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EVALUATION THE ABILITY OF SOME ORGANIC COMPOUNDS IS PROTECTING BEAN SEEDLING AGAINST INFECTION WITH *RHIZOCTONIA SOLANI*

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Abstract

The study was conducted to evaluate the efficiency of Coumaric acid, Fulvic acid and Mannitol, separately and in combination to restrict bean root rot disease caused by *Rhizoctonia solani*. The three Compound were separately mixed with PDA at 200, 400, 600 mg/L in 9cm dim Petri plates and inoculated with *R. solani*. Sterile mixed soils in pots were treated with the three compounds, seeded with bean seeds and contaminated with *R.solani* inoculums on millet seeds (20 g/ pot) after 10 days of seeding. Disease incidence, disease severity, fresh and dry weights were determined after 30 days of germination. Peroxides activity in the plants was estimated after 6 and 12 days of contamination with *R. solani*. Results showed that the three compounds caused significant inhibition in fungus radial growth on PDA. Coumaric acid was the more effective causing 100 % inhibition at 600 mg/L compared to 88.15% and 83..63% with Fulvic acid and Mannitol respectively. The combination, Coumaric acid and Fulvic acid was found to be the more effective in reducing disease development in pots under greenhouse conditions with disease incidence and severity 3.3% and 1.67% respectively compared with 73.3% and 69.99% respectively in control. The reduction in disease development was found associated with increases in plant fresh and dry weights attained to 59.97g and 18.34 g respectively compared with 25.0 g and 5.0 g respectively in control (infected). The same combination induced increase in peroxides activity that attained to 65.87 and 68.77 absorption variation / min / g fresh weight after 6 and 12 days respectively compared with 38-26 and 37-22 in control indicating to systemic resistance induction.

Key words: Cowpea, Induce systemic resistance, Mannitole, Fulvic acid, Cumaric acid, Mannitole.

Introduction

Bean, *Vigna unguiculata* is one of the most nutritive vegetable crops. Bean seeds reported to contain 23-4% protein, 56.8% carbohydrate as well as many nutritive mineral, calcium and ferrous and some vitamins, A1, B1, B2 (Agbicodact *et al*., 2009; Deboer and Lowenberg, 2004).

The total area cultivated with bean in Iraq attained to 17800 donums with total production range between 2600-2768 Kg/donum (Central Statistic System, 2018).

Bean was reported to be infected with many pathogens, of these pathogens, *Rhizoctonia solani* was the more destructive that causing seedlings death, stem and root rot, seed rot and wilting (AL-Mussawi, 2012).

The control of the disease was restricted for long time to chemical fungicides, Rhizolex and Benlate (AL-Shalah, 2005). But due to enormous problems created by the Fungicides for human and ecosystem as well as strains of pathogen resistant to fungicide were developed, therefore the efforts were focused on searching for after natives, innocuous and effectives, including oraganic acids to control the pathogen (Abdel- Monaim *et al*., 2012; Kamel *et al*., 2014).

It has been found that using Coumaric acid at 200 mg/L showed high activity in inducing resistance in pepper plants against root disease caused by *Fusarium oxysporum* and *F. solani* (Abdel –Monaim and Ismail, 2010). kamel *et al*., (2014) reported that using Fulvic acid at 150 mg/L induced high reduction in disease severity of powdery and downy mildews on cucumber associated with promotion of plant growth parameters, fresh and dry weights and increase in yield compared with control. The use of Mannitol at 200 mg/L caused

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inhibition to seeds and root rots causal agents, *R.solani*, *F.solani*, *F. oxysporum* on tomato and promote plant growth compared with control (Abdul-Monaim *et al*., 2012).

The study was conducted to evaluate the efficiency of Coumaric acid, Fulvic acid, Mannitol to protect bean plant, against *Rhizoctonia solani*, causal agent of root rot disease.

Material and Methods

Isolation and Identification of *R. solani*

Bean roots showing rot symptoms were cut to small pieces, 1-2 cm and surface sterilized with sodium hypochlorite 2% for 2 min. The pieces were rinsed with sterile distilled water, dried on filter papers and cultivated on potato dextrose agar (PDA) in 9 cm dim. petri plates (3 pieces / plate). The plates were maintained at 25±2°C for 5 days and a part of terminal growing mycelium was transferred in to new plate containing PDA for purification. The fungus was Identified as described by Parameter and Whitney, (1970), Balzar and Conway, (2004).

The fungus Inoculums was prepared as described by Dewan, (1989) on millet seeds, *Panicum miliaceum* Patho test Fungous inoculums on millet seeds was added to sterile mixture of soil and peat moss (1;1) in pots at 1%. Millet seeds were added to sterile soil in other pots at 1% as Control. The pots were seeded with bean seeds and the dead seedlings were estimated after 5 days of cultivation.

Efficiency of uniform fungicide in inhibition of *R. solani* growth on PDA

The concentration 50, 100, 200, 400, 600 mg/L of uniform 390 SE (Syngenta company) were prepared in PDA. The media containing the Fungicide were separately poured in 9cm dim Petri plates. The Center of each plate was inoculated with 0.5 cm disc of *R.solani* colony, 5 days old. The plates were maintained at 25±2°C for 5 days and the diameter of fungus colony was calculated when fungal growth in the control reach to plate border. The inhibition percentage was calculated by the equation:

$$\%\ \text{inhibition} = \frac{\text{Growth dim. in control - Growth dim. in treatment}}{\text{Growth dim. in control}} \times 100$$

Effect of coumaric acid, fulvic acid and mannitol on *R.solani* growth on PDA

The concentrations 200, 400, 600 mg/L of each compound were prepared and passed through 0.25 Mm Millipore filter. The three compounds were separately added into PDA and poured in 9cm dim Petri plates. The plates were inoculated in the center with 0.5 cm disc of *R. solani* colony at 5 days old. Discs of 0.5 cm dim of Fungous colony were inoculated on PDA in other plates as control. The plates were maintained at 25±2°C for 5 days and the diameter of growing colonies was calculated. The inhibition percentage of the fungus was estimated as previously described. Four replicates for each concentration were used.

Effect of treating plants with coumaric acid, fulvic acid, mannitol and Uniform on infection with *R.solani* and plant growth in pots

A mixture of mixed soil and peat moss (2:1) was autoclaved at 121°C and 1.5 kg /cm^2 for 20 min. Twice for two successive days and distributed in 25 cm dim pots (2kg /pot). The potting soils were treated with coumaric acid at 400 mg /L, (50 ml/ pot), fulvic acid and mannitol at 600 mg/ L (50 ml /pot), uniform at 1ml /L (50 ml/pot). The pots were seeded with surface steril bean seeds. Twenty grams of *R. solani* inoculums on millet seeds were added to each pot after10 days of seeding (Nadi *et al*., 2013). Un contaminated sterile millet seeds were added to other pots as control. The pots were distributed in green house in complete randomized designee (CRD) with 3 replicates. The results were observed after 3 days of contamination and the percentage of seedlings death was calculated as described by stanghellini and Phillips, (1975).

$$\%\ \text{seedlings death} = \frac{No.\ of\ dead\ seedlings}{Total\ Number} \times 100$$

The observation of results was followed for 30 days.The plants were removed out (3 plants / replicate) for determination disease severity due to scale of 5 degrees, where 0 = healthy plants, 1 = discoloring on 1-33% of root, 2 = 34 – 50% of root is rotten, 3 = 50 -75% of root is rotten, 4 = more than 80% of root is rotten.

The disease severity for each replicate was calculated as reported by Mckinney, (1923).

$$\%\ \text{disease severity} = \frac{No.of\ plants\ of\ 0\ degree\ X0 + \cdots . + No.of\ plants\ of\ 5\ degree\ x\ 5}{No.of\ tested\ plants\ x\ 5} \times 100$$

The plants were removed out and the fresh weights for foliage and root system were determined. The foliage and roots were oven dried at 40°C and the dry weights were determined. The peroxides activity in bean plants was determined as described by Hammershmidt, (1982) after 6 and 12 days of contamination with the fungus.

Table 1: *R. solani* pathogenicity in pots.

Treatments	% infection
Control	00.0
R.solani	96.7
L.S.D $_{0.05}$	9.25

Each value in the table represent mean of 3 replicates.

Table 2: Effect of uniform on *R.solani* growth on PDA.

Concentration (mg/L)	Mean of colony diameter (cm)	% inhibition
Control	9	0.0
50	6.53	27.4
100	3.26	63.7
200	1.80	80.0
400	0.0	100.0
600	0.0	100.0
L.S.D 0.05	-	7.33

Each value in the table represent mean of 3 replicates.

Results and Discussion

Rhizoctonia solani pathogenicity

Results (Table 1) showed that *R. solani* caused high percentage of seeds and seedlings infection under glass house conditions that attained to 96.7% compared with zero in control. The capacity of the fungus to infect and inhibit seeds germination may be due to its capacity to proud enzymes that in hydrolyze pectin and cellulose in the early stage of plant growth.

It was reported that *R. solani* produce several enzymes including pectinase, pectin methyl esterase and pectinlyase (Bertagnolli *et al*., 1996).

Activity of uniform to inhibit *R. solani* growth on PDA

It was found that uniform at 50, 100, 200, 400, 600 mg/L induced high percentage of *R. solani* growth inhibition compared with control, table 2. The concentration 200 and 400 mg/L caused the higher growth inhibition, 80 and 100% respectively while 50 and 100 mg/ L caused 27.4 and 62.7% respectively. The activity of uniform may came from the effect of azoxystrobin on respiration Leading to inhibit energy production in fungous cells (Bartholomaus *et al*., 2017).

Effect of Coumaric acid, Fulvic acid and Mannitol on *R. solani* growth on PDA

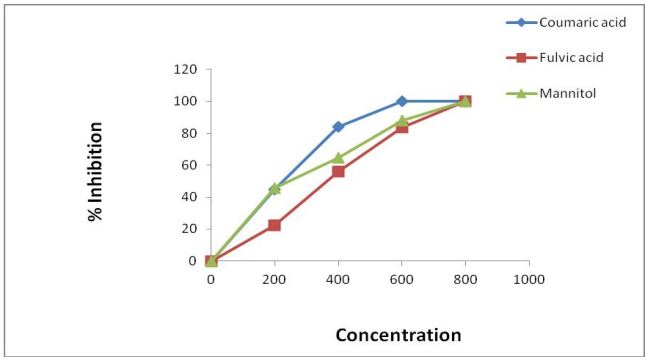

Fig. 1: Effect of coumaric acid, fulvic acid and mannitol on *R.solani* growth on PDA.

L.S.D $_{0.05}$ for treatments = 4.84; L.S.D$_{0.05}$ for concentration = 5.59; L.S.D$_{0.05}$ for interaction = 9.69. Each value in the table represent mean of 3 replicates.

Table 3: Induced systemic resistance by organic compound in cowpea against Rhizoctonia rots root disease.

% Infection	% Disease severity	Treatments
00.0	00.0	Sterilised soil
73.3	69.99	*R.solani*
00.0	00.0	Sterilised soil with cumaric acid
00.0	00.0	Sterilised soil with Fulvic acid
00.0	00.0	Sterilised soil with mannitole
13.3	6.67	C+ *R.solani*
26.7	16.67	F+ *R.solani*
40.0	30.55	M+ *R.solani*
3.3	1.67	F+C+ *R.solani*
10.0	3.33	M+C+*R.solani*
23.3	10.00	M+F+ *R.solani*
13.3	11.22	Uniform + *R.solani*
9.72	8.40	L.S.D $_{0.05}$

Each value in the table represent mean of 3 replicates.

The results in fig. 1 and 2, indicated that the three compounds caused significant inhibition in *R.solani* growth correlated with the concentration that attained to 100% at 800 mg/L. Coumaric acid was found to be the more effective with inhibition percentage 100% after 5 days at 600 mg/ L followed by Mannitol 88.15% and fulvic acid 83.63%.

The inhibition of *R. solani* by coumaric acid and Fulvic acid may be due to their acidity that affect fungous cell membrane, while the activity of mannitol may through affecting fungal cell membrane porosity. Several previous studies reported to the effect of organic compounds on fungous growth (Mostafa, 2006; Abdel-Monaim and Ismail, 2010; Wu *et al*., 2016).

Effect of treating bean plants with coumaric acid, fulvic acid, mannitol and uniform on disease development caused by *R. solani* and growth parameters under green house conditions

Result, in table 3, Revealed that all the compounds. Used caused high reduction in disease incidence and severity caused by *R.solani* on bean plants compared with the control.

The disease incidence values were found to be 13.3,

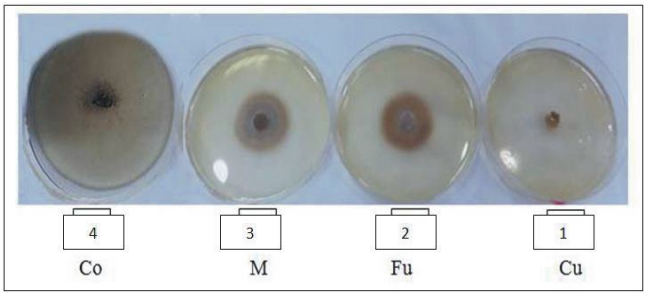

Fig. 2: Effect of coumaric acid(cu), fulvic acid (fu) and mannitol (m) on *R.solani* growth on PDA.

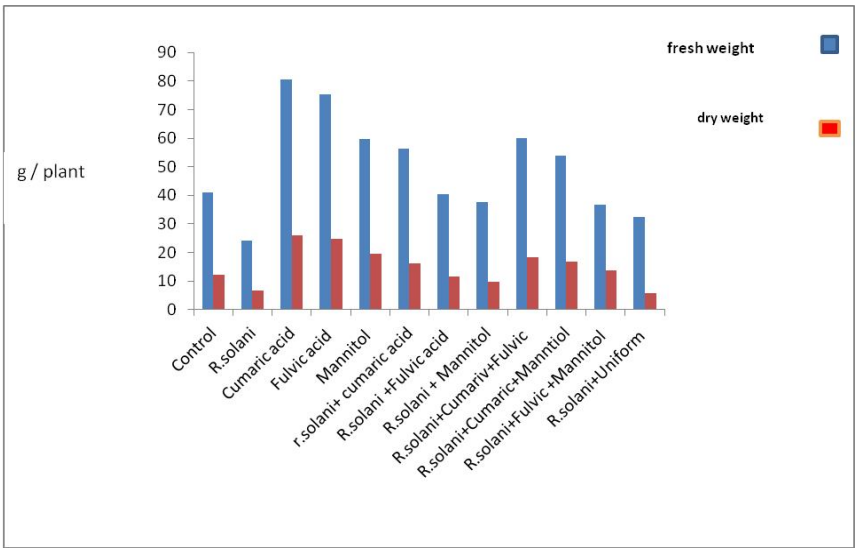

Fig. 3: Effect of coumaric acid, fulvic acid, mannitol and uniform on bean plant fresh and dry weights under greenhouse conditions.

L.S.D $_{0.05}$ for fresh weight = 2.04 and to dry weight = 4.15. Each value in this fig. represent mean of 3 replicates.

26.7, 40.0, 3.3, 10.0, 23.3, 13.3% compared with 73.3% in control, the disease severity values were 6.67, 16.67, 30.55, 1.67, 3.32, 10.0, 11.22% compared with 69.99% in control for the treatment C+*R.solani*, *F+R.solani*, *M+R.solani*, *F+C+ R.solani*, *M+C+ R.solani*, *M+F+ R.solani*, uniform+ *R.solani* respectively. The more effective treatment was the combination *F+C+ R.solani*. The reduction in disease incidence and severity was found associated with increase in fresh and dry weights of both of foliage and root system (Fig. 3). The combination C+F was the more effective with fresh and dry weights, 59.97 g and 18.34 g respectively followed by C and C+M with fresh and dry weights 56.43 g and 16.31 g, 53.91 g and 16.74 g respectively.

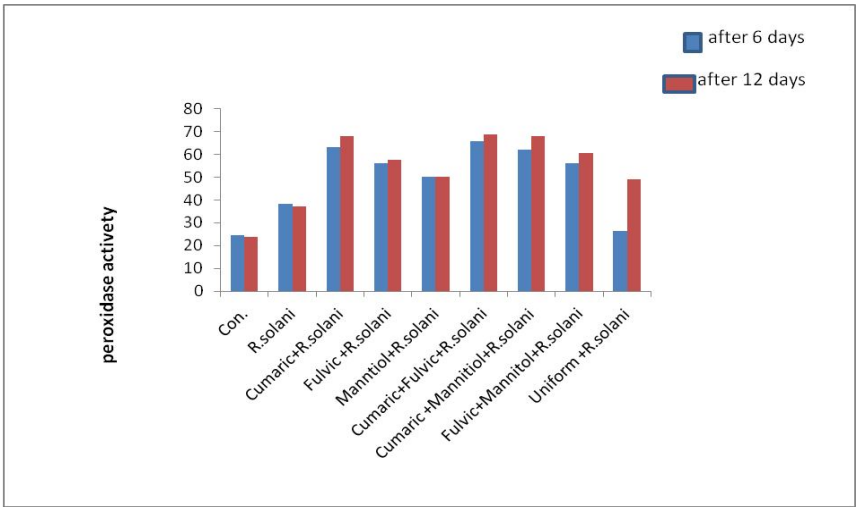

Fig. 4: Effect of coumaric acid, fulvic acid, mannitol and uniform on peroxidase activity (absorption varitions / min / g fresh weight).

Every value in the figure represent mean of 3 replicates, L.S.D $_{0.05}$ = 2.84 after 6 days and 3.52 after 12 days.

Peroxides activity

Significant differences in peroxidase activity were manifested in bean plants treated with coumaric acid, Fulvic acid, Mannitol and Uniform compared with *R.solani* infected plants (control), as determined by absorption variation min/g fresh weight.

The higher activity of the enzyme was manifested with combination C+F, 65.87 and 68.77 compared with 38.26 and 37.22 in control after 6 and 12 days respectively.

It was reported that peroxides with hydrogen peroxide inhibit and destroy the enzymes produced by the pathogen including pectinase as well as induced many plant defense mechanisms leading to enforce cell wall and producing phytoalexin, so peroxides is considered as indicator for induce resistance in the plant (siqueir *et al*., 2019).

The activity of these compounds in restriction *R. solani* growth and reduction of disease development may came from direct effects on the pathogen as previously reported and indirectly through activation plant defense mechanisms referred to as systemic resistance induction leading to production antifungal compounds proteins and lytic enzymes.

It has been reported that treatment plants with variety of agents, pathogenic, non- pathogenic, plant extracts synthetic chemicals, can lead to induce resistance in the plant to subsequent pathogen attack,that characterized by restriction of pathogen growth and suppression of disease development compared with non-induced plants infected with the pathogen (Walters, 2010).

Several previous studies reported that treating plants with coumoric acid, fulvic acid and mannitol induced systemic resistance associated with pathogenesis related proteins (PRP) including pectinases, glucanases that hydrolysis Fungal cell wall as well as increase peroxidase activity and accumulation of phytoalexins (clark *et al*., 2002; Abdul-Monaim, 2008; Wu *et al*., 2016).

The promotion of plant growth parameters could be resulted directly by

suppression *R. solani* development and indirectly through activation of root formation and improvement and regulation of plant rhizosphere conditions especially pH making some nutritive substances more available to plant and facilatid the uptake of others by plant roots (Abdul-Monaim *et al.*, 2012; Peictal, 2015; Wu *et al.*, 2016).

The efficiency of coumaric acid, fulvic acid and mannitol in restriction of *R. solani* disease development and promotion of plant growth parameters may be promising in *R. solani* disease management as efficient and innocuous alternatives to chemical fungicides.

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