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CONTROL OF SUNFLOWER POWDERY MILDEW USING RESISTANCE INDUCERS

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ABSTRACT

Values of area under powdery mildew progress curve values (AUPMPC) were significantly affected with response of sunflower cultivars. Giza.١ cv. was the most susceptible given a value of ٨٩٧ AUPMPC followed by cv. Miak ٦٩٧ AUPMPC value and cv.Giza.١٦١ was the least ٤٩٨ value AUPMPC. Thus, sunflower cv. Giza.١ was used throughout this study to induce resistance.

Powdery mildew was significantly affected by the type, concentration and application methods of resistance inducers used. Seed soaking and foliar spraying of resistance inducers resulted in resistant sunflower plants against powdery mildew. Efficiency of seed soaking and foliar spraying with benzothiadiazole (BTH) was most effective than other inducers. Since increasing concentration enhanced resistance of sunflower plants. The highest protection in sunflower plants obtained with benzothiadiazole at ٢٠٠ppm concentration (٩٤٪ and ٩٠.٨٪ protection by seed soaking and foliar spraying respectively). Resistance inducers gave various resistance levels according to application methods.

INTRODUCTION

The Sunflower (*Helianthus annuus* L.) is one of the most important oil crops word-wide with cultivated area over ٢٢ million hectares an production of ٢٦ million tonnes (Skoric *et al.,* ٢٠٠٧). Powdery mildew caused by the fungus (*Golovinomyces chicoracearum* formerly *Erysiphe chicoracearum*) is a widely distributed pathogen of cultivated sunflower, frequently causes economic loss in sunflower plants (Saliman *et al.,*١٩٨٢).

The incidence and severity of the Powdery mildew vary from one year to another depending on the growing season and prevailing enviromental conditions (Grau, ١٩٨٤).

Many studies have been published concerning the ability of plants to develop resistance to biotic (bacteria, fungi and viruses), physical and chemical agents (Tosi *et al.,* ١٩٩٩). Biochemical studies have shown that systemic acquired resistance (SAR) responses are correlated with the accumulation of some pathogenesis related (PR) proteins including ١,٣-glucanases, chitinases, cysteine rich proteins related to thaumatin and the PR-١ proteins (Cohen *et al.,* ١٩٩٤ and Kessmann *et al*., ١٩٩٤). The synthetic chemical benzo (١,٢,٣) thiadiazole-٧-carbothioic acid S-methyl ester (BTH) was also demonstrated to be a potent SAR activator (□Lawton *et al.* ١٩٩٦) that provides protection in the field against some diseases in several crops. Thus, BTH seems to be the proper compounds for practical agronomic use (Hafez *et al.* ٢٠٠٤). Geetha and Shetty (٢٠٠٢) found that chemical induction of resistance in pearl millet against downy mildew disease (*Sclerospora graminicola*) is possible by treating seeds of highly susceptible cultivars with the resistance activator benzothiadiazole (BTH) (CGA ٢٤٥٧٠٤), calcium chloride ($CaCl_{٢}$) and hydrogen peroxide ($H_{٢}O_{٢}$). BTH in ٠.٧٥٪, ٩٠ mM CaCl٢ and ١.٠ mM $H_{٢}O_{٢}$ were effective in managing the disease giving ٧٨٪, ٦٦٪ and ٥٩٪ protection, respectively.

The treatment of plants with certain synthetic chemical agents can also induce the SAR response (Brissert *et al.,* ٢٠٠٠ and Ismail *et al*., ٢٠٠٦). Many compounds provide good protection against fungal and bacterial pathogens of many crops in greenhouse as well as in field conditions. The synthetic chemicals Benzothiadiazole (BTH) and ٢,٦-dichloroisonicotinic acid (INA) have been used for induction of SAR in wheat, bean, soybean and barley against fungal and bacterial pathogens (Dann & Deverall, ١٩٩٥). Kogel *et al.*,(١٩٩٤) reported that INA (CGA ٤١٣٩٦) induced resistance in susceptible barley (*Hordeum vulgare* L.) cultivars to powdery mildew (*Erysiphe graminis* f.sp *hordei*). BTH, which was developed by Ciba-Geigy Cor. (Novartis) (Basel, Switzerland), has been tested under the experimental name CGA ٢٤٥٧٠٤ (benzo (١,٢,٣) thiadiazole-٧-carbothioic acid S methyl ester), triggers SAR in several crops [Ruess *et al*., ١٩٩٦].

In the present study the effect of BTH and some antioxidant compounds (ascorbic acid (AA), salicylic acid (SA), citric acid (CA) and propylgallate (PG), when applied as seed soaking and as foliar spray for protecting sunflower plants against powdery mildew were investigated.

MATERIAL AND METHODS

Source of inoculum:

Infected leaves with dense powdery mildew were collected from natural infected sunflower plants and used to infect the original plants cultivated in greenhouse (Fig.١).

Fig.١: Symptoms on sunflower plants cv. Giza. ١ infected by *Golovinomyces chicoracearum* (right infected and left control).

Pathogenicity tests:

Apparent healthy seeds of ٣ sunflower cultivars (Giza١, Giza ١٦١ and Miak) were surface disinfected by immersing them in ١.٠٪ sodium hypochlorite for one min, rinsed two times with sterile distilled water. Five seeds of sunflower were sown in each pot (٢٥cm) diameter with clay sandy soil (١:١ w: w) disinfected by ٥٪ formalin then irrigated and left up to three weeks. Twenty one days after sowing, the growing plants were artificially inoculated by dusting with conidia from powdery mildewed sunflower leaves using small brush and the inoculated plants kept in a glass box for ٢ days to avoid contamination (Alvaro, *et al.*, ٢٠٠٨). Five pots each contained ٥ plants, were inoculated for each cultivar. The experiment was repeated twice. The inoculated leaves were examined for the development of powdery mildew symptoms (Álvaro et al., ٢٠٠٨).

powdery mildew assessment:

As no standard scale was available for scoring the severity of powdery mildew on sunflower. The percent leaf area infected was recorded on the basis of visual observations. The visual scores showed a correlation of ٠-٥, where ٠ = no infection, ١ =١-٥٪ leaf area infected, ٢ = ٦-٣٠٪ leaf area infected, ٣ = ٣١-٦٥٪ leaf area infected, ٤ = ٦٦-٩٠٪ leaf area infected and ٥ = ٩١- ١٠٠٪ leaf area infected (Reddy *et al.*,١٩٩٤).

Powdery mildew severity was assayed ٤ times at ١٠ days interval started from ١٠ days after infection and area under powdery mildew progress curve values (AUPMPC) were calculated according to the method of Chiha *et al.*, (١٩٩٧) following this equation

$$AUPMPC = D\ (١/٢\ (Y_{١} + Y_{k}) + (Y_{٢} + Y_{٣} + \ldots\ldots Y_{k-١})$$

Where D= Time interval;$Y_{١}$= First disease score; Y_{k}= Last disease score; $Y_{٢}$,$Y_{٣}$ = Intermediate disease scores.

Induction of resistance in sunflower plants:

Unless otherwise stated, trials were conducted in the greenhouse using growing sunflower pots No.٢٥ (٥ seeds per each) and five pots were used per treatment and each experiment repeated twice.

Control of sunflower powdery mildew

Four antioxidant compounds; ascorbic acid (AA), citric acid (CA), propylgallate (PG), and salicylic acid (SA) were used. Beside these compounds the analoge of SA pathaway compound which named benzothiodiazole (acibenzolar-S-methyl) that used as resistance inducer under commercial name Bion, in Europe, or actiguard, in USA, was tested. Certain chemicals were dissolved in distilled water individually to obtain solutions with ٢ concentrations (١٠٠ and ٢٠٠ ppm) as reported by Ismail *et al.* (٢٠٠٦).

Effect of seed soaking:

Apparent healthy seeds of sunflower cv. Giza ١ were surface disinfected by immersing in ١٪ sodium hypochlorite for one min then washed thoroughly two times with sterile distilled water and soaked in different certain test solutions with various concentrations for ١٢ h. Control seeds were soaked in distilled water. After that, treated seeds were sowed in pots contained sterilized soil as mentioned above.

Twenty one day-old plants were subjected for inoculation similarly as described in pathogenicity test. Five pots (٥ plants per each) were used for each treatment and the experiment was repeated twice. Values of AUPMPC were assayed as described above.

Effect of foliar spraying:

Apparent healthy seeds of sunflower cv. Giza ١ after they have been surface sterilized as described previously. The tested inducers were used to induce resistance against powdery mildew under greenhouse conditions. Twenty one days- old sunflower plants cv. Giza.١ were sprayed with certain inducers at two concentrations (١٠٠ and ٢٠٠ppm). Five pots (٥ seeds per each) were used for each treatment. One day after spraying, sunflower plants were inoculated by powdery mildew as above described then AUPMPC values were calculated as described before.

Statistical analysis:

Standard deviation (SD) was calculated according to the methods described by Gomez and Gomez (١٩٨٤) to compare the variances between treatments.

RESULTS

Obtained data showed that all tested sunflower cultivars were susceptible to powdery mildew infection with varied degrees (Table ١). Plants of cv. Giza.١ were the most susceptible (٨٩٧ AUPMPC) followed by miak (٦٩٧ AUPMPC) and giza ١٦١ was the lowest ٤٩٨ AUPMPC value.

Table ١: Area under powdery mildew progress curve (AUPMPC) values to sunflower cultivars.

Sunflower cultivars	AUPMPC
Giza ١	٨٩٧±١٦
Giza.١٦١	٤٩٨±١٤
Miak	٦٩٧±١٢

Induction of resistance in sunflower plants against powdery mildew :

١- Using seed soaking in resistance inducers:

All resistance inducers tested caused significant reduction in AUPMPC values when sunflower seeds were soaked in a solution of these tested inducers before planting (Table ٢).

Table ٢: Area under powdery mildew progress curve values (AUPMPC) caused by *Golovinomyces chicoracearum* to sunflower plants cv. Giza ١ grown from soaked seeds by resistance inducers for ١٢h before planting.

Treatment	Conc.	AUPMPC	% Protection
AA♦	١٠٠	٣٤٨±١٢♦♦	٦٣.٢
	٢٠٠	٢٩٨±٨.٠	٦٨.٥
CA	١٠٠	٢٩٨±١٨	٦٨.٥
	٢٠٠	١٩٨±١٤	٧٩.١
BTH	١٠٠	٩٨±٨.٠	٨٩.٦
	٢٠٠	٤٨±٤.٠	٩٤.٩
PG	١٠٠	١٩٨±١١	٧٩.١
	٢٠٠	٩٨±٦.٠	٨٩.٦
SA	١٠٠	٢٤٨±١٦	٧٣.٨
	٢٠٠	١٤٨±١٢	٨٤.٣
Water (Control)		٩٤٧±١٤	٠.٠

♦ = AA = Ascorbic acid, BTH (benzothiadiazole), CA= citric acid, PG= propylgallate and SA = salicylic acid.

♦♦ = Data are means of AUPMPC values through ٢ experiments ± standard deviation (SD)

Control of sunflower powdery mildew

Increasing resistance inducers concentration significantly increased resistance of sunflower against powdery mildew infection. The highest protection (٩٤.٦٪ protection) was achieved by ٢٠٠ppm of BTH followed by ١٠٠ppm of BTH and ٢٠٠ppm of PG (٨٩.٦٪ protection). The least protection was obtained (٦٣.٢٪ protection) was obtained by ١٠٠ppm of AA.

٢- Using foliar spraying with resistance inducers:

Foliar spraying of sunflower plants with resistance inducers resulted in resistant plants against powdery mildew (Table ٣). However, BTH was the most effective to reduce AUPMPC, since it caused ٩٠.٨ % protection at ٢٠٠ ppm followed by SA which caused ٨٨.٤٪ protection at ٢٠٠ppm concentration. The least protection (٥٨.٩ % protection) was attained using ١٠٠ppm CA.

Table ٣:AUPMPC values caused by *Golovinomyces chicoracearum* to sunflower plants cv. Giza ١ as affected by foliar spray with resistance inducers

Treatment	Conc.	AUPMC	% Protection
AA♦	١٠٠	٢٩٨±١٨♦♦	٦٤.٨
	٢٠٠	١٤٨±١٥	٨٢.٥
CA	١٠٠	٣٤٨±٢٢	٥٨.٩
	٢٠٠	٢٤٨±٢٠	٧٠.٧
BTH	١٠٠	٢٩٨±١٢	٧٠.٧
	٢٠٠	٧٨±٦.٠	٩٠.٨
PG	١٠٠	٢٧٨±١٢	٦٧.٢
	٢٠٠	٢٥٧±١٠	٦٩.٦
SA	١٠٠	١٩٨±١٠	٧٦.٦
	٢٠٠	٩٨±٨.٠	٨٨.٤
Water (Control)		٨٤٨±٢٥	٠.٠

♦ = AA = Ascorbic acid, BTH (benzothiadiazole), CA= citric acid, PG= propylgallate and SA = salicylic acid.

♦♦ = Data are means of through ٢ experiments AUPMPC ± standard deviation (SD)

DISCUSSION

Powdery mildew (PM) appears to be an emerging disease in sunflower. Little information is available world-wide on potential yield losses or control measures – yield and varietal trials are needed to assess the effect of high levels of PM and investigate possible control measures (Gulya *et al.,* ١٩٩٤). In the present investigation, all sunflower cultivars were infected with the powdery mildew disease with different degree of infection where Giza.١ showed high susceptible (٨٩٧ AUPMPC value) followed by cv. Miak (٦٩٧ AUPMPC value) and Giza.١٦١ (٤٩٨ AUPMPC value) was the least susceptible. These results were confirmed with those reported by several researchers showing that plant cultivars vary in their reported to PM infection (Yurina, *et al.,* ١٩٩٣**;** Reuveni *et al.,* ١٩٩٥ Abd-El-Kareem, *et al.,* ٢٠٠١and Matsuda and Takamatsu ٢٠٠٣).

Induced resistance is a physiological "state of enhanced defensive capacity" elicited by specific environmental stimuli, whereby the plant's innate defenses are potentiated against subsequent biotic challenges (van Loon *et al*., ١٩٩٨). This enhanced state of resistance is effective against a broad range of pathogens and parasites, including fungi, bacteria, viruses, nematodes, parasitic plants, and even insect herbivores (Stenzel, *et al.,* ١٩٨٥; Lucas, ١٩٩٩; Cools and Ishii ٢٠٠٢; McCreight, ٢٠٠٦; and Faoro *et al.*, ٢٠٠٧).

The present study showed that application of resistance inducers (AA, CA, BTH, PG and SA) as seed soaking or foliar spraying significantly induced resistance in sunflower plants against powdery mildew infection. Data are consistent with those reported by several researchers using these compounds against several plant diseases caused by various pathogens (Galal and Abdou, ١٩٩٦; Stadnik and Buchenauer, ٢٠٠٠; Yu and Muehlbauer, ٢٠٠١; Cools and Ishii ٢٠٠٢; Van Loon and Pieterse, ٢٠٠٢; Johnson *et al.,* ٢٠٠٣; Faize, *et al*., ٢٠٠٤; Shaat and Galal, ٢٠٠٤; Sarwar *et al*., ٢٠٠٥; Nafie and Mazen ٢٠٠٨; El-Samawaty and Galal, ٢٠٠٩; Sparla, *et al*., ٢٠٠٩; Vimala, *et al.,* ٢٠٠٩; Skłodowska *et al*., ٢٠١٠). It worth mention that the efficiency of tested resistance inducers varied with concentrations and with application methods. Similarly as previously reported (Ismail *et* *al.,* ٢٠٠٦). Generally, BTH gave an stable effect to reduce AUPMPC values when it applied as seed soaking (caused ٩٤.٩٪ protection) or as foliar spraying (caused ٩٠.٨٪ protection) at ٢٠٠ppm concentration.

Systemic acquired resistance is an important component of the disease resistance of plants. In this study, a novel synthetic chemical, benzo (١,٢,٣) thiadiazole-٧-carbothioic acid S-methyl ester (BTH), was shown to induce acquired resistance in sunflower plants and gave a highest protection. This results agree with those reported by Gorlach *et al.,* (١٩٩٦) who assessment that BTH protected wheat also systemically against powdery mildew infection by affecting multiple steps in the life cycle of the pathogen.

However, the availability of chemical inducers of SAR, such as BTH, that make the applied use of induced resistance in conventional agriculture. Our results indicated that, all tested inducer caused reduction in the infection of powdery mildew on sunflower plants. Application of AA and SA as foliar spraying was more effective in this regard than seed soaking. Meanwhile, both PG and CA were most effective to induce resistance when they applied as seed soaking than foliar spraying. The mechanism of resistance has been well established in laboratory studies. BTH and INA are by far the best studied inducers available; both are considered functional analogs of salicylic acid, and elicit a systemic form of induced resistance across a broad range of plant-pathogen interactions (Ward *et al*., ١٩٩١; Friedrich *et al.,* ١٩٩٦; Lawton *al., et* ١٩٩٦; Mauch – Mani and Metraux, ١٩٩٨; Maleck *et al*., ٢٠٠٠). Plants exposed to high concentrations of BTH or INA may also exhibit signs of phytotoxicity, but this effect seems to be independent of the induced resistance response (Friedrich *et al.,* ١٩٩٦; Sticher *et al.,*١٩٩٧ and Hammerschmidt, ١٩٩٩).

It could be concluded from this study that BTH and antioxidants (AA, CA, SA and PG) at ٢٠٠ ppm, might be used as an alternative materials for disease management of powdery mildew of sunflower.

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مكافحة البياض الدقيقي لعباد الشمس باستخدام محفزات المقاومة

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اختلفت معنويا قيم منحنى تطور البياض الدقيقى باختلاف أصناف عباد الشمس المختبرة حيث كان الصنف جيزة ١ أكثر الأصناف قابلية للإصابة حيث أعطى (قيمة ٨٩٧ لمنحنى تطور البياض الدقيقى) تلاه الصنف مياك (٦٩٧) وأظهر الصنف جيزة ١٦١ أقل قابلية للإصابة (٤٩٨) ولذلك تم استخدام الصنف جيزة ١ لدراسة تحفيز المقاومة.

وتأثر البياض الدقيقى تأثيرا معنويا بنوع وتركيز وطريقة استخدام محفزات المقاومة المختبرة.

أدى غمر البذور أو رش نباتات عباد الشمس بمحفزات المقاومة إلى تحفيز المقاومة ضد الإصابة بالبياض الدقيقى وكان المركب بنزوثيوديازول هو الأكثر فاعلية عن باقي المحفزات المختبرة. أدت زيادة تركيز المحفز إلى زيادة مقاومة عباد الشمس وسجلت أعلى وقاية لنباتات عباد الشمس باستخدام ٢٠٠ جزء فى المليون من الـ بنزوثيوديازول (٩٠.٨% إلي ٩٤%) حماية في حالة غمر البذور أو رش النباتات على التوالي وأعطت محفزات المقاومة الأخرى اختلافا في مستوى المقاومة وفقا لطريقة الاستخدام.