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BIOCONTROL OF PEA ROOT-ROT USING SELECTED MICROBIAL STRAINS

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ABSTRACT

Fusarium solani, *Rhizoctonia solani*, *Fusarium oxysporum*, *Sclerotinia sclerotiorum*, *Macrophomina phseolina* and *Sclerotium rolfsii*, were isolated from diseased pea root rot from different localities, i.e., Giza, Ismailia, Sharkiya, Qalubiya and Behiera Governorates. Results indicated that *R. solani* and *F. solani* were the most dominant in all Governorates. All the isolated fungi were pathogenic to pea plants, however, they varied in their virulence. In general, *R. solani* and *F. solani* were the most virulent.

Some salts, bioagents and antioxidants were used as seed soaking as compared with the fungicidal Rizolex-T to control root-rot disease of pea, under greenhouse and field conditions. Results indicated that all the tested materials reduced pre- and post-emergence damping-off and increased the survival of plants as well as their yield and improved their quality (pod characteristics). These materials varied in their efficiency. The standard fungicide (Rhizolex-T) was the best in controlling the disease followed by acetyl salicylic acid (ASA), sodium salicylate (as antioxidant) and monopotassium sulphate (as salt), which gave good effects in controlling pea root rot disease as well as increased pea yield survival. Meantime, *Pseudomonas fluorsecens* and *Bacillus subtilis* (as bioagents) were moderately effective with Ca 64%, 78% and 75% for *Serratia*, *Pseudomonas fluorsecens* and *Bacillus subtilis*, respectively in comparison with 53% for control. On the other hand, calcium chloride (salt) and *Serratia* (bioagent) were the least effective ones. The best materials were considered non harmful control methods, which have advantage. Therefore, such materials should be used as effective and safe means for controlling soil-borne plant pathogens in order to avoid environmental pollution through decreasing chemical fungicides use.

Keywords: Pea root-rot, biocontrol, *Bacillus subtilis, Pseudomonas fluorescens, Serratia.*

INTRODUCTION

Pea (*Pisum sativum* L.) is considered one of the most important and popular leguminous crops in Egypt. It is considered also locally as green and/or dry seeds because of its protein content (Abdel-Al *et al*., 1980). It gained importance for both local consumption and exportation purposes. Pea plants are attacked by soil-borne pathogenic fungi causing root-rot diseases which seriously affects both plant stand and yield production. Root-rot diseases caused by *Rhizoctonia solani* and *Fusarium solani* were reported by many investigators as the most aggressive fungi attacking pea plants during the growing season (Abada *et al.*, 1992; Afifi *et al*., 1995 and Rauf, 2000).

Root-rot disease appears during growing season as both pre- and post-emergence stages of plant growth (Abada *et al.*, 1992 and Afifi *et al.*, 1995). Fungicides could successfully control root-rot diseases, however, they negatively affect human health and environment (Rauf, 2000). Recently there are several attempts to use fungicide alternatives for controlling plant diseases. In this concern some slats as potassium chloride, monopotassium sulphate and potassium sulphate are reported to have antifungal activities against several fungi (Abd-Alla, 2003 and El-Mougy *et al*., 2004).

El-Mougy (2004) used salicylic acid (SA) or acetyl salicylic acid (ASA) as seed dressing or soil drench, which reduced root-rot infection of lupin plants under greenhouse conditions. Dennis and Guest (1995) reported that treatment of tobacco plants with aqueous acetylsalicylic acid (aspirin) and beta-ionone reduced severity of symptoms caused by *Phytophthora parasitica* var. *nicotiana*, the causative agent of black shank, and tobacco necrosis virus (TMV). Spraying of cucumber plants with ASA induced resistance against downy and powdery mildews and increased fruit yield per plant under commercial greenhouse conditions (Abdel-Sayed, 2000).

Biological control of soilborne plant diseases by application of specific microorganisms to seeds or planting materials has been investigated by several researchers (Amer and El-Desouky, 2000; Luze, 2001 and Hassanein *et al.*, 2006). The systemic resistance induced by rhizosphere bacteria was reviewed by Van Loon *et al.* (1998).

Moreover, the concept of PGRBR created by Luze *et al*. (1998) was intended to encompass both Plant Growth Bioprotecting & Promoting Rhizobacteria. Those are rhizobacteria that promote beneficial effects on plant growth. In addition, co-inoculation of legumes and such PGPBR led to an enhanced yield productivity (Dileep Kumar *et al.*, 2001 and Abdel-Wahab and Said, 2004).

Clean agriculture is now occupying non-tiny place on the map of crop production in view of hazardous impacts of agrochemicals including mineral fertilizers and pesticides on the environment and marketing quality of agricultural products. The present study aims at evaluating the efficiency of some slats, antioxidants and bioagents as seed dressing of pea to control pea root-rot disease under the greenhouse and field conditions to protect human health and prevent the pollution of the environment.

MATERIALS AND METHODS

1. Survey of root-rot diseases of pea plants in different Governorates

Root-rotted of pea plants were collected from different Governorates, i.e., Giza, Qalubiya, Ismailia, Behaira and Sharkiya to determine the frequency of each causative organism. Isolation of the causative organisms were carried out to confirm disease incidence.

2. Isolation and identification of the causative organisms

Infected roots were cut into small pieces, washed thoroughly with tap water, surface sterilized with sodium hypochlorite (5% chlorine) for one minute, washed several times with sterilized water and then dried between folds of sterilized filter paper. Infected pieces were then placed onto PDA medium and incubated at 28^{o}C for 3-5 days. The growing fungi were then transferred to Petri-dishes containing plain agar. Purification was carried out through the transfer of hyphal tip and/or single spore techniques (Brown, 1924 and Hawker, 1960).

Purified fungi were placed on slants of potato dextrose agar (PDA) medium and kept for further studies. Identification of the isolated fungi was carried out according to cultural properties, morphological and microscopical characteristics described by Gilman (1957); Barnett (1960) and Singh (1982). Identification was confirmed through the Dept. of Fungal Taxonomy, Plant Pathology Institute, ARC, Giza, Egypt.

3. Pathogenicity tests

Inocula were prepared by inoculating the isolates in autoclaved sand corn medium (25g clean sand, 75g corn and enough tap water to cover the prepared mixture in 500ml bottle) using agar discs obtained from the periphery of 7 days old colony of the pathogenic fungus and incubated at 25±2 °C for two weeks.

Pots (20cm in diam.) were sterilized by immersing them in 5% formalin solution for 15 minutes and then left to dry for 7 days before use. Each pot was filled with Nile silt soil (2 kg), then inoculated with any of the desired inocula at the rate of 5% of soil weight. The inoculum was thoroughly mixed with the soil and watered regularly for one week before planting to insure the distribution and growth of the inoculum (Whithehead, 1957). Pots used for control were filled with the same soil and mixed with the same sterilized amount of autoclaved uninoculated sand corn medium and treated as the same way. Eight pots were used for each treatment and pots were completely randomized in the greenhouse. Pea seeds (Progress No.9 cultivar) were surface sterilized by immersing in 0.2% mercuric chloride for 2 minutes, washed several times with sterilized distilled water and dried using sterilized filter paper. Five sterilized seeds were planted in each pot and covered with thin layer of the same soil.

Data were recorded as pre-emergence damping-off percentages 15 days after sowing. Post-emergence damping-off and survived plants were counted after 30 days from sowing.

Disease assessment

1. Percentage of pre-emergence damping-off was determined as:

$$\textbf{\% of pre-emergence} = \frac{\textbf{No.of ungerminated seeds/pot}}{\textbf{No. of sown seeds/pot}} \times 100$$

2. Percentage of post-emergence damping-off was determined as:

$$\textbf{\% of post-emergence} = \frac{\textbf{No.of dead seedlings/pot}}{\textbf{No. of sown seeds/pot}} \times 100$$

3. Percentage of survival plants was determined as:

% survived plants = %100 - pre-emergence - post-emergence %

4. Control agents

a. Biocontrol

Microbial inocula prepraration

For bacterial inocula, conical flasks (250 ml) containing 100 ml of the specific liquid medium (King's medium) **(**King *et al*., 1954), were inoculated with a loop full of the bioagent and incubated at (28-30°C) for 3 days. Bacteria inoculation was performed through the seed soaking process.

Serratia sp., *Pseudomonas fluorsecens* and *Bacillus subtilis* were tested for their efficacy in controlling the disease at the indicated rates of application.

Treatment with biocontrol agents

Three bacterial strains grown on nutrient agar were suspended in sterile distilled water, then suspensions were standardized to A° 600±0.15 (a concentration of approximately 3 x 10^6 cells ml^{-1}). Bioagent-water suspensions were added over-head soil as 300 ml pot^{-1}, this is to secure not less than 10^3 cells g^{-1}.

b. Chemical salts

Calcium chloride (4.0 g/L water), monopotassium sulphate (4.0 g/L water) and Potassium sulphate 4.0 g/L were used in this work.

c. Antioxidants

Acetyl salicylic acid (ASA) 20.0 mM/L, sodium benzoate 20mM/L and sodium salicylate 20 mM/L were used in this work.

d. Standard fungicide:

Rizolex/Thiram 3.0 g/L water.

5. Control root-rot disease of pea

a. Under greenhouse conditions

The abovementioned materials and bioagents were used as seed soaking for 30 min. of (cv. Progress No.9).

Nile silt soil was artificially separately infested with the inoculum of *R. solani* strain (3) and *F. solani* strain (1). Inocula of both virulent strains were prepared and added for each pot as mentioned before.

Infested soil was placed in pots (20 cm in diam.) and sown with disinfected pea seeds. Two pots were used for each replicate (four replicates were used for each treatment). Disinfected pea seeds were sown in artificially infested soil, and served as a check treatment. Five pea seeds of (cv. Progress No.9) were sown in each pot.

Percentage of root-rot disease incidence was calculated as mentioned before.

b. Under field conditions:

Ten treatments of different materials, i.e., salts, biocides and antioxidant were used to control root-rot disease under field conditions.

Salts included calcium chloride 4.0 g/L water; monopotassium sulphate 4.0 g/L and potassium sulphate 4.0 g/L). Biocides included 3.0 ml/L *Serratia* sp.; 4.0 g/L *Pseudomonas fluorsecens* and 4.0g/L *Bacillus subtilis*). Antioxidants were acetyl salicylic acid 20 mlM/L, sodium benzoate 20 ml/L and sodium salicylate 20 ml M/L. Fungicide (Rizolex-T 3.0 g/L water) was also used as standard.

Pea Progress No.9 cv. seeds were used. Seeds were soaked for 30 min in the abovementioned solutions of different materials and thoroughly mixed in polyethylene bags. Four replicates plots ($^1/_{400}$ /fed.) were used for each treatment. The experiments were carried out at Qalubyia Governorate for two successive seasons (2005 and 2006). Pre- and post-emergence damping-off were recorded as mentioned before.

Yield characteristics (pod length, pod weight, No. of pods/plant and total green yield (kg/plot) were determined.

Also, the increase of yield was calculated from this formula:

$$\textbf{\% Yield increase} = \frac{\textbf{treatment yield– check yield}}{\textbf{Check yield}} \times 100$$

Sensitivity of pathogens to biocontrol agents

Sensitivity of pathogen to the biocontrol agents was monitored on potato dextrose agar (PDA) medium using the previously mentioned agar plate inhibition zone technique. Two of fungal pathogens responsible for root-rot and wilt diseases in a variety of legume and non-legume crops were tested.

Here, Petri dishes containing PDA medium amended with (g.l^{-1}), peptone 3.0, $CaCO_3$ 0.2 and $MgSO_4$, 0.2 were inoculated with the pathogenic fungi in centralized sites. Each fungal strain was separately introduced into one agar plate. Five to eight of the bioagants were inoculated into the agar plates. Plates were inoculated for 3-5 days at 28^{o}C, and inhibition zones diameter were measured.

Statistical analysis:

The obtained data were subjected to analysis of variance (Steel and Torrie, 1960 and Neler *et al.*, 1985), whereas the differences between treatments were tested by the calculated Least Significant Differences (LSD) at 5% level.

RESULTS AND DISCUSSION

Data in Table (1) show that the six fungal pathogens isolated from infected pea roots in the inspected locations of the five Governorates, however, with different frequencies. *Rhizoctonia solani* followed by *Fusarium solani* recorded the highest frequency percentages. On the other hand, *Macrophomina phaseolina* and *Sclerotium rolfsii* were the least ones and recorded (5.37& 6.29) and (5.87 & 5.48) during both successive seasons (2005 and 2006), respectively. Generally, the frequencies of the isolated fungi were higher during the second season than the first one (Oyarzun *et al*., 1993 and Khafagi *et al*., 1995).

Pathogenicity test

Data in Table (2) indicated that all the tested fungi were highly pathogenic to pea plants (cv. Progress No.9). However, *R. solani* followed by *F. solani* proved to be the most virulent ones resulting in highest percentages of pre- and post-emergence damping-off, subsequently, the lowest percentages of healthy survived plants. These results are in agreement with those of (Abada *et al*., 1992 and Faris *et al*., 1992).

T1

Table (2): Effect of isolated fungi on percentage of pre- and post-emergence damping-off as well as survived of pea plants cv. Progress No.9

Tested fungi	% pre-emergence damping-off	% post-emergence damping-off	% survived plants
Fusarium oxysporum	57.50	27.50	15.00
Fusarium solani	57.50	30.00	12.50
Sclerotinia sclerotiorum	50.00	27.50	22.50
Macrophomina phaseolina	35.00	17.50	47.50
Rhizoctonia solani	67.50	25.00	7.50
Sclerotium rolfsii	27.50	22.50	50.00
Check (without fungus)	0.00	0.00	100.00
LSD at 5%:	**1.78**	**1.96**	**1.51**

Greenhouse experiments

Different materials (salts, bioagents and antioxidants) compared with the standard fungicide Rhizolex-T were applied as seed soaking to evaluate their efficiency in controlling pre- and post-emergence damping-off of pea under greenhouse conditions. Data in Table (3) indicate that all treatments significantly reduced the percentages of occurrence of pea root-rot caused by *R. solani* and *F. solani* as pre- and post-emergence damping-off stages. Results also, indicate that the most effective treatments were Rizolex-T (standard fungicide) followed by acetyl salicylic acid (ASA), monopotassium sulphate and sodium salicylic. They raised the survival of plants infected with *Rhizoctonia* to 62.5, 57.50, 55.00 and 52.50%, respectively, compared with the check control (untreated) which gave 27.50% of survived plants. Meanwhile, the survival of plants infected with *Fusarium* were, 72.50, 65.00, 60.00 and 57.50% using Rizolex-T, acetylsalicylic acid monopotassium sulphate and sodium salicylate respectively, compared with control (check treatment) which gave 20.00% only.

Data in the same table indicated that the percentages of root-rot infection caused by *R. solani* showed higher disease readings than that recorded by *F. solani* in all treatments. Results also showed that calcium chloride and *Serratia* sp. gave the lowest survival pea plants. Meantime, *Bacillus* and *Psuedomonas* (as bioagents) were moderately effective. Similar results were found by Abd-El-Kader and Ashour (1999), Attia *et al.* (1997) and Bauf (2000).

Field experiments:

The promising treatments in the greenhouse experiments in addition to Rizolex-T at the rate of 3g/L for soaking seeds were applied during two successive seasons 2005 and 2006 to evaluate their efficacy against root-rot infection and producing green yield of pea plants under field conditions. The applied treatments during both seasons showed similar trend in reducing the incidence of pre- and post-emergence damping-off. The averages of the obtained results are present in Tables (4 and 5).

Data in Table (4) indicate that all the applied treatments could reduce the percentage of root-rot infection significantly in both pre- and post-emergence damping-off compared with untreated check.

T3-4

t5

The highest survived plants were observed with Rizolex-T followed by ASA, monopotassium sulphate and sodium salicylate (94.50, 87.00, 83.25 and 80.25%) and (95.25, 89.25, 85.50 and 82.75%) during the first and second seasons, respectively. Calcium chloride and *Serratia* sp. were the least effective ones. At the same time, *Bacillus* sp. and *Pseudomonas* sp. were moderately effective.

Reduction in disease occurrence increased in plant stand which reflected on the obtained green yield and improved pod characteristics during the two seasons. Results in Table (5) also indicate that Rizolex-T followed by ASA, monopotassium sulphate and sodium salicylate reduced root-rot occurrence followed by increasing green yield and improving yield characteristics (pod length, pod weight and No. of pods/plant), as compared with the untreated check during both successive seasons (2005 and 2006)**.** These results are in agreement with those obtained by El-Gamal *et al*. (2003), El-Mougy (2002& 2004) Ibrahim *et al*. (2003) and El-Mougy *et al*. (2004).

Results in the present study indicate that the tested materials such as salts, bioagents and antioxidants gave good control results. Rizolex-T as a standard fungicide against pea root-rot suppressed them. However, the later has a high risk due to environmental pollution and residual effects. The alternative materials are considered non harmful control methods which have many advantages. Therefore, such materials could be used as effective and safe method for controlling soil borne plant pathogens in addition to the avoidance of environmental pollution. The use of such fungicides alternatives may improve the control of soil borne diseases and should be considered in further investigations.

REFERENCES

Abada, K.A.; Aly, H.Y. and M.S. Mansour (1992). Pathological studies on damping-off and root-rot diseases of pea in ARE. Egypt. J. Appl. Sci. 7(9): 242-261.

Abdel-Al, Z.E.; A. Khalafallah; M. El-Shal and M. Abdel-Kader (1980). Vegetables, Part II: Protection. Dar-El-Matbouat El-Gadida, Alex., Egypt.

Abd-El-Kader, M.M. and A.M.A. Ashur (1999). Biological control of cowpea root-rot in solarized soil. Egypt, J. Phytopathol. 27: 9-18.

Abdel-Sayed, M.H. (2000). Studies on powdery midew disease of cucurbits under protected cultivation. M.Sc. Tesis, Fac. Agric., Cairo Univ. 108 pp.

Abdel-Wahab, A.F. and M.S. Said (2004). Response of faba bean to bio- and organic fertilization under calcareous soil conditions. Egypt. J. Appl. Sci. 19:305-320.

Abd-Alla, M.A. (2003). Effect of different salts on sour rot of lime fruits. Egypt. J. Appl: Sci., 18: 89-101.

Afifi, M.A.; W.M. Assal; Y.S. Khafagi Fatma S.A. Abd-El-Rahman and H.A. Mohamed (1995). Studies on damping-off and root-rot of pea seedlings in Egypt. Egypt. J. Appl. Sci. 10(12): 495-508.

Amer, G.A. and Sh.M. El-Desouky (2000). Suppression of bean damping-off caused by *Sclerotium rolfsii* using *Trichoderma* and *Gliocladium* species. Minufiya J. Agric. Res., 25:921-932.

Attia, M.F.; Abada, K.A.; Khfagi, Y.S. and Sahar, A. Zayan (1997). Control of pea downy mildew. 8th Congress of the Egyptian Phytopathol. Soc., Cairo. 211-226.

Barnett, H.J. (1960). Illustrated genera of imperfect fungi. Burgess, Minneapolis, USA. 225 pp.

Bauf, B.A. (2000). Seed-borne disease problems of legume crops in Pakistan. Pakistan. J. Sci. and Indust. Res. 27:18-21.

Brown, N. (1924). Two mycological methods II. A method of isolated single strain fungi by cutting hyphal tip. Ann. Bot., 38: 402-406.

Dennis, J.J.C. and D.I. Guest (1995). Acetylsalicylic acid and beta-ionne decreased the susceptibility of tobacco to tobacco necrosis virus and *Phytophthora parasitica* var *nicotiana*. Australian Plant Pathol. 24: 57-64.

Dileep Kumar, B.S.; I. Berggren and A.M. Martensson (2001). Potential for improving pea production by co-inoculation with fluorescent *Pseudomonas* and *Rhizobium*. Plant and Soil. 229:25-34.

El-Gamal, Nadia G.; Nehal S. El-Mougy and Badiaa R. Ismail (2003). Induction of resistance in bean against root-rot and leaf spot diseases incidence under field conditions. Egypt. J. Appl. Sci. 18: 47-67.

El-Mougy, Nehal S. (2002). *In vitro* studies on antimicrobial activity of salicylic acid and acetyl salicylic as pesticidal alternatives against some soilborne plant pathogenic. Egypt. J. Phytopathol. 30: 41-55.

El-Mougy, Nehal, S. (2004). Preliminary evaluation of salicylic acid and acetyl salicylic acid efficacy for controlling root-rot disease of lupin under greenhouse conditions. Egypt. J. Phytopathol., 32: 11-21.

El-Mougy, Nehal S.; F. Abd El-Kareem, Nadia G. El-Gamal and Y.O. Ftooh (2004). Application of fungicides alternatives for controlling cowpea root-rot disease. Egypt. J. Phytopathol. 21(1-2):23-35.

Faris, F.S.; Y.S. Khafaga and Amira S. El-Gizy (1992). Evaluation of bean (*Phaseolus vulgaris* L.) cultivars in relation to their susceptibility to root-rot and wilt diseases. Zagazig, J. Agric. Res. 19(4B): 1829-1841.

Gilman, J.C. (1957). A Manual of Soil Fungi. Second Ed. Iowa State College Press, Ames, Iowa, USA. 450p.

Hassanein, A.M.; A.M. El-Garhy and A.A. Mekhemar (2006). Symbiotic nitrogen fixation process in faba bean and chickpea as affected by biological and chemical control of root-rot. J. Agric. Sci. Mansoura Univ., 31:963-980.

Hawker, L.E. (1960). Physiology of Fungi. Univ. of London Press, LTD Warwish Square, London.

Ibrahim, A.S.; Y.S. Khafagi, A.M. Ghanim and I.H. El-Abbasi (2003). Integrated management of powdery mildew on cantaloupe. Egypt. J. Appl. Sci. 18 (58): 521-531.

Khafagi, Y.S.; A.M. Abo-El-Ela, Fatma S. Ahmed and Amira M. El-Gizy (1995). Varietal reaction and chemical control of pea powdery mildew. Egypt. J. Appl. Sci. 10: 146-157.

King, E.O.; M.K. Ward and D.E. Raney (1954). Two simple media for the demonstration of pyocyanin and flourescein. J. Lab. Clin. Med., 44:301-307 (c.f. El-Hadidy, 2003).

Luze, W.C. (2001). Evaluation of plant growth promoting and bioprotecting rhizobacteria on wheat crop. Fitopathol. Bras. 26:597-600.

Luze, W.C.; G.C. Bergstrom and C.A. Stockwell (1998). Seed applied bioprotectants for control of seedborne *Pyrenophora tritici-repentis* and agronomic enhancement of wheat. Can. J. Plant Pathol., 20:384-386.

Neler, J.; W. Wassermann and M.M. Kutner (1985). Applied Linear Statistical Models Regression, Analysis of Variance and Experimental Design 2nd Ed. Richard, D. (ed.) Irwin Inc., Homewood, Illinois, USA.

Oyarzun, P.; M. Gerlagh and A.E. Hoogland (1993). Pathogenic fungi involved in root rot of peas on the Netherlands and their physiological specialization. Netherlands Journal of Plant Pathology, 39 (1): 23-33 (c.f. Plant Pathol., 72 (11): 6792).

Rauf, B.A. (2000). Seedborne disease problems of legume crops in Pakistan. Pakistan J. Sci. and Indust. Res. 43: 249-254.

Steel, P.G.D. and Torrie, J.H. (1960). Principle and Procedures of Statistics. McGrawm Hill Book Co. Inc. New York, 481 pp.

Singh, R.S. (1982). Plant pathogen "the fungi" Oxford and IBH Publishing Co. New Delhi, Bombay, Calcutta, India. pp. 443.

Van Loon, L.C.; P.A.H.M. Bakker and C.M.J. Pieters (1998). Systemic resistance induced by rhizosphere bacteria. Ann. Rev. Phytopathol., 63:453-483.

Whithehead, M.D. (1957). Sorghum, a medium suitable for the increase of inoculum for studies of soil borne and certain other fungi. Phytopathology. 47: 450 (Abst.).

المقاومة الحيوية لعفن جذور البسلة باستخدام سلالات بكتيرية مختارة

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تم الحصول على عزلة من كل من الفطريات فيوزاريوم سولانى ورايزوكتونيا سولانى وفيوزاريوم اوكسيسبورم واسكليروتينيا اسكليروشيوم، ماكروفومينا فاسيولينا، اسكيروشيوم رولفزياى. وكانت من نباتات بسلة مصابة بأعفان الجذور وذلك من مناطق مختلفة فى محافظات الجيزة والقليوبية والشرقية والاسماعيلية والبحيرة.

تبين من تكرارية العزل أن الفطرين رايزوكتونيا سولانى وفيوزاريوم سولانى وكانا أكثر تكرارية فى محافظة القليوبية.

تم اختبار القدرة المرضية للفطريات والعزلات التى تم الحصول عليها على الصنف بروجرس رقم 9 وتبين أن جميع الفطريات أحدثت إصابة سواء فى مرحلة ما قبل الانبات أو بعده وبالتالى أثرت على النباتات المتبقية وقد كان الفطر رايزوكتونيا سولانى والفطر فيوزاريوم سولانى هما الأكثر قدرة على إحداث الإصابة.

تم اختبار بعض السلالات الميكروبية هى سيراتيا ، سيدوموناس فلوريسنس وباسيلس ساتلس لمقاومة أعفان الجذور على البسلة باستخدام الصنف بروجرس رقم 9 ، كما قورنت تلك المواد بالمبيد القياسى (ريزولكس ثيرام) وذلك تحت ظروف الصوبة والحقل.

أظهرت السلالات المستخدمة سواء تحت ظروف الصوبة أو الحقل انخفاضاً واضحاً فى نسبة الإصابة فى أعفان الجذور قبل وبعد الانبات وبالتالى زيادة فى النباتات المتبقية، و أيضاً أدت إلى زيادة معنوية فى الصفات المحصولية للقرون وكذلك زيادة المحصول الأخضر.

تفاوتت السلالات السابقة سواء فى المقاومة أو الصفات المحصولية إلا أن المبيد القياسى الريزولكس ثيرام كان أفضل منها جميعاً وتلاه كل من استيل سالسيلك اسيد ثم سالسيلات الصوديوم (مضادات أكسدة) ثم فوسفات البوتاسيوم الأحادية (كملح) حيث أدت إلى خفض نسبة الإصابة سواء قبل أو بعد الانبات وبالتالى زيادة المحصول زيادة معنوية. فى الوقت نفسه فقد كان سيدوموناس فلوريسنس وباسيلس ساتلس متوسطة فى مقاومتها للمرض وزيادة المحصول. والسيراتيا أقلهما كفاءة سواء فى المقاومة أو فى زيادة المحصول.

وقد أدى استخدام السلالات البكتيرية تحت الظروف الحقلية إلى الوصول إلى نسبة جيدة فى نجاة البسلة من الإصابة بنسبة 78% ، 75% ، 64% للسلالات سيدوموناس فلوريسنس وباسيلس ساتلس و سيراتيا على التوالى.

وعند مقارنة هذه الأرقام مع الكنترول والمعاملة القياسية بالمبيد ريزولكس ثيرام كانت النسبة 54% و 95% على التوالى.

من الدراسة السابقة يمكننا أن نخلص إلى أن استخدام حمض الأستيل ساليسليك أو ساليسلات الصوديوم أو فوسفات البوتاسيوم الاحادية وذلك بنقع بذور البسلة لمدة $^{1}/_{2}$ ساعة قبل الزراعة فإن ذلك يعتبر من الطرق الحديثة والآمنة وذات كفاءة فى مقاومة مسببات أمراض النبات الكامنة فى التربة وهى بالطبع تعتبر بدائل عن استخدام المبيدات التى تعمل على تلوث البيئة وكذلك الضرر الذى ينتج عنها على الانسان والحيوان والنبات والطيور والأسماك ... الخ.

Table (1): Frequency percentages of the fungi isolated from diseased root-rot of pea in five Governorates during both successive seasons 2005 & 2006

Isolated fungi	% of isolated fungi at Governorates:											
	First season 2005					Second season 2006					Mean	
	Giza	Qalubiya	Sharkiya	Behiera	Ismailia	Giza	Qalubiya	Sharkiya	Behaira	Ismailia	First season 2005	Second season 2006
Fusarium solani	10.33	10.68	10.25	10.49	10.17	10.55	11.44	10.38	10.68	11.07	10.35	10.81
Rhizoctonia solani	12.67	13.68	12.42	13.11	12.21	13.28	14.43	12.80	13.68	13.57	12.81	13.54
Fusarium oxysporum	7.67	7.26	7.76	7.49	7.85	7.42	6.97	7.61	7.26	8.21	7.61	7.49
Sclerotinia sclerotium	8.33	8.12	8.39	8.24	8.43	8.20	7.96	8.30	8.12	8.93	8.30	8.30
Macrophomina phaseolina	6.67	5.98	8.83	6.37	7.00	8.28	5.47	6.57	5.98	7.14	5.37	6.29
Sclerotium rolfsii	6.00	5.13	6.21	5.62	6.40	5.47	4.48	5.88	5.13	6.43	5.87	5.48

Table (3): Percentage of root-rot infection in response to some salts, bioagents and antioxidants compared with the standard fungicide Rizolex-T as seed soaking under the greenhouse conditions

Treatments	*R. solani*			*F. solani*		
	% pre-emergence damping-off	% post-emergence damping-off	% survived plants	% pre-emergence damping-off	% post-emergence damping-off	% survived plants
a. Salts:						
Calcium chloride	30.0	37.5	32.5	35.0	27.5	37.5
Mono-potassium sulphate	12.5	32.5	55.0	17.5	22.5	60.0
Potassium sulphate	35.0	25.0	40.0	37.5	17.5	45.0
b. Bioagents:						
Serratia sp.	37.5	27.5	35.0	40.0	17.5	42.5
Pseudomonas fluorescens	32.5	17.5	50.0	30.0	15.0	55.0
Bacillus subtilis	37.5	15.0	47.5	35.0	12.5	52.2
c. Antioxidants:						
Acetyl salicylic acid	25.0	17.5	57.5	22.5	12.5	65.0
Sodium benzoate	32.5	22.5	45.0	30.0	20.0	50.0
Sodium salicylate	10.0	37.5	52.5	15.0	27.5	57.5
Fungicide:						
Rizolex-Thiram	22.5	15.0	62.5	15.0	12.5	72.5
Control	35.0	37.5	27.5	37.5	42.5	20.0
LSD at 5%:	**2.16**	**2.71**	**2.16**	**1.80**	**2.18**	**1.64**

Table (4): Percentage of root-rot infection of pea in response to some salts, bioagents and antioxidants compared with Rizolex-T as seed soaking under the field conditions

Treatments	First season (2005)			Second season (2006)		
	% pre-emergence damping-off	% post-emergence damping-off	% survived plants	% pre-emergence damping-off	% post-emergence damping-off	% survived plants
a. Salts:						
Calcium chloride	25.25	15.50	59.25	20.75	17.50	61.75
Mono-potassium sulphate	10.25	6.50	83.25	5.00	9.50	85.50
Potassium sulphate	21.50	12.75	65.75	20.50	11.75	67.75
b. Bioagents:						
Serratia sp.	15.00	21.25	63.75	12.50	22.25	65.25
Pseudomonas fluorescens	12.00	10.75	77.75	12.00	8.75	79.25
Bacillus subtilis	10.25	15.00	74.75	13.75	10.75	75.50
c. Antioxidants:						
Acetyl salicylic acid	7.75	5.25	87.00	4.50	6.25	89.25
Sodium benzoate	20.50	10.50	69.00	21.00	9.00	70.00
Sodium salicylate	11.50	8.25	80.25	7.25	10.00	82.75
Fungicide:						
Rizolex-Thiram	3.00	2.50	94.50	2.25	2.50	95.25
Control	27.50	20.00	52.50	29.50	15.50	55.00
LSD at 5%:	1.93	1.36	1.04	1.15	0.93	2.03

Table (5): Pea pod characteristics and total green yield in response to seed soaking in solution of some salts, bioagents and antioxidants compared with the fungicide Rizolex-T during two successive seasons

Treatments	First season (2005)					Second season (2006)				
	Pod length (cm)	Pod weight (g)	No. of pods/ plant	Yield (kg)/ plot ($21m^2$)	Increasing %	Pod length (cm)	Pod weight (g)	No. of pods/ plant	Yield (kg)/ plot ($21m^2$)	Increasing %
a. Salts:										
Calcium chloride	8.5	4.6	13.8	14.7	17.60	8.3	4.5	13.6	14.5	20.83
Mono-potassium sulphate	10.9	7.9	16.0	29.3	134.40	10.8	7.8	15.6	29.0	141.67
Potassium sulphate	9.3	6.3	14.8	17.8	42.40	9.0	6.0	14.5	17.5	45.83
b. Bioagents:										
Serratia sp.	8.8	5.9	14.2	15.6	24.80	8.5	5.8	14.0	15.5	29.17
Pseudomonas fluorescens	10.3	7.2	15.7	23.8	90.40	10.1	7.0	15.3	23.5	95.83
Bacillus subtilis	9.9	6.9	15.3	21.2	69.60	9.8	6.8	15.0	21.0	75.00
c. Antioxidants:										
Acetyl salicylic acid	11.7	8.5	16.3	31.4	151.12	11.5	8.3	16.1	30.5	154.17
Sodium benzoate	9.7	6.6	15.0	19.3	54.40	9.4	6.5	14.8	19.0	58.00
Sodium salicylate	10.6	7.4	15.9	27.9	123.20	10.4	7.3	15.4	27.5	129.17
Fungicide:										
Rizolex-Thiram	12.3	9.3	16.9	33.8	170.40	12.0	9.0	16.6	33.5	179.17
Control	7.7	3.6	12.7	12.5	-	7.5	3.5	12.5	12.0	-
LSD at 5%:	0.54	0.52	0.60	0.67		0.36	0.59	0.64	1.12	