

**Controlling of Fungal Root Diseases of Pea (*Pisum sativum*L.)  
by Safe Alternatives Fungicides in Egypt and Ethiopia**

**By**

**Ibrahim Abd-El-Hady Ismail El-Nady**

**B. Sc. Agric. Sci., Fac. of Agric., Moshtohor,  
Benha Branch, Zagazig University, 2000**

**M. Sc. Agric. Sci. (Plant Pathology),  
Fac. of Agric., Mansoura University, 2010**

**THESIS**

**Submitted in Partial Fulfillment of the  
Requirements for the Degree of**

**DOCTOR OF PHILOSOPHY**

**In**

**AFRICAN STUDIES**

**(Natural Resources-Plant Resources)**

**“Plant Pathology”**

**Department of Natural Resources  
Faculty of African Postgraduate Studies  
Cairo University  
EGYPT**

**2022**

## CONTENTS

Content	Page
<b>LIST OF TABLES</b> .....	IV
<b>LIST OF FIGURES</b> .....	VI
<b>LIST OF ABBREVIATIONS</b> .....	VII
<b>1-INTRODUCTION</b> .....	1
<b>2-REVIEW OF LITERATURE</b> .....	6
2-1-pathogens and pathogenicity tests .....	6
2-2-Biological Control studies.....	8
2- 3-chemical inducer .....	20
<b>3-MATERIAL AND METHODS</b> .....	31
3-1- Location of experimental study.....	31
3-2- Isolation and purification of the causal pathogens .....	31
3-3-Identification of the causal pathogens .....	31
3-4- Pathogenicity tests .....	32
3-5-Cultivars Assay.....	34
3-6-Laboratory experiment .....	34
3-6-1-Isolation and identification of bioagents .....	34
3-6-2- In vitro Evaluation of bio control agents against linear radial growth of pathogenic fungi.....	35
3-7-Greenhouse experiment .....	36
3-7-1: Evaluation of BCAs individually or in amixture on the disease parameters of pea plants infected with R.solani on the disease parameters on pea plants under greenhouse.....	36
3-7-2-Evaluation of vesicular-arbuscular mycorrhizal inoculums for managing pathogenic fungi under greenhouse condition .....	37
3-7-3 Effect of seed soaking chemical inducer, and fungicide on disease parameters.....	38
3-8. Biochemical analysis.....	38
3-8-1. Estimate of photosynthetic pigments.....	38

Content	Page
3-8-2. Estimation of Enzymes Activity.....	39
3-8-3. Lipid peroxidation assay .....	40
3-8-4. Membrane permeability .....	40
3-8-5. Quality parameters .....	40
3-8-6. Anatomical parameters .....	40
3-8-7. Growth parameters.....	41
3-8-8. Yield Parameters .....	41
3-9. Field experiments.....	41
3-10. Statistical analysis .....	43
<b>4. RESULTS</b> .....	44
4.1. Isolation, purification, identification of fungi isolated from rhizosphere of healthy and diseased pea plants showing damping off and root rot symptoms in 10 localities .....	44
4.2. Pathogenicity of some pathogenic soil borne fungi on pea (master-B) Cultivar in pot experiment .....	45
4.3. Assay of some pathogenic soil-borne fungi on Cultivars in pot experiment.....	46
4.4. Dual culture technique.....	47
4.5. Evaluation of BCAs individually or in a mixture on pea plants under greenhouse .....	49
4.5.1. Disease assessment.....	49
4.5.2. Biochemical analysis .....	51
4.5.3. Anatomical features of the root.....	57
4.5.4. Effect of the treatment with BCAs individually or in a mixture on the vegetative and yield parameters on pea plants.....	61
4.6. Evaluation of chemical inducers on pea plants under greenhouse	66
4.6.1. Effect of chemical inducers on the linear growth of the pathogenic fungi.....	66
4.6.2. Effect of the treatment with chemical inducers on the disease parameters.....	68

<b>Content</b>	<b>Page</b>
4.6.3. Biochemical analysis .....	69
4.6.4. Anatomical features of the root.....	76
4.6.5. Effect of the treatment with chemical inducers on growth and yield Parameters of pea plants.....	82
4.7. Field experiments .....	86
<b>5-DISCUSSION</b> .....	96
<b>6- ENGLISH SUMMARY</b> .....	112
<b>7-REFERENCES</b> .....	126
<b>8-ARABIC COVER</b> .....	-

## LIST OF TABLES

No.	Title	Page
1.	Frequency (%) of fungi isolated from pea plants showing damping off and root rot symptoms at 10 localities.....	44
2	Pathogenicity of some pathogenic soil-borne fungi on Master-B cultivar in pot experiment.....	45
3	Assay of some pathogenic soil-borne fungi on cultivars in pot experiment.....	46
4	Antagonistic effect of BCAs isolates against pathogenic fungi.....	47
5	Evaluation of BCAs individually or in a mixture on the disease parameters of pea plants infected with <i>R. solani</i> .....	49
6	Evaluation of BCAs individually or in a mixture on the disease parameters of pea plants infected with <i>F. oxysporum</i> f.sp. pisi.....	50
7	Effect of the treatment with BCAs individually or in a mixture on the total phenol content and defense-related enzymes of pea plants infected with <i>R. solani</i> .....	51
8	Effect of the treatment with BCAs individually or in a mixture on the total phenol content and defense-related enzymes of pea plants infected with <i>F. oxysporum</i> .....	52
9	Effect of the treatment with BCAs individually or in a mixture on the photosynthetic pigments, membrane permeability% and lipid peroxidation $\mu$ moles/g FW of pea plants infected with <i>R. solani</i> .....	53
10	Effect of the treatment with BCAs separately or in a mixture on photosynthetic pigments, membrane permeability% and lipid peroxidation $\mu$ moles/g FW of pea plants infected with <i>F. oxysporum</i> .....	54
11	Effect of BCAs on some chemical compositions in pea fresh seeds under artificial infection by <i>R. solani</i> . .....	55
12	Effect of the treatment with BCAs individually or in a mixture on seed quality of pea seeds infested with <i>F. oxysporum</i> .....	56
13	Effect of the treatment with BCAs on anatomical features of pea roots infected with <i>R. solani</i> .....	57
14	Effect of the treatment with BCAs on anatomical features of pea roots infected with <i>F. oxysporum</i> .....	59
15	Mycorrhizal colonization frequency, % in pea root under artificial infection by pathogenic fungi .....	61
16	Effect of the treatment with BCAs individually or in a mixture on the vegetative and yield parameters on pea plants infested with <i>R. solani</i> ....	63
17	Effect of the treatment with BCAs individually or in a mixture on the vegetative and yield parameters on pea plants infested with <i>F. oxysporum</i> .....	65

No.	Title	Page
18	Effect of chemical inducers on the linear growth of the pathogenic fungi.....	66
19	Effect of chemical inducers seed treatments on the disease parameters of pea plants infested with <i>R. solani</i> .....	68
20	Effect of chemical inducers seed treatments on the disease parameters of pea plants infested with <i>F. oxysporum</i> .....	69
21	Effect of chemical inducers seed treatments on the total phenol content and defense-related enzymes of pea plants infested with <i>R. solani</i> .....	70
22	Effect of chemical inducers seed treatments on the total phenol content and defense-related enzymes of pea plants infected with <i>F. oxysporum</i> .	71
23	Effect of chemical inducers seed treatments on the photosynthetic pigments, membrane permeability % and lipid peroxidation $\mu$ moles/g FW of pea plants infected with <i>R. solani</i> .....	72
24	Effect of chemical inducers seed treatments on photosynthetic pigments, membrane permeability % and lipid peroxidation, $\mu$ moles/g FW of pea plants infected with <i>F. oxysporum</i> .....	73
25	Effect of chemical inducers seed treatments on seed quality of pea seeds infested with <i>R. solani</i> .....	74
26	Effect of chemical inducers seed treatments on seed quality of pea seeds infested with <i>F. oxysporum</i> .....	75
27	Effect of the chemical inducers seed treatments on anatomical features of pea roots infested with <i>R. solani</i> .....	77
28	Effect of chemical inducers seed treatments on anatomical features of pea roots infested with <i>F. oxysporum</i> .....	80
29	Effect of chemical inducers seed treatments on growth and yield parameters of pea plants infested with <i>R. solani</i> . .....	83
30	Effect of the treatment with chemical inducers seed treatments on growth and yield of pea plants infected with <i>F. oxysporum</i> .....	85
31	Effect of BCAs individually or in a mixture on the disease parameters on pea plants .....	87
32	Effect of BCAs individually or in a mixture on the growth parameters on pea plants .....	88
33	Effect of BCAs individually or in a mixture on the yield parameters on pea plants .....	90
34	Effect of the chemical inducer on the disease parameters on pea plants..	92
35	Effect of chemical inducers on the growth parameters on pea plants.....	93
36	Effect of chemical inducers on the yield parameters on pea plants .....	95

## LIST OF FIGURES

No	Title	Page
1	Individual and dual culture assay of <i>Rhizoctonia solani</i> as affected by BCAs .....	48
2	Effect of BCAs on <i>Fusarium oxysporum</i> under dual culturing .....	48
3	The anatomical changes in cross section through the root of pea plants infected with <i>Rhizoctonia solani</i> affected with bio-control agents under greenhouse conditions ( $\times 100$ ).....	58
4	The anatomical changes in cross section through the root of pea plants infected with <i>F. oxysporum</i> (Fo) affected with BCAs ( $\times 100$ ).....	60
5	Effect of Si-NPs on growth diameter (mm) and growth inhibition (%) of <i>R.solani</i> and <i>F. oxysporum</i> by dual culture assay .....	67
6	Effect of Chit-NPs on growth diameter (mm) and growth inhibition (%) of <i>R.solani</i> and <i>F.oxysporum</i> .....	67
7	The anatomical changes in cross section through the root of pea plants infected with <i>R. solani</i> (Rs) affected with chemical inducers under greenhouse conditions ( $\times 100$ ).....	78
8	The anatomical changes in cross section through the root of pea plants infested with <i>F. oxysporum</i> (Fo) affected with chemical inducers under greenhouse conditions ( $\times 100$ ).....	81

## LIST OF ABBREVIATIONS

Abb.	Full Form
%	Percent
°C	Degree Celsius
<b>BCAs</b>	Bio control agents
<b>TH</b>	<i>Trichodema harzianum</i>
<b>Ps</b>	<i>Pseudomonas fluorescens</i>
<b>AMF</b>	arbuscular mycorrhizal fungi
<b>FMOc</b>	frequency mycorrhizal root colonization
<b>Si</b>	Silicone
<b>Chit</b>	Chitosan
<b>PPO</b>	Polyphenol oxidase
<b>PO</b>	Peroxidase
<b>TSS</b>	Total soluble sugars
<b>SH</b>	Shoot height
<b>RL</b>	Root length
<b>LA</b>	leaf area
<b>NB</b>	number of branches
<b>SFW</b>	shoot fresh weight
<b>SDW</b>	shoot dry weight
<b>RFW</b>	root fresh weight
<b>RDW</b>	root dry weight
<b>PL</b>	Pod length
<b>NSP</b>	number of seed / pod
<b>NPP</b>	number of pod / plant
<b>W10P</b>	weight of 10 pod
<b>W100S</b>	weight of 100 seed
<b>YF</b>	yield of faddan



## 6- ENGLISH SUMMARY

Synthetic chemical fungicides seed treatments cause serious threats to environmental, human health and resistance to commercially available fungicides by phytopathogenic fungi has been increasing and has become a serious problem. Therefore there are mainly needed for safe alternative fungicidal seed treatments. Increasing the production of pea-green pods and dry seeds yield with high quality is an important goal and this goal can be achieved using various safe alternative fungicides as a seed treatment.

### **6-1- Fungi isolated from the rhizosphere of healthy and diseased pea plants showing damping-off and root rot symptoms in 10 localities:**

Isolation trails of fungal pathogens from rhizosphere of healthy and diseased pea samples plants collected from El Dakahlia governorates on PDA. The isolated pathogenic fungi were purified and identified as *F. oxysporum*, *F. solani*, *F. avenaceum*, *F. equiseti*, *R. solani*, *Pythium* spp, *Sclerotinia sclerotiorum*, *Trichoderma* spp and *Penicillium* spp. frequency percentages were calculated. *F. oxysporum*, showed the highest percentage followed by *R. solani*. *R. solani* and *F. oxysporum* were carefully chosen as the most aggressive pathogens in this evaluation.

### **6-2- Pathogenicity of some pathogenic soil-borne fungi on master-B Cultivar in pot experiment:**

*R. solani*-1 proved to be the most significant destructive fungus which caused a high percentage of both pre and post-emergence damping-off 23.33% and 53.33% of survival plants. *F.oxysporum*-1 showed (20, 26.66%) in pre and post-damping-off and 60% of survival plants. While *F. oxysporum*-1 showed a high significant percentage in disease severity followed by *R. solani*. *Pythium* spp., gave 16.66%, 13.33% in both pre and post damping-off and 70 % of

survival plants. *F. avencium* showed the lowest percentage of all disease parameters.

### **6-3-Assay of some Cultivars against pathogenic fungi in pot experiment:**

Master-B cultivar showed to be highly susceptible to infection with all tested root rot fungi. *R. solani* showed 23.33% in pre and post-emergence damping-off and 27.33% in disease severity. *F. oxysporum* showed 20 and 26.66% in pre and post-emergence damping-off and 28% in disease severity on Master-B cultivar. *Pythium* spp., fungi showed less effect in all disease parameters

### **6-4-Evaluation of The bio-control agents (BCAs):**

#### **6-4-1-In vitro experiment:**

All BCAs assessed significantly inhibited the growth of the pathogens and percent inhibition ranged between 39.99 to 73.33%. *T. harzianum* fungal bioagent showed maximum inhibition of 73.33% and 77.78% on growth of both pathogenic fungi followed by *T. virde* which showed 56.3% and 71.48%, respectively. Data also showed that in the case of bacterial bioagents, *P. fluorescens* inhibited fungal linear growth to the range of 60% and 72.22%, followed by *B. subtilis* with 39.99% and 46.29%.

#### **6-4-2- Greenhouse experiments:**

##### **6-4-2-1-Disease assessment:**

In infested soil by *R. solani* the triple mixture seed treatment showed a high significantly decreasing pre, post-emergence seedling damping-off and decreasing disease severity (75, 77.4 and 78%), followed by bilateral treatment decreasing pre, post-emergence seedling damping-off and decreasing disease severity (62% and 56% 78%). AM showed decreasing in disease severity of 58.5% followed by PS seed treatment which gave 46.3% and the less effective reduction of all disease parameters of single treatments. Mixed compatibility of BCAs was highly effective than single bio-treatments. Under infection with *F.*

*oxysporum* seed treatment of Ps (BCA) recorded a decrease of pre, post-emergence damping-off percentage and disease severity (38.7, 50, and 39.98%), respectively. However, the triple mixture seed treatment leads to high values of disease control nearby to that found with Topsin-M70 treatment. Triple mixture seed treatment showed the best decrease (75, 75, 79.99%) of pre, post-emergence damping-off and disease severity% meanwhile, (AM + T.H) of pre, post-emergence damping-off and disease severity% showed decrease (75, 62.4 and 72.27%), respectively.

#### **6-4-2-2 Biochemical analysis**

The maximum increase in total phenol and defense-related enzymes of pea plants PPO and PO was found with the triple mixture (AM+TH+Ps) seed treatment (119.8, 91 and 51.1%) followed by dual combination seed treatment. Though, the lowest increase was detected with *P. fluorescens* as a single treatment for total phenol and the two enzymes (20.3, 29.1 and 23.6%), respectively in plants sown in infested soil with *R. solani*. In the case of pea plants infested with *F. oxysporum*, the high increase was noted with the triple mixture treatment (AM+TH+Ps) on the content of total phenols and two enzymes PPO and PO (117.7, 136 and 57%), respectively, followed the dual synergistic (AM + TH) treatment which showed on both enzymes (116.8 and 30.7%). TH showed a moderate increase in both enzymes PPO and PO (56.6 and 28.5%) However, the lowest increase was detected with *P. fluorescens* as treatment for the two enzymes (44.5 and 23.5).

In general, pathogen markedly reduced the content of the tested photosynthetic pigments. It's noted clearly showed all seed treatments with BCAs singly or in combination showed a significant increase in chlorophyll content in leaves of pea plants infected with *R. solani* as a result of decreasing disease parameters, membrane permeability% and lipid peroxidation. The high increase was found with the triple mixture seed treatment on chlorophyll A, B, total and carotenoids (934.4, 7.79, 257.9 and 351.6%) and decreasing membrane

permeability 60.8% and lipid peroxidation 59.3%, followed by the dual combination (AM+TH) (731, 35, 221.4 and 201%) and decreasing membrane permeability and lipid peroxidation (40.3 and 53.7%), respectively this is as a result of increasing the synergistic combination effect. AM, TH and Ps single treatments gave the lowest level in increasing content of chlorophyll and less effect in protecting of cell membrane permeability (21.1, 17.9 and 2.9%) and lipid peroxidation (45.7, 40.7 and 21%), respectively of root pea plants. Under artificial infection with *F. oxysporum*, synergistic triple showed the highest ability in increasing in the content of leaves chlorophyll a, b, carotenoids (104.59, 46.9 and 174.4%), respectively and decreasing membrane permeability% and lipid peroxidation by (86.65 and 160.3%) followed by the dual synergistic (AM+TH). (TH+Ps) recorded reduction in the membrane permeability and lipid peroxidation by (26.3 and 52.1%) and Ps treatment singly gave (23.9 and 31.3%) in reducing the membrane permeability% and lipid peroxidation, respectively.

BCAs seed treatments significantly improved the content of T.S.S, protein, and carbohydrate, % over the untreated control. In this respect, the triple mixture seed treatment gave the greatest result in increasing all seed quality parameters as T.S.S, protein, and carbohydrate (56.7, 49.1, and 26.7%) of pea seeds followed by dual (AM+TH) (39.3, 46.7 and 24.4%). Also, BCAs singly seed treatments showed a moderate effect in rising of all quality seed parameters. Seed treatment with Ps suspension had less effective as compared to control which showed increase of T.S.S., protein, and carbohydrate (17.3, 29.7 and 18.4), respectively, in infested soil with *R. solani*. In the case of *F. oxysporum* the triple mixture treatment gave the greatest result in increasing all seed quality parameters (48.5, 61.6 and 24.8%) followed by the dual mixture (AMF+TH) treatment showed (36, 62.6 and 20.7%) increasing in T.S.S, protein, and carbohydrate,% respectively. Ps seed treatment had the less effect which showed an increase of T.S.S, protein, and carbohydrate (21.3, 40 and 11.1%),

respectively. (TH+Ps) the treatment gave an increase (32.9, 55.6 and 19.9%) in T.S.S, protein, and carbohydrate, respectively.

#### **6-4-2-3 -Anatomy of the root:**

It is realized that treatment with (AM+TH+Ps) improved thickness of section by 60.89% over the infested plants with *R. solani*. Increased in section thickness because of increase in cortex thickness by 94.50% and vascular cylinder thickness by 31.75%. Also, xylem vessels diameter was improved by 28.57% in comparison with the control (+).

Section thickness in (AM+TH) treatment was increased by 8.94% over the infested plants. Increased section thickness was due to increased thickness of cortex by 23.85%. While, vascular cylinder thickness and xylem vessels diameter reduced by 1.42, 8.93% below the control, respectively.

Also, the healthy plant was increased section, cortex, vascular cylinder thickness and xylem vessels diameter by 14.53, 18.81, 15.64 and 7.14%, respectively, more than control (+). Under infection with *F. oxysporum*, microscopical measurements of histological characters in transverse section in the pea primary roots of plants BCAs seed treatments.

It's noted that (AM+TH+Ps) seed treatment increased section thickness by 25.0% more than the infested plants (control+). It's clear that the increasing section thickness was due to the distinguished increasing cortex and vascular cylinder thickness. Cortex and vascular cylinder thickness were increased by 24.81 and 31.53% more than the control (+), respectively. Also, xylem vessels diameter was increased by 81.58% compared to the control (+).

As to the effect of (AM+TH) treatment increased section thickness by 1.04% over the infected plants. The increase in section thickness due to treatment with (AM+TH) could be attributed mainly to an increase in the thickness of the cortex by 3.88% more than control (+). Meanwhile, the vascular cylinder thickness was decreased by 0.49% less than the control. The diameter of xylem vessels was increased by 68.42% more than infected plants.

Also, healthy plant (control-) roots increased by 6.77, 0.39, 20.20 and 57.89% for section, cortex, vascular cylinder thickness and xylem vessels diameter, respectively over the control (+).

#### **6-4-2-4-Effect of the treatment with BCAs individually or in mixture on vegetative and yield parameters of pea plants**

under artificial infection *R. solani* under greenhouse, triple mixture (AM+TH+Ps) was the most superior seed treatment, recording the greatest increase in shoot length 150%, root length 98.1%, leaf area 161.8% plant biomass (shoot, root fresh weight 250%, 113,7% and dry weight 269, 72%), followed by the dual combination (AM+TH). In control + infested by *F. oxysporum* the triple combination seed treatments significantly increased the pod length, pod diameter, number of pods/plant, average 10 pod weight and weight of 100 seed (97.6, 33.3, 150.3, 111.1 and 26.3%). AM the best single seed treatment increased shoot length 136.3%, root length 79.6%, leaf area 77.9%, shoot fresh weight 217.8%, shoot dry weight 210.3%, root fresh weight 62% and root fresh weight 36.3%. (AM+ TH) seed treatment significantly increased pod length, pod diameter, number of pods/plant, average 10 pod weight and weight of 100 seed (71.4, 25, 137.9, 92.1 and 23.8%). Ps single seed treatment showed less stimulation of all the vegetative growth and yield parameters.

#### **6-5-Evaluation of chemical inducers:**

##### **6-5-1. *In vitro* experiment:**

Si-NPs and Chit-NPs showed significant effect as complete inhibition of mycelium linear growth of both pathogenic fungi at 150 ppm concentration. Silicone (Si) at 8 g/l showed 27.78% inhibition of linear growth of *R. solani* and 50.74% inhibition of mycelium linear growth of *F. oxysporum*. Chitosan at 8 g/l showed 42.96% inhibition of linear growth of *R. solani* and complete inhibition of mycelium linear growth of *F. oxysporum*. Gradual increases of the

concentration of treatments lead to a significant decrease in mycelium linear growth of both pathogenic fungi.

### **6-5- 2- Greenhouse experiments:**

#### **6-5- 2- 1 -Disease assessment:**

Under artificial inoculation by *R. solani* in greenhouse Si 8 g showed (50, 62.4 and 70.7%) on pre and post-emergence damping off and disease severity%, followed by chitosan 8 g/l which showed (37.5, 100 and 70.7%) at pre, post-emergence damping-off and disease severity%, respectively. Si-NPs the best seed treatment showed (75,62.4 and 78% ) on pre and post-emergence damping-off as fungicide and disease severity%, showed the best seed treatment followed by Chit-NPs which showed (50, 50 and 78%) in pre and post-emergence damping off and disease severity. In pea plants infested with *F. oxysporum* under greenhouse, Si-NPs was the most effective and decreased mean percentage of pre, post-emergence damping-off and disease severity(75,57.1, and 72.7%) and this was not significantly different from the fungicide effect followed by Chit-NPs effect (50, 57.1 and72.2%). Si 8 g showed a reduction in all disease parameters as a percentage of pre, post-emergence damping-off and disease severity (75, 42.8 and 60.6%), followed by chit 8 g/l.

#### **6-5- 2- 2 Biochemical analysis**

The high increase was noted with the chemical inducers Si-NPs treatment and this was on the content of total phenol (258.1%) and defense-related enzymes of pea plants PPO (270.5%) and PO (63.3%) infected with *R. solani* under greenhouse respectively, followed by Chit-NPs treatment (243.3, 249 and 66.9%) at three parameters respectively. However, the lowest increase was detected with chitosan 8 g/l as a seed treatment for the two enzymes. Si showed a moderate increase in total phenol and on both enzymes PPO and PO (228.6, 245 and 47.3%). In pea plants infested with *F. oxysporum* Si-NPs increased total phenols (116.8. %), PPO (102 %) and PO (31.4%) followed by Chit NPs increased total phenols (106. 6, 72.1 and 27.9%). Si 8 g/l increases the total

phenols, PPO and PO (229.7, 125 and 22.4%), respectively followed by Chitosan.

In general, the presence of the pathogen reduced the content of the tested photosynthetic pigments in pea leaves. under the stress of infection with *R. solani*, seed treatments with Si-NPs showed the best significance increasing in chlorophyll content as chl a, chl b, total chl and carotenoids (52.7, 36.5, 161 and 37.1%) and significance decreasing in both membrane permeability (34.6%) and lipid peroxidation  $\mu\text{moles/g FW}$  (58.9%), in compared to in infested control and Chit -NPs decreasing in both membrane permeability (28.2%) and lipid peroxidation  $\mu\text{moles/g FW}$  (51.4%). Chitosan treatment at 8 g/l showed an increased in chlorophyll content as chl a, chl b and total chls (11.6, 4.5 and 138.9%) and decreasing in both membrane permeability (22.9%) and lipid peroxidation (41.9%), respectively. Under the stress of infestation with *F. oxysporum* of pea plants seed treatments with Si-NPs showed the best significance increasing in chlorophyll a, chlorophyll b, total chlorophyll content and carotenoids (108.9, 43.75, 111.7 and 69.2%), significance decreasing in both membrane permeability and lipid peroxidation  $\mu\text{moles/g FW}$  (37.1 and 69.8%) in compared to in infested control followed by Chit-NPs showed increasing chlorophyll content and carotenoids (99, 15, 98 and 66.6%) decreasing in both membrane permeability and lipid peroxidation (31.1 and 51.2%). Gradual increasing of the concentration of Si and chitosan inducers lead to a significant increase chlorophyll content and carotenoids and decreasing in both membrane permeability and lipid peroxidation.

In general, The NPs tested increased quality parameters as T.S.S, protein% and carbohydrate%. Data revealed a similar trend for three parameters. However, Si-NPs showed the highest effect and increased T.S.S., protein% and carbohydrate (39.5, 35.7 and 21.9%) followed by Chit-NPs which showed (36.2, 34, 20.8%) compared to (12.4, 15.86% and 36.89%) for three parameters, respectively in the untreated control. Si at 8 g/l increased three parameters (33.8,



32.5 and 20.4%) respectively followed by chitosan at 8 g/l in control infested by *R. solani*. Under the stress of infection with *F. oxysporum* of pea fresh seed compared to the untreated infested control, Si-NPs showed the highest effect and increased T.S.S, protein, and carbohydrate% compared to (39.6, 32.3 and 18.9%) for three-parameter, respectively followed by Chit-NPs showed for T.S.S, protein and carbohydrate (34.9, 36.2 and 17.3%). The NPs tested are the most effective in increasing quality parameters than silicon and chitosan. Silicon showed (34.1, 27.8 and 16.6%) for three parameters, respectively followed by chitosan.

#### **6-5- 2- 3 -Anatomy of the root:**

It's obvious that Si at 8 g/l treatment on pea plants infested with *R. solani* induced an increase in section thickness by 31.84% more than the (control+). The increase in section thickness was observed in the root mainly due to the increase in cortex and vascular cylinder thickness. The increments over those of (control+) were 56.88 and 15.17%, respectively. But, xylem vessels diameter was decreased by 3.57% comparison with (control+).

Si-NPs level at 150 ppm increases section thickness by 41.90% over the (control+). The increased in section thickness was due to the increase in cortex and vascular cylinder thickness by 50.46 and 34.12% more than control (+), respectively. Xylem vessels diameter increased by 16.07% over the infested plants (control+).

Results showed that section thickness in plants treated with 8g/L chitosan was increased by 14.53 % over the (control +). An increased in section thickness was due to an increase in cortex thickness by 50.46 % and vascular cylinder thickness by 2.37 %. Meanwhile, xylem vessels diameter decreased by 10.71% less than (control +).

Also, the section thickness of plants treated with 150 ppm chit-NPs was increased by 34.08 % over the (control +). The increment in section thickness is attributed to an increase in cortex and vascular cylinder thickness. The increases

were 66.06 % for cortex thickness and 4.74 % for vascular cylinder thickness more than the control (+). Vessel diameter was increased by 1.79% more than (control +).

Results illustrated the effect of treatment with Nanoparticles (Si-NPs and chit-NPs) on certain histological characters measurements in the transverse section through the primary roots of pea plants infected with *F. oxysporum*.

It's noted that 8 g/l Si treatment, it is realized that such treatment increased section thickness by 6.25% more than the control. It's clear that the section thickness induced by Si was mainly due to an increase of cortex and vascular cylinder thickness by 8.14 and 8.37% over the control, respectively. Xylem vessels Diameter increment by 78.95% more than (control +).

Concerning the effect of Si-NPs at 150 ppm treatment, it is obvious that such treatment produced an increase of section thickness by 15.63% more than (control+). Section thickness induced by Si-NPs was mainly due to the increase of cortex and vascular cylinder thickness. The increase over the control was 12.40% for cortex thickness and 20.69% for vascular cylinder thickness. It's clear that Si-NPs increased xylem vessels diameter by 86.84% compared to control (+).

As to the effect of chitosan at 8g/L, treatment increased section thickness by 11.46% over the infested plants. The increase in section thickness due to chitosan could be attributed mainly to an increase of cortex and vascular cylinder thickness by 6.20 and 19.70% more than infected plants, respectively. Also, of xylem vessels diameter was increased by 47.37% more than control (+).

Data also revealed that chit-NPs concentration 150 ppm on pea infected with *F. oxysporum* induced a prominent increase in section thickness by 13.54 % compared to control (+). It is clear that the increase in section thickness was accompanied by 7.75 and 18.72 % increments in the cortex and vascular cylinder thickness over control (+), respectively. Xylem vessels diameter increased by 60.53% more than (control +).

#### **6-5- 2- 4 -Effect on the vegetative growth and the yield Parameters**

Data revealed that all tested soluble sources of silicon significantly increased growth and yield parameters compared with the control infected with *R. solani*. Si at 4, 6 g/l and chitosan 4, 6 g/l showed less increase in all parameters. Si 8g increased yield parameters (41.8, 21.9, 128.7, 60 and 20.4%), respectively followed by chitosan at 8 g/l which increased the yield parameters *i.e.*, pod length, pod diameter, number of pod plant, weight of 10 pods and weight of 100 seed (81.7, 18.6, 85.8, 55.3 and 13.7%). Si-NPs were the best treatments in increasing percentages of growth parameters *i.e.*, recording the greatest shoot, root length, leaf area, shoot fresh, dry weight and root fresh and dry weight (51.3, 53, 150.5, 120.5, 78, 246.1 and 244.4%) and yield parameters *i.e.*, pod length, pod diameter, number of pod plant, weight of 10 pods and weight of 100 seed (54.5, 24.3, 142.9, 67.3 and 34.1%) followed by Chit-NPS significantly increased yield parameters (48, 24.3, 100, 60.6 and 23.4%), respectively.

Seed treatment of pea with chemical inducers and infested with *F. oxysporum*, clearly stimulated the vegetative growth and yield of pea plants when compared with control treatments. Si-NPs was the most superior treatment, recording the greatest shoot, root length, leaf area, shoot fresh, dry weight and root fresh and dry weight (52.9, 63.1, 175.4, 172.4, 120.4, 126.6 and 350%) and yield parameters *i.e.*, pod length, pod diameter, number of pod plant, weight of 10 pods and weight of 100 seed (77.1, 21.9, 112.7, 75, 18.1%), respectively, followed by Chit-NPs which increased yield parameters (60, 18.6, 100.3, 69.4, and 11.26%). Si at 8g/L increased the yield parameters (62.8, 16.2, 87.9, 66.1 and 18.1%) followed by chitosan 8g/L.

## **7-Under Field conditions:**

### **7-1-Evaluation of BCAs under field conditions:**

#### **7-1-1-Effect of BCAs on disease parameters:**

The (AMF+TH+Ps) treatment caused high values in reducing of pre, post-emergence damping-off %, disease severity (66.7, 75 and 71.4%) in the first season and (80, 77.8 and 75%) in the second season followed by the dual combination AM+TH (66.7, 75 and 71.4%) and (70, 66.7 and 71.8%), respectively. It is clear that mixed of BCAs were highly effective for decreasing damping-off and D. severity% than single treatments. Single Ps seed treatment clearly showed less effect on decreasing D. severity in two seasons (50, 33.3 and 50%) and (66.6, 50 and 18.4%), respectively.

#### **7-1-2-Effect of BCAs on the growth parameters:**

Among the treatment, maximum shoot length, root length, shoot fresh weight, shoot dry weight and root fresh weight and root dry weight ( 88.1, 62.5, 261.6, 125.5, 205.7, 150.4, 225.5 and 168.1%) were recorded in the first season and (77.4, 116.6, 189.5, 233, 253.8, 213.4, 125 and 148.1%) in the second season by synergistic triple seed treatment followed by synergistic bilateral (AM+T.H ) which showed (82.2, 69, 46.9 and 91.6%) increasing in shoot and root length of two seasons. AM is the best single seed treatment increased vegetative growth parameter (70.3, 34.4, 159.3, 50.3, 129.2, 84.6, 155.5, and 235.4%) in the first season and (64.8, 79.1, 108.6, 100, 167.1, 142.9, 50.8, and 88.8%) in the second season, respectively, followed by PS single seed treatment in all vegetative growth parameters.

#### **7-1-3-Effect of BCAs on the yield parameters:**

In combined seed treatments the maximum increase was observed for triple combination treatment on pod diameter, pod length, the number of pod plant, the weight of 10 pods, the weight of 100 seed and yield of faddan in (38.4, 118.8, 140, 125, 106.9, 50 and 32.6%) in the first season and (54.4, 107.4, 136.8, 115.4, 101.9, 61 and 31.7% ) in the second season followed by dual

combination (AMF +TH) treatment increased yield of faddan (23%) in two seasons, respectively. However, in single seed treatment, AMF treatment showed the best one increasing yield of faddan (20.7 and 21.2%) although *P. fluorescens* as an individual treatment showed the lowest increase in all yield parameters compared with other treatments.

## **7-2-Evaluation of the chemical inducers under field conditions:**

### **7-2-1. Disease parameters:**

Seed treatment with all the tested NPs significantly decreased the mean percentage of pre and post-emergence damping-off compared to the untreated control and this was not significantly different from the fungicide. On the other hand, concerning the root rot severity, all NPs significantly decreased it where Si-NPs was the most effective and decreased pre, post-emergence damping and disease severity (61.5, 71.4 and 71.8%) in the first season (59.9, 66.4 and 75.4%) in the second season, respectively, and this was not significantly different from the fungicide effect. Chit-NPs decreased disease severity in the first season (49.9, 57.1 and 62, 4%) and (70, 77.8 and 69.2%) in the second season. Si decreased three disease parameters (49.9, 57.1 and 53.1%) in the first season (50, 55.5 and 66.2%) in the second season followed by Chitosan.

### **7-2-2. Effect of the chemical inducers on the growth parameters:**

Si-NPs and Chit-NPs treatments clearly stimulated the vegetative growth parameters of pea plants during both two seasons when compared with control treatments. Si-NPs was the most superior treatment, recording the greatest increase in shoot length (61 and 51%), root length (95.2 and 100%), leaf area (205.3 and 144.5%), the average number of branches/plant (70 and 200%) as well as shoot fresh weight (120.1 and 110), and root fresh weight (128.5 and 88.9%) and dry weight/plant (143.4 and 106.8%) in both two seasons followed by Chit-NPs. Si seed treatment showed a moderate effect in increasing as shoot fresh weight (100.9 and 89%), shoot dry weight (100 and 89.1%), (100 and 55.6%), root dry weight/plant (104.3 and 82.7%) followed by chitosan.

### **7-2- 3-Effect of the chemical inducers on the growth parameters:**

It was observed that Si-NPs showed the best significant improvement of plant yield parameters in two seasons on pod diameter, pod length, pod seed, weight of 10 pods, weight of 100 seed and faddan yield in the first season (30.7, 46, 42.8, 152.2, 123.1, 46.1 and 21.8%) in the second season, respectively, (21.4, 100, 85.8, 68.2, 88, 35.5 and 22.6%) followed by Chit-NPs which increased yield of faddan (23, 61.9, 42.8, 122.1, 109.7, 42.2 and 20.5%) in the first season (14.2, 96.2, 78.7, 68.2, 64.1, 27.4 and 17.33%) in the second season). Si at 8 g/l increased the yield of faddan (19.9% in the first season and the second season 17%) followed by chitosan which showed (19.3%), in the first season and second season (17.3%) less increase in all yield parameters. Si- NPS and Chit-NPS treatments clearly stimulated the crop parameters of pea plants during both two seasons when compared with control treatments.

### **8-Conclusions and Recommendation:**

- Application of seed treatment using the triple mixture of AM+TH  $3 \times 10^6$  cfu/ml + Ps  $10^9$  cell/ml, Si-NPs 150 ppm and Chit-NPs 150 ppm treatments can provide a high level of protection against fungal root rot disease of pea plants.
- This protection was equal to the control that was treated with the fungicide.
- These pea seed treatments may be safely used commercially as traditional synthetic fungicides for controlling seed, and soil-borne plant pathogens and stimulation vegetative growth, yield, seed quality, physiological and anatomical characteristics of the root of the pea plant.