Journal

J. Biol. Chem. Environ. Sci., 2010, Vol. 5(1):505-516
www.acepsag.org

INDUCED RESISTANCE IN SWEET PEPPER PLANTS AGAINST POWDERY MILDEW DISEASE USING SOME CHEMICAL INDUCERS AND MICROELEMENTS UNDER GREENHOUSE AND FIELD CONDITIONS

SAHAR, A.M. ZAYAN and TOMADER, G. ABDEL RAHMAN.

Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.

ABSTRACT

Powdery mildew of sweet pepper is devastating disease of peppers and other crops under greenhouse and field conditions in Egypt. This study targeted to find out the effect of some chemical inducers i.e. ascorbic acid (AA), Dichloro- isonicotinic acid (INA), ethylene diamine tetra acetic acid (EDTA), and certain microelements i.e. Iron (Fe), Zinc (Zn) and Manganese (Mn), as foliar sprays, singly and in combination were evaluated as an alternative control substance against sweet pepper powdery mildew under both greenhouse and field conditions in 2007 and 2008 successive growing seasons. Results under greenhouse conditions indicated that the highest reduction in disease severity % was obtained by INA and AA followed by EDTA respectively. As well as Fe, Zn followed by Mn respectively. In particular, combined treatments between (INA 100 mM + AA 2.0 g/L) and (Fe + Zn + Mn), had much better record as the lowest average of disease severity % respectively. Similar trend was observed under field conditions. This result suggested that an applicable, safe, cost effective and fungicide alternative might be used for controlling sweet pepper powdery mildew.

Keywords: *Leveillula taurica,* sweet pepper, powdery mildew, chemical inducers, microelements.

INTRODUCTION

Sweet pepper (*Capsicum annuum* L.) is a nutritious vegetable that is gaining its popularity throughout the world. It is considered one of the most important vegetable cash crops in Egypt for both local consumption and export purposes. Powdery mildew caused by *Leveillula taurica* (Lev.) Arn. is a devastating disease of sweet pepper under protected cultivation and open field conditions (Palti, 1988). Pepper powdery mildew does not infect the fruit or stems but can quickly destroy unprotected leaves and eventually the entire pepper crop. Furthermore, increasing resistance to fungicides by pathogens has greatly increased pre- and post harvest losses (Braun, 1987 and Correll *et al*., 1987). Premature leaf shed caused by the disease strongly affects production and makes fruits unmarketable. Whereas most powdery mildews are ectoparasites, the mycelium from *L. taurica* grows intercellularly within the host tissues limiting the chemical control efficiency. Very little literature is available concerning pepper resistance to *L. taurica*. (Ullasa *et al*., 1981 & Desphande *et al*., 1985). Pepper growers need to follow an intensive disease prevention plan relay mainly on chemical control because it is very important that powdery mildew never gets out of hand. Therefore, and for aforementioned facts there has been an urgent need to develop the substitutes or alternatives for fungicides, which are eco-environmentally safe materials. Some chemicals were reported as resistance inducers against plant diseases. In this regard, the content of ascorbic acid in plant tissues has been associated with resistance to some diseases. Nofal and Hagag (2006) demonstrated that foliar applications of mango with ascorbic acid solution were effective in reducing powdery mildew disease. Moreover, EDTA (Ethylene diamine tetra acetic acid) was reported to induce resistance in broad been against rust disease. While Dicloro-isonicotinic acid (INA) is efficient agent that protects several crops against viruses, bacteria, and fungal infection (Nielsen *et al*., 1994). A number of studies have shown the capability of certain mineral nutrients to induce the activation of specific defense mechanisms developed by plants against the attack of pathogens. They reported that the mineral nutritional status of plants with micronutrients (Fe, Zn and Mn) is a crucial component in whole resistance mechanism of plants against pathogens and should be considered in all type of management systems (Graham, 1983; Sindha, 1989; Graham & Webb, 1991; Maharana *et al*., 1993 and Reuveni *et al*., 2004).The main goal of the present investigation was to study the efficacy of some chemicals and microelements as resistance inducers against sweet pepper powdery mildew disease severity under greenhouse and field conditions. Looking for safe alternatives materials to manage such disease, avoiding human hazards and environment pollution.

MATERIALS AND METHODS

1. Greenhouse Experiments:

1.1. Inoculum Preparation:

Infected leaves samples, were showing typical powdery mildews symptoms obtained from diseased pepper plants during autumn of 2006 season. Disease plants of highly pepper susceptible cultivars (California Wonder) was inoculated with conidia obtained from the same host in isolated polyethylene cages and maintained as source of inoculum for such study under greenhouse conditions.

Effect of Chemical Inducers:

This experiment was performed under greenhouse at El-Haram (Giza). Four pots (15 cm) contained soil consisted from sandy soil and peat moss (3:1 v/v) mixture were used for each tested concentration of chemical inducers. Therefore, 40 plants of pepper California Wonder cv. were sprayed with each tested concentration of both individual chemical inducer and in combined (Ascorbic acid (AA) + Dichloro-isonicotinic acid (INA)), then kept under greenhouse conditions for 45 days. In addition, control (sprayed only with tap water) with the same number of pots was included. Seedlings were divided into two treatments; the first served as individual treatments, for different concentrations of chemical inducers, *i.e.* AA at 1.0, 2.0 and 3.0 g/l; EDTA at 0.25, 0.50 and 1.0 g/l and INA at 50.0, 100.0 and 150.0 mM. The second served as in combined treatments, for different concentrations of Ascorbic acid, *i.e.* 1.0, 2.0 and 3.0 g/l combined with each of 50.0, 100.0 and 150.0 mM of INA (pepper plants were sprayed first with AA and then sprayed with INA after 7 days). All chemical inducers were applied as foliar spraying after 4 days of inoculation with conidia of *L. taurica* by using dusting method (Palti, 1988) to study their effects against powdery mildew of sweet pepper plants. Traditional agricultural practices, *i.e.* irrigation and fertilization were carried out as needed.

Effect of microelements:

The effect of certain microelements on the disease severity of powdery mildew on sweet pepper plants were performed following the previous design as mentioned above. The microelements used were as follows: Iron chelate (Fe- EDTA 6%) at 2.5g/l, Zinc chelate (Zn-EDTA 12%) at 1.0g/l and Manganese chelate (Mn-EDTA 12%) at 1.0g/l. Inoculated sweet pepper seedlings with powdery mildew conidia, were done after 4 days of treatment with Fe, Mn and Zn singly or in combination together [Fe + Zn, Fe + Mn, Zn + Mn and Fe + Zn + Mn].

Disease assessment:

Powdery mildew disease severity was Evaluated according to a rating scale, adopted modified from Reuveni *et al*., (1998). Ten plant leaves were weekly selected randomly for each particular treatment and were classified to 0-4 Plants of each particular treatment were classified into five categories as follows:

0 = no powdery mildew colonies observed on tissue.

1 = 1-10% of the area of selected leaves was covered with sporulating colony of mildew.

2 = 11-25% of the area of selected leaves was covered with sporulating colony of mildew.

3 = 26-50% of the area of selected leaves was covered with sporulating colony of mildew.

4 = > 50% of the area of selected leaves was covered with sporulating colony of mildew.

The percentage of disease severity for each particular treatment was calculated by using the following formula:

$$P= \text{sum of } [(n \times v) / Zn] \times 100$$

Where: P= percentage of disease severity, n= number of leaves in each category, v= numerical value of each category, Z= numerical value of the highest category and N= total number of leaves in sample.

2. Field Experiments:

The promising treatments, in pot experiments were applied under field conditions to evaluate their efficiency against powdery mildew diseases in the field in addition to its effect on sweet pepper fruit yield. Field experiments were carried out at Ebshoway (El-Fayoum Governorate) through out 2007 and 2008 of sweet pepper growing (Nilli seasons) in commercial pepper fields of highly susceptible cultivars (California Wonder). The trail design was randomized complete blocks with four replications per treatment. Plot area was 1/400 of feddan (for each particular treatment) was separated at each end by 0.5 m alleys. Cultural practices were done as usual under natural infection. Two concentrations of each chemical inducer, *i.e.* AA at 2.0 and 3.0 g/l; EDTA at 0.50 and 1.0 g/l and INA at 50.0 and 100.0 mM, in addition to combined treatments between ascorbic acid at 2.0 g/l and INA at 50 or 100 mM and in addition, the microelements in mixture [Fe + Zn + Mn] were tested. The different concentrations of chemical inducers and microelements as mentioned, either individually or in combination, were sprayed every 15 days for three times after the clear detection of the early sign of powdery mildew appeared through the daily field inspection. However, the combined treatments of chemical inducers were applied weekly at the same time of other treatments started with ascorbic acid followed by INA respectively. Sweet pepper plants were sprayed with some specific guidance; that sprays should cover pepper plants, particularly on the under surface of the foliage and the lower plant canopy and should not be used during very warm, sunny weather to prevent phytotoxicity. Recommended fungicide (Sumi - 8) 50 cm / 100 L water was applied as comparison treatment. Disease severity according to the previously modified scale of Reuveni *et al.* (1998) was measured, two weeks after the last spray. Meantine Fruit yield of sweet pepper (Kg / plant) for each treatment was determined.

RESULTS AND DISCUSSION

Results

1. Greenhouse experiments:

Efficacy of some chemical inducers on powdery mildew severity of sweet pepper plants under greenhouse conditions was evaluated. Results presented in Table (1) show that all treatments significantly reduced the powdery mildew disease severity on sweet pepper plants compared with the control. The highest reduction was obtained with INA at 50.0 & 100 mM and AA at 2 & 3 g/l; they reduced powdery mildew as (89.4 to 90.8 %) and (84.4 to 88.3 %), respectively. Meanwhile, EDTA showed less effect on the diseases severity which ranged between (76.5 to 78.6 %).

Table 1: Effect of some chemical inducers, as foliar spray treatments, on powdery mildew disease severity of sweet pepper plants, under greenhouse conditions.

Chemical inducers	Concentration	Disease severity%	Efficiency %
Ascorbic acid (AA)	1.0 g/l	9.2	83.5
	2.0 g/l	6.5	88.3
	3.0 g/l	8.7	84.4
Ethylene diamine tetra acetic acid (EDTA)	0.25 g/l	13.1	76.5
	0.50 g/l	11.9	78.6
	1.0 g/l	12.8	77.0
Dicloro-isonicotinic acid (INA)	50mM	5.9	89.4
	100mM	5.1	90.8
	150mM	7.2	87.0
Control		55.7	

T= treatment C= concentration
L.S.D at 5% T= 0.82 C= 0.71 TC= ns

The effect of combined treatments with Ascorbic acid and INA inducers was also recorded. Results in Table (2) indicate that all treatments reduced powdery mildew severity. The great reduction was obtained with combined treatments between AA at 2 g/l with INA at 100 and 50 mM followed by treatments of AA at 3 g/l and INA at 50 or 100 mM, which reduced the powdery mildew diseases severity as (89.5 - 90.1 %) and (86.6 - 87.2 %), respectively compared with untreated plants. Meanwhile single treatments showed moderate less effect.

Table 2: Effect of combination of the chemical inducers Ascorbic acid and Dichloro- isonicotinic acid, on disease severity of sweet pepper powdery mildew, under greenhouse conditions.

Ascorbic acid (g/l)	INA (mM)	Disease severity (%)	Efficiency (%)
1.0	0.0	8.9	85.6
	50	10.6	82.8
	100	10.5	83.0
	150	11.4	81.5
2.0	0.0	8.2	86.7
	50	6.5	89.5
	100	6.1	90.1
	150	7.2	88.3
3.0	0.0	9.1	85.3
	50	8.7	86.6
	100	8.3	87.2
	150	7.9	85.9
0.0	0.0	61.8	----
	50	9.6	84.5
	100	8.4	86.4
	150	9.2	85.1

A=AA = Ascorbic acid I = INA= Dichloro- isonicotinic acid
L.S.D at 5% A= 0.44 I= 0.44 AI= 0.89

Data reported in Table (3) show that, foliar application of microelements singly or in mixture reduced powdery mildew disease severity compared with the control. Fe was more efficient than addition of other microelements individually followed by Zn and Mn. In this regard, the averages of disease severities were 12.6, 13.4 and 15.2% respectively. Furthermore, the lowest average of disease severity was recorded when plants were sprayed with combination of 3 microelements (Fe + Zn + Mn), and disease severity was 7.6%. While another combinations of (Fe + Zn), (Fe + Mn) and (Zn + Mn) reduced disease severity more than individual applications of these microelements and the average of disease severities were 10.9, 11.7 and 12.1%, respectively.

Table 3: Effect of some microelements on powdery mildew disease severity of sweet pepper plants under greenhouse conditions.

Microelements	Disease severity (%)	Efficiency (%)
Zn	13.4	80.0
Mn	15.2	77.3
Fe	12.6	81.2
Fe + Zn	10.9	83.7
Zn +Mn	12.1	81.9
Fe + Mn	11.7	82.5
Fe + Zn + Mn	7.6	88.6
Control	66.9	------
L.S.D at 5%	1.78	------

2. Field experiments:

Results in Table (4) indicate that all treatments reduced the powdery mildew disease severity during two successive Nili grown seasons. High reduction was obtained with combined treatments between AA at 2.0 and INA at 50.0 or 100 mM, which reduced the disease severity between 87.8 and 90.3 %. Individual treatment of AA at 2.0 & 3.0 g / l and INA at 100 mM reduced the powdery mildew disease severity between 82.0 and 85.7 %. The fungicidal treatment recorded 86.8 - 87.7 % reduction in disease severity, which was superior to microelements in mixture (Fe +Zn +Mn) treatment. However EDTA showed the lowest reduction records which were between 76.2 to 78.6 %.

In addition, to disease suppression, data indicated that all treatments increase sweet pepper fruit yield during the two Nilli growing seasons. The highest increase was obtained with combined treatments between AA at 2.0 and either INA at 50.0 or 100 mM, which recorded as 3.10 up to 3.95 Kg/plant. Individual treatments of INA at 50 &100 mM and AA at 2.0 & 3.0 g/l increased fruit yield between (2.39-2.73) and (2.25-2.58) Kg/ plant, respectively. The moderate increase was obtained with the other treatments as well as fungicidal application. EDTA showed the lowest increase of fruit yield which was between 1.33 to 2.0 Kg / plant at the two seasons.

Table 4: Effect of some chemical inducers and microelements, applied singly or in combinations as foliar spray treatments on powdery mildew disease severity and fruit yield / plant compared with the fungicide, sumi-8 under field conditions during Nili seasons of 2007 and 2008 of sweet pepper.

Treatment	Dose	2007			2008		
		Disease Severity (%)	Efficiency (%)	Yield Kg/plant	Disease Severity (%)	Efficiency (%)	Yield Kg/plant
AA	2.0 g/l	13.3	84.4	2.58	15.5	82.5	2.47
	3.0 g/l	14.2	83.4	2.25	16.0	82.0	2.33
EDTA	0.5 g/l	19.4	77.3	1.48	21.1	76.2	1.33
	1.0g/l	18.3	78.6	2.00	19.1	78.5	1.92
INA	50mM	13.8	83.8	2.39	15.5	82.5	2.41
	100mM	12.2	85.7	2.73	13.9	84.3	2.66
AA + INA	2.0g/l + 50mM	10.1	88.2	3.15	10.8	87.8	3.10
	2.0g/l + 100mM	9.3	89.1	3.84	8.6	90.3	3,95
Fe+ Zn+ Mn		15.9	81.4	1.77	18.2	79.6	1.60
Fungicide Sumi -8	50cm/100Lwater	11.3	86.8	3.00	10.9	87.7	2.95
Control	0.0	85.4	-------	0.26	88.7	--------	0.23

T= treatment C = concentration

	2007	2008
L.S.D at 5%	T = 1.38	T = 0.94
	C = 0.21	C = 0.437
	TC = 0.534	TC = 1.138

Discussion

Sweet pepper powdery mildew, caused by *Leveillula taurica,* is considered one of the most serious diseases attacked Sweet pepper plant (Palti, 1988). The control of plant diseases using classical fungicides could successfully control most of plant diseases. However, they have raises serious concerns about food safety, environmental quality and fungicides resistance, which have dictated the need for alternative disease management techniques. There are several fungicidal alternatives commercially used for induction of plant resistance against diseases. In this respect, Dicloro-isonicotinic acid (INA) was reported for inducing resistance in several plants against viruses, bacteria, and fungal infection (Nielsen *et al*., 1994). The obtained results in the present study indicate that INA at 50.0 or 100 mM could reduce the severity of powdery mildew disease and increase the Fruit yield during the two successive cultivation seasons. The role of INA might be due to increase the enzymes activities and stimulated the defense reactions, or due to its fungicidal properties (Ward *et al*., 1991). On the other hand, ascorbic acid was reported as plant resistance inducer in many plants and was successful for controlling several plant fungal diseases. (Metraux *et al*., 1991) reported that the content of ascorbic acid in plant tissues has been associated with resistance to some diseases. Similar results are recorded in the present investigation that sweet pepper plants sprayed with ascorbic acid caused significant reduction in disease severity of powdery mildew under greenhouse and field conditions. Furthermore, EDTA was reported to induce resistance in broad bean against rust disease (Walters & Murray, 1992). These reports support the present findings that EDTA at 0.5 and 1.0 g/l reduce powdery mildew severity under greenhouse and field conditions. EDTA could still affect intracellular functions by binding apoplectic calcium, which is critical for maintaining membrane stability and selective ion permeability (Clarkson & Hanson, 1980).Moreover, foliar application of microelements (Fe, Zn and Mn), singly or in mixture reduced powdery mildew disease severity, and the highest percentage reduction was obtained with the mixture (Fe + Zn +Mn), under greenhouse and open field conditions. In this concern, results presented in this study were in agreement with those of Sindha, (1989), Graham, (1983), Graham & Webb, (1991), Maharana *et al*. (1993) and Reuveni *et al*. (2004).

On the light of the results obtained in the present study, it could be suggested that combined treatments with ascorbic acid and INA waned be a good alternated of fungicide used for suppressing the severity of powdery mildew disease of sweet pepper plants and stimulate fruit yield under field conditions, however it considered as safe, cost-effective and easily applied diseases control measure.

REFERENCES

Agrios, G.N. 1997. Plant Pathology. 4th Edition. Academic Press, San Diego, USA, pp: 93-114.

Braun, U. 1987. A monograph of the *Erysiphales* (powdery mildew). Nova Hedwigia, n.89, p.1-700.

Clarkson, D.T. and Hanson, J.B. 1980. Mineral nutrition of higher plants. Annu. Rev. Plant Physiol., 31: 239-298.

Correll, J.C.; Gordon, T.R.; Elliott, V.J. 1987. Host range, specificity, and biometrical measurements of *Leveillula taurica* in California. Plant Dis. 71: 248-251.

Deshpande,N. G.; Eilam, G.; Barger, V. and Halzen, F. 1985. Number of Neutrinos in the Standard Model and its Extensions to Super symmetry. Phys. Rev. Lett. 54, 1757 – 1760.

Graham, R. D. 1983. Effects of nutrient stress on susceptibility of plants to disease with particular reference to the trace elements. Adv. Bot. Res. 10: 221-276.

Graham, R.D. and Webb, M.J.1991. Micronutrients and disease resistance and tolerance in plants. Pp. 329-370. In J.J. Mortvedt, F.R. Cox, L.M. Shuman, and R.M. Welch Eds.) *Micronutrients in Agriculture*. 2nd edition. Soil Science Society of America.

Maharana, D.P.; Sarengi, S.K.; Singh, R.N.B. and Ali, M.H. 1993. Proceeding of the workshop on micronutrients. 22-23 January, 1992. Bhubaneswar, India. Pp 228-238.

Metraux, J.P.; Signer, H. J.; Ryals, E.; Ward, W.M.; Benz, H. and Inverardi, B., 1990. Increase in salicylic acid at the onset systemic squired resistance in cucumber. Science, 250: (4983) 1004- 1006.

Nielsen, K.K.; Bojsen, K. and Mikkelsen, D.1994. Induced resistance in sugar beet against *Cercospora beticola* induction by dichloroisonicotinic acid is independent of chitinase and glucanase transcript accumulation. Physiol. Mol. Plant Pathol., 45: 89-99.

Nofal, M. and Hagag, W., 2006. Integrated management of powdery mildew of mango in Egypt.

Palti, J., 1988. The *Leveillula* mildews. *Bot. Rev.* 54:423-535.

Reuven, R.2004. A foliar spray of micronutrient solutions induces local and systemic protection against powdery mildew (*Sphaerotheca fuliginia*) in cucumber plants. Euro. Plant Pathol. 103, 581-588.

Reuveni, R.; Dor, G. and Reuveni, M. 1998. Local and systemic control of powdery mildew (*Leveillula taurica*) on pepper plants by foliar spray of mono-potassium phosphate. Crop protection 17:703-709.

Sindha, G. S. 1989. Effect of macro and micro nutrients on the development of powdery mildew of pea. Indian J. Myco. Plant Pathol., 19(2): 219-221.

Ullasa, B.A.; Rawal, R.D.; Sohi, H.S.; Singh, D.P. 1981. Reaction of sweet pepper genotypes to Anthracnose, Cercospora leaf spot, and Powdery Mildew. Plant Dis., 65, :600-601.

Walters, D.R. and Murray, D.C. 1992. Induction of systemic resistance to rust in Vicia faba by phosphate and EDTA: Effects of calcium. Plant Pathol. 41: 444–448.

Ward, E.R.; Uknes, S.J. and Williams, S.C. 1991. Coordinate gene activity in response to agents that induce systemic acquired resistance. Plant Cell, 3: 1085-1094.

المقاومة المستحثة في الفلفل لمرض البياض الدقيقي باستخدام بعض محفزات المقاومة والعناصر الصغرى تحت ظروف الصوبة والحقل

سحر عبده زيان و تماضر جمعه عبد الرحمن

معهد بحوث أمراض النبات – مركز البحوث الزراعيه

يسبب الفطر ليفيلبولا نوركيا مرض البياض الدقيقي لمحصول الفلفل وبعض المحاصيل الأخرى تحت ظروف الصوبة والحقل وذلك في جميع انحاءالعالم ومصر ، وتهدف هذه الدراسة إلي معرفة تأثير بعض المستحثات الكيميائية مثل حمض الأسكوربيك ، وحمض داي كلورو أيزونيكوتيتنك ، حمض تترا إيثيلين داي أمتين وكذلك بعض العناصر الصغري مثل الحديد ، الزنك ، المنجنيز وذلك عند استخدامها رشا علي المجموع الخضري بمفردها أو بخلطها مع بعضها لمقاومة المرض بهدف استخدامها كبدائل للمبيدات تحت ظروف الصوبة والحقل خلال موسمي 2007،2008 . وقد دلت النتائج تحت ظروف الصوبة أن أعلي هذه المواد مقاومة للمرض حمض الأيزونيكوتينيك، حمض الأسكوريبك ثم حمض الترا إيثيلين دايأفين بالتتابع كما تم الحصول علي نفس النتائج باستخدام عناصر الحديد ، الزنك يلهم عنصر المنجنيز .

أدي إستخدام المخاليط المكونة من 100 فللميكرون من حمص دايكلورو أيزونيكوتينيك + 2.0جم / لترحمض أسكوربيك ، (حديد + زنك + منجنيز) للحصول علي نتائج ممتازة بالنسبة لمتوسط نسبة الاصابة إلي أقل حد ممكن وذلك بالتتابع . وقد تم الحصول علي نتائج مشابهة عند استخدامها بالحقل ، وهذه النتائج تبين أنه في الإمكان الحصول علي طرق مقاومة آمنة ، فعالة باستخدام مواد بديلة للمبيدات في مقاومة مرض البياض الدقيقي علي الفلفل .