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INFECTION SUPPRESSION OF VERTICILLIUM WILT DISEASE IN EGGPLANT AS AFFECTED BY SOME FUNGICIDES, BIOCIDES AND SALICYLIC ACID

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ABSTRACT: The ability of some modern fungicides, biocides and organic acids to reduce verticillium wilt in Eggplant was carried out in this study. Eleven fungicide (active ingredient: Fludioxnil + Tebuconazole), Vincint (a.i.: Flutriafol) and Meta Z (a.i.: Metalaxyl + Thiram), Clean root (*Bacillus subtilis*) and salicylic acid were investigated by treating transplants of eggplant through dipping their roots in the recommended doses of these biocides. The disease incidence was significantly reduced in case of fungicides followed by clean root and salicylic acid in comparison with control treatment. The fungicides Vincint and Meta Z recorded the highest efficacy as 77% and 76.6%, respectively, hence both of them are systemic fungicides. They followed by the fungicide eleven which gave 70% efficacy. Meanwhile, salicylic acid was the least effective where it revealed 44% efficacy. Growth parameters and yield of eggplant measurements revealed high record with fungicides more than the other treatments. Concerning to peroxidase and polyphenoleoxidase activities in leaves of eggplant, it was observed that clean root and the fungicides Vincint, Meta Z and Eleven ones recorded high levels of activity for both enzymes.

Key word: Eggplan, verticillium wilt, systemic fungicides, biocides, salicylic acid, oxidative enzymes.

INTRODUCTION

Eggplant (*Solanum melongena* L.) is an important vegetable crop that is widely cultivated in the tropical and subtropical areas in Asia. Globally, as of 2013, the top three eggplant producers are China with 18 million ton, India with 8.5 million tons, and Egypt with one million ton **(Choudhary and Gaur, 2013)**. The cultivated eggplant is a popular vegetable crop worldwide, especially in Asia and Africa **(Collonnier *et al*., 2001)**. China being the largest producer and consumer of eggplants. In China, eggplants are planted both in open fields and in greenhouses. In fact, it is one of China's most widely grown greenhouse vegetables. verticillium wilt is a soilborne disease caused by *Verticillium dahlia* that greatly reduces the yield and quality of eggplants, especially in greenhouse production. verticillium wilt is a vascular wilt disease that cannot be sufficiently controlled by many fungicides, and there are few fungicides that can rid infected eggplants of the disease (**Fradin *et al.*, 2009**). Other approaches, such as grafting and soil fumigation, are effective. However, they are either expensive or harmful to the environment (**Lee, 1994; Fradin and Thomma, 2006; King *et al*., 2010**). Therefore, the best way to control verticillium wilt is to develop disease resistant varieties. Fungicides are either chemicals or biological agents that inhibit the growth of fungi or fungal spores. New fungicides do not kill fungi, they simply inhibit growth for a period of days or weeks. Fungi can cause serious damage in agriculture, resulting in critical losses of yield, quality and profit. Fungicides can either be contact, translaminar or systemic. Contact fungicides are not taken up into the plant tissue and only protect the plant where the spray is deposited; translaminar fungicides re-distribute the fungicide from the upper, sprayed leaf

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surface to the lower, unsprayed surface; systemic fungicides are taken up and re-distributed through the xylem vessels to the upper parts of the plant. New leaf growth is protected for a short period. Most fungicides that can be bought retail are sold in a liquid form **(Carisse, 2010)**.

Developing new cultivars with improved resistance is an important consideration in disease control (**Panthee and Chen, 2010**). There has been a growing interest in the use of biological control methods and practices. In a study by **Elmer and Ferrandin (2009),** an increase in the size of snail populations improved the biological state of soil, thus stimulating the growth of eggplant and reducing the symptoms of verticillium wilt. Strains of *Bacillus* spp., applied to soil or root surface, provided good control of pathogens, such as *Verticillium albo-atrum, V. dahliae* and *V. tricorpus* (**Tjamos *et al*., 2004**).

The aim of this study was to manage verticillium wilt of eggplant cultivation by using resistance inducer, commercial biocides and compare their efficacy with fungicides and their effect on yield. Also, the activity of oxidative enzymes which responsible of resistance to the disease were determined.

MATERIALS AND METHODS

In *Vivo* Studies (Greenhouse Trial)

Under greenhouse condition an experiment was carried out in Agricultural Res. Center, Giza April 2015. Five weeks old transplants Eggplants (*Solanum melongena* L.) cv. Black Beauty, were planted in pots (30 cm) containing loam sandy soil; 2 transplants in each. The pots were divided into six groups, each group 9 pots (3 replicates, each replicate 3 pots). The pots were arranged in complete randomized block design. Transplants eggplant dipping with treatments *e.g* fungicides (Eleven, Vincint and Meta Z) and salicylic acid about 2 houres whereas, the treatment biocide (Clean root) the transplants were dipped 24 hours before transplanting. Pots with unprotected and infested soil served as control. After 45 days from the transplanting the disease incidence, was recorded. Samples of fruits were harvested after 70-85 days and number and weight of fruits were recorded, fresh weight, long of both stem and roots were also recorded. After 30 days from transplanting the activity of polyphenoloxidase and peroxidase enzymes, were determined.

Commercial bioagent

1- Biocide Clean root 2.5 g/l

Each 1 g contains 30×10^6 CFU of wild isolates of *Bacillus subtilis*

Organic acid used

1- Salicylic acid 3g/l $C_7H_6O_3$

Pathogenic fungi

The casual agent *Verticillium dahliae* kindly taken from Dr Hala Ali Eldakar chef of research plant pathology institute. ARC, Giza, Egypt.

Fungal Inoculum Preparation

Fungal inoculum was prepared by growing the *Verticillium dahlia* on autoclaved sorghum grains in 500 ml glass bottles and incubated at 27 ± 2ºC for 2 weeks until sufficient growth of the fungus **(El-Shafey *et al.*, 1979)**. Contents of the bottles were poured out and mixed to get homogenized inoculum, and then inoculum was used for soil infestation at the rate of 3 g /kg soil.

Disease Assessment

Vegetative growth was recorded at 45 days after transplanting. Pots containing non-treated transplants act as control with infested soil. Disease incidence was estimated as percentage of dead plants.

Assessment of Dead Plants

Verticillium wilt was measured as percentage of dead plants at 45 days after transplanting **(Booth, 1971)** as follow:

$$\text{Percentage of dead plants (\%)} = \frac{\text{Number of dead plants}}{\text{Total number of plants}} \times 100$$

Efficacy of treatment (%)

= C-T/C×100

C= control;

T= treatment.

Table 1. Investigated fungicides used

Trade name	Active ingredient	Common name	Chemical name	Recommended dose
Eleven	10% (Fs)	Fludioxnil 4% + Tebuconazole 6%	4-(2,2-difluoro-1,3benzodioxol-4-yl)1-H-pyrrole-carbonitrile	2.5cm/ l
Vincint	25% (Sc)	Flutriafol	(±)-α-(2-fluorophenyl) -1-H-1,2,4 triazole-1-ethanol	4cm/ l
Meta Z	38%(WP)	Metalaxyl 8%+ Thiram 30%	Methyl-N-(2,6methyldiphenyl)-N-(methoxyacetyle) DL-alaninate	2.5cm/l

Growth Parameters and Yield Traits

The growth parameters and yield were recorded after harvest [fresh weight of plant (gram), shoot length (cm), root length (cm), number of fruits and their weight (gram)].

Determination of Peroxidase Enzyme Activity

The peroxidase activity was determined according to **Allan and Hollis (1972)** by measuring the oxidation of pyrogallol to pyrogallin in the presence of H_2O_2 at 425 nm. using a UV spectrophotometer. The enzymes was expressed as the change in the absorbance of the mixture every 15 sec./min. for 5 min. period.

Determination of polyphenoloxidase enzyme activity

The crude enzyme was prepared as aforementioned determination of peroxidase activity. The activity of polyphenoloxidase was expressed as the change in absorbency of 1 ml of extract per min. at 495 nm. using a UV spectrophotometer.

Statistical Analysis

Data were statistically analyzed as complete randomized blocks design and least significant differences (LSD 0.5) was calculated according to **Fisher (1948)** and **Snedecor and Cochran (1967)** and Multiple range and multiple F test **(Duncan, 1955**), using Web Agri Stat Package computer program (WASP).

RESULTS

Effect of Fungicides and Bioagents on Verticillium Wilt of Eggplant

Results in Table 2 show that, all fungicides were effective in controlling the disease compared with the control and there were significant differences between them. Fungicides Vincint (Flutriafol) and Meta Z (Metalaxyl + Thiram) were the most effective ones in reducing Verticillium wilt resulting disease control efficacy of 77% and 76.6%, respectively. Whereas, Eleven (Fludioxnil 4% +Tebuconazole 6%) recorded 70% efficacy, followed by Clean root (60% efficacy) in reducing wilt incidence. Meanwhile, salicylic acid was the least effective in reducing the Verticillium wilt resulting 44% efficacy. It was clear that there was significant differences between all treatments in comparing with the control.

Growth Parameters and Yield Traits of Eggplant Treated with some Fungicides and Bioagents in Greenhouse Infested with *Verticillium dahlia*

Results in Table 3 indicate that, treatment with fungicides, highly increased the plant growth parameters and yield traits values comparing with clean root, salicylic acid and control. Fungicides vincint and meta Z were the best ones revealed 45.6 g and 43.3 g of plant weight, respectively; followed by fungicide Eleven which gave 41.6 g. However Clean root and Salicylic acid exhibit 30.3g and 16 g, respectively; if compared with control (15.6 g)

All growth parameters display the same trend and that was clear in number of fruits. Vincint and Meta Z were the best of all resulting an average of 14 and 13.3 fruits followed by Eleven 9 fruits. Also, the fruit weigh were of 253 g, 210 g and 199 g, respectively; comparing with control (3.6 fruits) in average and the weight was 32.3 gm. Meanwhile, all the other treatments were almost similar whereas Clean root and Salicylic acid recorded averages of 7 and 5.6 fruits and weights of 139.3 and 86.6 g, respectively. Thus, revealed the least values in all plant growth parameters nearly by of control.

Table 2. Effect of treating eggplant with commercial bioagent, salicylic acid and some fungicides in controlling Verticillium wilt and their efficiency under greenhouse conditions

Treatment	Mean of disease incidence (%)	Disease control efficacy (%)
Clean root	31	60
Salicylic acid	43	44
Eleven	23.3	70
Vincint	17.6	77
Meta Z	18	76.6
Control	77	--
LSD 0.05	3.993	

Table 3. Effect of treating eggplant (Black beauty cv.) with commercial bioagent, salicylic acid and some fungicides on some crop parameters under greenhouse conditions of soil infested with *Verticillium dahliae*

Treatment	Foliage fresh weight/plant (g)	Plant height (cm)	Root length/cm	Number of fruits	Weight of fruits (g)
Clean root	30.3a	29b	18.6c	7bc	139.3c
Salicylic acid	16 b	27b	17.6c	5.6cd	86.6d
Eleven	41.6a	41.3a	27.3b	9.3b	199b
Vincint	45.6a	50.6a	41.6a	14a	210.6b
Meta Z	43.3a	43a	27.6b	13.3a	253.3a
Control	15.6b	23.3b	14c	3.6d	32.3e

Polyphenoloxidase and Peroxidase Enzymes Activity in Eggplant after Treating the Plants with the Investigated Fungicides and Bioagents

Results in Fig. 1 show that the activity of oxidative enzymes *i.e.,* polyphenoloxidase and peroxidase was greatly increased in eggplants treated with the investigated fungicides and bioagents. Plants treated with the Clean root revealed highest enzyme of both estimated peroxidase being 2.832 after 30 sec. Meanwhile, Meta Z resulted in the highest activity of polyphenoloxidase 2.016 after 30 sec.

DISCUSSION

Vascular wilts caused by members of the genus Verticillium are among the most devastating fungal diseases worldwide. The genus Verticillium consists of a relatively small group of soil borne ascomycete fungi and several of them cause wilt disease on a variety of plant hosts in many parts of the world. Causal agents of Verticillium wilt diseases are globally distributed, most prevalent in temperate and subtropical regions and rare in tropical regions. The consequences of infection can be far-reaching, leading to huge yield losses **(Pegg and**

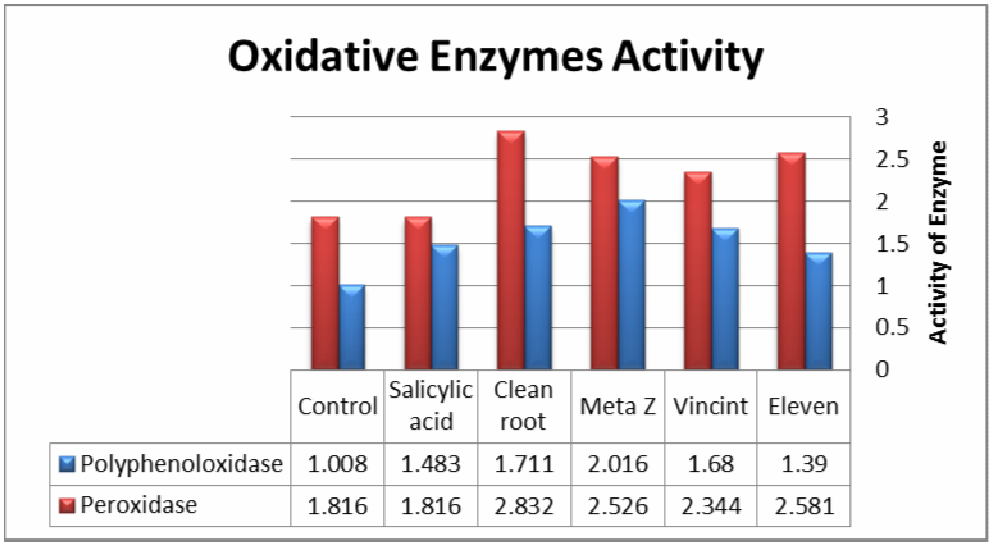

Fig. 1. The activity of polyphenoloxidase and peroxidase enzymes in eggplants leaves treated with fungicides and bioagents

Brady, 2002). Currently, 10 species are defined within the Verticillium genus of which *Verticillium dahliae* has the broadest host range and infects over 200 plants pecies (**Inderbitzin *et al*., 2011 ; Inderbitzin and Subbarao, 2014).** *Verticillium* species produce long-lasting resting structures such as microsclerotia, chlamydospores, and resting mycelium in dead or dying plant tissues. The fungus usually infects the host through entering young roots and growing into the water-conducting vessels. The vessels become plugged and collapse, blocking the plant's water supply. Consequently, the leaves wilt. This wilting may be in the top or bottom leaves. Leaf tips often dry and turn pale or brown, but frequently no symptoms are seen until the season is well under way. The visible wilting may partially recover, particularly during nighttime **(Noble**, **2014)**.

Control of Verticillium disease is difficult due to the long persistence of the resting structures in the field and the broad host range of some species. Moreover, the pathogen is difficult to manage once it reaches the vascular plant tissue and fungicides appear to be ineffective. Reducing the primary inoculum in the soil has been considered as an important goal and can be accomplished by several management strategies. Chemical fumigants can reduce the inoculum of Verticillium in soil, however, their use is restricted because of the detrimental effect on the environment. Chemical soil disinfestations (solarization, soil steaming, anaerobic disinfestation, inundation, and biofumigation) can be used to control the disease incidence. Those management strategies have been implemented into agricultural production and all of them have their specific concerns and limitations (**Depotter *et al.*, 2016)**.

Concerning to the use of fungicides in this study for controlling Verticillium wilt in eggplant under greenhouse conditions the fungicide Vincint revealed the highest effect in reducing the disease, it was observed that this fungicide belonged to the (Triazoles**)** family which introduced in the 1980s, consists of numerous members: difenoconazole, fenbuconazole, myclobutanil, propiconazole, tebuconazole, tetraconazole,triadimefon, and triticonazole. This family is promising tools against diseases of many crops and vegetables. They are applied as foliar sprays or seed treatments, but are diverse in use, may be applied as protectant or curative treatments. If applied as a curative treatment, triazole application must be used early at the fungal infection process, up to the fungus begin to produce spores on an infected plant. The

triazoles are very specific in their mode of action where they inhibit the biosynthesis of sterol, which is a critical component for the integrity of fungal cell membranes (**Fishel, 2005**). This study refers that Vincint as a systemic fungicide belong to Triazoles was the highest effect on wilt and this is in harmony with (**Mihajlović *et al.*, 2017**). They found that fungicides that effectively reduce soil borne pathogens of some crops by soil and plant applications are the dicarboximide, benzimidazole and triazole chemical groups were very effective in reducing the severity of Verticillium wilt in eggplant under field and greenhouse conditions.

Meta Z (metalaxyl) was effective also in reducing the disease, due to its action as systemic fungicide. The mode of action of metalaxyl is due to impede protein formation by interfering with the biosynthesis of nucleic acids and inhibit polymerase enzyme action in ribonucleic acid synthesis (**Agrios, 2005**).

The fungicide Eleven is belong to family (Fludioxnil), is protective fungicide so it mixed with (Tebuconazole) to be more effective but the aforementioned fungicides were more effective than it.

The biocide Clean root reduced the wilt percentage comparing with the control and that was in harmony with **Tjamos *et al*. (2004)** who mentioned that the *Bacillus* strains (*Bacillus amyloliquefaciens* and *Bacillus subtilis*) reduced the percentage of *V. dahliae* disease in eggplant by 40–70% in the greenhouse and could reduce disease incidence with more than 50% in a field experiment of potato. **Rais *et al.* (2017)** found that the activation of antioxidant system in rice by the antagonistic *Bacillus* sp in response to blast pathogen *Pyricularia oryzae* through the quantification of defense enzymes involved in induction of systemic resistance in the host. These findings clearly demonstrate that *Bacillus* sp. enhanced the activity of peroxidase polyphenoloxidase, Also, **Zongzheng *et al.* (2009)** found that the inoculation of *B. subtilis* SY1 strengthened the plant pathogen resistance, which was expressed in terms of increase in antioxidant enzymes activity and that is in harmony with our results which showed an increasing in activity of oxidative enzymes with biocide clean root treatment.

Salicylic acid also in this study recorded a positive effect on Verticillium wilt whereas, it investigated as an endogenous plant growth of phenolic nature that possesses an aromatic ring with a hydroxyl group or its hormone plays a vital role in plant growth, ion uptake and transport (**Hayat *et al.*, 2010**). Salicylic acid enhanced germination percentage and seedling growth of wheat, when the grains were subjected to pre-sowing seed-soaking treatments in salicylic acid (**Shakirova, 2007**). In cucumber and tomato, the fruit yield enhanced significantly when the plants were sprayed with low concentrations of salicylic acid (**Larque-Saavedra and Martin-Mex, 2007)**.

Meta Z was found to enhance the activities of antioxidant enzymes such as peroxidase and polyphenoloxidase hence, it revealed high level of them and that was in harmony with (**DeSousa *et al.*, 2013**) they mentioned that metalaxyle increase the activity of those enzymes in plants.

In conclusion, this study reported that the systemic fungicides are the most effective chemical ways of controlling Verticillium wilt comparing with biocide Clean root and Salicylic acid. Using systemic fungicides in spite of fumigation compounds in the soil and avoiding its costs and their pollutant to the environment.

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تثبيط العدوى بمرض الذبول الفيرتسليومي في الباذنجان متأثرا ببعض المبيدات الفطرية والمبيدات الحيوية وحامض السالسليك

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تم دراسة مقدرة بعض المبيدات الفطرية الحديثة، المبيدات الحيوية، حمض السالسليك على خفض الإصابة بالذبول الفيرتسليومي في الباذنجان، مبيد إليفين (المادة الفعالة: فلوكسودينيل + تيبيوكونازول)، المبيد الفطري فينزينت (المادة الفعالة: فلوترايافول) والمبيد ميتازد (المادة الفعالة: ميتالاكسيل + ثيرام)، المبيد الحيوي كلين رووت (باسيلاس سايكس) وحامض السالسليك وتم استخدامهم في معاملة شتلات الباذنجان بغمر جذورها في تلك المواد بالمعدلات الموصى بها، انخفضت شدة الإصابة بشكل معنوي في حالة المبيدات الفطرية يليها المبيد الحيوي كلين رووت وأخيرًا حامض السالسليك بالمقارنة بالكنترول، أظهر المبيدين فنزينت وميتا زد أعلى كفاءة حيث كانت ٧٧%، ٧٦,٦% على الترتيب حيث أنهما من المبيدات الجهازية يليهم المبيد الفطري إليفين حيث كانت كفاءته ٧٠% في حين كان أقلهم حامض السالسليك ٤٤%، تم تقدير قياسات النمو والمحصول وأعطت المبيدات الفطرية أعلى قيم في المعاملات، عند تقدير نشاط إنزيمي البيروكسيديز والبوليفينول أوكسيديز في أوراق الباذنجان تم ملاحظه إرتفاع نشاطها مع المبيد الحيوي كلين رووت والمبيدات فنزينت وميتازد.

المحكمـــــون:

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