Minufiya J. Agric. Res. Vol.39 No. 3(1): 887-893 (2014) "http://www.mujar.net"

CONTROL OF *FUSARIUM* WILT OF PEPPER BY SOME PLANT MATERIALS AMENDMENT AND *STREPTOMYCES* SPP.

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(Received: Apr. 8, 2014)

ABSTRACT: *This work was conducted to study the effect of Datura stramonium , Nerium oleander dry leaves powder and Allium cepa dry peel powder at rate 1,2 % of soil weight, Streptomyces phycochromgenes and Streptomyces exfoliatus (45 ml bacterial suspension per pot) applied as soil drench on the control of pepper Fusarium wilt under greenhouse conditions. Application of plant leaf, peel powders and Streptomyces to soil infested with Fusarium oxysporum significantly reduced Fusarium wilt incidence and improved plant growth components. Generally, the D. stramonium powder at the rate of 2 % gave the best result in reducing wilt disease, while A. cepa powder at rate 1% gave the least effect on reducing disease incidence.*

Key words: *Fusarium oxysporu, Streptomyces spp, organic amendments*

INTRODUCTION

Sweet pepper (*Capsicum annuum* L.) is one of the most important vegetable crops in Egypt as well as in many other countries of the world. Pepper plants are vulnerable to infection by several soil-borne pathogenic fungi, which cause considerable loss in plant mortality and consequently in the quantity and quality of fruit yield. (Abada, 1994 and Mushtaq and Hashmi,1997) .

Fusarium oxysporum Schlecht. is a fungus that causes serious disease in many important crops , with symptoms that include vascular wilt, stunting, chlorosis and eventual plant death (Kaur,2003). *Fusarium* root rot and wilt caused by *Fusarium solani* and *F. oxysporum* are the most important diseases in sweet pepper plants (Abdel-Monaim and Ismail, 2010)

Use of fungicides may cause hazards to human health and many directly increase environmental pollution. In addition, some fungicides may not readily be biodegradable and tend to persist for years in environment (Brady, 1984). Some fungi have developed resistance to chemicals. Because of these associated problems, soil organic amendment and biological control has received attention as the environmentally safe alternative methods for plant pathogenic fungal control.

Strepyomyces have been estimated to produce up to 100,000 distinct antimicrobial compounds, of which only a small number have been identified to date (Watve *et al.*, 2001). When inoculated into soil (or) on seeds, many isolates of *Streptomyces* spp play a role in protecting plants from fungal diseases by produce multiple antibiotics (Jones and Samac , 1996 and Challis and Hopwood , 2003).

Organic amendments with botanical toxicant have shown promising results in the control of root infection of crop plants. Soil amendment with *Datura* powder significantly reduced root rot disease in okra and mungbean (Ehteshamul-Haque *et al.*,1996 and Javaid and Saddique, 2011). Likewise, water extract and leaves powder of *Nerium oleander* exhibit some level of toxicity toward juveniles of *Meloidogyne javanica* (Mohammad , 2012) and protected lupine plants against damping- off and wilt diseases (Abdel-Monaim *et al.* , 2011).

The alliaceous crops onion (*Allium cepa* L.) and garlic (*Allium sativum* L.) exhibit multiple bioactive properties as stimulatory and inhibitory effects on variety of soil organisms (Bianchi *et al.*, 1997). Although, Clarkson *et al*, (2006) found that the onion and / or garlic extracts or amendments were inhibitory to many soil and root organisms.

The present work aimed to study the efficacy of *Streptomyses phycochromgenes* and *Streptomyces exfoliates as* biocontrol agents *and ,Datura stramonium* L. *, Nerium oleander* L. dry leaves powder and *Allium cepa* dry peel powder as soil amendment to control *Fusarium* wilt disease of pepper.

MATERIALS AND METHODS

This work was conducted under greenhouse conditions at the Experimental farm, Faculty of Agriculture, Menoufia University, Shibin El-Kom,Egypt during two winter seasons of 2012 and 2013.

Isolation and cultures of *Fusarium oxysporum*.

samples of pepper plant showing wilt diseases symptoms were collected from Vegetable Experimental farm, Faculty of Agriculture, Shibin El-Kom.Samples were subjected to isolation trials for the pathogenic fungi according to the method devised by (Sahi and Khalid, 2007).The developed fungal colonies were purified onto Potato Dextrose Agar (PDA) medium by hyphal tip techniques. Purified isolated fungi were identified as *Fusarium oxysporum* according to (Nelson *et al*., 1983). Subcultures of the obtained isolate were kept on PDA slants and stored at 5° c until used.

Preparation of plant amendments.

The leaves of *Datura stramonium*, *Nerium oleander* and *Allium cepa* peel were washed and dried in the shade for three weeks then finely ground into powder using a home blender according to (Mohammed, 2012).

Preparation of fungal inoculums.

Inoculums of the obtained isolate of *F. oxysprum* were prepared on autoclaved Sand/wheat bran medium (75g wheat bran, 100g dried coarse sand and 75 ml tap water) in 500 ml glass bottles. Each bottle was inoculated with five discs (o.5 cm in diameter) of 10-days-old culture of isolate. Bottles were incubated at 25 ± 1c°. For 15 days and manually homogenized for final use.

Preparation of *Streptomyces* spp inoculums.

Streptomyces. phycochromgenes and S. exfoliatus isolates provided by Dr, Sabha Mahmoud, Botany Department, Faculty of Sciences, Menoufia University. were maintained on Starch Nitrate medium (SN) in slants (Küster and Williams, 1964).The liquid suspension (used as inoculums) was prepared by transferring a loop of 7 days old slant grown on SN medium to a 500 ml Erlenmeyer flask containing 150 ml of Starch Nitrate broth medium. Flasks were then incubated for 7 days on a rotary shaker at 100 rpm at room temperature30°±5. After that, cells were removed by centrifugation at 2000 rpm for 20 minutes. The resulted pellets of each isolate were suspended in phosphate buffer and adjusted to 1.8 x 10^7 cfu/ ml.

Soil preparation.

Clay-sand mixed soil (1:1, v/v) were sterilized by adding 5 % formalin solution. Consecutively, soil was covered with polyethylene sheet for 7 days to retain the gas then left 2 weeks until all traces of formaldehyde disappeared (Abdel-Monaim *et al.,* 2011).

Effect of plant materials amendment and *Sterptomyces* spp on pepper *Fusarium* wilt control.

D. stramonium leaves, *N. oleander* leaves, *A. cepa* peel dry powder and *Streptomyces* spp. were tested for controlling *Fusarium* wilt on pepper seedling cv.*Baladi* (35.Days old) under greenhouse condition. Plastic pots 25-cm in diameter was filled with sterilized soil at 4kg/pot. Pots were inoculated with *F.oxysporum* prepared on sand/wheat bran medium at rate of 3% of soil weight, while control pots were inoculated with the same medium without fungus. Pots were left for 1 week under greenhouse, after irrigation, for the establishment of fungal inoculums. Pots were amended with 1.0 or 2.0 % (w/w) of plants amendment. Likewise, pots were irrigated and left 10 days for materials decomposition. *Streptomyces* spp. were

added by drenched 45 ml of liquid bacterial suspension (1.8 x 10^7 cfu / ml) around the growing plant roots per pot.

Treatments were arranged in a completely randomized design. Five replicates were used for each treatment. Each replicate consisted of three plants. Pots were irrigated as needed and fertilized every three weeks Greinzet NPK solution (50 ml/10 liters water) either added to the soil or sprayed on the leaves (50 ml per pot).The experiment was terminated 60 days after planting.

The percentage of disease incidence was recorded using the formula Disease incidence = (Number of diseased plants/number of plants assessed) x 100.

The severity of the disease was scored using 1-5 scale developed by (DeCal *et al.*, 1995):

1- All leaves green
2- 2- Lower leaves yellow
3- Lower leaves dead
4- lower leaves dead and upper leaves wilted
5- Dead plants

The data on plant height, fresh shoot weight and fresh root weight were also recorded.

Statistical analysis.

Average data, obtained from the two seasons, were subjected to analysis of variance (ANOVA) using Costat software. The mean differences were compared to Duncan's Multiple Range Test (DMRT).

RESULTS

Results in Table (1) indicate that all tested plant materials and *Streptomyces* spp. significantly reduced the disease incidence of *Fusarium oxysporum* with different degree. The highest reduction in disease incidnce was recorded by *D.stramonium* at rate (2%) 83.34 % followed by *D .stramonium* (1%) and *N.oleander* (2%) 75%, while the lowest reduction was recorded by A.cepa peel at rate (1%) 50% compared to 83.33% in *F.oxysporum* treatment only. Also, disease severity was significantly reduced by application of plant materials amendment and *Streptomyces* spp. (Table. 1). The greatest reduction was achieved by *D. stramonium* (2%) 0.97 then *D .stramonium* (1%) 1.11, while the least reduction was 1.99 by A. cepa peel (1%). *F .oxysporum* treatment showed 3.94 disease severity .

As showed in Table. (2) All treatments significantly increased pepper plant height compared to pathogenic inoculated control only. The height was between 26.92-29.46 cm, while it was 24.30 cm in *F.oxysporum* treatment. The most effective treatments was *D.stramonium* (2%) followed by *S. phycochromgenes*, while the least effective was observed by *A.cepa* peel(1%). Likewise, all treatments significantly increased shoot and root fresh weight (Table 2). The shoot fresh weight was between 28.13 and 32.48 g and root fresh weight was between 3.04 to 4.08 g after application amendments and bacteria to the soil, while it was 26.13g (shoot weight) and 2.89 g (root weight) at pathogenic fungi treatment only.

DISCUSSION

The application of the *Streptomyces* spp as biocontrol agents, *D.Stramonium, N,oleander* dry leaves powder and *A. cepa* peel dry powder as soil amedment controlled *Fusarium* wilt fungus by reduced disease incidence as well as disease severity under greenhouse condition and in turn increased plant growth.

Streptomyces might inhibit pathogens in soil via several mechanisms i.e.; antibiosis, nutrient competition, production of extracellular hydrolytic enzymes (Deshpande *et al.,* 1988 Nahadevan and Crawford, 1997 and Mahmoudi *et al.,* 2011) or produce nitrous oxide which activate plant defenses against pathogens. (Cohen *et al.*, 2005). Because of their ability to produce spores, *Streptomyces* spp. are resistant to desiccation and nutrient stress. These characteristics make these bacteria attractive candidates for biological control agents against soil-borne plant pathogens (Samac and Kinkel, 2001). The structure of S. *exfoliatus* antifungal compound is diphenethyl tetradecahydrophenazine-2,8-dicarboxylate. This compound belongs to

phenazine derivatives, a group of compounds are known to possess a broad-specturm of antibiotic activity toward bacteria and fungi.(Saba, Heba, 2012)

Organic matter amendment to soil has beneficial effects on soil nutrients, soil physical conditions, soil biological activity and crop viability (oka, 2010). Many plant diseases such as *Vertticillium* wilt of cotton (Huang *et al*, 2006) and *Fusarium* root and stem rot of cucumber (Pavlou and Vakalounakis, 2005) have been controlled with the incorporation of plant materials to the soil. Furthermore, some plant materials have good effects on yield when incorporated into soil (Ingemarsson, 2004). *N.oleander* (shoot plus leaves) and *D. metel* (seeds) have the potential of controlling RKN and can be efficiently used as soil amendments (Mohammad, 2012).

Table (1): Effect of plant materials amendment and *Streptomyces* spp on *Fusarium* wilt disease incidence and disease severity on pepper plants.

Treatment	Disease incidence (%)	% of Reduction	Disease severity (1-5)	% of Reduction
S. phycochromgenes	41.66^{c}	50.20	1.89^{bc}	52.03
S. *exfoliatus*	33.33^{cd}	60.11	1.58^{e}	59.89
D. stramonium (1%)	25.00^{de}	70.11	1.11^{fg}	71.82
D. stramonium (2%)	16.66^{e}	80.08	0.97^{g}	75.38
N.oleander (1%)	33.33^{cd}	66.64	1.63^{de}	58.62
N.oleander (2%)	25de	70.11	1.24^{f}	68.52
A.cepa peel (1%)	50^{b}	40.23	1.99^{b}	49.49
A.cepa peel (2%)	41.66^{c}	50.20	1.74^{cd}	55.03
F.oxysporum (only)	83.66^{a}	-	3.94^{a}	-
Control**	0^{f}	-	0^{h}	-

Control**: Healthy plants
*Duncan's multiple range test was used. Values followed by the same letters are not significantly differed ($p \leq 0.05$)

Table (2): Effect of plant materials amendment and *Streptomyces* spp on some vegetative parameters of pepper plants.

Treatment	Plant height (cm)	% of Increase	Fresh shoot weight (g)	% of Increase	Fresh root weight (g)	% of Increase
S. phycochromgenes	29.07^{b}	19.62	28.70^{e}	9.83	3.50^{e}	21.10
S. *exfoliatus*	28.08^{bc}	15.55	31.08^{c}	18.94	3.81^{d}	31.83
D. stramonium (1%)	27.98^{bc}	15.14	32.04^{b}	22.61	4.06^{c}	40.48
D. stramonium (2%)	31.46^{a}	29.46	29.67^{d}	13.54	3.39^{e}	17.30
N.oleander (1%)	27.44^{bc}	12.92	32.48^{b}	24.30	4.61^{b}	59.51
N.oleander (2%)	29.06^{b}	19.58	30.73^{c}	17.60	4.29^{c}	48.44
A.cepa peel (1%)	26.98^{c}	11.02	29.22^{f}	11.82	3.34^{c}	15.57
A.cepa peel (2%)	26.92^{c}	10.78	29.22^{f}	11.82	3.04^{f}	5.19
F.oxysporum (only)	24.30^{d}	-	26.13^{g}	-	2.89^{f}	-
Control**	32.34^{a}	-	34.77^{a}	-	4.61^{b}	-

Control**: Healthy plants
Duncan's multiple range test was used. Values followed by the same letters are not significantly differed ($p \leq 0.05$)

While decomposing in soil, *N.oleander* and *D. stramonium* leaves release compounds; glycosoides, alkolides(hyoscine -hysocyamine)These compounds have inhibiting effects on plant pathogenic fungi(Hussain, 1981).

As with the *Brassicaceae, Alliums* pp. produce numerous sulfur- containing chemical products, including volatile compounds arising via cleavage of certain s-alk(en)yl cysteine sulphoxides(Jones *et al*, 2004).which can act upon a variety of soilborne bests, including fungi, bacteria and nematodes (Clarkson et al, 2006).*A. cepa* peel showed good results in controlling *Fusarium* wilt fungus. These results may be referred to flavonoids compound in the peel, which inhibited the pathogenic fungi (Hasen, 2000).

In conclusion, the present study demonstrated that some plant materials amendment and two isolates of *Strptomyces* can be used for control of pepper *Fusarium* wilt. Thus, this method can contribute to minimizing the risk and hazards of fungicides. Further research should be done to use these amendments and biocontrol agent under field condition.

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مقاومة مرض الذبول الفيوزاريومى فى الفلفل بأستخدام بعض الاضافات النباتية و بكتريا الأستربتوميسس

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يعتبر مرض الذبول الفيوزاريومى من أهم الأمراض التى تصيب محصول الفلفل حيث يتسبب فى خسائر اقتصادية كبيرة للمحصول الناتج. تم استخدام ثمانى معاملات خلال الدراسة و هى البودرة الجافة لكل من اوراق نباتى الداتورة و الدفلة و قشر البصل الأحمر بتركيزى ٢% و ١% من وزن التربة و ايضا استخدم عزلتين من بكتريا الأستربتوميسس (٤٥ مللى من معلق البكتريا ١.٨ * ١٠^{٧} خلية لكل مللى) فى كل اصيص لدراستها على مقاومة المرض و بعض الصفات المرتبطة بنمو النباتات. و قد أوضحت النتائج أن جميع المعاملات كان لها تأثير فعال فى مقاومة المرض من حيث خفض الشدة المرضية و نسبة حدوث المرض و كاننت أفضل المعاملات كفاءة هى بودرة أوراق الداتورا بتركيز ٢ % (٨٠.٨ – ٧٥.٣٨ % انخفاض فى نسبة حدوث المرض و الشدة المرضية على التوالى). بينما كانت اقل المعاملات كفاءة هى بكتريا استربتوميسس فيكوكروموجين (٥٠.٢٠- ٥٢.٠٣ % انخفاض فى نسبة حدوث المرض و الشدة المرضية على التوالى). ايضا كانت للمعاملات دور واضح فى زيادة بعض الصفات الخضرية لنمو للنباتات وكانت أفضل زيادة فى طول النبات عن طريق بودرة أوراق نبات الداتورا بنسبة ٢٩.٤٦% . بينما كانت بودرة أوراق الدفلة الأفضل فى زيادة الوزن الطازج للمجموع الخضرى بنسبة ٣٢.٤٨ % و الوزن الطازج للمجموع الجذرى بنسبة ٥٩.٥١% . و كانت أقل المعاملات كفاءة هى بودرة قشر البصل بتركيز ٢% بنسبة ١٠.٧٨% فى طول النبات – الوزن الطازج للمجموع الخضرى بنسبة ٢٩.٢٢% – الوزن الطازج للمجموع الجذرى بنسبة ٥.١٩ %.