STUDIES ON SEEDLINGS DAMPING-OFF AND ROOT ROT DISEASES OF PEA I. DISEASE AGENTS AND VARIETAL RESPONSES

BY

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ABSTRACT

Isolatation from damping-off pea seedlings was carried out from different locations of Alexandria and El-Behaira governorates. The obtained fungal isolates were purified and identified as *Pythium debaryanum* (PD), *Rhizoctonia solani* isolates (RSI and RSII), *Fusarium solani* isolates (FSII and FST). All tested fungal isolates were proved to be pathogenic producing different degrees of pre- (PRD), and post-emergence (PTD) damping off and root rot symptoms on pea cultivars, namely; Victory Freezer (VF), Lincoln (L), Masher-B (M-B) and Sinnary (S). The highest PRD and PTD values were obtained in case of Lincoln pea cultivar, whereas Sinnary cultivar was less compatible with the tested damping-off agents. *F. solani* I and *R. solani* II were more pathogenic as PRD agents, whereas the highest PTD values were obtained in case of *P. debaryanum* (56.79).Three categories of root rot infection % were identified; 1st category: > 85% (PD); 2nd category: 70-84% (RSI, RSII and FSI); 3rd category: 55-69% (FSII and FST).

INTRODUCTION

Pea (*Pisum sativum* L.) is considered one of the most important leguminous crops cultivated not only in Egypt but also in many other countries all over the world. It is considered as an economical crop due to its high protein content, balanced amino acid composition and good digestibility.

According to statistical estimates carried out in 2002 season (The Annual Statistical Book, 2002), cultivated area of pea in Egypt was about 55997 feddans, whereas the total peas crop produced was about 352267 tons.

Pea plants are commonly exposed to attack by many serious soil-borne fungi, i.e. *Aphanomyces euteiches, F. semitectum, Fusarium solani, Pythium debaryanum, P. ultimum, P. dissotocum, P. oligandrum, P. violae, Rhizoctonia solani, , Sclerotinia sclerotiorum* and many species of *Verticillium* and *Cladosporium*. (Chen and McBeath, 1993 and King and Parke, 1993). Most of them cause damping-off and root rot diseases (Abada *et al.*, 1992), leading to great economic losses in crop yield and quality.

Therefore, the objectives of this work were to: (1) Survey the most common damping-off and root rot fungal agents of pea in Alexandria and Behera governorates; (2) Check the relative pathogenicity of such agents; (3) Study the susceptibility and resistance of some common cultivated pea cultivars to infection with the isolated damping-off and root rot pathogens.

MATERIALS AND METHODS

Collection of samples

Samples of pea (*Pisum sativum* L.), showing different degrees of root-rot and damping-off symptoms were collected from different locations in Alexandria governorate, i.e. El—Sabahia and the farm of Faculty of Agriculture and in Beheira governorate, i.e. Noubaria Research Farm, Moderiet El-Tahrir and Abo-El-Matamir regions.

Isolation and identification

Discolored roots were cut into small fragments, surface sterilized by immersing them in 1% sodium hypochlorite for 2 minutes and then washed several times in sterilized distilled water. Surface sterilized root fragments were dried between two sterilized filter papers then transferred to potato dextrose agar (PDA) medium (4 pieces/dish). Plates were incubated for 5 days at 25 ±

2°C. Any developed fungus was transferred to new PDA plates. Hyphal tips or single spore technique were used for purification. Pure cultures were kept on PDA slants at 5°C.

Identification of the isolated fungi was carried out, then identification was verified by the phytopathological staff of Plant Pathology Research Institute, Agricultural Research Center (ARC) Sabahia, Alexandria, Egypt. Using characteristics of mycelia and spores of fungi as described by Gilman (1957) and Barnett and Hunter (1972).

Inoculation and determination of pathogenicity:

Throughout this study six fungal isolates from diseased pea plants were used, two of *Rhizoctonia solani*, two of *Fusarium solani*, one of *Pythium debaryanum* and one isolate of *Fusarium semitectum*.

These isolates were individually tested for their pathogenicity on Lincoln, Master-Bean, Victory Freezer and Sinnary pea cultivars under greenhouse conditions. Pots (20 cm in diameter) were sterilized by immersing them in 5% formalin solution (were left 3 weeks to allow formaldehyde evaporation) and filled with autoclaved aerated sandy clay soil (1:1 w/w). Fungal inocula were grown on sterilized barley grains- sand medium (30 gm barley grains + 10 gm sand + 30 ml water) at 25 ± 2°C for 2 weeks. Soil infestation was carried out using the inoculum of each fungus at the rate of 4% of soil weight. Inoculum was mixed thoroughly with the soil in each pot, watered and left for one week to secure establishment of the inoculated fungi. Control pots were filled with the same soil mixed with the same amount of sterilized barley grains-sand medium (non-infested soil). A set of four pots, with 10 seeds per pot, was used for each tested fungus. Pea cultivar seeds were surface- sterilized using 1% sodium hypochlorite solution for 2 minutes, washed with sterilized water, dried and sown at a depth of 2 cm and watered regularly every 3 days under greenhouse conditions.

Determination of the number of pre and post emergence damping-off, seedling survival and percentage of root-rot were recorded after 45 days from planting. Root-rot of diseased plants were determined by using 45-days old plants carefully removed, washed with tap water and examined for root-rot symptoms. Determination of the root-rot disease severity index (DSI) was carried out based on a scale from 0 (non visible damage) to 5 (completely destroyed roots) according to Salt (1981). Percentage of root-rot was recorded according to the formula.

$$\% \text{ Root - rot} = \frac{\text{No. of infected plants}}{\text{Total plant number}} X100$$

Statistical analysis

A completely randomized design with 4 replicates were used in the present study. Percentage data were transformed into arcsine angles (Snedecor and Cochran, 1981) before carrying out analysis of variance (ANOVA) to produce approximately constant variance. Least significant difference (LSD) at 5% level of probability was applied for comparing treatment means (Duncan, 1955).

RESULTS AND DISCUSSION

Isolation and Identification

Isolation and purification yielded a number of fungal isolates related to the genera *Pythium*, *Fusarium* and *Rhizoctonia*. Recovered isolates were preliminarily identified in the laboratory of Plant Pathology, Dept. of Agricultural Botany, Faculty of Agriculture, Saba Bacha, Alexandria University. Preliminary identification was carried out according to cultural and morphological characteristics given by Booth (1971), Ellis (1971) and Gillman (1957). The obtained fungal isolates were identified as one isolate of *Fusarium semitectum* (FST), two isolates of *Fusarium solani* (FSI and FSII), one isolate of *Pythium debaryanum*, and two isolates of *Rhizoctonia solani* (RSI and RSII). Identification was verified by the help of the phytopathological staff Plant Pathology Research Institute, Agricultural Research Center (ARC), Giza, Egypt.

These fungi were recorded as damping-off and root rot pathogens of pea (Harman *et al.*, 1981 and 1989; Burke and Miller, 1983; Kraft and Papavizas, 1983; Shehata *et al.*, 1983; King and Parke, 1993; Bowers and Parke, 1993). Moreover, most of these fungi were isolated from many vegetable and field crops, other than pea, as damping-off and root rot pathogens, (Omar, 1986; Harman *et al.*, 1989; Muyolo *et al.*, 1993; Stephens *et al.*, 1993; El-Gantiry *et al.*, 1994; Omar *et al.*, 1995; Ibrahim, 1996; Abdel Mageed and Zaghloul, 1997 and Mao *et al.*, 1998).

Pathogenicity and varietal responses

I. Damping-off incidence

A. Pre-emergence damping-off (PRD)

From data presented in Table (1) and illustrated in Fig. (1), the followings could be concluded:

(1) All the tested isolates induced significant pre-emergence damping-off symptoms on Victory Freezer pea cultivar, however, the infection percentage differed according to the tested isolate. Infection values were significantly higher in case of *Pythium debaryanum, Fusarium solani* I and *Rhizoctonia solani* I (39.23, 39.23 and 35.22, respectively) compared with control experiment (9.097). The least virulent isolate was *F. semitectum* (33.21).

(2) All the tested isolates induced (PRD) on Lincoln pea cultivar. The highest infection values were obtained by *F. solani* I and *R. solani* II (43.08 and 41.15, respectively), whereas *F. semitectum* treatment gave the least infection values (31.00).

(3) In Master-B pea cultivar, the highest pre-emergence damping-off occurred by *R. solani* II, *F. solani* I and *P. debaryanum.* Moreover, higher values of infection % were obtained with *R. solani* II (41.15%) followed by *F. solani* I (35.21%) and *P. debaryanum* (33.21%). The least infection value was induced by *F. semitectum.*

(4) Sinnary pea cultivar was significantly affected by the tested isolates, except for *F. solani* II, *R. solani* I and *F. semitectum* (26.57%).

Finally, it could be concluded that the most virulent isolates inducing pre-emergence damping-off were *Pythium debaryanum* and *Rhizoctonia solani* II and *F. solani* I isolates, whereas *F. semitectum* was the least virulent isolate tested. Moreover, Lincoln pea cultivar was the most compatible cultivar with PRD agents, especially with FS I and RS II compared with the other tested cultivars, whereas Sinnary pea cultivar showed the lowest compatibility.

B. Post-emergence damping-off (PTD)

Post-emergence damping-off infection index was estimated 20 days after planting according to the formula described in detail in the part of "Materials and Methods". Data were presented in Table (1) and illustrated in Fig. (1). The obtained results revealed the followings:

(1) The highest levels of infection percentage values of post-emergence damping-off (PTD) were obtained with *P. debaryanum* isolate. This was true for all the tested cultivars. Infection % values were 50.77 for Victory Freezer and 56.79 for Lincoln, Master-B and Sinnary, respectively.
(2) In addition to *P. debaryanum* each of Victory Freezer and Lincoln cultivar showed relatively higher rates of compatibility to *R. solani* II isolate. Infected percentage values were (37.22) and (33.21) for these cultivars.
(3) Both Master-B and Sinnary cultivars were relatively compatible to *F. solani* I isolate, which induced infection values 46.92 and 33.21, respectively.

Data obtained on the effect of the tested isolates on inducing post-emergence damping-off on different cultivars of peas indicated that *P. debaryanum* was the most virulent isolate in inducing post-emergence damping-off (PTD), compared with the other tested fungal isolates, whereas *F. solani* II and *R. solani* I isolates were the least virulent ones. This was true for all the tested cultivars.

C. Seedling survival

Seedling survival was estimated 45 days after planting different pea cultivars in soil inoculated with each of the six tested isolates. Data were presented in Table (1) and Fig. (1).

From data presented in Table (1) the followings could be concluded:

(1) All the tested cultivars inoculated with *P. debaryanum* showed survival not more than 9.097 value.

(2) The highest survival rates were recorded when Victory Freezer, Master-B and Sinnary cultivars were inoculated with *F. solani* II (48.93% and 50.77%, respectively) and as a result of inoculation of Victory Freezer, Lincoln, Master-B and Sinnary cultivars with *F. semitectum* (45, 45, 43.08 and 56.79, respectively).

These data showed that *P. debaryanum* caused not only pre-emergence damping-off but also severe post-emergence damping-off and severe decrease in percentages of surviving seedlings.

D. Root rot

An experiment was designed to measure the effect of the six tested isolates on inducing root rot symptoms on different pea cultivars. Root rot index was estimated 45 days after planting in inoculated soil.

Results were presented in Figs. (2). Data indicated that all the tested isolates could induce root rot symptoms on all the tested cultivars. However, root rot index differs according to the tested isolate and the inoculated cultivar. In this respect, three artificial categories could be recommended (based on the mean root rot index of each isolate on different tested cultivars): Category A, includes isolates that induce root rot on more than 85% of seedlings such as *P. debaryanum*; Category B, includes isolates that induce root rot on 70-84% of seedlings such as *F. solani* I, *R. solani* I and *R. solani* II; Category C, includes isolates that induce root rot on 55-69% of seedling such as *F. solani* II and *F. semitectum*.

Tab le (1) : Infection index of some pea damping-off and root rot pathogens on different pea cultivars.

Tested Isolates	Index values														
	Pre-emergence (PRD)					Post- emergence (PTD)					Survivor				
	Cultivars														
	Victory freezer	Lincoln	Master-B	Sinnary	Mean	Victory freezer	Lincoln	Master-B	Sinnary	Mean	Victory freezer	Lincoln	Master-B	Sinnary	Mean
Pythium debaryanum	39.23	33.21	33.21	33.21	34.72	50.77	56.79	56.79	56.79	55.29	9.097	9.097	9.097	9.097	9.097
Fusarium solani I	39.23	43.08	35.21	30.99	37.13	33.21	28.78	46.92	33.21	35.53	33.21	33.21	21.14	34.89	30.13
Fusarium solani II	31.00	33.21	26.57	26.57	29.34	23.76	26.57	26.57	26.57	25.87	48.93	39.31	50.77	50.77	47.45
Rhizoctonia solani I	35.22	33.21	31.00	26.57	31.50	26.57	26.57	26.77	18.43	24.54	43.08	45.00	46.92	63.43	49.61
Rhizoctonia solani II	33.21	41.15	41.15	35.21	37.68	37.22	33.21	33.00	26.57	32.50	35.22	24.73	30.29	54.78	36.26
Fusarium semitectum	33.21	31.00	28.78	26.57	29.89	26.57	28.78	33.00	18.43	26.70	45.00	45.00	43.08	56.79	47.47
Control	9.097	9.097	9.097	9.097	9.097	9.097	9.097	9.097	9.097	9.097	80.90	80.90	80.90	80.90	80.90
Mean	31.90	32.44	29.29	26.89		29.60	29.97	33.14	27.01		42.21	39.61	40.31	50.09	
L.S.$D_{0.05}$ (Fungi)	1.940					3.439					3.878				
L.S.$D_{0.05}$ (Varieties)	1.467					2.600					2.932				
L.S.$D_{0.05}$ (Interaction)	2.123					3.687					4.242				

* values are means of 4 replicates.

* Values are the arcsine square root of transformation percentage of data.

* V F: Victory Freezer ; L: Lincoln ; MB: Master B ; S: Sinnary

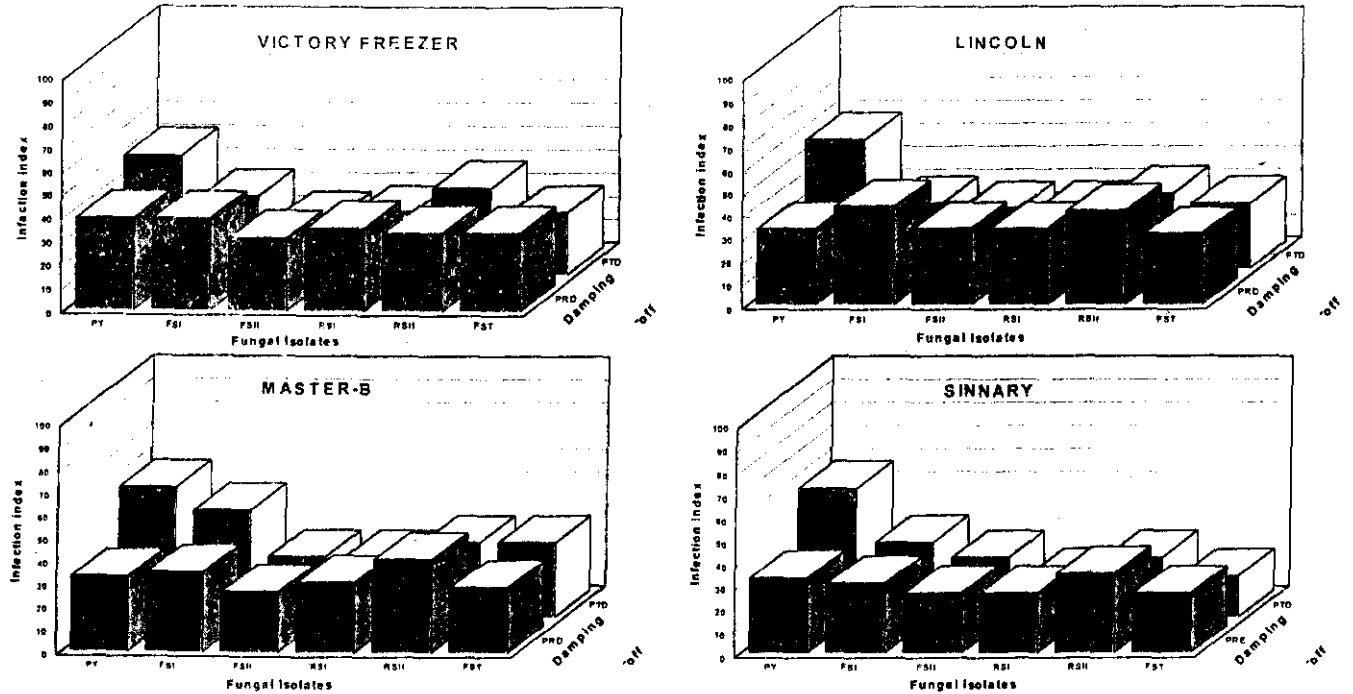

Fig. (1): Infection index of pre- and post-emergence damping-off agents on Victory freezer, Lincoln, Master-B and Sinnary pea cultivars. PRD = pre-emergence damping-off. PTD = post-emergence damping-off. PY : *Pythium debaryanum*; FSI : *Fusarium solani* I; FSII : *Fusarium solani* II; RSI : *Rhizoctonia solani* I; RSII : *Rhizoctonia solani* II; FST : *Fusarium semitectum*

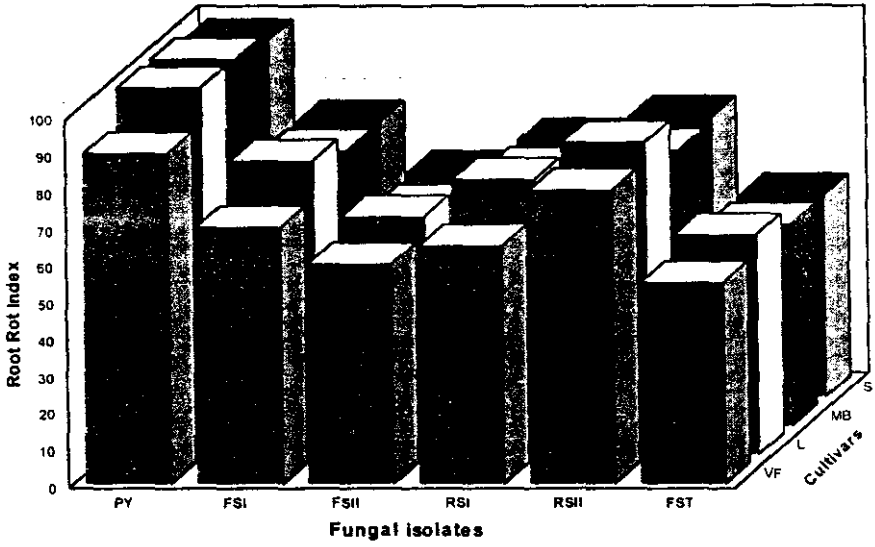

Fig. (2): Root rot index (%) of some root rot agents on different cultivars.

VF : Victory freezer; L : Lincoln; MB : Master-B; S : Sinnary. PD : *Pythium debaryanum*; FSI : *Fusarium solani* I; FSII : *Fusarium solani* II; RSI : *Rhizoctonia solani* I; RSII : *Rhizoctonia solani* II; FST : *Fusarium semitectum.*

The present study showed that *R. solani* was highly pathogenic causing high rates of PRD and PTD on pea seedlings. These findings were similar to those found on bean seedlings (El-Farnawany and Shama, 1996 and Abdel Mageed and Zaghloul, 1997) and Broad bean (Sabet *et al.*, 1998). *P. debaryanum* was the most virulent throughout this study in decreasing seedling survive ones, compared with the other tested isolates. Quiet similar observations were recorded by many authors (Kraft and Pepavizas, 1983; Wolffechel and Funckjensen, 1992; Abdel-Kader, 1997 and Sabet *et al.*, 1998).

Sinnary pea cultivar was the most incompatible with many of the tested pathogens, whereas Lincoln was the most compatible. Accordingly, the author recommend Sinnary pea seeds for the wide commercial production of pea in Egypt, in particular in the Northern governorates, where humid weather is prevailing.

Detailed symptoms produced due to inoculation with the isolated pathogens were in harmony with those recorded by Shama (1987 and 1988) on cowpea and Shama (1989) on bean.

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الملخص العربـــى

دراسات على أمراض الذبول الطرى وأعفان الجذور فى بادرات البسلة

١. المسببات المرضية و إستجابات الإصناف

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تـم العـزل مـن بادرات بسلة تعانى من الإصابة بالذبول الطرى وأعفـان الجـذور وذلـك مـن مناطق مختلفة من محافظتى الأسكندرية والبحـيرة. ولقـد تم تنقية العزلات الفطرية التى تم الحصول عليها وتم تعريفها وشملت بيثيوم ديباريانم ، ريزوكتونيا سولانى (عزلة I ، وعزلة II) ، فـيوزاريوم سولانى (عزلة I ، وعزلة II) ، فيوزاريوم سيميتكتم. ولقد أثبتت جميع العزلات الناتجة مقدرتها المرضية على إحداث مستويات مخـتلفة مـن الذبـول الطـرى ما قبيل وما بعد ظهور البادرات وكذلك أعـراض أعفـان الجذور وذلك على أصناف البسلة فيكتورى فريزر ، لنكولن، ماشر – ب ، سنارى. ولقد تم الحصول على أعلى نسبة من ذبول مـا قبل وما بعد ظهور البادرات على الصنف لنكولن بينما كان الصنف سـنارى أقل توافقاً مع مسببات الذبول الطرى. ولقد أثبتت كل من عزلة فـيوزاريوم سـولانى رقم (I) وعزلة ريزوكتونيا سولانى رقم (II) أنها أكثر العزلات مقدرة على إحداث أعراض ذبول ما بعد ظهور البادرات ، بيـنما أعطى البيثيوم أعلى نسبة إصابة ذبول ما بعد ظهور البادرات. تم تصـنيف إصابات أعفان الجذور إلى ثلاثة أقسام: القسم الأول أعلى من ٨٥% ويشـمل البيثـيوم ؛ القسـم الثانى من ٧٠-٨٤% ويشمل كل من عـزلات الريزوكتونـيا والعزلة رقم (I) من فيوزاريوم سولانى ؛ القسم الثالـث مـن ٥٥-٦٩% ويشمل كل من العزلة رقم (II) من فيوزاريوم سولانى وعزلة فيوزاريوم سيميتكتم. ولقد تسببت العدوى بالفطر بيثيوم إلى ظهور أقل نسبة من البادرات الناجية من الإصابة.