J. Agric. Sci. Mansoura Univ., 26 (11): 6867 - 6875, 2001.

BIOLOGICAL AND CHEMICAL CONTROL OF SOYBEAN DAMPING-OFF

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ABSTRACT

An aggressive isolate of *Fusarium solani* was obtained from damped-off soybean plants cultivated in the experimental farm, Faculty of Agriculture, Alexandria.

Pathogenicity tests show that the isolated *F.solani* cause damping-off symptoms. The seedling emergence of the two soybean cultivars, Crowford and Clark was reduced by 79.5, 82.7 % respectively. Tric*hoderma harzianum* and Vitavax-Thiram reduced the damping-off percentage and increased the fresh and dry weight of the two tested cultivars. No significant difference was observed in the treatment with *T. harzianum* and Vitavax-Thiram.

INTRODUCTION

Soybean (*Glycine max L.*) is a popular crop in Egypt. Seedling disease complex of soybean causes annual economic losses in U.S.A. (Sinclair,1982). *Fusarium* spp. are the major fungal pathogens, Leao, et al.,(1998); Romero, et al.,(2000); Sinclair,(1982) and Warren and Kommadahl, (1973).

Nelson and Windels (1992) reported that 10% of 476 *Fusarium* isolates from soybean roots collected from Minnesota and North Dakota in U.S.A. were *F. solani* (Mart.) Appel & Wollenweb. emend. Synd. & Hans. They added that *F. solani* is frequently isolated from soybean seedlings, but there are conflicting reports regarding its pathogenicity (Grant, et al.,(1981) ; Schlub, *et al.,* (1981) and Zakaria, (1981). French and Kennedy (1963), isolated *F. solani* from soybean roots but those isolates were non- -pathogenic. Other investigators have reported *F. solani* causing root rot of soybean Killebrew, *et al.,* (1988); Killebrew, *et al.,* (1993) and Nelson *et al.,* (1997). Killebrew et al. (1993) mentioned that root rot and damping – off was the most prevalent caused by *F. solani* isolates when inoculated on soybean. Also, Nelson et al. (1997) Indicated that isolates of *F. solani* from Minnesota and North Dakota cause root rot on soybean and cultivars grown in this region lack resistance to this pathogen, and these isolates were not identical to those associated with sudden death syndrome disease (SDS). The most work done has focused on SDS (Jin *et al.,* 1996 ; Luo *et al.,* 2000; Pennwpacker,1999 and Roy *et al.,* 1989). There have been few in-depth studies on the biology or importance of root rot of soybean caused by isolates of *F. solani* that do not cause SDS (Killebrew, *et al.,* (1993) ; Killebrew, *et al.,* (1988) Leao *et al.,* (1998); Romero et al.,(2000) and Roy *et al.,* (1989).

Major symptoms of SDS include root rot, crown necrosis and vascular discoloration of roots and stem, but leaf symptoms are the most

conspicuous phase of the disease, these include interveinal chlorosis and necrosis, defoliation and pod abortion (Luo *et al.,* 2000 ; Pennypacker, 1999 and Roy *et al.,* 1989). Symptoms typical of SDS have not been reported in Egypt, although root rot and seedling damping-off caused by *F. Solani* has been observed.

The role of antagonistic microorganisms in the control of plant diseases as a seed treatment has been applied. Pieta (1998), used the spores of *Trichoderma* spp. as a seed treatment of soybean, pea and common bean against infection by soil borne pathogenic fungi. Also, Pastucha (1998), mentioned that *Trichoderma* spp. was the most effective fungal in protecting germinating seeds and roots of soybean plants.

The purposes of this research were to identify symptoms produced by *F. solani*, compare the efficiency of biocontrol seed treatment with *T. harzianum and the fungicidal treatment .*

MATERIALS AND METHODS

Isolation was carried out from damped-off soybean seedling Crowford and Clark cultivars. Seedlings were washed under current tap water, cut into small pieces, surface sterilized by dipping for 3 minutes in 1% sodium hypochlorite solution, plated on PDA medium, then incubated at 25°c. Isolation was also made from seeds of the two forementioned cultivars. The obtained *Fusarium was identified* based on description reported by Booth, (1971) and Nelson et al. (1983).

Seed Health Testing: -

Seed samples of soybean cultivars were obtained from Agricultural Res. Center Giza, Egypt. Two hundred seeds from each sample were tested by the standard agar method according to the International Seed Testing Association (I.S.T.A.), (1966). For isolation purposes seeds were washed in running tap water for 10 minutes, then surface sterilized in 1% sodium hypochlorite solution for 3 minutes, placed on PDA medium at the rate of 10 seeds/dish, and incubated at 20°C for seven days. The developed colonies were examined using the compound microscope. The infection percentage was recorded.

Natural Infection: -A field experiment was conducted in The Experimental Farms, Faculty of Agriculture, Alexandria University during growing seasons 1999 and 2000. The field plot 4x15 meters was divided into twenty-five rows. The rows were sown with ether soybean seeds Crowford or Clark cvs. at the rate of 10 seeds/row. Four replicates were done from each cultivar. Cultivars were arranged in completely randomized design (CRD). Number of damped-off seedlings were recorded after 21 days, Isolation was made from the naturally infected seedlings.

Data were statistically analyzed using analysis of variance in CRD, Steel and Torrie (1980).

Pathogenicity Tests: -

For Pathogenicity tests, *Fusarium* isolate was grown on sterilized barley grains in 250 ml flasks for 10 days. Inoculum was transferred from the flasks to autoclaved aerated potted soil at the rate of 5g/15cm pot, the Inoculum was mixed with the soil, watered every day for 10 days, then sown with soybean seeds at the rate of 10 seeds/pot of either Crowford or Clark cvs.. Ten replicates (pots) were served for each cultivar. In check treatment, the seeds were sown in autoclaved aerated soil . Pots were arranged in CRD on greenhouse Pre- and Post- emergence damped-off were recorded after 21 days.

Biological Control: -

1. *In vivo*,tests: 7 mm diameter were taken from the growing margin of *Fusarium* and *Trichoderma harzianum* , transferred to PDA medium in 9 cm diameter petri dishes. Inocula of the antagonist and *Fusarum* were placed on opposite side PDA medium. *Fusarium* discs were plated 2 days after *T. harzianum* . Inhibition zone was measured.
2. *In vitro* tests : The isolated *Fusarium* was grown on autoclaved barely grains for 10 days and then applied to the sterilized soil at the rate of 5g/15cm pot. *T. harzianum* was grown on PDA medium at 25°c for 7 days then flooded with sterile water, conidia were gently harvested from the culture surface with a brush. The concentration of conidia was adjusted to 10^8 conidia/ml with the aid of hemocytometer. Seeds of the two tested soybean cultivars were coated with *T. harzianum*. The treatments were: 1) Pots containing sterilized soil and non-treated seed , as check 2) Pots with *Fusarium* only and non-treated seed , 3) Pots which received the pathogen and seeds were coated with *T. harzianum*, 4) Pots that received the pathogen and seeds were coated with Vitavax-Thiram at the rate of 3g/kg seeds. Ten replicates were used. Number of damped-off seedlings, fresh and dry weight was calculated . Treatments were arranged in CRD and statistically analyzed.

RESULTS

Isolation and Identification:

Isolation trials from both damping-off soybean seedlings and seeds of Crowford and Clark cvs yielded *F. solani* (Nelson et al.,1983).

Seed Health Testing:

Data Table1, show that *F. solani* was detected on the seed of the two soybean tested cultivars. Clark cv. showed the highest infection percentage (11%), while the lowest (7%) were found in Crowford cultivar. Associates *Alternaria* spp., *Colletotrichum* sp., *Macrophomina* sp., *Aspergillus* spp. and

Penicillium spp., were obtained from the two tested cultivars, and were detected on Clark cv..more than Crowford cv.

Natural infection:

1. No significant difference was detected in Pre-emergence damping-off among the two tested soybean cultivars ,on the other hand ,post emergence was higher in Clark cv .

Table 1: Fungi isolated from the two tested soybean cultivars on PDA medium (200 seeds/cultivars)

Fungi	Infection percentage (%)	
	Crowford cultivar	Clark cultivar
Fusarium solani	7	11
Alternaria spp.	4	8
Colletotrichum sp.	2	4
Macrophomina sp.	2	3
Aspergillus spp.	3	5
Penicillium spp.	2	3

Table 2: Pre-and post-emergence damping-off of Crowford and Clark cvs. 21 days after sowing.

Cultivar	Pre-emergence damping-off	Post-emergence damping-off	Total	Survival
Crowford	22.3 a	8.7b	31.3a	197.0 a
Clark	19.3 a	23.3 a	42.6 b	170.4 a

Means of ten replicates.
Means in a column followed by the same letter are not significantly different (p=0.05) according to Duncan's multiple range test.

Pathogenicity Tests:

Artificial inoculation carried out in pots, elucidate that, *F. solani* reduced seedling stand in Crowford and Clark cvs. It causes, pre-and post-emergence damping-off root rot within 3 weeks to soybean seedling grown in the greenhouse. Reisolation tests from diseased seedlings of the tested cultivars proved the presence of the pathogen.

Biological Control:

1. *In vitro*: no inhibition zones were observed in case of *T. harzianum* and *F. solani*. Microscopic preparations from the meeting line of *F. solani* and *T. harzianum* showed abnormal growth of *F. solani*, as a result of hyphal collapse .

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2. In vivo: data Table 3, indicated the following: pot experiment. The results, presented in Table3, indicated the following: -
   1) Coating soybean seeds with *T. harzianum* or Vitavax reduced damping-off from 79.5% to 31.3, 30.1% respectively in Crowford cultivar, and from 82.7% to 29.6, 30.9% respectively in Clark cultivar when planted in infested soil inoculated with *F. solani, the no. of healthy seedling was also increased*.
   2) No significant difference was detected in seedlings fresh or dry weight in both the two tested cultivars grown in infested soil when the seed treated with *T. harzianum* or Vitavax-Thiram.

DISCUSSION

The present work showed that *F.solani* is a seed borne pathogen. Seed health testing showed that the fungus was detected on Clark and Crowford soybean cultivars, the infection percentage reached 11, 7% respectively.

Pathogenicity tests showed that the infection by *F. solani* induce root rot and damping-off symptoms this is in the line with Killebrew,et al.,(1988).
Roy, *et al.,* (1989) mentioned the presence of two morphological distinct forms of *F. solani*, designated FS-A and FS-B,isolated from soybean plants. FS-A caused the symptoms characteristics of SDS, FS-B caused root rot and reducing stand, but no other symptoms characteristics to SDS. The results obtained in pathogenicity tests show that isolate of *F. solani* isolated from soybean in Egypt is highly aggressive on the two tested soybean cultivars(79.5, 82.7%) and induce SDS symptoms, this is in the line with Romero, et al. (2000).Many investigators studied the role of *Trichoderma* spp. in protecting soybean plants ,Pastucha(1998) and pieta(1998). Pastucha (1999) found that isolates of *T. Koningii* and *T. Viride* were antagonistic to pathogenic *F. solani*. Data obtained show that seed coated by *T. harzianum* or Vitavax reduced damping-off percentage in the two soybean tested cultivars, and increased the plant fresh and dry weight . But no significant difference were observed among T.*harzianum a*nd Vitavax treatments in infested soil. Further studies on biological control of *F.solani* which do not cause SDS is needed.

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تمكن الباحثون من الحصول على عزلة من الفطر فيوزاريوم سولانى تسبب موت بادرات فول الصويا وذلك من العينات التى تم الحصول عليها من مزرعة كلية الزراعة بالإسكندرية٠ أوضحت القدرة المرضية لعزلة هذا الفطر أنها ذات قدرة مرضية عالية حيث أحدثت موت لبادرات صنفي فول الصويا كراو فورد و كلارك تحت ظروف الصوب الزجاجية كما أنها قللت من عدد البادرات بنسبة ٧٩,٥ و ٨٢,٧ %على التوالى.

اتضح من تجارب الصوب الزجاجية آن المعاملة بالفطر ترايكودرما هارزيانم او الفيتافكس أديا إلى خفض نسبة الإصابة بموت البادرات لصنفي فول الصويا المستخدمان في الدراسة وكذلك زيادة كل من الوزن الرطب و الجاف لكليهما٠