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EFFECT OF SEED TREATMENT WITH FUNGICIDE (RIDOMIL) COMBINED WITH RHIZOBIAL INOCULATION ON ROOT-ROT DISEASES AND GROWTH OF FABA BEAN PLANTS.

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ABSTRACT

Broad bean seeds were treated by Ridomil combined with seeds inoculation with specific strain of *Rhizobium leguminosarum* biovar *viceae* to study the effect of fungicide application and rhizobial inoculation on *Rhizoctonia solani* causing damping-off and root-rot diseases.

Data show that soil infested with *R. solani* alone caused the highest percentage of damping-off and root-rot diseases. Above mentioned diseases significantly decreased by using either *Rh. leguminosarum* biovar *viceae* or Ridomil in combination with *R. solani* and the lowest percentage of disease severity was obtained in the treatment included *R. solani* + *Rh. leguminosarum* + Ridomil. Nitrogenase activity and root nodulation reached their highest values with rhizobial inoculation alone. Soil infestation with *R. solani* combined with rhizobial inoculation significantly reduced N_2-ase activity and root nodulation, while above mentioned parameters were non-significantly decreased with the application of fungicide in combination with *R. solani* + *Rh. Leguminosarum*.

There was a significant decrease in studied growth characters due to soil infestation with *R. solani* alone. On the contrary, studied growth characters were significantly increased with rhizobial inoculation alone as well as when *Rh. leguminosarum* combined with either *R. solani* or Ridomil and all of them. Soil infestation with *R. solani* alone gave the lowest values of chemical constituents of faba bean plants. In contrast, N, P, K, chlorophyll and total carbohydrates were increased with either rhizobial inoculation or fungicide application when used alone or in combination with *R. Solani*.

INTRODUCTION

Faba bean (*Vicia faba* L.) is an important seed legume in Egypt used for human consumption. To face its high consumption, it is necessary to raise the yield of faba bean by different agricultural methods as the control of root-rot

pathogenic fungi. The most common and economically important pathogen causing damping-off and root-rot diseases on broad bean is *Rhizoctonia solani* (Nofal *et al.*, 1982; Gowily, 1987 and Eisa *et al.*, 1994). On the other hand, broad bean crop was reported among the leguminous crops which suffer from damping-off and root-rot diseases that reflecting on losses of both yield quality and quantity. Chemical control including the use of seed dressing compounds (fungicides) with specific reference to those of systemic action was found as effective to eliminate these severe losses due to soil-borne diseases fungi.

Regarding the relation between rhizobial inoculation and fungal infection, Chakraborty & Chakraborty (1989) found that bacterization of seeds with *Rh. leguminosarum* biovar *viceae* was highly effective in reducing the severity of root-rot of pea. Ehteshamul-Haque & Ghaffar (1993) found that *R. meliloti* and *Rh. leguminosarum* inhibited the growth of soil-borne (root-rot) fungi *Macrophomina phaseolina, R. solani* and *F. Solani*.

This research aims to study the relation between rhizobial inoculation and fungal infection in the presence of the fungicide (Ridomil plus Cu^{++}) in broad bean plants.

MATERIALS AND METHODS

A pot experiment was carried out under greenhouse condition at the Fac. Agric., Moshtohor, Zagazig Univ. to study the interaction between broad bean inoculation with *Rh. leguminosarum* biovar *viceae* and root-rot pathogenic fungus (*R. solani*) in the presence of the fungicide (Ridomil plus Cu^{++}) and their effects on the growth and some chemical constituents of faba bean plants.

Source of rhizobial strain :

An effective strain (*Rhizobium leguminosarum* biovar *viceae*) was supplied by the Dept. of Microbiology, Water and Soil Res. Inst., Agric. Res. Center, Giza, Egypt.

Source of pathogenic agents :

An effective strain of *Rhizoctonia solani* was kindly supplied by Plant Pathology Institute, Agric. Res. Center, Giza, Egypt.

Rhizobial inoculum preparation :

Preparation of rhizobial inoculum was carried out with using the yeast extract mannitol agar medium according to Gohar *et al.*, (1991).

Fungal inoculum preparation :

Preparation of fungal inoculum was carried out on sterilized sand sorghum grain medium (Whitehead, 1975).

Fungal infestation :

For each treatment into which the *R. solani* was included, sterilized soil was infested with inoculum of *R. solani* at a rate of 3% of soil weight (Zaghloul & Abd El-Mageed, 1996). Water was applied to the soil and all were thoroughly mixed to ensure even distribution for fungal inoculum, then left for one week to fungal activation.

Rhizobial inoculation :

Rhizobial inoculum was mixed with the carrier (peat) containing $CaCO_3$ (50% w/w) as a neutralizing material. Rhizobial suspension : peat ratio was 2:1. Seeds were mixed with suitable amount of Arabic gum, then thoroughly mixed with the carrier containing the specific rhizobial strain to ensure sufficient coating by inoculum. This process was carried out in the greenhouse just before sowing.

Fungicide seed dressing was carried out according to Gamal El-Din *et al*., (1990) using Ridomil plus Cu at a rate of 2 g/kg seeds.

Cultivation :

A clay soil was autoclaved at 15 lb/in^2 for 3 hr. Thirty cm diameter pots were filled with the sterilized soil (5 kg/pot). Cultivation process was performed by sowing ten inoculated or uninoculated seeds of broad bean Giza-2 cv. in each pot. This experiment included the following treatments:

- Control.
- Infection with *R. solani* only.
- Inoculation with *R. leguminosarum* only.
- *R. solani* + *Rh. leguminosarum*.
- *R. solani* + Fungicide.
- *Rh. leguminosarum* + Fungicide
- *R. solani* + *Rh. leguminosarum* + Fungicide

Generally, all pots had been supplied with equal amounts of N and P_2O_5 as ammonium sulphate and calcium super-phosphate at a rate of 15 and 30 kg/fed., respectively in two equal doses at vegetative and flowering stages.

Treatments were arranged in a randomized complete block design and each treatment was replicated four times.

Sampling and determinations :

A- Disease assessment :

1- Percentage of post-emergence damping-off was estimated after 45 days from sowing.

2- Percentage of root-rot disease was estimated after 60 days from sowing. Plants were carefully removed, thoroughly washed with tap water and examined for root-rot according to Salt (1982).

B- Nitrogenase activity :

N_2-ase activity was immediately assayed after sampling at flowering stage in nodules according to Hardy *et al.*, (1973).

C- Growth characters :

- Plant height (cm).
- Dry weights of root system (g/plant).
- Dry weights of shoot system (g/plant).
- Tillers number/plant.
- Flowers number/plant.
- Dry weights of pods (g/plant).
- Dry weights of nodules (g/plant).

D- Chemical analysis :

1- Total nitrogen was estimated in the dry matter of shoot system according to Chapman & Pratt (1961).
2- Total phosphorus was colourimetrically determined in the dry matter of shoot system according to American Public Health Association (1989).
3- Total potassium was estimated in the dry matter of shoot system by flame-photometer apparatus according to the method described by Brown & Lilliland (1946).
4- Chlorophyll a and b as well as carotenoids were estimated in the 3rd leaf of the plant according to Wettstein (1957).
5- Total carbohydrates content was determined in dry leaves by the phenol sulphuric acid method described by Michel *et al.*, (1956) and calculated as mg/g dry weight.

Statistical Analysis:

All data presented in percentages such as disease assessments were transformed to arcsin as well as data of growth characters and subjected to analysis of variance according to Snedecor & Cochran (1989).

RESULTS AND DISCUSSION

Effect of different treatments on disease severity, N_2-ase activity and nodulation :

Data in Table (1) show that soil infestation with *R. solani* alone gave the highest percentage of post-emergence damping-off and root-rot of faba bean plants. This result is in harmony with those obtained by Omer (1986), Eisa *et al.*, (1994) and Zaghloul & Abd El-Mageed (1996) who found that *R. solani* was the most virulent in causing post- emergence damping-off and root-rot diseases.

The percentage of post- emergence damping-off and root-rot diseases significantly decreased by using either *Rh. leguminosarum* or fungicide (Ridomil plus Cu^{++}) in combination with *R. solani*. The lowest percentage of post-emergence damping-off and root-rot was recorded in the treatment included *R.*

solani + *Rh. leguminosarum* + Ridomil. These results are in agreement with those obtained by Chakraborty & Chakraborty (1989) who reported that seed bacterization with *Rh. leguminosarum* biovar *viceae* was highly effective in reducing the severity of root-rot of pea. Also, similar results were observed by Ehteshamul-Haque & Gaffar (1993) who reported that rhizobial inoculation either as seed dressing or as soil drench reduce the infection with root-rot causing fungi in leguminous plants.

Regarding the effect of fungicide Gamal El-Din *et al.*, (1990) found that seed treatment with either Benlate 50% or Ridomil plus Cu^{++} increased survival of bean plants. Also, Eisa *et al.*, (1994) reported that root-rot caused by *R. solani* and *F. solani* in broad bean (*Vicia faba*) was significantly reduced by the application of fungicides.

Table (1): Effect of different treatments on disease severity, N_2-ase activity and nodulation.

Treatments	%Post-emergence damping-off	% Roor-rot	N2-ase activity (n moles $C_2H_4g^{-1}h^{-1}$)	Dry weight of nodules g/plant
Control	0.00	0.00	0.00	0.00
R. solani	45.00	60.30	0.00	0.00
Rh. leguminosarum	0.00	0.00	160.00	1.68
Ridomil	0.00	0.00	0.00	0.00
R. solani + *Rh. leg.*	12.00	9.60	112.00	1.14
R. solani + Ridomil	6.30	6.60	0.00	0.00
Rh. leg + Ridomil	0.00	0.00	136.00	1.36
R. solani + *Rh. leg.* + Ridomil	3.30	3.90	148.00	1.24
L.S.D. at 0.05	18.20	20.40	40.10	0.34

Data in Table (1) also emphasize that rhizobial inoculation alone gave the highest value of nitrogenase activity. While, the lowest value of N_2-ase activity was recorded in the treatment included *R. solani* + *Rh. leguminosarum* and this result clearly indicates that *R. solani* can antagonize *Rh. leguminosarum* biovar *viceae* and consequently reduce its nitrogen fixation activity. Nitrogenase activity was non-significantly decreased with the application of fungicide in combination with rhizobial inoculation. This result is in accordance with Gamal El-Din *et al.*, (1990) who found that using of benzimidazol fungicides as seed dressing together with rhizobial inoculation were very effective in controlling of damping-off and root-rot diseases of peas and soybean, enhanced nodulation and increased nitrogenase activity.

Also, the same trend of results was observed in the treatment included *R. solani* + *Rh. leguminosarum* + fungicide and this may be due to the inhibitory effect of fungicide on *R. solani* and consequently reduce its harmful effect. These results are in accordance with Radhakrishnan & Chatrath (1991) and Tu (1993).

As regard to dry weight of nodules, data in Table (1) also show that dry weight of nodules significantly increased with rhizobial inoculation solely and this result is in harmony with Zahra *et al.*, (1990), Gohar *et al.*, (1991) and Hussein *et al.*, (1993) who found that rhizobial inoculation of legumes significantly increased numbers and dry weights of nodules/plant.

Whereas, dry weights of nodules were significantly decreased when *R. solani* was used in combination with *Rh. leguminosarum*. This result is in line with Manninger *et al.*, (1985) and Bhattacharyya & Mukherjee (1990) who reported that the numbers and weights of *Rhizobium* nodules were greatly reduced by root-rot causing fungi i.e. *R. solani*, *F. solani* and *Sclerotium rolfsii* in leguminous plants.

Dry weights of nodules was non-significantly decreased with the application of fungicide combined with *Rh. leguminosarum* either alone or in a combination with *R. solani* + fungicide.

Effect of different treatments on growth parameters of faba bean:

Data presented in Table (2) indicate that soil infestation with *R. solani* alone gave the lowest values of all studied growth characters. Similar results were obtained by Amer *et al.*, (1983), El-Faham (1993) and Zaghloul & Abd El-Mageed (1996) who reported that fungal infection lead to stunting of the vegetative parts of the plant, reduced the fresh and dry weights of root and shoot systems. They also found significant decrease in the number of leaves and flowers/plant. On the contrary, highest significant increase of studied growth characters was obtained with rhizobial inoculation alone and these results are in harmony with Abdel-Nasser *et al.*, (1988) and Hussein *et al.*, (1993) who reported that rhizobial inoculation of legumes gave significant increase in plant height, number of leaves and flowers as well as crop yields.

Table (2): Effect of different treatments on growth parameters of faba bean.

Treatments	Plant height (cm)	Dry weights (g/plant)			No. of Tillers/ plant	No. of /plant
		Root system	Shoot system	Pods		
Control	65	0.96	5.73	2.55	1.60	22.30
R. solani	61	0.68	4.68	2.10	1.33	18.60
Rh. leguminosarum	98	1.92	9.62	4.73	3.00	40.30
Ridomil	85	1.28	6.35	3.40	2.21	30.30
R. solani + *Rh. leg.*	84	1.48	7.26	2.98	2.33	26.60
R. solani + Ridomil	81	1.36	6.43	3.26	2.33	28.30
Rh. leg. + Ridomil	91	1.61	8.12	4.12	2.60	36.60
R. solani + *Rh. leg.* + Ridomil	94	1.82	8.24	4.48	3.00	36.30
L.S.D. at 0.05	8.30	0.42	1.52	0.98	0.70	4.82

Data in Table (2) also show that compared with soil infested with *R. solani* alone, plant height, dry weights of root and shoot system, weights of pods and number of flowers were significantly increased when *Rh. leguminosarum* combined with either *R. solani* or Ridomil were used. These results are in agreement with several investigators. As regard to the interaction between rhizobial inoculation and fungal infestation, Ehteshamul-Haque & Ghaffar (1993) and El-Faham, (1993) found that application of *Rh. leguminosarum*, *Rh. meliloti* and *Bradyrhizobium japonicum* as seed-dressing combined with infestation by *R. solani*, *Macrophomina phaseolina* or *Fusarium* spp. improved plant growth characters and gave increase in shoot length and dry weights of plant organs as compared with uninoculated ones. As regard to *Rhizobium* and fungicide interaction, Gamal El-Din *et al.*, (1991) reported that the using of benzimidazole fungicides as seed-dressing together with rhizobial inoculation improved growth characters of the leguminous plants.

Regarding the rhizobial inoculation in combination with *R. solani* + Ridomil, obtained data clearly indicate that the above mentioned studied growth characters were significantly increased in the treatment included *R. solani* + *Rh. leguminosarum* + Ridomil as compared to the application of either *R. solani* or Ridomil separately. This result is in line with Radhakrishnan & Chatrath (1991) who obtained significant increase in growth characters of groundnut by seed inoculation with *Rhizobium* in combination with fungicide (Carbendazim) and soil infested with *Macrophomina phaseolina*.

Effect of different treatments on some chemical constituents of faba bean.

Data in Table (3) clearly indicate that, except the control treatment, total nitrogen percentage of faba bean plants was the lowest in case of soil infestation with *R. solani* alone. This result is in harmony with Amer *et al.*, (1983), Gamal El-Din *et al.*, (1991) and Zaghloul & Abd El-Mageed (1996) who reported that fungal infection greatly diminished total nitrogen content. In contrast, rhizobial inoculation alone gave the highest percentage of total nitrogen. This result is in conformance with those reported by Gohar *et al.*, (1991), Hussein *et al.*, (1993) and Monib *et al.*, (1994).

Compared with soil infested with *R. solani* alone, total nitrogen percentage moderately increased when *R. solani* combined with either *Rh. leguminosarum* or Ridomil.

Regarding the effect of fungicide, data show that application of Ridomil alone or in combination with either *Rh. leguminosarum* biovar *viceae* or *Rh. leguminosarum* + *R. solani* gave a high increase in total nitrogen as compared to control and *R. solani* alone treatments. The increase in total nitrogen content in plants raised from seed inoculated with *Rhizobium* in combination with seed-dressing by fungicides may be attributed to a synergistic effect. It is possible that the fungicide which ultimately reaches to the soil may be reduce the numbers of soil-fungi and their colonization of nodules and roots. The soil fungi are known

to be compete with rhizobia populations and resulting an inhibition of growth and nitrogen fixation of host plants (Wildin & Kennedy, 1983). Also, the biological efficacy of the *Rhizobium* was not found to be affected by fungicide application and this had been reported by Kataria *et al.*, (1985). Therefore, Ridomil fungicide can be recommended as seed treatment in combination with rhizobial inoculation.

Data in Table (3) also show that total phosphorus and potassium were the lowest values in case of fungal infestation alone as compared to other treatments. On the contrary, rhizobial inoculation alone gave the highest values of total phosphorus and potassium. While, total phosphorus and potassium content increased when *R. solani* was used in combination with either Ridomil or *Rh. leguminosarum* as well as all of them. These results are in accordance with those obtained by Husein (1996) who found that fungal infection of leguminous plants gave higher decrease of phosphorus and potassium content than uninfected ones. In contrast, Hammouda *et al.*, (1991) and Zaghloul & Abd El-Mageed (1996) found that rhizobial inoculation of leguminous crops gave higher increase of phosphorus content than uninoculated ones.

Regarding the chlorophyll's level in leaves data recorded in Table (3) show that chlorophyll a, b and c content was highly reduced in the treatment in which soil was infested with *R. solani* alone and this result is in accordance with Gamal El-Din *et al.*, (1990) and Zaghloul & Abd El-Mageed (1996). While, chlorophyll a, b and c level increased when *R. solani* was used in combination with either *Rh. leguminosarum* or Ridomil as well as in the treatment included all of them. This result clearly indicates that *Rh. leguminosarum* and fungicide (Ridomil) can antagonize or inhibit *R. solani* and reduce its harmful effect on chlorophyll's biosynthesis. The highest level of chlorophyll a, b and c was recorded in the treatment included rhizobial inoculation solely.

Table (3): Effect of different treatments on some chemical constituents of faba bean.

Treatments	Total N	Total P	Total K	Chlorophyll			Total carbohydrates
				a	b	c	
	%	%	%	mg/g fresh matter			mg/g dry matter
Control	2.31	0.380	2.15	0.290	0.196	0.168	24.10
R. solani	2.50	0.304	2.05	0.261	0.184	0.152	19.20
Rh. leguminosarum	4.80	0.780	4.25	0.704	0.428	0.386	58.60
Ridomil	4.12	0.443	3.10	0.511	0.233	0.255	39.20
R. solani + *Rh. leg.*	3.92	0.582	3.50	0.560	0.304	0.292	46.50
R. solani + Ridomil	3.86	0.460	3.27	0.589	0.312	0.236	36.40
Rh. leg. + Ridomil	4.62	0.560	3.62	0.595	0.258	0.301	44.32
R. solani + *Rh. leg.* + Ridomil	4.32	0.623	3.84	0.610	0.420	0.340	52.80

Data in Table (3) also show that the lowest level of carbohydrates was detected in case of soil infestation with *R. solani* alone. Similar results were detected by Amer *et al.*, (1983) and Gamal El-Din *et al.*, (1990) who found that fungal infestation decreased the carbohydrates content of leaves. Whereas, the highest levels of carbohydrates was obtained from seed inoculated with *Rh. leguminosarum* alone. Moreover, it is importance to notice that rhizobial inoculation in combination with either *R. solani* or Ridomil, gave higher carbohydrates content than the application of Ridomil either alone or in combination with *R. solani*. The treatment included *R. solani* + *Rh. leguminosarum* + Ridomil raised the carbohydrates content in faba bean plants as compared with rhizobial inoculation in combination with either *R. solani* or Ridomil separately.

Generally, obtained results emphasize that total carbohydrates was almostly proportionated with chlorophyll's level in various treatments since the chlorophyll's pigments are responsible of photosynthesis and consequently carbohydrate formation.

CONCLUSION

Obtained results clearly indicate that the using of Ridomil plus Cu fungicide as seed-dressing together with seed bacterization by specific strain of *Rhizobium leguminosarum* biovar *viceae* were very effective in controlling damping-off and root-rot diseases of faba bean. Nevertheless, root nodulation and nitrogenase activity were significantly increased. Also, rhizobial inoculation combined with *R. solani* + Ridomil improved faba bean as growth features and increased chemical components compared to rhizobial inoculation combined with either *R. solani* or Ridomil separately.

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تأثير معاملة البذور بالمبيد الفطري (ريدوميل) في وجود التلقيح بالرايزوبيا على أمراض أعفان الجذور والنمو في الفول البلدي

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في هذا البحث تم معاملة بذور الفول البلدي بالمبيد الفطري (ريدوميل) في وجود التلقيح بسلالة الرايزوبيوم *Rh. leguminosarum* biovar *viceae* لدراسة تأثير المعاملة بالمبيد الفطري والتلقيح بالريزوبيوم على فطر الـ *R. solani* المسبب لمرض سقوط البادرات وعفن الجذور. وقد أوضحت النتائج أن العدوى بفطر *R. solani* أدى إلى حدوث أعلى نسبة إصابة بمرض سقوط البادرات وعفن الجذور ، بينما قلت نسبة الإصابة معنوياً عند التلقيح بالرايزوبيوم أو عند استخدام المبيد الفطري كل على حده مع فطر *R. solani* ، وكانت أقل نسبة إصابة بكلا المرضين عند معاملة البذور بالمبيد الفطري مع التلقيح بالرايزوبيوم.

أوضحت النتائج أن أعلى نشاط لإنزيم النيتروجينيز وكذلك الوزن الجاف للعقد الجذرية لوحظ عند التلقيح بالرايزوبيوم منفرداً ، بينما لوحظ نقص معنوي عند العدوى بفطر *R. solani* في وجود التلقيح بالرايزوبيوم. عند استخدام المبيد الفطري والعدوى بفطر *R. solani* في وجود التلقيح بالرايزوبيوم لم يلاحظ نقص معنوي في نشاط إنزيم النيتروجينيز والوزن الجاف للعقد الجذرية.

أوضحت النتائج أيضاً نقص معنوي في صفات النمو التي درست عند العدوى بفطر *R. solani* منفرداً . على العكس من ذلك أدى التلقيح بالرايزوبيوم سواء منفرداً أو مع كلاً من فطر *R. solani* أو الريدوميل أو كلاهما معاً إلى زيادة معنوية في صفات النمو للفول البلدي.

عند عدوى التربة بفطر *R. solani* منفرداً أوضحت النتائج إنخفاض في المكونات الكيماوية للفول البلدي ، بينما حدثت زيادة في النيتروجين والفوسفور والبوتاسيوم والكلوروفيلات وكذلك محتوى الكربوهيدرات الكلية لنباتات الفول البلدي عند التلقيح بالرايزوبيوم أو استخدام المبيد الفطري سواء عند استخدام كل منهما منفرداً أو مع العدوى بفطر *R. solani* .

عموما أوضحت النتائج المتحصل عليها في هذا البحث أن معاملة بذور الفول البلدي عند الزراعة بالمبيد الفطري ريدوميل مع التلقيح بالرايزوبيا أدى إلى إنخفاض نسبة الإصابة بأمراض سقوط البادرات وعفن الجذور . كذلك لوحظ زيادة في نشاط إنزيم النيتروجينيز مما أدى إلى تحسين صفات النمو والمكونات الكيماوية لنباتات الفول البلدي.