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In Vitro Evaluation of Bacterial Bioagents and Fungicides Efficiency Against *Rhizoctonia Solani* Isolates the Causal agent of Black Scurf Disease of Potato

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ABSTRACT

The efficacy of two isolates of bacterial bioagents and two fungicides via, Rhizoctonia solani isolates were evaluated in vitro. In the dual culture assays, significance inhibition ratio of R. solani hyphal growth was obtained in all treatments of Pesudomonas fluoresnces and Bacillis subtilis compared to control. Also, Bacillus subtilis exhibit the highest significant suppression for the mycelial growth of R. solani isolates more than Pseudemonas fluorescens, and the ratios of inhibition differed according to the bioagents and R. solani isolates similar to those results in antifungal activity technique.

The volatile metabolite studies revealed that in the first period (three days) inhibition percentage significantly differed according to the tested isolates of R. solani and the applied bioagents, B. subtilis gave the highest inhibition (57.41%) in R. solani isolate 3. Similarly in P. fluoresnces treatments, the highest inhibition was (57.41%) was detected in isolate 3 of R. solani considering that the second tested time period (five day inoculation). B. subtilis showed highest inhibition value on isolate 1, on the other hand P. flouresnce showed high value of inhibition on isolate 2, whereas the lowest inhibition values were produced on isolate 1.Non volatile activity both bacterial bioagents isolates showed different values of inhibition on R. solani isolates. On the other hand, Rezolex was most effective against R. solani at two concentrations (0.2 and 0.3), however Topsin-M showed fluctuate inhibition values at two concentration used (0.04% and 0.075%). Further incubation of plates showed suppressed the formation of sclerotia by all the antagonists tested.

The results implied that the extent of inhibition by B. subtilis and P. fluoresnces rovides the use of excellent potential antagonists capable of controlling the R. solani in vitro.

1 Introduction

Rhizoctonia solani, is the most widely recognized species of the genus *Rhizoctonia* which originally first described by Julius Kühn on potato in 1858, which is one of the most prevalent and important soil borne fungal pathogen causing destruction of a wide range of economically important crops such as rice, wheat, tomato, potato etc. (Gull, 2008 and Murphy and Riley, 1962). This fungus does not produce asexual spores, and exists as mycelium and sclerotia or sexual spores called basidiospores (Keijer, 1996). Potato (*Solanum tuberosum* L.) is one of the most important crops worldwide belonging to the family Solanaceae grown in around 150 countries spread through the world in both temperate and tropical regions (Adebola et al., 2020 and Siddique et al., 2020).

Unfortunately, commercial cultivars of potato are susceptible to fungal and bacterial diseases leading to

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big losses in yield and quality of products (Walter et al., 2001and Khan et al., 2008). Among the different fungal diseases of potato crop, black scurf disease of potato caused by, *Rhizoctonia Solani*, is the most commonly observed disease with the characteristic of black scurf symptoms on tubers and stem canker are the result of *Rhizoctonia* disease complex in potato. Black scurf disease increases gradually and sclerotia may develop on tuber surface even under low infection, .resultantly the control through fungicidal chemistries is not always useful especially when levels of infection are high (Malik Owais et al., 2014). In addition to that, overuse of chemical pesticides has caused soil pollution and harmful effects on human beings. Accordingly, biological control of soil borne diseases has been attracting attention. Many reports or reviews in this area have already appeared. So far, gram-negative bacteria, especially Pseudomonas strains, have been intensively investigated as biological control agents with regard to the production of antimicrobial metabolites, genetic analysis and regulation of some metabolites, and ecological fitness of soil. The gram-positive bacteria, like *Bacillus* spp., however, have been studied less intensively than the gram-negative bacteria. *Bacillus* subtilis is considered to be a safe biological agent, but evaluation of *Bacillus subtilis* has focused primarily on the degree of disease suppression (Asaka and Shoda, 1996).

Therefore, the objective of the present work was to evaluate the antagonistic effect and to determine the efficacy of volatile and non- volatile activity of bioagents *Bacillus subtilis* and *Pseudomonas fluorescens* on mycelial growth of *R. solani* isolates, and to evaluate the effectiveness of two fungicides viz., Rezolex and Topsin M70 at two different concentrations against five isolates of *R. solani* the causal agent of black scurf of potato under in vitro condition

2 Materials and Methods

The antagonistic bacteria Bacillus subtilis and Pseudomonas fluorescences used in the present study, were identified registered with the aid of members of the Botany Department at Saba Bacha Agriculture Faculty, Alexandria University.

2.1 Source of tested bioagents isolates:

The antagonistic bacteria *B. subtilis* and *P. fluorescens* used in the present study were obtained from the Botany Department at Saba Bacha Agriculture Faculty, Alexandria University.

Sclerotia of *Rhizoctonia solani* were collected from infected potato tubers cv. Spunta, showing typical black scurf symptoms, were first washed free of excess soil in tap water. A portion of each tissue was transferred to 2 min in 2% sodium hypochlorite solution then rinsed twice in sterile water. The treated tissue pieces with sclerotia were blot dried and then transferred to petri plates containing sterilized potato dextrose agar (PDA) medium with five pieces per plate. All plates were incubated at 25°C±2 for 7 days. The pure cultures of different isolates of *R. solani* were stored on PDA medium at 4°C in the dark for experimentation.

2.2 Isolation and Identification of pathogen:

For the isolation of *R. solani*, sclerotia grown on the skin of infected potato tubers cv. Spunta, showing typical black scurf symptoms were first washed free of excess soil in tap water. A portion of each tissue was transferred to 2 min in 2% sodium hypochlorite solution then rinsed twice in sterile water. The treated tissue pieces with sclerotia were blot dried and then transferred to petri plates containing sterilized potato dextrose agar (PDA) medium with five pieces per plate. All plates were incubated at 25 ± 2°C for 7 days. The pure cultures of different isolates of *R. solani* were stored on PDA medium at 4°C in the dark for experimentation. The fungal isolates were identified observing the morphological and microscopic characteristics described by (Parmeter, 1970; Sneh et al., 1991and Tredway & Burpee, 2001).

2.3 Antagonistic Activity of Bacterial Biocontrol Agents:

Dual culture technique:

Antifungal activity of the bacterial bioagents was tested against *R. solani* isolates using dual culture technique (Rabindran and Vidyasekaran, 1996). In this technique *B. subtilis* and *P.fluorescens* were streaked at one side of Petri dish (1 cm away from the edge) containing PDA medium. A disc (5mm in diameter) from seven days old of *R. solani* isolates were placed on the opposite side of the petri dish perpendicular to the bacterial streak and plates were incubated at room temperature (28℃ ± 2) for 7 days. Plates inoculated with fungus only served as control. Three replicates were maintained for each isolate. The experiment was repeated three times. In in vitro tests, inhibition zone was measured when the mycelium of control was completely filled the Petri dishes. The measurements of inhibition zone were taken from edge side of mycelium of bacteria colony. Percent of inhibition is determined according to Cubukcu (2007).

Percentage Inhibition = C – T/C × 100

Where: C = Colony diameter (cm) of the control T = Colony diameter (cm) of the test plate

Antifungal activity of *B. subtilis* and *P. fluorescens* can be tested by dual culture:

The antifungal activity of *B. subtilis* and *P. fluorescens* can be tested by dual culture technique. PDA disk (5 mm) with active mycelium of the pathogen is placed in the center of a Petri dish with PDA; on the same plate, at a distance of 1.5 cm in the four cardinal points, a loopful of antagonistic bacterial isolates is placed. Plates inoculated with the pathogen culture

serve as controls. In order to quantify the antagonistic potential of bacterial isolate, the size of growth inhibition zones measured after 7 days of incubation at 25°C±2 and the percent of radial growth inhibition was calculated according to Jimenez et al. (2018) .

The radial growth of the pathogen was measured and the percentage of inhibition was calculated by adopting from the following formula.

R= CD –TD/ CD X 100

Where,

R= percentage inhibition of test pathogen

CD = Radial growth of test pathogen in control

TD = Radial growth of test pathogen in treatment

Volatile activities:

Petri plates containing 20 ml of PDA were inoculated separately with 5 mm disc of antagonists and incubated at 28°C ±2 for 24 hours. After this lid of each plate was replaced by a bottom containing PDA previously inoculated with the disc of the pathogen and sealed together with paraffin film. The cultures were incubated at 25 °C ± 2, three replicates were used in this experiment. Radial growth was measured at 3and 5 days intervals and inhibition percent was determined using the following formula: Percent of inhibition = C2 − C1/C2 × 100.

Where, C2 means growth of *R. solani* in control and C1 means growth of *R. solani* according to Dennis and Webster (1971).

Non-volatile activities:

The method of Jariwala et al. (1991) with small modifications was used to evaluate the non-volatile activities of bacterial bio-agents. In this method, loop full of bacterial biocontrol agents were inoculated in nutrient broth and incubated for 48 hours at room temperature (25° C ± 2). After the incubation, cultures were centrifuged at 3000 rpm for15min. and the supernatant was used for antibiotic activity. Culture filtrates were added to PD Agar medium at 25%, 50%, 75% and 100% concentration, the pH was adjusted to 6.8 ± 0.2. Then the medium was sterilized and poured in sterile petri plates.7day old actively growing *R. solani* cultures were removed from the edge of the colony using 5mm diameter cork borer and placed at the center of these culture medium and the plates were incubated at room temperature. Three replicates were maintained for each concentration. Plates containing PDA medium with pathogens alone served as control. Radial growth of the fungal colony was measured on 5 day after incubation. Percent of inhibition was determined using the following equation:

Percent of inhibition = C2 − C1/C2 × 100

Where, C2 means growth of *R. solani* in control and C1 means growth of R. *solani* in treatment.

Evaluation of the effectiveness of two fungicides against *Rhizoctonia solani*

This experiment was conducted in vitro in plant pathology lab, dep of plant protection, Faculty of Agriculture, University of Tripoli to evaluate the efficacy of two fungicides belonging to various of chemical groups; namely Rizolex and Topsin-M (Table,1) aginst *R. solani* isolates,by using poisoned food technique (Hawamdeh & Ahmad 2001), in this technique, different fungicide concentrations were prepared in flasks by dissolving requisite quantities of each fungicide in warm media (50ºC). The fungicides were added after the media had been autoclaved. Twenty ml of amended medium was poured in 90 mm sterilized petri dishes and allowed to solidify. Mycelial discs of 5 mm diameter from 7day old culture was inoculated at the center of the petri plate and then incubated at 25°C ± 2 for 7days.Control was maintained without fungicide. Three replicated plates were used for each concentration of every fungicide with completely randomized design (CRD).The inhibition percentage of growth was calculated by using the formula I = (C-T/C) x 100

Where, I = Per cent inhibition of mycelial growth

C = Colony diameter in control (cm)

T = Colony diameter in treatment (cm) that given by Vincent (1927)

Table 1. Fungicides used for in vitro evaluation:

Common name	Trade name	Formulation	IUPAC name	concentrations
Tolclofos-methyl	Rizolex	50%WP	(2,6-dichloro-4-methylphenoxy)-dimethoxy-sulfanylidene-λ^5-phosphane	0.2, 0.3%
Thiophanate-methyl	Topsin-M	70%WP	methyl *N*-[[2-(methoxycarbonylcarbamothioylamino)phenyl]carbamothioyl]carbamate	0.04 , 0.075%

Statistical Analysis:

All experiments were set up in a complete randomized design. One-way ANOVA was used to analyses the differences between antagonistic inhibitor effect and linear growth of pathogenic fungi in vitro. A general linear model option of the analysis system SAS was used to perform the ANOVA (SAS, 1996).

3 Results

3.1 Antagonistic Activity of Bacterial Biocontrol Agents

Dual culture method

The bacterial antagonists *P. fluorescens*, *B. subtilis* showed inhibition in the mycelial growth ratios compared to control, though, inhibition ratios differed according to the bacterial isolates the *R. solani* tested isolates.

The highest inhibition ratio was obtained by B. subtilis (74.10%) on isolate No.3 of *R. solani*, while, the lowest inhibition ratio was obtained on isolate No.5 of *R. solani*. Otherwise the highest inhibition ratio (68.13%) was obtained by *P. fluoresces* on isolate No.3 of *R. solani*, although the least inhibition ratio (13.8%) was obtained in *R. solani* No.1. The formation of sclerotia has been suppressed by all the antagonists used (Table 2 and Fig 1).

Table 2. Effect of *Bacillus subtilis* and *Pseudomonas fluorescens* on mycelial growth inhibition of *Rhizoctonia solani* isolate

Bioagent (B)	Growth inhibition % Isolates of *Rhizoctonia solani* (I)					Mean
	1	2	3	4	5	
B. subtilis	45.00	50.00	74.10	60.56	38.33	53.60
P. fluorescens	13.89	50.56	68.13	58.06	41.67	46.46
Control	0.00	0.00	0.00	0.00	0.00	0.00
Mean	19.63	33.52	47.41	39.54	26.67	33.35
LSD0.05 (B) = 1.054	**LSD0.05 (I) = 1.36**		**LSD0.05 (B x I) =2.355**			

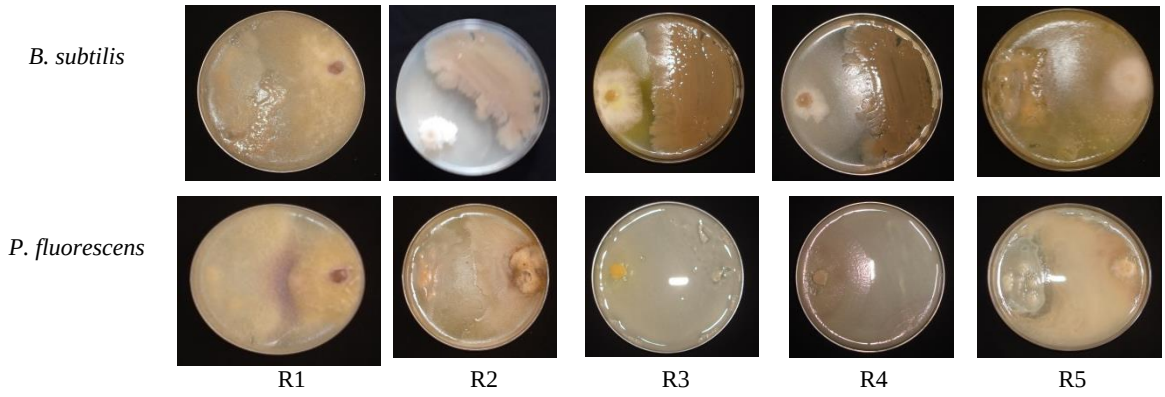

Fig. 1. The antagonistic effect of the tested bacteria (BACs) against *R. solani* isolates 1,2,3,4,5

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Antifungal activity of *B. subtilis* and P. fluorescens canbe tested by dual culture:

Significant effect on all *R. solani* isolated used in this experiment was obtained by the two bacterial agents. *B. subtilis* gave highest inhibition value (66.44%) on isolate No.5 of *R. solani*, moreover, the lowest value was detected in isolate No.1 of *R. solani*. Finally, *P. fluoresscens* treatment showed high inhibition level on isolate No.4 of *R. solani* although, the lowest inhibition level was (6.11%) on isolate No.5 of *R. solani* compared to control (Table 3 and Fig. 2)

Table 3. Effect of *Bacillus subtilis* and *Pseudomonas fluorescens* on mycelial growth inhibition of *Rhizoctonia solani* isolates

Bioagents (B)	Growth inhibition % Isolates of *Rhizoctonia solani* (I)					Mean
	1	2	3	4	5	
B. subtilis	31.39	38.33	57.47	34.67	69.44	46.26
P. fluorescens	22.22	52.78	33.89	77.22	6.11	38.44
Control	0.00	0.00	0.00	0.00	0.00	0.00
Mean	17.87	30.37	30.45	37.30	25.19	28.23
LSD0.05 (B) =1.05	**LSD0.05 (I) =1.36**		**LSD0.05 (B x I) =2.36**			

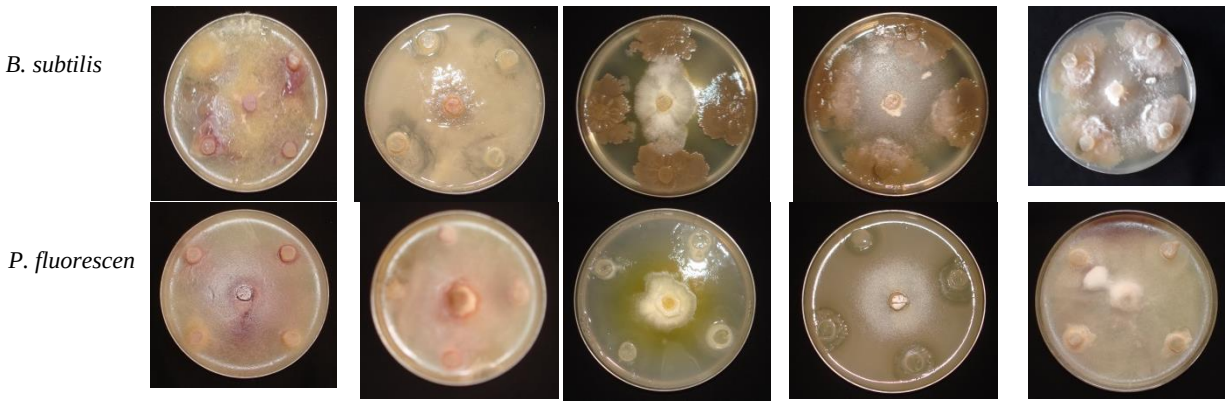

Fig. 2. The antagonistic effect of the tested bacteria (BACs) against *R. solani* isolates by dual culture technique .

Effect of volatile substance produced by *P. fluorescens* and *B. subtilis* on growth of the pathogen.

Two time-periods i.e. three days and five days after inoculation were carried out to determine the percent of inhibition. Inhibition percentages significantly differed according to the tested R. solani isolates and the applied bioagents in the first period. In B. subtilis treatment, the highest inhibition (57.41 %) was recorded in R. solani isolate 3, since, the least inhibition (30.30%) was detected in isolate 5. Similar to those in P. fluorsences treatment, the highest inhibition (57.41%) was detected in isolate 3 of R. solani, and the least percent was obtained on isolate 1 (33.3%). In the second tested time period (five days of inoculation), the highest inhibition value was showed by B. subtilis on isolate 1 (37.08%), meanwhile the least inhibition value was (15.54) on isolate no 5 of R. solani. Moreover, P. flouresences showed high value of inhibition on isolate 2 (39.33), though, the lowest inhibition values were detected on isolate 1 (13.89%) (Table 4)

Tale 4 .Volatile activity of *B. subtilis* and *P. fluorescens* against *R. solani* isolates in two-time period:

Bioagent (B)	Growth inhibition (%) 3 days after inoculation (T) Isolates of *Rhizoctonia solani* (I)					Mean	Growth inhibition (%) in the 5 days after inoculation(T) Isolates of *Rhizoctonia solani* (I)					Mean
	1	2	3	4	5		1	2	3	4	5	
B. subtilis	42.04	25.68	57.41	32.20	30.30	37.53	37.08	17.59	35.56	23.44	15.54	25.84
P. fluorescens	33.3	55.37	57.41	43.33	46.44	47.18	13.89	39.33	24.69	22.20	19.74	23.97
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	25.12	27.02	38.27	25.18	25.58	28.23	16.99	18.97	20.08	15.21	11.76	16.6
LSD0.05 B=0.16	**LSD0.05 I= 0.24**		**LSD0.05 T=0.10**									
LSD0.05 IxB=0.37	**LSD0.05 IxT=0.30**		**LSD0.05 BxT = 0.23**		**LSD0.05 IxBxT =0.52**							

Non-Volatile activity

Different concentrations of *bacterial bioagents* (25%, 50%, 75% and 100%) were tested on mycelial growth of *R. solani* isolates (Table 5).

Results obtained by this technique showed that: *B. subtilis* gave different inhibitions values between all tested isolates. The concentrations 75 and 100% gave the highest inhibition values in isolate 2 (64.63 and 70.42% respectively) followed by 60.74% in isolate 4 at concentration 100% considering that the least inhibition value were detected at concentration 25% in isolate 2 (4.17%). Results similar to those obtained by *P. fluorsences*, which showed different inhibition values on *Rhizoctonia* isolates tested. The highest values of inhibition were obtained by concentrations (75, 100%) in isolate (2) 66.22 and 69.37% respectively, followed by 60.37% in isolate 4 at concentration 100%, while the least inhibition value were detected at concentration 25% in isolate (1) 3.3

Table 5. Effect of different concentrations of *Bacillus subtilis* and *Pseudomonas fluorescens* on mycelial growth of *R. solani* isolates.

	Bioagent (B)	Concentration % (C)	Isolates of *Rhizoctonia solani* (I)					
			1	2	3	4	5	
	B. subtilis	**25**	**5.833**	**4.17**	**35.56**	**39.27**	**9.34**	**18.734**
		50	**7.500**	**56.71**	**42.22**	**54.07**	**14.07**	**34.491**
		75	**19.028**	**64.63**	**47.00**	**55.18**	**18.15**	**37.167**
		100	**29.167**	**70.42**	**49.33**	**60.74**	**19.63**	**45.899**
	Control	**-**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
	Mean		**12.305**	**39.18**	**34.82**	**41.85**	**12.24**	**27.258**
	P.fluorescens	**25**	**3.355**	**10.74**	**5.17**	**3.70**	**6.22**	**5.837**
		50	**4.889**	**55.00**	**5.43**	**49.26**	**12.70**	**25.455**
		75	**15.926**	**66.22**	**5.76**	**52.22**	**17.44**	**31.513**
		100	**24.889**	**69.37**	**7.23**	**60.37**	**19.63**	**36.297**
	Control	**-**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
	Mean		**9.812**	**40.27**	**4.72**	**33.11**	**11.20**	**19.820**
	$LSD_{0.05}$ C= 1.427		**$LSD_{0.05}$ I =1.009**			**$LSD_{0.05}$ CXI= 2.257**		
	$LSD_{0.05}$ B= 0.638		**$LSD_{0.05}$ BXCXI = 3.192**					

Evaluation of the effectiveness of two Fungicides on *R. solani* isolates

Two fungicides were used at two concentrations in vitro to control *R. solani*.

Results showed that Rezolex was the most effective at concentrations (0.2 and 0.3%) on all *R. solani* isolates tested with inhibition values (82.41%) in isolate 1 at concentration 0.2% followed by 79.81% in isolate 1 at concentration 0.3%, where as the least inhibition value was (63.33%) at concentration 0.3% in isolate 4. While Topsin M-70 showed fluctuated inhibition values at two concentrations tested (0.04-0.075%) which was (83.11%) at concentration 00.075% in isolate 3 mean while 2.20 % inhibition value was detected at concentration 0.04% in isolate (1) Table 6. (Fig4&5).

Table 6. Effect of two concentrations of the tested Fungicides on mycelial growth of *R. solani* isolates.

Fungicides	Tretements (T) Concentration %(C)	Growth inhibition (%) 7 days after inoculation Isolates of *Rhizoctonia solani* (I)					Mean
		1	2	3	4	5	
Rezolex	0.2	82.41	69.07	78.15	65.37	75.74	74.148
	0.3	79.81	65.37	63.59	63.33	72.96	69.012
Control	-	0.00	0.00	0.00	0.00	0.00	0.00
Mean		54.07	44.81	47.25	42.90	49.57	47.719
		1	2	3	4	5	
Topsin – M70%	0.04	2.20	47.61	52.78	31.37	29.07	32.606
	0.075	2.75	52.41	83.11	53.70	41.48	46.674
Control	-	0.00	0.00	0.00	0.00	0.00	0.00
Mean		1.65	33.34	45.30	28.36	23.52	26.426
		$LSD_{0.05}$ C= 0.894		$LSD_{0.05}$ I = 1.145		$LSD_{0.05}$ CXI= 1.632	

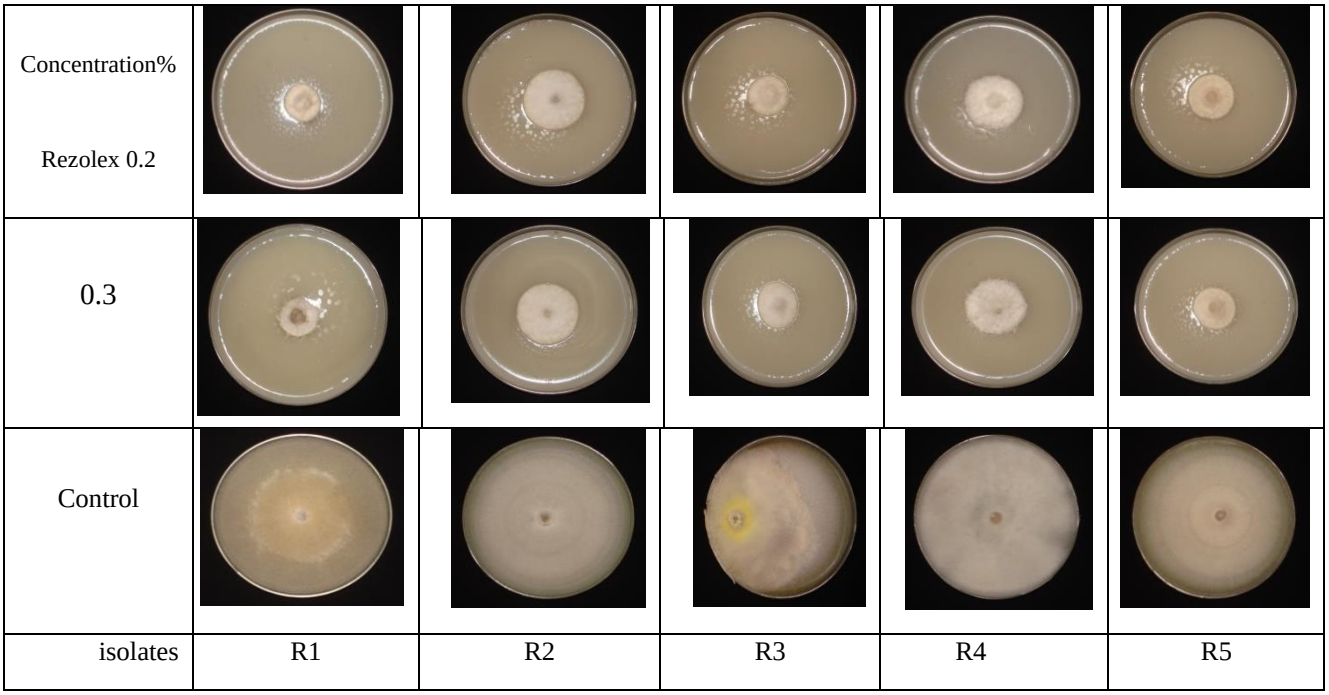

Fig. 4. Effect of two concentrations of the Rezolex on linear growth of *R. solani* isolates

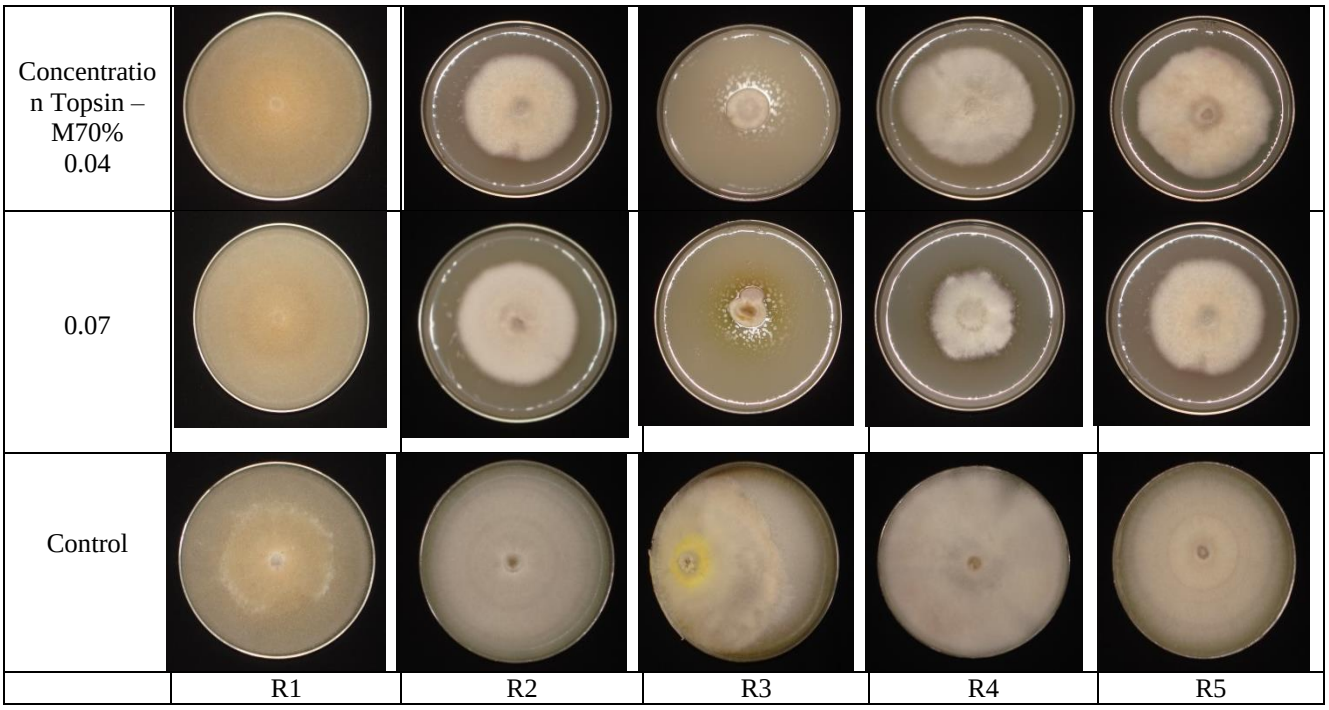

Fig. 5. Effect of two concentrations of the Topsin - M Fungicide on linear growth of *R. solani.*

4 Discussion

Results of the antagonism tests showed that *B. subtillis* was able to inhibited the the mycelium of *R. solani* isolates , this antagonistic action of *Bacillus subtilis* might be due to the antibiotic secretion (Siahmoshteh et al., 2019) enzymes and metabolites which reflect permeability changes in the protoplasmic membrane; competition for nutrients and bulb formation and lysis of the fungal hyphae. Several research workers have been reported that *B. subtilis* produces antibiotics which include iturin A and surfactin and Bacillopeptins and Bacillomycin (Cavaglieri et al., 2005 and Cheng et al., 2019) and this could have contributed to the biological control activity observed in the present study. The antibiotics produced in vitro were generally assumed to be the compounds responsible for biocontrol in vivo. *Bacillus* spp., however, produce a range of other metabolites including biosurfactants (Edwards and Seddon 1992), chitinase and other fungal cell wall-degrading enzymes (Pelletier and Sygusch 1990; Frandberg and Schnurer 1994), volatiles (Fiddaman and Rossall 1993) and compounds which elicit plant resistance mechanisms (Kehlenbeck et al. 1994)

Some isolates of bacteria change the media colour especially near the inhibition zone resulting in mycelial growth retardation *B. subtilis* showed the inhibition and the color of the media turned brown at the antagonized portion on the 7 day after . This may be due to the presence of the antibiotic like Inturin A and Surfactin produced by *B. subtilis*. Akihiro et al. (1993) has reported that *B. subtilis* produced antifungal peptide antibiotic Inturin A and Surfactin. Inturin A has a strong antifungal activity when compared to Surfactin. A purple pigment consistently produced in culture by strain Pseudomonas cepacia, strain 5.5B was isolated and identified as 4,9-dihydroxyphenazine-1,6-dicarboxylic acid dimethyl ester, a phenazine (Kelly Cartwright et al.,1995). On the other hand, Pal et al.(2000) reported that Pseudomonas fluorescens isolate EM85,produced the fluorescent pigment in PDA and antifungal antibiotics.

Moreover, Production of heat labile antifungal substances by *Bacillus* to control different fungal pathogens was reported by several workers (Podile et al., 1987 and Dileepkumar et al., 1988). Cyanides were also considered as volatile metabolites produced by bacterial biocontrol agents (Laha et al., 1996). During antibiosis, both volatile and nonvolatile secondary metabolities have been implicated in restricting the vegetative growth of pathogenic fungi.

In addition to that several studies described that the use of *P. fluorescenes* as a biocontrol agent against plant diseases. The activity of antifungal or antibacterial pseudomonads is traced back to the production of phenazines, 2-4-diacetyl phloroglucinol, pyrrolnitrin, pyoluteorin, cyclic lipopeptides (Liu et al., 2007), and rhizoxin (Loper et al., 2008). In addition to that they play a role in ecological fitness (Chin-A-Woeng et al., 2003).

Moreover, the suppression of *Rhizoctonia* diseases by bacterial bioagents usually attributed to the production of secondary metabolites which are toxic to the pathogen (Whipps, 2001). The results of dual culture studies showed that *P. fluorescenes* inhibited the growth of *R. solani* isolates.Member of the genus pseudomonas spp. Are well known antagonistic fungi (Kriet Low., 1949) .They are known to produce volatile compounds such as hydrogen cyanide (Castric et al.,1983). In vitro inhibition of *R. solani* occurred with the phenazine. Ester Dimethyl Pseudomonas Secondary Metabolite Liquid Culture (Kelly Cartwright et al.,1995).on the otger hand, compounds implicated in biocontrol of *R. solani* are usually antibiotics or fungal cell-wall degrading enzymes. It has been demonstrated that the ability of many strains of rhizobacteria to suppress plant pathogens is dependent on their capacity to produce secondary metabolites which directly inhibit the pathogens, and these include antibiotics, siderophores, bacteriocins, volatile compounds cyanide,enzymes and phytohormons (Homma, 1996).

On the other hand, all chemical fungicides used in this experiment prevent mycelial growth in vitro, but the inhibition rate was different between selected fungicides. In this study, tolclofos-methyl (TME) was the most effective of *R. solani* isolates, and suppressed of sclerotial production. However, Csinos (1985) found that 0.1 ug of TME/ml PDA suppressed sclerotia formation by 40% or more. (Ammar, 2003 and Korra, 2005) reported the inhibitory effect of Topsin M70 against many plant soil-borne pathogenic fungi. In other studies by Srinivas et al, 2014 and Rajput et al, 2014 reported the effectiveness of the active ingredient. Thiophanate methyl in the control of *R. solani* The different levels of reductions of radial growth by the fungicides was more likely due to varying extent of interference of these chemicals with the metabolism of the pathogen involved. In addition to genetic requirement, Deacon (1980) reported that the metabolism of pathogen depends, among other factors, on the substrate composition which could have been the active principles in the fungicides that affect the qualitative state of the fungus. The fungus genetics can affect its sensitivity toward the fungicides as a sensitive or insensitive to a chemical molecule (McGrat, 2009 and Reis et al., 2010), also the difference in the chemical group and the active ingredient of the used fungicides are much supported for the potential variation .The obtained results agreed with Rajput et al., 2016. Considering several systemic as well as non-systemic fungicides have been used which possess good control against *R. solani* (Madhavi et al., 2018).

The difference between both fungicides under study could be attributed to one or more factors, as mode of action of the fungal cell (Watkins et al., 1977), degree

of permeability of cell wall and/or plasma-lemma of fungi for uptake and passage of the fungicide into the fungal cell (Giffin, 1981). The similarity was obvious in terms of response of several isolates to the fungicides and those first reported by Dimond et al. (1941) for thiram. This response may be due to alterations in cell membrane permeability, storage of non dissociated fungicide or detoxification by the fungus (Horsfall, 1956).

5 Conclusion

We conclude that the success of *B. subtilis* and *P. flouresnces* as a potential biocontrol agents encouraged research into new microbial agents as alternatives to chemical control compounds. Whereas application of fungicides is the most convenient and predominant way for disease control. Their use has made it feasible to enhance crop yields and food production. The efficacy of fungicides is influenced by many biological and environmental factors that directly influence the metabolic activities of fungal cells. Sometimes critical concentrations are not effective long-term, as the fungus can become resistant to the fungicide. In the present study, two fungicides showed as effective control agents against *R. solani*, though their efficacy varied among fungicides. These findings need further verification by application of these treatments in infected host plants and to find out the degree of control over the pathogen in vivo conditions.

Conflict of interest: The authors declare that there are no conflicts of interest.

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