

PATHOLOGICAL STUDIES ON ROOT-ROTS OF BEAN

THESIS

Presented to the Graduate School Faculty of Agriculture, Damanhour University in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

In

Agricultural Sciences

(Plant Pathology)

By

Somaia Abd El-Ghany Abd El-Halim Mohamed

B.Sc (1999) & M.Sc.(2007) Faculty of Agriculture (Damanhour) Alexandria University

> Department of Plant Pathology Faculty of Agriculture Damanhour University

CONTENTS

	LIST OF TABLES	Ι
	LIST OF FIGURES	li
	ABBREVIATIONS CONTENTS	iiii
	INTRODUCTION	1
	REVIEW OF LITERATURE	3
	MATERIALS AND METHODS	11
	EXPERIMENTAL RESULTS	16
	Laboratory Experiments	16
1-	Fungi associated with root rot of bean	16
2-	Variation among <i>R. solani</i> isolates for the <i>in vitro</i> colony growth at	
		19
	Greenhouse Experiments	21
3-	Pathogenicity tests and varietal reaction	21
3-1	Variation among <i>R. solani</i> isolates for pre-emergence damping	
		21
3-2	Variation among <i>R. solani</i> isolates for post-emergence damping	• •
~ ~		21
3-3	Bean varietal reaction.	21
3-4	Biochemical changes associated with cultivar resistance and	<u> </u>
	susceptibility to root fot disease parameters	25
3-4-1	Total phenolic content	25
3-4-2	Peroxidase and polyphenol oxidase	25
3-4-3	Correlation between chemical constituents and bean root rot	
	parameters	28
	Field Experiments	29
4-	Effect of NPK fertilization on root rot and growth traits of	
		29
4-1	Effect of NPK on damping off incidence of bean	29
4-1-1	Effect of NPK on pre-emergence damping off	29
4-1-2	Effect of NPK on post-emergence damping-off	29
4-2	Effect of NPK on growth traits of bean	32
4-2-1	Effect of NPK on root traits	32
4-2-2	Effect of NPK on shoot traits	32
4-2-3	Effect on yield and yield component	32
5-	Effect of compost application on root rot and growth traits of bean	32
5-1	Effect of compost on damping off incidence	34
5-1-1	Effect of compost on pre-emergence damping off	34

5-1-2	Effect of compost on post-emergence damping off	34
5-2	Effect of compost on growth traits of bean	36
5-2-1	Effect of compost on root traits of bean	36
5-2-2	Effect of compost on shoot traits of bean	36
5-2-3	Effect of compost on pod yield of bean.	36
6	Effect of certain resistance inducers on root rot and growth traits of	
	bean	38
6-1	Effect of resistance inducers on damping off incidence	38
6-1-1	Effect on pre-emergence damping off	38
6-1-2	Effect on post-emergence damping off	38
6-2	Effect of certain resistance inducers on bean growth traits and	
	yield	41
6-21	Effect on root traits of bean	41
6-2-2	Effect on shoot traits of bean	41
6-2-3	Effect on pod yield of bean	41
7	Effect of certain fungicides on root rot and growth traits of	
	Bean	43
7-1	Effect of fungicides on damping off	43
7-1-1	Effect of fungicides on pre-emergence damping off	43
7-1-2	Effect of fungicides on post-emergence damping off	43
7-2	Effect of fungicides on growth traits of bean	46
7-2-1	Effect on root traits of bean	46
7-2-2	Effect on shoot traits of bean	46
7-23	Effect on yield and yield component of bean	46
	DISCUSSION	48
	SUMMARY	52
	REFERENCES	56
	الملخص العربي	66

LIST OF FIGURES

Fig.	No PA	GE
1-	Frequency of fungal species recovered from root rot bean samples collected from different governorates during the 2012-2013 summer seasons.	18
2-	Number of fungal isolates recovered from bean root rot samples collected from the five surveyed governorates during 2012-2013 summer	10
3-	Effect of different degrees of temperature on colony growth of <i>Rhizoctonia solani</i> (Rs1 isolate) recovered in a survey conducted for associated bean rot fungi, during 2012-2013 summer seasons, six days after inoculation	18
4-	Effect of different degrees of temperature on colony growth (diameter) on PDA of <i>Rhizoctonia solani</i> isolates recovered in the conducted survey, seven days after inoculation	20
5-	Variation in pathogenicity of <i>Rhizoctonia solani</i> isolates recovered from different surveyed governorates during the 2012-2013 summer seasons and tested on cv. Giza 6 of bean, 45 days after inoculation	20
6-	Mean percentage of pre- and post-emergence damping off on different bean cultivars grown in potted soil infested with <i>R. solani</i> isolates recovered in the survey	34
7-	Chemical constituents of bean cultivars grown in potted soil inoculated with <i>R. solani</i> (isolate Rs1) under greenhouse conditions, five days after inoculation	27
8-	Correlation between chemical constituents of bean roots and root rot disease parameters of bean grown in potted soil infested with <i>R. solani</i> (Rs1,isolate), five days after inoculation under greenhouse conditions	
	conditions	28
9-	Effect of NPK fertilization at different rates on percentage of pre- and post- emergence damping off of bean (cv. Bronco) grown in field naturally infested with root rot fungi of bean during the 2016 and 2017 summer seasons	31
10-	Effect of compost application at different rates on mean pre- and post- emergence damping off of bean (cv. Bronco) grown in field naturally infested with root rot fungi of bean during the 2016 and 2017 summer season.	35
11-	Effect of some resistance inducers on pre- and post-emergence damping off on bean (cv. Bronco) grown in field naturally infested with bean root rot	55

fungi during 2016 and 2017 summer seasons.....

Effect of seed dressing of bean cv. Bronco with certain fungicides on percentage of pre- and post-emergence damping off on bean grown in field naturally infested with root rot fungi during the 2016 and 2017 summer seasons.

LIST OF TABLES

TABLES NO

1-	Number of isolates and frequency of fungal species recovered from root rot bean samples collected from different governoates during the 2012-2013	
	summer seasons	17
2-	The <i>in vitro</i> colony diameter (cm) of <i>Rhizoctonia solani</i> isolates recoverd from the surveyed governorates during 2012-2013 summer seasons, on PDA at different degrees of incubation temperature	20
3-	Pre- and post-emergence damping off of <i>Rhizoctonia solani</i> isolates, representing the different surveyed governorates, on four bean cultivars under greenhouse conditions	23
4-	Biochemical constituents of bean cultivars grown in potted soil inoculated with R . solani (isolate Rs1) under greenhouse conditions, 5 days after inoculation	26
5-	Effect of NPK fertilization on percentage of pre-and post-emergence damping off on bean cv. Bronco grown in field naturally infested with root rot function during the 2016 and 2017 summer seasons	30
6-	Effect of NPK fertilization at different rates on growth characteristics of bean cv. Bronco grown in field infested naturally with root rot fungi during 2016	50
7-	Effect of compost application at different rates on incidence of damping off of bean cv. Bronco grown in field infested naturally with bean root rot fungi	33
8-	Effect of compost application on growth characteristics of bean cv. Bronco grown in field naturally infested with bean root rot fungi during 2016 and 2017 summer seasons.	34
9-	Pre- and post-emergence damping off incited on bean cv. Bronco grown in field naturally infested with bean root rot fungi and treated with certain resistance inducers, during the 2016 and 2017 summer seasons	39
10-	Effect of resistance inducers (at 4mM/L) on growth traits of bean cv. Bronco grown in field naturally infested with bean root rot fungi during 2016 and 2017summer seasons	42
11-	Effect of seed dressing of bean cv. Bronco with certain fungicides on damping off incidence on bean during 2016 and 2017 summer seasons	лл
12-	Effect of fungicides on growth traits of bean cv. Bronco grown in field naturally infested with bean root rot fungi during the 2016 and 2017 summer	44
	seasons	47

SUMMARY

The present study was conducted in both Faculty of Agriculture, Damanhour University, and Etay El-Baroud Agriculture Research Station during the 2012-2017 period and the obtained results can be summarized as follows:

Laboratory Experiments:

- Fifteen fungal species were found to be associated with bean root rot samples collected from five surveyed governorates, *i.e.*, El-Beheira, El-Sharqia, El-Monufia, El-Gharbia and Kafr El-Sheikh during 2012-2013 summer seasons. These fungal species were *Rhizoctonia solani*, *Fusarium oxysporium, Fusarium roseum, Fusarium solani, Alternaria alternata, Asperigillus niger, Penicillium digitatum, Pythium debaryanum, Pythium ultimum, Trichoderma viride, Asperigillus flavus, Macrophomina phaseolina, Mucor sp., Rhizopus stolonifer and Trichoderma harzianum* where *Rhizoctonia solani was* the most prevalent fungal specie over the surveyed governorates.
- Isolates of the most common fungal species recovered in the survey *i.e. Rhizoctonia solani* were analysed for their *in vitro* variations for colony growth (on PDA) as well as their pathogenicity on four commonly cultivated common bean cultivars (*i.e.* Nebraska, Contender, Giza 6 and Bronco). Considerable variations were revealed among the *R. solani* isolates for the *in vitro* colony growth at the different degrees of temperature. The tested El-Beheira isolate showed the highest diameter of growth values in all the tested degrees of temperature. This was mostly followed by El-Sharqia, El-Monufia and El-Gharbia tested isolates while Kafr El-Sheikh showed the lowest colony growth among the tested isolates. However, at the 30°C all isolates colonized the 9-cm plate and no significant differences were recorded between them.
- The optimum temperature for *R. solani* isolates colony growth was 30° C as values of colony growth of the tested isolates increased with increasing temperature from 5oC to 30oC then decreased with increasing temperature where there was no growth at 45° C.

Greenhouse Experiments:

- In pot experiment all *R. solani* isolates tested were pathogenic and incited pre- and postemergence damping-off on the four tested common bean cultivars (*i.e.* Giza 6, Nebraska, Contender, and Bronco) to different degrees. However, El-Beheira *R. solani* isolate incited the highest mean pre- and post-emergence damping off, which was followed by El-Gharbia and El-Monufia isolates while, the lowest damping off was obtained with El-Sharqia and Kafr El-Sheikh isolates over the four tested common bean cultivars. Also, three isolates out of the five isolates analyzed were not significantly different for these disease parameters.

- All tested common bean cultivars were susceptible to the tested *R. solani* isolates to different degrees. However, cv. Giza 6 was the most tolerant as exhibited 15.5% mean damping off (pre- and post-emergence). Meanwhile, cv. Bronco was the most susceptible and exhibited 23.5% mean damping-off. The other two bean cultivars tested (*i.e.* Nebraska and Contender) showed intermediate damping off values.

- Total phenolics in common bean tested cultivars significantly increased with inoculation with *R.solani* in all tested cultivars compared to the un-inoculated control. However the most tolerant cultivar, *i.e.*, Giza 6, showed the highest values of total phenolics (12.48 mg/g.f.w.), while, the most susceptible cultivar, *i.e.* Bronco, showed the lowest total phenolics with intermediate values for the other two cultivars.

- Infection with *R. solani* isolate significantly increased activity of peroxidase (PO) and polyphenol oxidase (PPO) in the inoculated common bean cultivars compared to the uninoculated control. However, the most tolerant cv. Giza 6 exhibited the highest activity of PPO (0.91 absr./min/g f.w.) and PO (0.086 absr./min/g f.w.). Also, in the most susceptible cv. Bronco, PPO and PO increased with R. *solani* inoculation but at lower values compared to cv. Giza 6. The other two cultivars tested which exhibited intermediate susceptibility also showed intermediate values for both enzymes activity.

- Negative correlations were revealed between severity of pre- and post- emergence damping, incited by *Rhizoctonia solani* (Rs1) on common bean cultivars, and the phenolic content, and PO and PPO activity of bean roots to different degrees. However, at the pre-emergence damping off stage, there was a moderate correlation (r=456) with phenolic content and also with PO and PPO where correlations were r=0.637 and r=638, respectively. Concerning the post-emergence damping off stage, there were strong correlations for all parameters being r=0.795-0.975.

Field Experiments:

Effect of NPK fertilization on root rot and growth traits of bean.

- The NPK fertilization at 150-200-50 kg/fed, to bean grown in field naturally infested with root rot fungi, was most effective and decreased mean pre-emergence damping-off on the tested cv. Bronco of bean to 5.5% compared to 22.5% for the untreated (unfertilized) bean control over the two seasons of the study (2016&2017).

- Absence of one of NPK nutrient led to mean pre-emergence damping-off ranged between 7.25% and 10.25% while absence of two nutrients exhibited pre-emergence ranged between 8.75% and 14.75%, compared to 22.5% for the untreated control, with the highest percentage for N fertilization only, and the lowest for K fertilization only.

- The NPK fertilization at 150-200-50 kg/fed was, also, the most effective and decrease mean post-emergence damping off on cv. Bronco of bean to 2.75% compared to 19.5% for the untreated infected control over the 2016 & 2017 seasons. Absence of one nutrient led to post-emergence damping-off ranged between 5.25% and 9.38% while absence of two nutrients exhibited post-emergence ranged between 7.63% and 11% with the highest percentage for N fertilization only, and the lowest for K fertilization only.

- The NPK at 150-200-50 kg/fed significantly enhanced growth of beans cv. Bronco, growing in field naturally heavily infested bean root rot fungi, and increased values of root traits, shoot traits, and pod yield of bean compared to the untreated infected control. Absence of one nutrient or two nutrients exhibited trait values still significantly higher than the untreated infected control but lower than the NPK treatment.

Effect of compost application on root rot and growth traits of bean.

-Application of compost at 2, 4, and 6 ton/fed to bean grown in field naturally infested with root rot fungi significantly decreased percentage of pre-emergence damping off on bean (cv. Bronco) compared to the untreated infected control. However, the 4 ton/fed rate treatment was the most effective as decreased mean pre-emergence damping off to 8.5% over the two 2016, 2017 successive seasons compared to 26.3% for the untreated control. This compost rate was even better than the higher compost rate of 6 ton/fed where mean pre-emergence damping off was 12.8%, while the application of the low rate of 2 ton/fed exhibited 16.3% mean pre-emergence damping off.

- Application of compost significantly, also, decreased post-emergence damping off of bean. Meanwhile, the 4 ton/fed was the most effective and decreased mean post-emergence damping off to 4.6% compared to 18.7% for the untreated control over the two seasons of 2016 and 2017. This rate was even better than the higher rate of 6 ton/fed where mean post-emergence damping off was 8.35% while, the low rate of 2 ton/fed was of the least effect as mean post-emergence damping off was 9.3% over the two seasons of the study.

- Compost application significantly enhanced bean growth (cv. Bronco), grown in field naturally infested with root rot fungi, and significantly increased bean root, shoot and pod parameter values compared to the untreated control. However, the different compost rates treatments, *i.e.* 2 ton/fed, 4 ton /fed and 6 ton/fed, were not significantly different for most of the tested root, shoot, and pod traits in the two seasons of the study but still significantly higher the untreated control.

Effect of certain resistance inducers on root rot and growth traits of bean.

- The four tested resistance inducers *i.e.*, boric acid, sodium sulphate, magnesium sulphate and sodium mono-phosphate at both rates of 2 mM/L and 4 mM/L applied to bean (cv. Bronco), grown in field naturally infested (naturally) with root rot fungi of bean, significantly decreased pre-emergence damping off of bean over the two season of 2016, 2017 compared to the untreated control. However, the 4mM/L rate was the most effective for all the tested resistance inducers. Sodium sulphate showed the highest effect (at 4 mM/L) and decreased mean pre-emergence damping off to 8.9 % compared to 22.5% for the untreated control. This was followed by Boric acid which exhibited 9.75% mean pre-emergence damping off being 11.55%, 13.75% respectively, over the two seasons of the investigation (2016 & 2017).

- All the tested resistance inducers significantly decreased post-emergence damping off on bean grown in field naturally infested with bean root rot fungi over the two years of the study. The higher rate of 4 mM/L was consistently more effective than 2 mM/L and all tested inducers were not significantly different at 4mM/L for this disease parameter in both seasons (2016 & 2017) of the study.

- All tested resistance inducers (at 4 mM/L tested rate) enhanced bean growth (cv. Bronco), grown in field naturally infested with root rot fungi, and significantly increased root, shoot, and pod growth parameters compared to the untreated control with no significant differences between most treatments particularly for pod yield/plant.

Effect of certain fungicides on root rot and growth traits of bean.

- Treatment of bean seeds (cv. Bronco) with the tested fungicides *i.e.* Moncut (25%), Rhizolex-T (50%), Mega top (70%) and Captan (75%), significantly decreased mean preemergence damping off of bean (cv. Bronco) grown in field naturally infested with root rot fungi. Rhizolex-T (50%) was the most effective and decreased pre-emergence damping off to 8.7% compared to (25%) for the untreated control. The other three fungicides showed 10-13.2% mean pre-emergence damping off over the two seasons of the study (2016 & 2017).

- All tested fungicides significantly decreased post-emergence damping off compared to the untreated control. Rhizolex-T (50%) was the most effective and decreased mean post-emergence damping off to 5.4% compared to 18.3% for the untreated control. The other three

fungicides showed 5.8% - 8.0% mean post-emergence damping off over the two seasons of the investigation.

- All the tested fungicides improved bean (cv. Bronco) growth grown in field naturally infested with root rot fungi and significantly increased root, shoot, and pod yield parameter values. Rhizolex T (50%) consistently exhibited the highest values followed by Moncut (25%) while the other fungicides showed significantly lower values for plant growth and pod yield over the two years of the study (2016 & 2017).

.