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INDUCED RESISTANCE IN CUCUMBER AGAINST RHIZOCTONIA DAMPING-OFF DISEASE USING A BIOTIC AND BIOTIC AGENTS

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ABSTRACT

The study was conducted to evaluate the efficiency of three control agents, Preservepro,Biaclean , and Biohealth, separately or in combination, for inducing systemic resistance in cucumber plants against *Rhizoctonia solani*, the causal agent of root rot disease . It was found that the three agents induced significant reduction in pre and post emergence damping off and in disease severity compared with control. The applications of these agents in combination were found to be more efficient in reducing pre and post emergence damping off and in disease severity that attained to 0.00, 3.33, 1.33% and 3.33, 3.70, 4.00% and 3.33, 13.70, 13.33% with Preservepro + Biaclean, Preservepro + Biohealth, Biaclean +Biohealth respectively, compared with 3.33,13.70,14.67% and 6.67, 24.81, 25.33% and 13.33, 15.74, 24.0% with Preservepro, Biaclean, Biohealth respectively when applicated separately at 25 ml/pot (50mg/L), 2.5 g/Kg soil and 600 ml/Kg respectively and with 40.00, 39.50 and 72.00% in control. The reduction in disease incidence and severity was found associated with increases in root and foliage dry weights and in peroxides antioxidant enzyme in cucumber plants. These results may be promising in IPM program to manage root rot disease.

INTRODUCTION

Rhizoctonia solani Kühn is considered as one of the most important pathogenic fungi surviving in the soil and does not form asexual spores (Simsek *et al., 2009*). It has been reported that *R. solani* infect a wide host range including corn (Ogoshi, 1987), rice (Ou,1985), lawn grass (Parmeterand, 1970) and cucumber (Strashnov *et al.*, 1985), causing sheath blight. The control of the fungus was constricted for long time on chemical fungicides, but the excessive application of the fungicides caused enormous problems to ecosystem. Human and animals as well as new strains of the pathogen resistance to fungicide were appeared (Parry, 1990). So, the efforts of searchers were oriented toward searching of alternatives to fungicides, safe and effective, capable of inducing systemic resistance in the plants against pathogens. The plants possess numerous defense mechanisms to protect themselves against pathogens attack, some of these mechanisms are inducible and become activated after pathogen infection including synthesis of phytoalexins and production of anti-pathogen proteins (Mehdy, 1994, Jackson and Tylor, 1996, Mohammed *et al*., 2017, Escherichia et al., 2017). This resistance referred to as systemic acquired resistance (SAR) (Rayals *et al.*, 1996). The resistance induced may be localized at the site of application or may be transmitted systemically to other plant tissues (Walters, 2007, Aalaa, 2017). Many substances were reported to induce Systemic resistance including chemical compounds, metabolic substances of the host

plants or microorganisms through activate plant signaling way such as salicylic acid pathway (Canakci, 2011). Some biocontrol agents, including Preservepro were shown to induce resistance in tomato against *Fusarium oxysporum f. sp. Lycopersici* in tomato (Juber *et al.*, 2014). This study was conducted to evaluate the activity of Preserve pro, Biaclean and Biohealth to inhibit *R. solani* growth in vitro and as SAR inducers in cucumber plants against *R. solani*.

MATERIALS AND METHODS

Isolation and Identification of the Pathogen: Cucumber plants (*Cucumis sativus*) showing symptoms of root rot disease were collected from five locations around Baghdad governorate (Abu Ghraib, Al Radwania, Latifia, Al Za'faraniya and Al Madain). The infected roots were cut into 0.5-1cm and washed under running tap water for 30 minutes, pieces of infected root were surface sterilized in 2% sodium hypochlorite for 2 minutes, rinsed with sterile water and dried by clean filter paper in the laminar flow cabinet. Four pieces were placed on Potato Dextrose Agar (PDA) medium containing 100 µg ml^{-1} of streptomycin sulfate in Petri-dish of 9cm diam and maintained at 25±2˚C for three days. Fungal hyphae from the margin of developing colony were transferred into PDA to obtain pure culture. The isolates were identified to species level based on cultural and

morphological characteristics as described by Parameter and Whitney (1970) and Sneh *et al.,* (1996).

Pathogenicity of *Rhizoctonia solani* isolates to Cucumber: Sterilized soil (1 kg/ pot) was distributed in 14 cm diameter pots and five of *R. solani* isolates (Rh1 - Rh5) grown on Millet seeds were added into the potting soil at 1% W: W. The pots were irrigated and kept for 3 days before sowing. Seeds of Cucumber (Beta alfa) were surface sterilized in 1% sodium hypochlorite solution for two min., followed by extensive rinsing in sterile distilled water and sown in pots (5 seeds/pot). Seeds were sown in uncontaminated soil serve as control. The pots were distributed in the greenhouse at complete randomized design with 4 replicates. The percentage of Pre and Post emergence damping-off was estimated after four and 30 days of inoculation. The percentage of seed germination was computed according to the following formula:

$$\text{Germination \%} = \frac{\text{number seeds germinated}}{\text{Total number of seeds}} \times 100$$

The higher pathogenic isolate was used in the next experiments.

Preparation of fungal inoculums: Fife isolates of *R. solani* grown on millet seeds. Fifty gram of clean Millet seeds were placed in each of 250 ml flasks. The seeds were autoclaved twice at 121°C and 1.5 kg /cm^2 for one hour for two successive days. Then, 5mm diameter discs from the margin of the colony of the fungal pathogens grown on PDA were added to each flask containing sterilized millet seeds. Flasks were shaken every 2 days to ensure uniform colonization, incubated at 25 ±2°C for 2weeks.and used for soil infestation.

Effect of Systemic acquired resistance inducers on mycelia growth of *Rhizoctonia solani:* Preserve pro product was provided by Arysta life science (2% Ascorbic acid). Biaclean SL was provided by Bayer (3%oligo saccharin and Trace elements 10%), and Biohealth WSG (75% Humic acid, 5% Sea weed extracts and Trichoderma product was provided by Arysta life science.

The center of each Petri dish was inoculated with 5mm discs of fungal isolates taken from edges of actively growing cultures on PDA, 5days old, amended with 0, 300, 400, 500, 600, 700 and 800 mg L^{-1} of each product. The Petri dishes were incubated at 25± 2˚C for 5 days, and the percent of growth inhibition was calculated by the formula I= C-T/ C× 100, where I= percent of growth inhibition, C= radial growth in control, and T= radial growth in treatment. Four replications of each concentration were used.

Activity of Systemic acquired resistance inducers against *R. solani*on cucumber under greenhouse conditions: Cucumber seedlings, in pots of 14 cm diameter, were treated with 25ml/pot of Preserve pro solution at 50 mg/ml as soil drench, Biohealth (WSG), 2.5 g / Kg soil and Bioclean (SL) at 600 ml/Kg soil. *Rhizoctonia solani* grown on millet seeds were added into potting soil potting soil at 1% (w/w).The experiment has included 12 treatments (Table2), T1=Non-treated – non-inoculated soil / control, T2= *R. solani* inoculated– non treated soil/control, T3= Biacleanin non-inoculated soil, T4= Biohealthin non- inoculated soil, T5= Preserveproin non-inoculated soil, T6= Biacleanin inoculated soil , T7= Biohealthin inoculated soil, T8= Preserveproin inoculated soil, T9 Biaclean + Biohealthin inoculated soil, T10= Biaclean + Preserve pro in inoculated soil, T11= Biohealth +Preserve pro in inoculated soil, T12= Beltanol + *R. solani*. The pots were distributed in the greenhouse / plant protected dept. / College of Agriculture / University of Baghdad, in complete randomized designee with four replicates. The disease incidence was recorded after 7 days of inoculation with the pathogen, while the disease severity was determined after one month depending on the width of infection lesions on root, as described by Jaiswal *et al*., (2014). Shoots and roots were separately weighted before and after dry weight at 70˚C. The old leaves were collected from cucumber plants, treated and non-treated, after 7 days of inoculation for peroxidase (PO) determination as described by Hassan (2013). One-gram of leaves was ground in one ml of 0.1 M phosphate buffer, pH 7.0, in cold pestle (4˚C). The homogenate was transferred into 1.5 ml centrifugation tube and centrifuged at 15.000 g for 15 min at 4˚C. The supernatant was immediately used for the assay.

RESULTS

Pathogen isolation identification: The cultural and morphological characteristics on PDA media indicated that the isolates from cucumber plants showing slightly-melanized hyphae and irregularly-shaped and brownish sclerotia. Microscopic observation showed short-branched young hyphae produced. The hyphal width ranged from 5.0 to 8.0 µm. Also, slightly melanized mycelium with

irregular shaped seclerotia were appeared on PDA cultivated with infected pieces of cucumber roots. The microscopic observation showed short branched hyphae with width of 5.0 – 8.0 µm. These characters indicated that the causal agent of root rot disease on cucumber is *Rhizoctonia solani*. Similar results concerning *R. solani* were reported by several searchers (Carling, 1996, Fenille *et al.*, 2000)

Pathogenicity of *Rhizoctonia solani* isolates to Cucumber. Results the pathogenicity test revealed that all the isolates were pathogenic to cucumber seedling after 10 days of inoculation with 10.0-53.3 and 26.1-51.1% of pre and post emergence damping off (Table 1).

Table 1: Effect of *Rhizoctonia solani* isolates on disease incidence of *R. solani* isolates on cucumber seedling

Isolates	Disease Incidence (%)	
	Pre-emergence damping- off	Post emergence damping-off
Control	0.0	0.0
Rh1	30.0	33.1
Rh2	43.3	51.1
Rh3	10.0	26.1
Rh4	26.7	41.1
Rh5	53.3	50.0
L.S.D=0.05	12.58	21.80

*Three replicates for each treatment.

Effect of SAR inducers on mycelia growth of *Rhizoctonia solani*: Results showed that all the concentration of SAR agents (Preserve pro, Biaclean, Biohealth) were effective and caused significant reduction in *R. solani* growth on PDA (Table 2). The reduction in fungal growth was found to be correlated with the concentrations. The percentage of mycelium growth inhibition was attained to 100% at 600 mg/L of Preserve Pro, and 800 mg/L of Biaclean and Biohealth.

Table 2: The inhibition activity of Preservpro, Biaclean and Biohealth against *R. solani* on PDA

Treatments	Concentration (mg/ L)				
	200	400	600	800	Control
Preserve pro	45.88	76.74	100.00	100.00	0.00
Biaclean	20.63	44.96	81.22	100.00	0.00
Biohealth	0.00	26.81	55.63	100.00	0.00

*Three replicaties for each treatment.

$L.S.D_{=0.05}$ to Treatments = 0.89, $L.S.D_{=0.05}$ to Concentration = 1.15 , and $L.S.D_{= 0.05}$ to Interaction = 1.99

Activity of resistance inducers against *rhizoctonia solani* on cucumber plants under greenhouse conditions: the addition of sar agents to the contaminated soil with *r. Solani* separately or in combination have induced significant reduction in disease incidence (di),disease severity index(dsi), and increased plant dry weight compared with untreated plants (control)table (3&4).the disease incidences for pre and post emergence damping off , were found to be 3.33.13.70;13.33,15.44; and6.67,24.8% on the plants in soil treated with preserve pro + rh., biaclean + rh. and biohealth + rh. Respectively compared with 40.00, 39.50 % of plants in soil treated with the pathogen only (control). The disease severity was attained to 14.67, 24.0 and 25.33% for the same treatment respectively compared with 72.0% in control. It has been found that the reduction in disease incidence and severity were associated with increase in root and shoots dry weights that attained to 1.625,1.230 and 1.142 g/plant and 0.503, 0.521, and 0.493 g/plant for the above treatments respectively compared with 0.203 and 0.100 g/plant in control (table 3). Additional reduction in pre and post emergence damping off and disease severity with increase in root and shoots dry weights ere manifested on plants in soil treated with combination of control agents. The di values were found to be 3.33, 3.70; 0.00, 3.33 and 3.33, 13.70 % for the treatments, preserve pro + biohealth +rh, preserve pro + biaclean + rh and biaclean + biohealth +rh respectively compared with 4.00 and 39.50% respectively in control. the dsi were 4.0, 1.33 and 13.33% for the treatments before respectively compared with 72.0% in control. The shoot and root dry weights were 2.089, 0.635, 1.96, 0.592 and 0.755, 0.320 g/ plant for the same treatments respectively compared with 0.203 and 0.100 g/plant respectively in control (table 4)

Table 3: Activity of Systemic acquired resistance (SAR) inducers against *R. solani* on cucumber under greenhouse conditions.

Treatment	% Disease Incidence		% Disease severity
	Pre emergence damping off	Post emergence damping off	
Control(uninoculated)	0.00	0.00	0.00
R.solani	40.00	39.50	72.00
Biaclean only	0.00	0.00	0.00
Biohealth only	0.00	0.00	0.00
Preserve Pro only	0.00	0.00	0.00
Biaclean + *R.solani*	13.33	15.74	24.0
Biohealth . + *R.solani*	6.67	24.81	25.33

Preservepro + *R. solani*	3.33	13.70	14.67
Biaclean + Biohealth + *R.solani*	3.33	13.70	13.33
Biaclean+Preservepro +*R.solani*	0.00	3.33	1.33
Biohealth + Preser-vepro +*R.solani*	3.33	3.70	4.00
Beltanol + *R.solani*	13.33	25.92	28.00
L.S.D= 0.05	8.42	9.43	8.33

Three replicaties for each treatment

Table 4: Effect of SAR inducers on shoot and root dry weights of cucumber seedling, infected with *Rhizoctonia solani* in pot experiments.

Treatment	Growth Parame-ters Dry Weight	
	Shoot (g)	Root (g)
Control(uninoculated)	1.021	0.483
Rhizoctoniasolani	0.203	0.100
Biaclean only	2.090	0.802
Biohealth only	1.494	0.730
Preserve Pro only	1.828	0.702
Biaclean + *R.solani*	1.230	0.521
Biohealth . + *R.solani*	1.142	0.493
Preservepro + *R.solani*	1.625	0.503
Biaclean+Biohealth+ *R.solani*	1.96	0.592
Biaclean + Preservepro + *R.solani*	2.216	0.670
Biohealth + Preservepro + *R.solani*	2.089	0.635
Beltanol + *R.solani*	0.755	0.320
L.S.D= 0.05	0.019	0.010

*Three replicaties for each treatment

Effect of SAR inducers on Peroxidase (Po) activities in leaves (10 day old): Results presented in figure (1) indicated that, all tested SAR inducers increased the activity of peroxidase enzymes in cucumber leaves compared tothose grown from untreated seeds (Control) in this respect, activity of Po showed the highest increase when a combination of Preserve Pro + Biaclean was used followed by the combination of PreservePro + Biohealth and Biaclean +Biohealth.

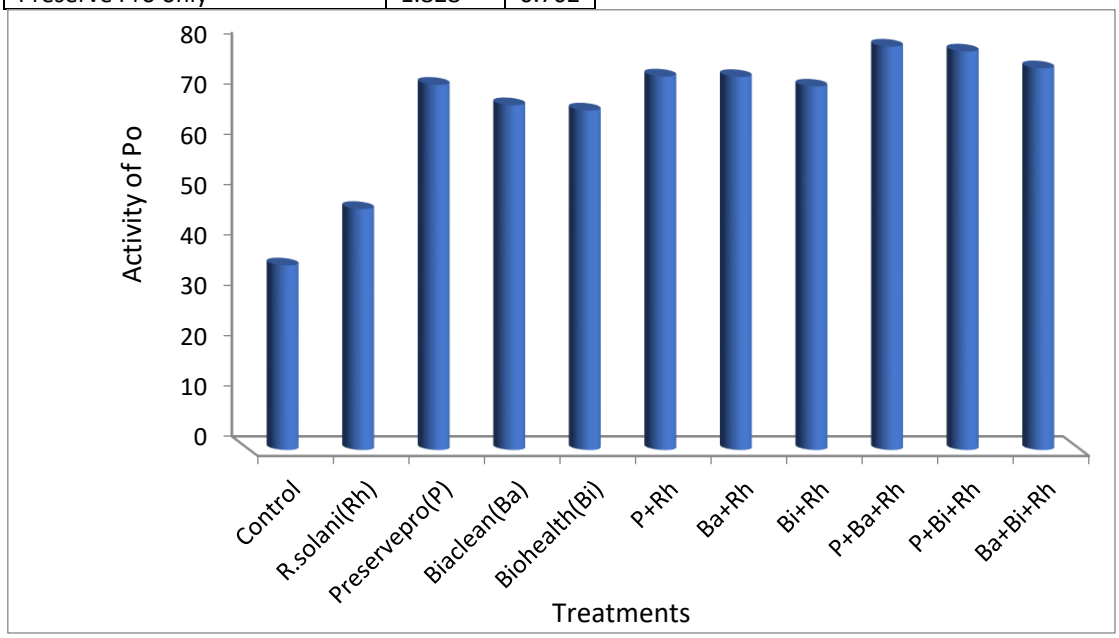

Figure 1: Effect of SAR compounds, on peroxides activity of cucumber plants infected with *Rhizoctoniasolani*.

Discussion

The results of this study showed high inhibition activity of Preserve pro, Biaclean and Biohealth against *Rhizoctonia solani* growth and enhanced plant resistance towered the fungus characterized by restriction of fungus growth and reduction disease development in cucumber compared with untreated plants.

The activity of the control agents may have conected to their components that may acts directly against the fungus or indirectly through inducing systemic resistance in the plant as proved by the reduction of disease incidence and disease severity. It has been reported that treated plants with vaccines agents, biotic (non-pathogenic microorganisms) or non – biotic (plant extracts, synthetic chemicals), rendered the plants more resistance to pathogens attack (Hammerschmidt, 1999, Walters *et al.*, 2005).

The resistance induced in the plants treated with the control agents may be through activation of genes in the plant encoding for proteins acts directly as antifungal or induce variation in plant tissue structure making the plant more resistance. Several previous studies repotted that plant synthesize phytoalexins, deposit of callus

around the penetration site, induce cell collapse around the site of infection, and produce anti-pathogen proteins after infection with pathogen (Mehdy, 1994, Jocksen and Taylor, 1995). Results also showed that the induction of systemic resistance in cucumber plant was found associated with plant growth promotion as proved by the increase in plant shoots and roots dry weight, this may come from the suppression of *R. solani* (VanLoon *et al.*, 1998). The results of this study could be promoting in developing effective integrated program for managing root rot diseases.

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