.

CONCLUSION

It is recommended to use the combination of hydroquinone, salicylic acid and chelated iron (HQ+SA+Fe) for treating faba bean seeds at concentration of 15 mM, 3 mM and 4 g L^{-1} before sowing followed by three times of spraying applications on the growing plants of 30 days old to overcome the disease severity caused by *B. fabae* and increase the quality of the crop.

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