Bicontrol of *R. Solani* AG-4 with Piperitone Product from *Cymbopogon Proximus* and Comparison with Bicontrol Agents (*Trichoderma* Spp)

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ABSTRACT

In vitro **antagonism experiments were carried out to study the ability of five biocontrol agents (: *Trichoderma harzianum, T. asperellum, T. hamatum, T. virenes* and *T. viride*) and fourteen plant extracts for their effects on the growth of mycelia of *R. solani* AG-4. *T. harzianum* was recorded the maximum inhibitor of mycelial growth followed by *T. asperellum* with average of the inhibition growth 56.82 % and 54.48 %, respectively. Results showed also that significant antifungal activity was detected for the extracts of *Cymbopogon proximus*, and *Ruta chalepensis*.. These extracts were effective in inhibiting the mycelial growth of *R. solani* AG-4, (at amount/disc, 4 mg) with an average of 22.59and 21.48 respectively. Comparison studies of the inhibitory effects of piperitone, biocontrol agents and fungicide (Rizolex) on DSI, length (cm), fresh and dry weight (gm) of bean plants were also recoded. Treatments of piperitone in the presence of *R. solani* Ag-4 showed the best control of dry root rot and stem bean disease there are also reduction effect in disease severity with average 1.00, compared with inoculated bean plants with *R. solani* AG-4 alone with average 3.65. *R. solani* AG-4. In the presence of *T. asperellum* or *T. harzianum* or Fungicide (Rizolex) were also recorded reduction in disease severity with average of 2.41 and 1.66, 1.91, respectively.**

INTRODUCTION

Cymbopogon spp (Poaceae /Graminae) represent an important genus of about 120 species and several varieties. *Cymbopogon* species was known as a source of commercially valuable compounds (like , pipertone) which were either used as such in perfumery and allied industries or as starting materials for the synthesis of other products commonly used in perfumery Shahi and Tava, (1993**).**This genus was distributed in the tropical and subtropical parts of the world, more than 52 types in Africa, 45 in India, six in South America and Australia , four in Europe, and two in North America Bhan, *et.al,* (2005**).** Screening of some aromatic plants for fungitoxicity of their volatile oils, *Cymbopogon pendulus*, exhibited strong activity as fungicidal, completely inhibiting the mycelial growth of the tested fungi at its minimum inhibitory concentration of 200ug /ml, inhibiting heavy inocula of the tested fungi, Pandey, *et.al*., (1996**).** Valarini, *et.al*., (1994). found that The essential oil of extract of *Cymbopogon citrates* leaves, completely controlled mycelial growth *Rhizoctonia solani* on P.D.A. medium. Bankole and adebanjo, (1995)**.** found that the *in-vitro* and *in- vivo* efficacy of leaf extracts from 5 plants including *Cymbopogon citrates* were evaluated in inhibiting the growth of four plant pathogenic fungi (*Macrophomina phaseolina*, *Fusarium moniliforme*, *Fusarium solani*, and *Botryodiplodia theobromae*). Aqueous extracts of *C. citrates* completely inhibited the growth of *M.phaseolina* and *B. theobromae*, and also significantly reduced the growth of *F. moniliforme* and *F. solani*.

Sangwan., *et al.,* (2000) showed that the extract of Cymbopogon grass was analysed by gas-Liqid Chromatography (GLC), GC-MS and peak –enrichment method. The oil from the *Cymbopogon* spp was identified ,as, varian, and piperitone, the cyclic monoterpene ketone , formed a significant percentage. Singh, *et.al*. (1998) showed that, the essential oil extracts from varirous parts of 11 higher plants which were screened *in-vitro* for their fungitoxicity against fungal pathogens: *Colletotrichum falcatum*, *Fusarium moniliforme*, *Ceratocystis paradoxa*, *Rhizotonia solani* , *Curvularia lunata* , *Periconia atropurpuria* and *Epicocum nigrum* GC-MS analysis of *Lippia alba* essential oil showed Limonene (at 12.6%) and piperitone (at 19%) to be the major chemical constituents. Piperitone was strongly toxic even at 200ppm., Menut, *et. al*. **(**2000), found that The essential oil from leaves and flowering staks of the three Cymbopogon species (*C. citratus*, *C.giganteus*, and *C.Proximus)*, were analysed by GC and GC-MS, the main component of the leaf and flower esstial oil of *C. proximus* was piperitone (59.1% and 55.6% repectively). *C. proximus* showed also the strongest antioxidant. Dhar, *et. al*.(1997)**.** studied the variation in essential oil in five genotypes of *C.Jwarancusa*, from stage 1 (green plant spikes initiated to stage 9 (30-50 leaves brown) the essential oil content ranged from 0.5 to1.64%. The main components were Piperiton (44.9-66.8%) and delta 2-carene(8.3- 23.5%). Lohani, *et .al.,* 1986., found that of the 17 compounds identified from essential oil of Cymbopogon, Piperitone (47%) and car-2-ene (29%)predominated. Shahi and Sen, (1989). Found that forty compounds in the essential oil extracted for herbage collected from 3 sites in western Rajasthan, the main constituent was piperitone (47.57- 64.37%). Singh, and Pathak, (1994). found that, the growth

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performance of three cultivars of *Cymbopogon jwarancusa* were proved herb yield (21.1 t / ha.) oil content (1.6%) and oil composition (83% piperitone).

The well known antagonistic fungus *Trichoderma* spp, which is commercially available in several countries, can be used also to suppress soil-bore pathogens that cause diseases such as damping –off, root rot, stem rot, and wilting in many vegetables and has also a good effect on plant growth. *Trichoderma* spp can be used also to treat vegetables seed or mixing it into the soil a few days before planting as a protection against seed-borne and soil –borne fungal diseases Punja, 1997 and Kubicek *et al* (2001) The efficiency of *T. harzianum* as a seed treatment was assessed against stem rot of soyabean caused by *Rhizoctonia solani* in sterilized soil in pot tests. The lowest diseases severity index was observed when seeds were treated with *T. harzianum* plus the carrier of methyl cellulose, Dutta, and Das, (1999). Biological control of *Rhizoctonia solani* on *Phaseolus vulgaris* was investigated in greenhouse trials. *Trichoderma harzianum* was the most effective *in- vitro* and in pot experiment , also reduced post emergence root rot and proved useful controlling root rot of French bean caused by *Rhizoctonia solani* Mathew and Gupta (1998). Hazarika and Das, (1998) showed that Both *Trichoderma harzianum* and *T. viride* were effectively controlled root rot disease of *Phaseolus vulgaris* L. when applied as seed or soil treatments. Mathew and Gupta, 1998 found that *Trchoderma harzianum* was the most effective in vitro and in pot experiments in greenhouse trails against *Rhizoctonia solani* on Phaseolus vulgaris.

The objective of this study is to;

1- Compare antagonistic effect of fourteen plant extracts as natural chemical agents, and five *Trichoderma* spp. as biological control agents, against *R. solani* AG-4 in *in-vitro* studies.

2- Evaluate the disease severity index(DSI) of dry root rot and stem necrosis of bean (*Phaseolus vulgaris*), and some growth characteristics of affected bean plants after inoculation with *R. solani* AG-4 alone, or with biocontrol agents under greenhouse condition.

3- Comparison of the inhibitory effects of piperitone (isolatied from *Cymbopogon proximus*) , biocontrol agents and fungicide (Rizolex) on DSI, length (cm), fresh and dry weight (gm) and some growth characteristic of affected bean plants after inoculation with *R. solani* AG-4 under greenhouse condition..

MATERIALS AND METHODS

An isolate of *Rhizoctonia solani AG-4* obtained from naturally infected bean plants from Riyadh region was used throughout this study. The isolate was maintained in the dark at 25ºC on potato dextrose agar. Five biocontrol agents: *Trichoderma harzianum, T. asperellum, T. hamatum, T. virenes* and *T. viride* were isolated in the Plant Pathology Laboratory, College of Agriculture, King Saud University from local soil or plants grown in Riyadh region from commercial field and greenhouse and identified by Gary Samuels, United States Department of Agriculture, Systematic Botany and Mycology Laboratory.

Plant extracts:

In – Vitro experiments:

In-vitro experiments were carried out to study the ability of fourteen plant extracts using a randomized block design with three replicate plates for each plant extracts used in this study show in (Table 1).These plants were obtained from the local market and 100g of each were ground to a fine powder and extracted twice with 90% aq. ethanol. The alcohol was evaporated under vacuum and the remaining extracts were weighed and kept in the refrigerator at 10ºC until used.

Table1. Effect of fourteen plant extracts in three concentrations (amount/disc (1,2, 4 mg) against inhibition of mycelial growth of *R.solani* AG-4

Plant extracts	%Growth inhibition		
	Amount/disc Concentration		
	1 mg	2 mg	4mg
Artemisia abysinica	00.00 *f*	00.00 *f*	00.00 *d*
Clutia myricoides	00.00 *f*	00.00 *f*	00.00 *d*
Cymbopogon proximus	9.57 *c*	21.12 *a*	22.59 *a*
Eucalyptus spp	00.00	00.00 *f*	00.00 *d*
Ficus palmata	5.55 *d*	12.06 *d*	14.46 *b*
Juniperus polycaepus	00.00	00.00 *f*	00.00 *d*
Lavandula dentate	0.48 *f*	3.40 *e*	5.82 *c*
Lavandula pubescens	1.37 *e*	2.77 *e*	4.72 *c*
Lawsonia inesmis	12.47 *b*	13.87 *c*	14.52 *b*
Leptadenia protechnica	00.00 *f*	00.00 *f*	00.00 *d*
Nepeta difflersiana	00.00 *f*	00.00 *f*	00.00 *d*
Plectranthus spp	00.00 *f*	00.00 *f*	00.00 *d*
Ruta chalepensis	15.91 *a*	18.12 *b*	21.48 *a*
Sagaretia thea	00.00 *f*	00. 00 *f*	00.00 *d*

*Values within a column followed by the same letter are not significantly
(*P= 0.05*) different

At the time of the experiment, a stock solution of each extract at a concentration of 100 mg/ml was prepared. Aliquots of 1, 2 and 4 mg/ml of each extract were applied on sterilized filter paper discs and placed on the inside edge of a petri dish. Treatments also included an untreated filter paper disk (treated with 90% ethanol/water) and served as a control. Three replicate petri dishes containing 20 ml of PDA were used for each plant extract. Culture of *Rhizoctonia solani* AG-4 was grown on PDA for 1 week and 8mm diameter mycelial plugs were cut from the margins of the colony. These plugs were transferred to the inside edge of a petri dish (8 cm away from filter paper disc) containing the PDA medium. Plates were also incubated at 25ºC in the dark, and the growth area of the fungus was measured 4 days after planting.

Isolation and identification of piperitone:

The procedure for isolation and identification of piperitone from *Cymbopogon proximus was done as follows*: One kg of *Cymbopogon proximus* was purchased from the local market (local name "halfa bar"), and ground into a fine powder. The powder was then extracted twice with 90% aq. ethanol, filtered in a büchner funnel, and evaporated under vacuum. The oily aromatic residue (150 g) was refrigerated at -10^{o}C until further fractionation.

Antifungal tests showed that the extract of *C. proximus* was most effective on *R. solani* AG-4. It was therefore subjected to further fractionation to isolate the active compound(s). Five g of the alcoholic extract were dissolved in the least amount of petroleum ether and subjected onto the top of a glass column (100 cm x 5 cm i.d.) packed with Silica gel 60 A (230 to 400 mesh) in petroleum ether. The column was eluted with a solvent gradient consisting 500 ml of: petroleum ether (5 fractions), 5% ether in petroleum ether (3 fractions), 25% ether in petroleum ether (4 fractions), 50% ether in petroleum ether (4 fractions), 75% ether in petroleum ether (4 fractions), 100 % ether (4 fractions) , 50% ether in acetone (5 fractions) , 100% acetone (4 fractions), 50% acetone in methanol (5 fractions), and finally 100 % methanol (8 fractions). All fractions were evaporated under reduced pressure to remove eluting solvents.

The course of chromatographic separation was followed by thin-layer chromatography (TLC) coated with silica gel and developed by the solvent mixture of hexane, ether, acetic acid (90:10:1). Spots were visualized after spraying with anisaldeheyde/sulfuric acid and heating in the oven at 100^{o}C. Similar fractions were combined into 10 fractions according to their profile on the thin layer chromatograms. The combined fractions were tested against *R. solani* AG-4 to determine the active fractions. The antifungal assay showed that fractions 3 and 4 possessed 100% inhibition of fungal growth. These 2 fractions were combined and further fractionated on a reversed phase (C-18) column (250 mm x 9 mm) and eluted with CH_2Cl_2:MeOH:H_2O (90:10:1) with the aid of a medium pressure pump. Fractions were eluted 20 ml each and collected in test tubes. Similar fractions were also combined according to their TLC separation profile. The combined fractions were then subjected to antifungal tests to determine the most effective fraction. Fractions 3-4 were found to contain the same major compound and were the most active.

Re-chromatography of these two fractions combined following the same conditions above revealed the isolation of a pure compound (340 mg) of an aromatic oily substance.

This fraction was subjected to GC/MS, on an Agilent 6890 gas chromatograph equipped with a 5973 mass selective detector MSD chromatograph and attached to HP-5MS capillary column (30m, 0.25 mm i.d., 0.25 mm film) with flow rate 1 ml/min helium. Injections were split and the injector temperature was 150 ^{o}C. The oven's initial temperature was 45 ^{o}C, held for 1.5 min., then programmed to 190 ^{o}C at 30 ^{o}C/min., then to 240 ^{o}C at 3 ^{o}C/min. The MSD interface temperature was 280^{o}C and the dwell time in the mass analyzer was 40 msec/ion. Peak identification was performed by comparing the retention times of each peak with those of known standard compounds and by the NIST 98 mass spectral library. The mass spectral library showed that the isolated compound has a similar mass spectrum to piperitone, a known monterpenoid, with the molecular formula $C_{10}H_{14}O$.

Astandard substance of piperitone was purchased from Chem Service, USA then injected to the GC/MS system and it gave the same retention time and mass spectral fragmentation as the isolated active compound alone and upon admixture of both substances. Therefore, the isolated compound responsible for the antifungal activity of *Cymbopogon proximus* is identified as piperitone.

1- Laboratory experiments:

In vitro antagonism experiments were carried out to study the ability of five biocontrol agents show in Table (2) for their effects on the growth of mycelia of *R. solani* AG-4, following the dual culture technique (Hudec, 2000),. The experiment was performed twice. A mycelial disc (8mm diameter) from 4-day-old cultures of *R. solani* AG-4 was placed on the inside edge of a petri dish. Then a mycelial disc (8mm diameter) obtained from 4-day-old cultures of each *Trichoderma* spp. was placed 8 cm away from the inoculum of the pathogen, the plates were incubated at 25ºC. The growth

area of the fungus was measured 4 days after planting. The growth inhibition of *R. solani* AG-4 was calculated in relation to the growth of the control in all treatments according to the method of Abdulsalam *et. al* 1993. (%, Growth inhibition = 100 - fungal growth (treated) X 100 / fungal growth (control) Data were then analyzed using the Statistical Analysis System software (SAS Institute Inc.,1988**)**. Analysis of variance and least significance differences (LSD) P=0.05 were used to detect differences among means.

Table2. Effect of five biocontrol agents on inhibition of mycelial growth of *R.solani* AG-4

Biocontrol agent:	%Growth inhibition
T. asperellum	54.48 *ab*
T. harzianum	56.82 *a*
T .hamatum	52.62 *ab*
T. viride	50.13 *b*
T . virens	43.62 *c*

*Values within a column followed by the same letter are not significantly (*P= 0.05*) different

2- Greenhouse experiments :

Two experiment designs in a randomized complete block with six replicates was carried out in the greenhouse at the college of Food Science and Agriculture, King Saud University . Treatments of the first experiment show in (Table. 3) to evaluate the disease severity of dry root rot and stem necrosis of bean (*Phaseolus vulgaris*), and determine some of the growth characteristic of affected bean plants after inoculation with *R. solani* alone or with biocontrol agents.

Table3. Comparison of the inhibitory effects of plant extracts and bicontrol agent on growth of *R. solani* AG-4

Treatment	Amount/disc Concentration	%Growth inhibition of *R. solani*
Plant Extracts:		
Cymbopogon proximus	4mg	22.59 *d*
Ficus palmate	4mg	14.46 *e*
Lavandula dentate	4mg	5.82 *f*
Lavandula pubescens	4mg	4.72 *f*
Lawsonia inesmis	4mg	14.52 *e*
Ruta chalepensis	4mg	21.48 *d*
Biocontrol agents:		
T. asperellum	--	54.48 *a*
T. harzianum	--	56.82 *ab*
T .hamatum	--	52.62 *ab*
T. viride	--	50.13 *b*
T . virens	--	43.62 *c*

*Values within a column followed by the same letter are not significantly different according to the L.S.D. test (*P= 0.05*)

Treatments of the second experiment show (in table 4) to compare the inhibitory effects of plant extracts, bicontrol agent and fungicide (Rizolex) aganist *R. solani* AG-4 causing dry root rot and stem of bean. **a) Inoculum of *R. solani* AG-4**

Inoculum of *R. solani* AG-4 was prepared by the whole grain method of (Gaskill, 1968). The inoculum was thoroughly mixed added to the pot soil surface at the rate of 0.5% w/w per pot (Papavizas and Dvery, 1962) and then covered with a thin layer of sand. The control consisted of sterilized ground wheat grains with no pathogen.

a) Inoculum of *R. solani* AG-4

Inoculum of *R. solani* AG-4 was prepared by the whole grain method of (Gaskill, 1968). The inoculum was thoroughly mixed added to the pot soil surface at the rate of 0.5% w/w per pot (Papavizas and Dvery, 1962) and then covered with a thin layer of sand. The control consisted of sterilized ground wheat grains with no pathogen.

b) Seed treatment:

Bean seeds (*Phaseolus vulgaris* L.) were surface disinfested in 1.5% sodium hypochlorite solution for 10 min, washed in sterilized water and air dried. Conidia of *Trichoderma* spp 7 day old Petri cultures grown on Potato Dextrose Agar, were flooded with sterile water and spores were scraped from the agar surface, sieved through three layers of sterile cheesecloth and counted in a haemacytomter. Spores were then resuspended in sterile water containing 1.4%CMC to give a final concentration of 1.0×10^{8} spores per ml. Bean seeds were pergerminated in the CMC spore suspension for 24 h at 25 ºC before sowing. The fungicide(Rizolex) was applied at doses recommended by the manufacturers to simulate the actual field doses. Four bean seeds were planted in 12 cm diameter plastic pots containing steam sterilized soil. The soil consist of clay loam and sand in a 1:1 ratio by volume. Control pots containing autoclaved soil alone were included for comparison. Temperatures during the experiment ranged from 18-27 ºC. Disease Severity Index (DSI) based on a scale 0-5 where 0= No apparent infection, 1= light discoloration of crown and root tissue approximately 10%, 2= 11-20% crown and root tissue covered with dark brown lesion, 3= 21-50 crown and root tissue covered with large dark brown lesion, 4= 51-75%crown and root tissue covered with large lesion and decay of root tissue, and 5 = dead plant (Baudion, 1988).At the end of experiment differences parameter of plant growth (plant height, fresh and dry weight (g) of root and shoots) were assessed. Data were analyzed using the Statistical Analysis System (SAS Institute,Inc.1988) Analysis of variance and Least Significance Difference values

Table4. Effects of biocontrol agents (*T. asperellum* (T.1) and *T. harzianum* (T.2) on DSI, length (cm), fresh and dry weight (gm) of bean plants after inoculation with *R. solani* AG-4

Treatment	**DS	Length (cm)	Fresh weight (g)		Dry weight (g)	
			Shoot	Root	Shoot	Root
Control	00.00	20.43*a	13.75 a	1.85 a	3.95 a	0.69 a
***T. asperellum* (T.1)**	00.00	19.23 a	13.55 a	1.76 a	3.92 a	0.65 a
***T. harzianum* (T.2)**	00.00	19.90 a	14.17 a	1.93 a	4.20 a	0.70 a
***R. solani* + (T.1)**	1.66 b	17.40 b	9.55 c	1.30 b	2.95 b	0.72 a
***R. solani* + (T.2)**	1.53 b	18.96 b	11.10 b	1.45 b	3.00 b	0.50 b
R. solani	3.58 a	15.23 c	8.03 c	1.05 c	1.41 c	0.30 c

*Values within a column followed by the same letter are not significantly (*P= 0.05*) different

** DS = Disease Severity

(LSD),(P=0.50) were used to detect differences among treatment means.

RESULTS AND DISCUSSIONS

Results showed that significant antifungal activity was detected for the extracts of *Cymbopogon proximus*, and *Ruta chalepensis*.. These extracts were found effective in inhibiting the mycelial growth of *R. solani* AG-4, (at Amount/disc, 4 mg) with an average of 22.59and 21.48 respectively. *Lavandula* spp was found to be a weaker antifungal agent under the tested concentration, (at Amount/disc, 4 mg) which recorded only 4.72% and 5.82 % (*L. pubescens and L.. dentate*, respectively), mycelial growth inhibition of *R. solani* AG-4. No evidence of growth inhibition of *R. solani* when used plant extracts of *Artemisia abysinica***,** *Clutia myricoides*, *Eucalyptus* spp, *Juniperus polycaepus*, *Leptadenia protechnica*, *Nepeta difflersiana*, *Plectranthus* spp, and *Sagaretia thea* . (Table 1) Other studies support these results in which *Cymbopogon pendulus*, exhibited strong activity as fungicidal, completely inhibiting the mycelial growth of the tested fungi at its minimum inhibitory concentration of 200ug /ml, inhibiting heavy inocula of the tested fungi, (Pandey, 1996). The *in-vitro* and *in- vivo* efficacy of leaf extracts from 5 plants including *Cymbopogon citrates* were evaluated in inhibiting the growth of four plant pathogenic fungi (*Macrophomina phaseolina, Fusarium moniliforme, Fusarium solani*, and *Botryodiplodia theobromae*). Aqueous extracts of *C. citrates* completely inhibited the growth of *M.phaseolina* and *B. theobromae*, and also significantly reduced the growth of *F. moniliforme* and *F. solani* Bankole and adebanjo, (1995). Data in (Table:2) show significant differences between all treatments in the growth inhibition of *R. solani* AG-4. All tested biocontrol agents except *T. viride* were the most effective in inhibiting the pathogen growth as compared with tested plant extracts. *T. harzianum*, *T. asperellum* and *T .hamatum* were found highly effective against *R. solani* AG-4, than other tested *Trichoderma* spp. in the dual culture technique. *T. harzianum* was recorded the maximum inhibition of mycelial growth followed by *T. asperellum* with average of the inhibition growth 56.82 % and 54.48 %, respectively. Durman *et al* 1999 suggested that dual cultures in Petri dishes may be useful for detecting isolates as biological control agents. Chet, I., (1987). showed that the species of *Trichoderma* are commercially applied as biological control agents against fungal pathogens.Plant pathogenic fungi such as *Botryodiplodia, Colletotrichum, Gliophalotrichum, Fusarium, Pythium, Rhizoctonia, Sclerotium* spp. and others were successfully controlled by *Trichoderma* spp. Sivakumar *et. al*.(2000) and Sanjay *et,al*.,(2001) found that the inhibition of *R. solani* AG-4 by *Trichoderma* spp., especially *T. harzianum*, *T. asperellum* and *T .hamatum*, which provided evidence of its applicability to control this pathogen. Data in (table.3) show the comparison of the inhibitory effects of six plant extracts at the concentration (4mg, amount/disc) and bicontrol agent on inhibition growth of *R. solani* AG-4. Significant antifungal activity was detected for the extracts of *Cymbopogon proximus*, and *Ruta chalepensis* with average of the inhibition growth of *R. solani* AG-4, 22.59 % and 21.48%, respectively**) .***T. harzianum*, *T. asperellum* and *T .hamatum* were found highly effective on inhibition growth *R. solani* AG-4 with average of the inhibition growth 56.82 % ,54.48 %, and 50.13 % respectively.

Data in (Table. 4), show the effects of biocontrol agents: (*T. asperellum* (T.1) and *T. harzianum* (T.2) on diseases severity index, plant length (cm), fresh and dry weight (gm) of bean plants after inoculation with *R. solani* AG-4. Symptoms and signs of disease on inoculated bean plants were identical of those observed on naturally infected plants. Dark brown in the crown region or just below the soil surface, frequently girdled the basal stem causing stem and dry root rot bean disease. Disease Severity Index showed highly significant differences among all treatments (Table.4). *T. harzianum* (T.2) plus *R. solani* AG-4 and *T.*

asperellum (T.1) plus *R. solani* were the most effective to control disease with the average of disease severity of 1.53 and 1.66, respectively, as compared with inoculated bean plants with *R. solani* AG-4 alone with the average of disease severity of 3.58. Significant differences in plant height were observed, plants inoculated with *R. solani* AG-4 alone showed reduction in plant height (cm) with average 15.23 and reduction in fresh and dry weight (g) of shoot and root with the average of 8.03, 1.05, 1.41, and 0.30 respectively, as compared with inoculated bean plants with *R. solani* AG-4 in the presence of *T. harzianum* (T.2),which recorded the highest value in fresh and dry weight (g) of shoot and root with the average of 11.10,1.45,3.00, and 0.50 respectively. Data in (Table. 5), show the comparison of the inhibitory effects of piperitone , biocontrol agents and fungicide (Rizolex) on DSI, length (cm), fresh and dry weight (gm) of bean plants after inoculation with *R. solani* AG-4. Disease Severity Index showed highly significant differences among all treatments (Table.5). Treatments of piperitone in the presence of *R. solani* Ag-4 showed the best control of dry root rot and stem bean disease and reduction effect in disease severity with average 1.00, as compared with inoculated bean plants with *R. solani* AG-4 alone with average 3.65. *R. solani* AG-4 in the presence of *T. asperellum* or *T. harzianum* or Fungicide (Rizolex) were also recorded reduction in disease severity with average of 2.41 and 1.66, 1.91, respectively. Significant differences in plant height were observed, plants inoculated with *R. solani* AG-4 alone showed reduction in plant height (cm) with average 15.33 and reduction in fresh and dry weight (g) of shoot and root with the average of 6.13 , 2.67, 1.40, and 0.20 respectively, as compared with inoculated bean plants with *R. solani* AG-4 in the presence piperitone in plant height (cm) with average 27.50 and recorded considerable value in fresh and dry weight (g) of shoot and root with the average of 16.00, 7.10 , 2.10 c, and 1.00, respectively. Treatments of *T. harzianum* was also recorded considerable value in plant height (cm) with average 26.50 and recorded considerable value in fresh and dry weight (g) of shoot and root with the average of 13.00, 6.80, 2.90 and 0.87, respectively.

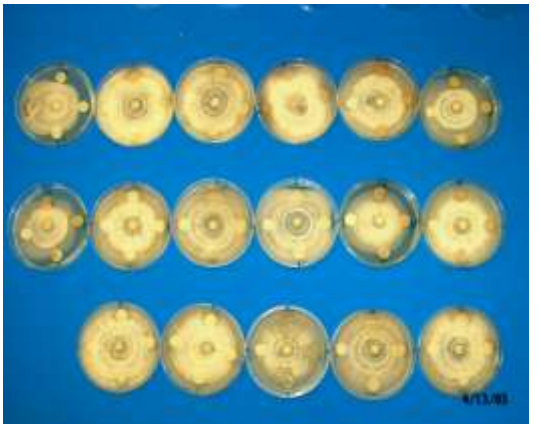
Table5. Comparison of the inhibitory effects of piperitone, biocontrol agents and fungicide (Rizolex) on DSI, length (cm), fresh and dry weight (gm) of bean plants after inoculation with *R. solani* AG-4

Treatment	**DSI	Length (cm)	Fresh weight (g)		Dry weight (g)	
			Shoot	Root	Shoot	Root
Control	00.00 e	29.20 a	14.30 abc	7.06 ab	3.00 b	1.20 a
Piperitone (4 ppm)	00.00 e	29.30 a	15.66 a	7.40 a	3.40 a	1.40 a
Piperitone (blank)	00.00 e	29.33 a	14.33 abc	7.26 a	2.80 b	1.35 a
T. asperellum	00.00 e	28.16 ab	15.33 ab	6.43 b	3.10 a	0.99 b
T. harzianum	00.00 e	27.50 bc	16.00 a	7.23 a	3.60 a	1.30 a
Rizolex (Fungicide)	00.00 e	16.33 e	8.50 e	4.30 d	2.30 c	0.45 c
Piperitone + *R. solani*	1.00 d	27.50 bc	11.66 d	5.50 c	2.10 c	0.75 b
T. asperellum* + *R. solani	2.41 b	24.83 d	13.00 cd	6.80 ab	2.80 b	0.87 b
T. harzianum* + *R. solani	1.66 c	26.50 c	13.66 bc	7.10 ab	2.90 b	1.00 b
Rizolex + *R. solani*	1.91 c	15.33 e	7.66 ef	3.86 d	1.60 d	0.72 c
R. solani	3.65 a	15.33 e	6.13 f	2.67 e	1.40 d	0.20 d

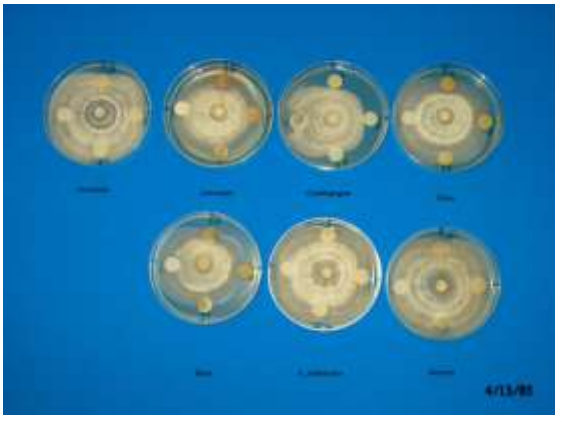
*Values within a column followed by the same letter are not significantly different according to the L.S.D. test(*P= 0.05*)

DSI = Disease Severity Index

a)



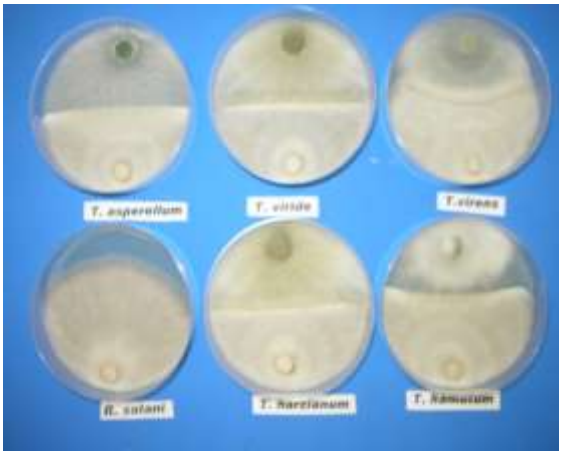
b)



C)

D)

E)



F)

Plate 1. a) Effect of *Cymbopogon proximus* on inhibition of mycelial growth of *R.solani* Ag-4.

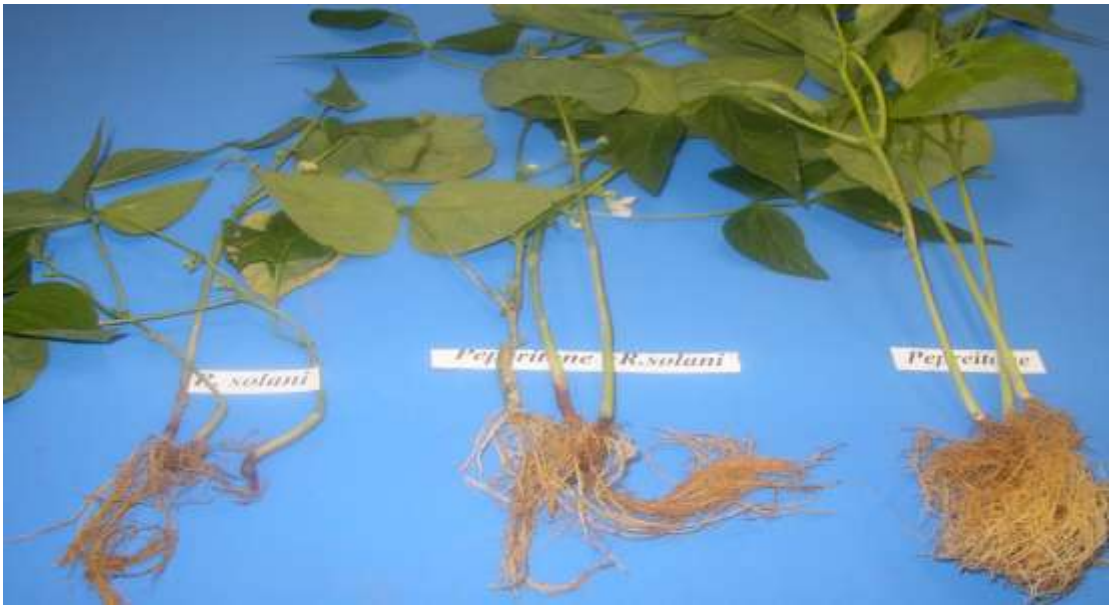
b) Effect of tested plant extracted in three concentration (amount /disc(1,2,4 mg) on inhibition of mycelial growth of *R.solani* Ag-4.

C) Active tested plant extracts.

D) Non active plant extract of *Leptadenia protechnica*

E) Five tested biocontrol agents : *T. asperellum, T. harzianum, T.hamatum, T. viride , and T.virens*

F) Effect of five biocontrol agents on inhibition of mycelial growth of *R.solani*



a) R.solani - ***R. solani + Piperitone -*** ***Piperitone***

a) control- ***T. harzianum -*** ***T. asperellum*** **b)** ***T. harzianum*** ***T. harzianum*** **alone**
+ R. solani

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الملخص العربي

المكافحة الحيوية للفطر ريزوكتونيا بواسطة البيريتون المستخلص من نبات الحلفا بر والمقارنة بفطريات المكافحة الحيوية التابعة للجنس تريكوديرما

يونس مولان، علاء كامل،صلاح الحسيني

اجريت تجارب تضاد معملية لدراسة قدرة خمس عوامل حيوية (تريكوديرما هيرزيانيم – أسبريليم – هماتم – فيرنس–فيريدي)، 14 مستخلصاً نباتياً في تأثيرهم على النمو الميسليومي للفطر ريزوكتونيا سولاني . سجل الفطر تريكوديرما هيرزيانم أعلى تثبيط يليه الفطر تريكوديرما اسبيريليم بمتوسط 82 و56 ، 48و 54 على التوالى ظهرت النتائج ايضاً فروق معنوية في نشاط التضاد الفطري لمستخلص نبات الحلفابر ونبات Ruta حيث وجدت انها فعالة في تثبيط النمو الفطري عند تركيز 4مجم / Disc بمتوسط 59و22، 48و 21 على التوالي.

سجلت دراسة مقارنة في تأثير البيريتون والعوامل الحيوية والمبيد الفطري ريزولكس على شدة الإصابة وطول النباتات والوزن الرطب والجاف لنباتات الفاصوليا. معاملات البيريتون في وجود الفطر ريزوكتونيا سولاني اعطى افضل مكافحة لمرض عفن الجذور والساق في الفاصوليا مع انخفاض في شدة الإصابة بمتوسط 00و 1 بالمقارنة بنباتات الفاصوليا الملقحة بالفطر ريزوكتونيا بمفرده بمتوسط 65 و 3. في وجود الفطر تريكوديرما اسبريليم او هيزيانيم او في وجود المبيد الفطري ريزولكس سجل انخفاض في شدة المرض بمتوسط 41و 2 ، 66 و 1 ، 91 و 1 على التوالي.