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Antifungal activity of bioagents and plant extracts against certain fungal diseases of potatoes

Ahmed B. Mohamed*, Mohamed M. El-Sheikh Aly, Rafeek M. I. El-Sharkawy

Agricultural Botany Department, Faculty of Agriculture, Al-Azhar University, 71524 Assiut, Egypt

Abstract

Twenty-six fungal isolates were obtained from potato plants and tubers growing in different localities in Egypt. The isolates were identified as 11 *Rhizoctonia solani*, 8 *Sclerotinia sclerotiorum* and 7 *Fusarium* spp. The 26 isolates were screened due to their pathogenic capabilities and the most pathogenic isolate among each of the three obtained genera was selected for this study. *In vitro* studies included the effect of 7 bacterial isolates, 6 *Trichoderma* isolates, as well as 6 plant extracts at four rates of application against the three fungal pathogens, *Trichoderma harzianum* (T5) achieved the highest mycelial growth inhibition, followed by *T. asperellum* (T34) and *T. harzianum* (T10) isolates. Additionally, *Bacillus subtilis* (BS2) recorded the best mycelial growth inhibition against the three tested fungi, followed by *B. subtilis* (BS1) and *B.megatirum*(BM2). On the subject of plant extracts, garlic extract gave the greatest reduction of the mycelial growth with all rates of application, followed by henna and ginger extracts. Field experiments were conducted during 2018/2019 and 2019/2020 growing seasons to evaluate bioagent activities as well as plant extracts in reducing disease severity caused by the three fore-mentioned pathogenic fungi. *Trichoderma harzianum* (T5) exhibited the highest disease reduction *in vivo*, followed by (T34) and *Pseudomonas fluorescens* (PF2), as compared with the control. Under greenhouse conditions, garlic extract decreased disease severity of both *Fusarium* sp and *S. sclerotiorum*, followed by henna and ginger extracts. On the other hand, henna extract came in the first order in reducing disease severity caused by *R.solani*, followed by ginger and garlic, as compared with the control. On the whole, *Trichoderma harzianum* (T5) and *T. asperellum* (T34) were the best treatments, those reduced diseases severity to the greatest extent if compared with the other treatments and the control.

Keywords: potato, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Fusarium* spp., bioagents.

*Corresponding author: Ahmed B. Mohamed,
E-mail: ahmedmohamed.5419@azhar.edu.eg

1. Introduction

Potato (*Solanum tuberosum* L.) is one of the most important crops in Egypt as well as all over the world and produces a tuber very rich in starch that it ranks as the world's fourth most paramount food crop, after maize, wheat and rice (Cunnington, 2008). Annual production of potato in Egypt during 2019 was about 5078347 tones (FAOSTAT, 2019). Potato crop is susceptible to diseases caused by several fungi, bacteria and other pathogens, leading to considerable losses in yield and quality (Khan et al., 2008; Walter et al., 2001). Potato plant was infected by *Rhizoctonia solani*, which causes black scurf of potato tubers, belong to commonly appearing potato pathogens. Scleroses of the mentioned fungi occurring on sets can be the source of infection for plants and descendant tubers. Moreover, it makes their quality worsen (Ahrenniemi et al., 2005). Potato yield losses caused by this disease amounted even to 50 % (Häni et al., 1998). Besides, rhizoctoniosis constitutes distinct aesthetic defect, which decreases market value of potatoes intendent for consumer purpose or for food industry (Lutomirska, 2007). *Sclerotinia sclerotiorum* is an ascomycetous phytopathogenic fungus, which can infect over 400 plant species in the world (Boland and Hall, 1994). The pathogen causes white mold in many potato producing areas of Egypt, mainly in fields irrigated by sprinkler systems (Ojaghian, 2009). Although a few reports are available showing severe damage of white mold on potato, the pathogen frequently causes substantial yield losses in fields (Ojaghian, 2011). The first infection of potato plants by *S. sclerotiorum* is initiated by ascospores which, can infect only senescent tissues (Atallah and Johnson, 2004). The secondary spread of the pathogen resulted from direct contact between healthy and infected tissues, when the pathogen colonized onto lower branches and leaves (Abawi and Grogan, 1979). Dry rot was caused by a number of *Fusarium* spp affecting sprouting and emergence at the beginning of the season, which results in yield losses and damage to the quality of daughter tubers, especially during storage (Borca and Carmen, 2013; Al-Mughrabi, 2010; Corsini and Pavek, 1986; Hooker, 1981). Biological control of dry rot has been demonstrated in laboratory studies using bacterial antagonists (Kiewnick and Jacobsen, 1997; Schisler et al., 1997; Schisler and Slininger, 1994; Slininger et al., 1994). The renewed interest in biocontrol among agriculture biologists is due to its eco-friendly protection against weeds, insects, and plant diseases, a long-lasting effect, and safety features. Some of the bacterial antagonists, however, also have been found to show direct growth promoting effects on crop plant inoculants (Deshwal *et al*. 2003; Barker and Paulitz, 1996). Studies on the mechanisms of disease control by plant extracts revealed that their biologically active constituents may have either direct antimicrobial activity (Amodioha, 2000; Ansari, 1995) or induce host plants defense response resulting in reduction of disease development (Schneider and Ullrich, 1994). Natural plant extracts have been found effective against a wide range of plant pathogens (Feng and Zheng, 2007; Amodioha, 2003; Wilson *et al*. 1997). This work aimed to study the effect of biological control and different plant extracts against several fungal diseases of potato which lead to considerable losses in yield and quality.

2. Materials and methods

2.1 Isolation and identification of the causal pathogens

For isolation of fungal pathogens, collected samples of potato plant and tubers were washed carefully under running tap water to remove the adjacent soil particles, followed by sterile water. Infected parts (stems or tubers) were cut using sterilized scalpel into small pieces, surface sterilized by dipping in 70% ethyl alcohol for 2 minutes, dried well between two sterilized filter paper, then transferred to plates contained PDA medium. Petri dishes were incubated at 25°C for 5 to 7 days, daily inspected for fungal growth. The developed fungal colonies were picked on PDA medium and purified using hyphal tip or single spore techniques. The purified fungi were identified according to fungal morphological and microscopical characteristics as described by Barnett and Hunter (1986), Booth (1977) and Sneh et al. (1991).

2.2 Pathogenicity tests

The pathogenic capability of the isolated fungi was conducted under greenhouse conditions in the Farm of Faculty of Agriculture, Al-Azhar University (Assiut branch), Egypt during 2017/2018 growing season.

2.3 Inoculum preparation

The fungal inoculum was grown in 250 ml jars containing the following substrate per jar (75 g grain barley, 25 g coarse sand and 25 ml tap water to cover the mixture in each jar). The jars were autoclaved at 121°C for 30 minutes, left to cool, then inoculated with the tested fungi and incubated at 25°C for 15 days to obtain sufficient growth of each fungus. Then, sterilized plastic pots in 5% formalin solution (40 cm in diameter) were filled with sterilized soil with formalin solution at 5% (10 Kg /pot). After that, the inoculum was mixed with the soil at the rate of 3% (w/w) of soil, then irrigated three times a week before sowing to ensure even distribution and growth of each particular fungus. Other sterilized pots were filled with sterilized soil and un-infested with the tested fungi were kept as control. Three seeds pieces were planted in each pot and three pots were used as replicates for each treatment.

2.4 Disease assessment

Black scurf disease severity was determined by using 0- 5 grade scale based on the percent of tuber surface showing disease symptoms, where 0 = no symptoms on potato tubers; 1 = less than 1 % tuber area affected; 2 = 1-10 % tuber area affected; 3 =11-20 % tuber area affected; 4 =21-51 % tuber area affected; 5 = 51 % or more tuber area affected, according to Ahmad et al. (1995). Dry rot disease severity was determined visually using a 0-5 scale where "0" represented no disease symptoms and a "5" represented 100% dry rotted tissue. (Schisler et al., 2000). White mold disease severity was estimated as following., 0-4 scale of where: 0 = 0 percent, 1 = 1–25 percent, 2 = 26–50 percent, 3 = 51–75 percent and 4 = 76–100 percent of the surface area of the shoot with symptoms of white mold, according to Morton and Hall (1989).

2.5 Evaluation of antagonistic bacteria against the pathogenic fungi *in vitro*

The antagonistic bacteria were obtained from MERCIN, Faculty of Agriculture, Ain Shams University, Egypt. The used bacteria in this study were two isolates of *Bacillus subtilis* (BS1 and BS2), two isolates of *B. megaterium* (BM1 and BM2), one isolate of *Penibacillus polymyxa* (BP*)* as well as two isolates of *Pseudomonas fluorescens* (PF1 and PF2). These isolates were tested against the pathogenic fungi *R. solani, S. sclerotiorum* and *Fusarium.* sp *in vitro.* The antagonistic bacteria were grown on nutrient sucrose agar medium (NSA), which consisted of peptone 5gm, beef extract 3gm, sucrose 5gm, yeast extract 2gm, agar 20gm and distilled water per/ Liter for 5 days at 25±2°C. A 5mm mycelial disc in diameter of the pathogenic fungus taken, of advancing zone of growing hyphae was transfered at the center of 9 cm diameter Petri plates containing PDA medium. On the other hand, the antagonistic bacteria were streaked at a distance of 2-3 cm either in semicircular pattern. The culture plates were incubated at 25±2°C and inhibition zone was checked after 24, 48, 72 and 96 hours. Three replicate plates were used for each treatment. The inoculated plates with tested fungi only were served as control. Percentage of growth inhibition of the pathogen was calculated, when pathogen achieved full growth in the check by the following formula:

$$\text{Mycelial growth inhibition (\%)} = (A-B\ /A) \times 100$$

Where: A= The diameter of the mycelial growth in control. B= The diameter of the mycelial growth in treated Petri plates.

2.6 Evaluation of antagonistic activity of *Trichoderma* isolates against the pathogenic fungi *in vitro*

The antagonistic. *Trichoderma* isolates and the tested pathogenic fungi *R. solani*, *S. sclerotiorum* and *Fusarium* sp. were grown on PDA medium for 7 days at 25±2°C to study the efficacy of *Trichoderma* bioagents against the pathogenic fungi. Discs (5 mm in diameter) from each isolate of *Trichoderma* were cut and placed on PDA medium in one side of Petri dish and the opposite side was inoculated with the pathogenic fungal isolates. Three replicate plates were used for each treatment. The inoculated plates with the tested fungi only, without *Trichoderma* isolates served as control. The inoculated Petri plates were incubated at 25±2°C until fungal growth of control grew to full Petri plates. At the same time, the fungal growth was measured in two dimensions. The percentage of mycelial growth inhibition was calculated according to the following formula:

$$\text{Mycelial growth inhibition (\%)} = (A-B\ /A) \times 100$$

Where: A= The diameter of the mycelial growth in control. B= The diameter of the mycelial growth in treated Petri plates.

2.7 Evaluation of antagonistic bacteria against the tested fungi under greenhouse conditions

Pot experiments were carried out during 2018/2019 and 2019/2020 growing seasons to study the antagonistic effect of the antagonistic bacteria against fungal pathogens causing the diseases of potato

foliage and tubers (Cara cultivar) caused by the pathogenic fungi under greenhouse conditions. Bacterial isolates were applied as soil treatment, 15 days before planting by adding 100 ml of bacterial suspensions (10^8 cfu /ml) for each pot, which previously infested with the pathogenic fungi. to study the effects of the selected fowr antagonistic bacteria., *Penibacillus. polymyxa* (BP*), B. subtilis* (BS2), *B. megaterium* (BM1) and *Pseudomonas fluorescens* (PF2) for controlling potato disease incidence. Cara cv. were planted (3 seed tubers /pot) in infested soil with the pathogenic fungi as mentioned before. After 100 days from planting, diseases severity was recorded. The inoculum of *Trichoderma harzianum* isolates (T5, T7, T10 and *Trichoderma asperellum* (T34) were used as antagonistic fungi against *R. solani, S. sclerotiorum* and *Fusarium* sp which grown on barley medium as mentioned before in the pathogenicity tests. The antagonistic *Trichoderma* bioagents and the fungal pathogens were mixed with sterilized soil at the rate of 3% (w/w) of soil for each antagonistic and pathogenic fungi, 15 days before planting. Each pot was planted with 3 potato seeds (Cara cv.). The infested pots with the pathogenic fungi only served as control. Each treatment was replicated three times. Percentage of potato disease was calculated after 100 days as previously mentioned in the pathogenicity tests.

2.8 Comparative effectiveness of plant extracts on potato disease incidence

2.8.1 Preparation of plant extracts

Fifty grams from each leaf, or parts of garlic cloves and rhizomes of Basil, Blue gum, Garlic, Henna, Thyme and Ginger were washed several times with tap water, rewashed with sterilized distilled water and left to air dry at room temperature. Then, the plant parts were cut into small pieces and crushed separately in a porcelain mortar with 100 ml of ethyl alcohol (70%). The grinded material was passed through six layers of cheesecloth and Whatman filter paper No. 1. The filtrate was centrifuged at 3000 rpm for 10 minutes and the supernatant was filtered with sterilized centered glass funnel (El- Shaer, 1998). Four dilutions were prepared from each crude extract using sterilized distilled water. The dilutions were 5, 10, 15 and 20% culture filtrates.

2.8.2 Effect of different concentrations of plant extracts on the mycelial growth inhibition of the pathogenic fungi *in vitro*

Different concentrations of Basil, Blue gum, Garlic, Henna, Thyme and Ginger extracts prepared as previously mentioned were used to study their effects on the mycelial growth inhibition of *R. solani, S. sclerotiorum* and *Fusarium* sp. Dilution of an extract was separately mixed with PDA medium before solidification, then poured in sterilized Petri dishes. Three replicate plates were used for each concentration. Petri plates were inoculated at the center with equal disc, 5mm in diameter, taken from 7 days old culture of any of the tested pathogens. The Petri plates were incubated at 25°C. Sterilized distilled water was mixed with the same dilutions on PDA medium instead of any extract served as control (Singh et al., 1986). The mycelial growth inhibition of the

tested fungi was measured after the growth completely covered the control plates by taken middle of two axis of growth. The reduction of mycelial growth inhibition was calculated using the following formula:

$$\text{Mycelial growth inhibition } (\%) = (A-B\ /A) \times 100$$

Where: A= The diameter of the mycelial growth in control. B= The diameter of the mycelial growth in treated Petri plates.

2.8.3 Effect of mixing plant extracts with the infested soil on the disease incidence under greenhouse conditions

Leaves, cloves and rhizome extracts of basil, blue gum, garlic, henna, thyme and ginger were used to study their efficacy on controlling potato diseases under greenhouse conditions. This experiment was carried in the Farm of Fac of Agriculture, Al-Azhar University (Assiut branch), Egypt during 2018/2019 and 2019/ 2020 growing seasons. The plant extracts were added to the infested soil with the pathogenic fungi at the rate 3% (w/w) as fore mentioned described. Two different concentrations of plant extracts *i.e.* 15 and 20% (v/w) of soil were added and poured in the upper layer of the infested in pots (40 cm in diameter). Three potato seeds of Cara cv. were planted in each pot. Three replicate pots were used for each treatment. The infested soil with the pathogenic fungi and without any additive of plant extracts served as control. The experiment was irrigated and fertilized regularly. Data were recorded after 100 days from planting as disease severity as previously mentioned.

2.9 Statistical analysis

Analysis of variance of the data was carried out on the mean values of the tested treatments according to the procedures described by Gomez and Gomez (1984). The least significant difference (L.S.D) at 5% probability was used for testing the significance of the differences among the mean values of the tested treatments for each character.

3. Results and Discussion

3.1 Isolation and identification of the associated fungi with the infected potatoes

Different fungal isolates representing three genera *i.e. R. solani, S. sclerotiorum* and *Fusarium* sp. were isolated from potato plants and tubers collected from different locations of Menofeya, Behera and Minia governorates, Egypt. The fungal isolates were identified by using the morphological features of mycelium and spores as described by Barnet and Hunter (1986), Booth (1977) and Sneh et al. (1991) and confirmed by Agric. Botany Department, Faculty of Agriculture, Al-Azhar University (Assiut Branch), Egypt.

3.2 Pathogenicity tests

Twenty-six fungal isolates were tested to study their pathogenic capabilities on potato plants and tubers of (Cara cv.) under greenhouse conditions during 2018 growing season. Data in Table (1) illustrated that all tested fungal isolates were able to infect potato seedlings

causing black scarf, white mold and dry rot diseases. All tested isolates significantly increased the infection potato compared with the control. *R. solani* (R8), (R1), *S. sclerotiorum* (S4) and *R. solani* (R4), gave the highest disease severity as reached 71.73, 68.93, 64 and 58.36 %, respectively. On the other hand, isolates *S. sclerotiorum* (S3), *Fusarium* sp. (F2), *S. sclerotiorum* (S5), *R. solani* (R6), followed by *S. sclerotiorum* (S1), Showed moderate disease severity as reached 51.8, 48.13, 45.8, 44.7 and 44 %, respectively While *R. solani* (No. 5, 7 and 10) and *Fusarium* sp. (No. 5 and 4), gave the lowest disease severity as reached 15.2, 20.5, 21.5, 22.4 and 27.3 %, respectively. Virulence of *R. solani* isolate on potato may be depending on the source of isolates from lesions or sclerotia, as reported by Carling and Leiner (1990).

Table 1: Pathogenicity tests of 26 fungal isolates on potatoes under greenhouse conditions during 2018 growing season.

The tested isolates	Isolate code	Disease severity (%)		
		Black scarf	Dry rot	Whit mold
R. solani	R1	68.93	0	0
R. solani	R2	41.13	0	0
R. solani	R3	35.23	0	0
R. solani	R4	58.36	0	0
R. solani	R5	15.26	0	0
R. solani	R6	44.7	0	0
R. solani	R7	20.56	0	0
R. solani	R8	71.73	0	0
R. solani	R9	32.53	0	0
R. solani	R10	21.56	0	0
R. solani	R11	42.73	0	0
S. sclerotiorum	S1	0	0	44.06
S. sclerotiorum	S2	0	0	37.86
S. sclerotiorum	S3	0	0	51.8
S. sclerotiorum	S4	0	0	64
S. sclerotiorum	S5	0	0	45.83
S. sclerotiorum	S6	0	0	32.53
S. sclerotiorum	S7	0	0	38.63
S. sclerotiorum	S8	0	0	55.4
Fusarium sp.	F1	0	32.66	0
Fusarium sp.	F2	0	48.13	0
Fusarium sp.	F3	0	30.76	0
Fusarium sp.	F4	0	27.33	0
Fusarium sp.	F5	0	22.46	0
Fusarium sp.	F6	0	36.76	0
Fusarium sp.	F7	0	28.16	0
Control		0	0	0
LSD at 5%		3.78	3.31	3.09

3.3 Effect of *Trichoderma* spp on the mycelial growth inhibition of the tested fungi *in vitro*

The efficacy of different bioagents on the mycelial growth inhibition of the tested fungi was evaluated to study their antagonistic effects *in vitro*. The inhibitory effect of biological control agents, *Trichoderma* spp. was shown in Table (2). Data clearly indicated that all the tested *Trichoderma* isolates were

significantly decreased the mycelial growth of the three pathogenic fungi on PDA medium compared with the check. In this respect, *T. harzianum* (T5), *T. asperellum,* (T34), followed by *T. harzianum* (T10), exhibited the greatest mycelial growth inhibition of *R. solani*. as reached 78.9%, 77.8% and 73.2%, respectively. *Trichoderma harzianum* isolates (No. 7 and 8) showed moderate inhibition against *R. solani*, while *T. harzianum* (T9) gave the lowest reduction of the mycelial growth of the same fungus. Meanwhile, *T. harzianum* isolates (No. 10 and 7) and *T. asperellum,* (T34) gave the best effect in reducing the mycelial growth inhibition, of *S. scleroturium*.as reached 75.8%, 69.9% and 69.6%, respectively, *Trichoderma harzianum* (No. 5 and 8) showed moderate inhibition against *S. sclerotiorum*, while *T. harzianum* (T9) gave the lowest reduction of the mycelial growth of *S. sclerotiorum*. On the other hand, *T. harzianum* isolates (No. 5 and 7) and *T. asperellum,* (T34), gave the greatest inhibition of the mycelial growth of *Fusarium* sp. as reached 81.3%, 78.13%, 74.4%, respectively. At the same time *T. harzianum* isolates (No. 8 and 7) showed moderate growth inhibition against *Fusarium* sp., while *T. harzianum* (T9) gave the lowest reduction of the mycelial inhibition of *Fusarium* sp. Similarly, this study showed that the mycelium growth inhibition of *R. solani* of 75.8%, 69.9% and 69.6% by *Trichoderma* isolates, however, in another study, three different isolates of *Trichoderma* against soil borne pathogen *R. solani* was observed to be 74.4-67.8% (Asad et al., 2014). Whatever, some of the antagonists tested were not found to be very effective against the pathogens under *in vitro* observations but they may show better result under their natural field conditions as their activities depend on the physico-of the environment (Burgess and Griffin, 1967).

Table 2: Effect of different isolates of T*richoderma harzianum* sp and *T. asperellum* on mycelial growth inhibition of the tested fungi *in vitro*.

Bioagents	Mycelial growth inhibition (%)		
	R. solani	*S. sclerotiorum*	*Fusarium* sp.
Trichoderma harzianum (T5)	78.96	65.80	81.30
Trichoderma harzianum (T7)	67.36	69.96	78.13
Trichoderma harzianum (T8)	63.53	56.30	73.0
Trichoderma harzianum (T9)	57.86	48.90	69.76
Trichoderma harzianum (T10)	73.20	75.83	70.03
Trichoderma asperellum (T34)	77.86	69.60	74.46
Control	0	0	0
LSD at 5%	3.64	3.84	3.91

3.4 Effect of certain bacterial bioagents on the mycelial growth inhibition of the tested fungi *in vitro*

Different bacterial bioagents were evaluated on the mycelial growth of the tested fungi to study their antagonistic effects under Lab. conditions. The inhibitory effect of bacterial agents was obtained in Table (3). Data clearly indicated that all the tested bacterial bioagents significantly decreased the

mycelial growth inhibition of the three pathogenic fungi on PDA medium compared with the check. In this respect, *B. subtilis* (Bs1), *B. megaterium* (Bm2), followed by *B. subtilis* (Bs2), showed that highest reduction of the mycelial growth of *R. solani*, as recorded 74.76%, 73.0% and 71.1%, respectively. As mean, *B. megaterium* (Bm1) gave moderate inhibition against *R. solani*, while *P. fluorescens* (Pf1) gave the lowest reduction of the mycelial growth inhibition of *R. solani*. At the same time, *B. subtilis* (Bs2), *B. megaterium* (Bm1) and *B. megaterium* (Bm2) gave the best effect in reducing the mycelial growth of *S. sclerotiorum*, as recorded 53.43%, 52.8% and 51.6%, respectively. As regard, *B. subtilis* (Bs1) showed moderate inhibition against *S. sclerotiorum*, while *Penibacillus polymyxa* (Bp) gave the lowest reduction of the mycelial growth of *S. sclerotiorum*. It was shown from the same Table That *P. fluorescens* (Pf2), *P. fluorescens* (Pf1) and *B. subtilis* (Bs2), gave the best results in inhibition of the mycelial growth of *Fusarium* sp., as reached 80.83%, 78.26%, 66.30%, respectively. Meanwhile, *B. subtilis* (Bs1) gave the same trend in inhibition against *Fusarium* sp., while *Penibacillus polymyxa* (Bp) gave the lowest effect in of the mycelial growth of *Fusarium* sp. Among different biological approaches, the use of the microbial antagonists like fungi and bacteria offers an effective, similar study safely and ecofriendly strategy to control many of soil-borne pathogens (Gravel et al., 2004). The isolates and strains of *Trichoderma spp*. and *Bacillus spp*. have been proven to be effective in suppressing plant diseases caused by *Fusarium* spp. (Kahkashan and Bokhari, 2012; Abdel-Monaim, 2010).

Table 3: Effect of different bacterial bioagents on the mycelial growth inhibition of the tested fungi *in vitro*.

Bacterial bioagents	Mycelial growth inhibition (%)		
	R. solani	*S. sclerotoirum*	*Fusarium* sp.
P. fluorescens (Pf 1)	20.23	33.66	78.26
P. fluorescens (Pf 2)	22.83	42.43	80.83
Pb. polymyxa (Bp)	28.23	26.16	32.80
B. megaterium (Bm1)	68.90	52.80	48.83
B. megaterium (Bm2)	73.03	51.63	46.96
B. subtilis (Bs1)	74.76	46.26	53.16
B. subtilis (Bs2)	71.10	53.43	66.30
Control	0	0	0
LSD at 5%	3.87	3.80	3.73

3.5 Effect of microbial bioagents on incidence of potato diseases under greenhouse conditions

In pot experiment, the efficacy of using *B. subtilis, B. megaterium, Penibacillus polymyxa, P. fluorescens, T. harzianum,* (No. 5, 7 and 10) and *T. asperellum* (T34) on potato diseases in drenched soil with the previous bioagents and artificially infested with the tested fungi was carried out under greenhouse

conditions during 2018/2019 and 2019/2020 growing seasons. Data obtained in Table (4) clearly showed that the tested biocontrol agents significantly reduced the disease severity of potato diseases compared with control. *Trichoderma harzianum* (T5), *T. asperellum* (T34), *Penibacillus polymyxa* (Bp), *P. fluorescens* (Pf 2) and *T. harzianum* (T7) exhibited the highest reduction of disease severity caused by *R. solani* which recorded 16.1%, 18.5%, 22.8%, 23.4% and 28.2%, respectively, followed by *T. harzianum* (T10) and *B. subtilis* (Bs2) 30.8% and 38.3% during 2019 growing season. While. *Trichoderma harzianum* (T5), *T. asperellum* (T34), *P. fluorescens* (Pf2) and *T. harzianum* (T7) as recorded 18.53%, 21.26%, 22.96% and 25.86% respectively. Also, *Penibacillus polymyxa* (Bp), *T. harzianum*, (T10) and *B. subtilis* (Bs2) gave 26.33%, 32.93% and 37.26% disease severity during 2020 growing season. Whatever, *B. megaterium* (Bm1) recorded the lowest reduction of disease severity, reached at 44.2% and 42.96% during 2019 and 2020 growing seasons. The same data showed that, *T. harzianum* (T5), and *T. asperellum* (T34), exhibited the greatest reduction of disease severity caused with *S. sclerotiorum*, as reached 22.9% and 25.4% during 2019 growing season, as well as reached at 20.60% and 27.83% during 2020 growing season.

Table 4: Effect of microbial bioagents on incidence of potato diseases under greenhouse conditions.

Bioagents	Disease severity%					
	R. solani		*S. sclerotiorum*		*Fusarium* sp.	
	2019	2020	2019	2020	2019	2020
P. fluorescens (Pf2)	23.4	22.96	33.2	31.2	23.26	24.16
B. polymyxa (Bp)	22.8	26.33	35.26	33.1	20.33	24.3
B. megaterium (Bm1)	44.23	42.96	42.7	38.16	36.1	33.667
B. subtilis (Bs2)	38.33	37.26	35.46	31.26	31.46	36.33
T. harzianum (T5)	16.1	18.53	22.93	20.6	9.6	11.1
T. harzianum (T7)	28.26	25.86	35.5	32.33	27.13	25.83
T. hamatum (T10)	30.8	32.93	32.8	28.46	31.3	28.06
T. asperellum (T34)	18.56	21.26	25.43	27.83	13.1	10.26
Control	73.66	72.03	62.4	60.13	44.33	43.36
LSD at 5%	3.68	3.20	3.30	3.52	3.54	3.36

In this regard, *T. harzianum* (T10), *P.fluorescens* (Pf 2), *B. polymyxa* (Bp), *B. subtilis* (Bs2) and *T. harzianum* (T7) gave moderate effects in disease reduction, while *B. megaterium* (Bm1) recorded the lowest reduction, of disease severity as reached 42.7% and 38.16% during 2019 and 2020 growing seasons. On the other hand, *T. harzianum* (T5), *T. asperellum* (T34), *B. polymyxa* (Bp), and *P. fluorescens* (Pf 2) gave the highest reduction of disease severity of dry rot disease caused by *Fusarium* sp as reached 9.6%, 13.1%, 20.3% and 23.2%, during 2019 growing season. As mean disease severity reached it 11.10%, 10.26%, 24.30% and 24.16% during 2020 growing season. The same trend was observed by using *T. harzianum* (T7) and *T. harzianum* (T10) and as recorded 27.1% and 31.3% during 2019 growing season, 25.83% and 28.06%

during 2020 growing season. Meanwhile, *B. subtilis* (Bs2) and *B. megaterium* (Bm1) recorded the lowest reduction of disease severity. Most frequently species of *Bacillus*, *Pseudomonas* and *Trichoderma* are used for biological control of fungal pathogens. Among them, one of the fungal biocontrol agents used in this study is *Trichoderma* species. They are common saprophytic fungi found in almost any soil and rhizospheric microflora. The reason for choosing *Trichoderma* species as potential biocontrol agents because of their ability to reduce the incidence of disease caused by plant pathogenic fungi (Dubey et al., 2007).

3.6 Comparative effectiveness of using plant extracts against potato diseases

3.6.1 *In vitro* test

Ethanolic extracts of six plant leaves parts and cloves with four different concentrations *i.e.* 5, 10, 15 and 20% prepared from Basil, Blue gum, Garlic, Henna, Thyme and Ginger were tested to study their effectiveness on the mycelial growth inhibition of the pathogenic fungi under lab. conditions. The inhibitory effect of plant extracts on the tested fungal growth are shown in Table (5). The decreasing of the mycelial growth was increased with increasing the extract concentrations.

Table 5: Effect of different concentrations of six plant extracts on the mycelial growth inhibition of the pathogenic fungi.

Plant extracts (A)	Concentration (B) (%)	Mycelial growth inhibition (%)		
		R. solani	*S. sclerotiorum*	*Fusarium* sp
Basil	5	0	0	0
	10	0	0	0
	15	17.167	15.233	18.167
	20	22.967	26.333	29.367
Blue gum	5	35.433	28.367	40.333
	10	62.367	58.233	64.133
	15	93.8	86.133	89.567
	20	100	100	100
Garlic	5	20.9	37.233	46.933
	10	51	70.967	80.667
	15	76.433	100	100
	20	100	100	100
Henna	5	54.3	49.5	60.967
	10	84.567	77.267	76.167
	15	100	92.867	100
	20	100	100	100
Thyme	5	0	0	0
	10	0	0	35.167
	15	33.9	23.167	72.067
	20	61.067	54.967	100
Ginger	5	63.467	54.9	82.733
	10	86.333	76.967	94.6
	15	95.7	88.333	100
	20	100	100	100
Control	-	0	0	0
LSD at 5%	A	1.78	2.01	2.07
	B	0.98	0.91	0.84
	A × B	2.6	2.42	2.2

All the tested plant extracts with any concentration significantly decreased the mycelial growth of the pathogenic fungi, except Basil and Thyme at 5 and 10% on *Fusarium* sp. Among the plant extracts tested, Henna gave the highest value of mycelial growth inhibition which recorded (84.5and 100%) at 10 and 15% concentrations, Ginger (86.3, 95.7 and 100) followed by Blue gum (62.367, 95.7, and 100) and garlic cloves extract as reached (51, 76.4and 100%) at 10,15 and 20% concentrations respectively. Basil and thyme revealed that lowest mycelial growth inhibition of *R. solani* reached (22.9 and 61.0 %) at 20% concentrations, respectively. Garlic cloves extract gave the highest value of mycelial growth inhibition (70.9 and 100%) at 10, and 15% concentration, Henna (77.2, 92.8 and 100) followed by ginger (76.9, 88.3and 100) and blue gum (58.2, 86.1, and 100) at 10,15 and 20% concentrations respectively. Basil and thyme (26.3 and 54.9) at 20% concentration showed the lowest mycelial inhibition of *S. sclerotiorum*, ginger gave the highest mycelial inhibition value (94.6 and 100%), garlic (80.6 and 100%), Henna (76.1 and 100%) at 10, and 15% concentration respectively followed by blue gum (64.1, 89.5, and 100) and thyme (35.1, 72.0 and100%) at 10,15 and 20% concentration respectively, Basil (29.3) at 20% concentration showed the lowest mycelial inhibition of *Fusarium* sp. The same effect was obtained by using Henna extract which strongly retarded and inhibited the fungal growth. These results are in line with those reported by Latif et al. (2006), Abd-El Khair et al. (2007) and Salim (2011) who mentioned that plant extracts had a good potential to control various fungal diseases and the inhibitory effect of the plant extracts might be attributed to the presence of some antifungal toxicants.

Table 6: Effect of plant extracts on potato diseases incidence under greenhouse conditions during 2019 and 2020 growing seasons.

Plant extracts (A)	Concentration (B) (%)	Disease severity%					
		R. solani		*S. sclerotiorum*		*Fusarium* sp	
		2019	2020	2019	2020	2019	2020
Basil	15	61.36	58.23	43.16	40.3	38.06	36.3
	20	56.16	53.26	40.53	36.7	35.6	32.6
Blue gum	15	55.5	53.4	38.36	42.5	33.43	30.16
	20	52.2	50.46	34.46	37.36	30.0	28.63
Garlic	15	27.53	24.96	23.03	20.26	18.1	20.56
	20	22.7	23.43	20.46	19.33	16	18.56
Henna	15	23.13	25.43	31.8	28.43	22.8	24.16
	20	20.56	24.83	28.13	25.16	20.6	17.46
Thyme	15	46.2	40.3	33.5	35.86	27.93	23.63
	20	43.66	38.33	31.06	33.4	25.8	22.6
Ginger	15	24.53	23.13	27.43	25.1	32.76	35.33
	20	23.0	20.96	24	20.63	30.43	33.46
Control		73.66	72.03	62.4	60.13	44.33	43.36
LSD at 5%	A	2.64	2.40	1.34	2.54	2.48	1.64
	B	1.29	1.09	1.60	1.20	1.48	1.12
	A x B	ns	ns	ns	ns	ns	ns

3.6.2 Comparative effectiveness of plant extracts on potato diseases incidence

Testing the effectiveness of the plant extracts was conducted to find out the best plant extract and concentration that may inhibit the fungal infection caused by *R. solani*, *S. sclerotiorum* and *Fusarium* sp. under greenhouse conditions. Data obtained in Table (6) demonstrated that all the tested plant extracts at 15% and 20% significantly reduced the infected plants of potato Cara cv. if compared with the check during 2019 and 2020 growing seasons. Henna followed by garlic cloves and ginger each at 15 and 20 % concentrations gave higher effect in reducing the infected plants caused by *R. solani*. While, thyme, blue gum and basil gave the lowest effect in reducing disease severity during 2019 growing season. On the other hand, ginger followed by garlic cloves and henna each at 15 and 20 % concentrations revealed higher effect in reducing the infected plants caused by *R. solani* more than, thyme, blue gum and basil, which showed lower effect in reducing disease severity during 2020 growing season. Garlic cloves extract followed by ginger and henna each at 15 and 20 % had higher effect in reducing the infected plants caused by *S. sclerotiorum*. While, thyme at 20% concentration exhibited moderate effect in reducing disease severity, while blue gum and basil were less effective in reducing disease severity during 2019 and 2020 growing season. On the other hand, garlic cloves extract followed by henna and thyme each at 15 and 20 % gave higher effect in reducing the infected plants caused by *Fusarium* sp., While, ginger at 20% concentration gave moderate effect in reducing disease severity, while blue gum and basil showed the lowest effect in reducing disease severity during 2019 growing season. At the same time, Henna followed by garlic cloves and thyme extract each at 15 and 20 % concentrations resulted the highest value in reducing the infected plants caused by *Fusarium* sp. On the contrary, blue gum, ginger and basil gave the converse effect in reducing disease severity during 2020 growing season. Although some researchers who only used the aqueous extracts in their studies reported antifungal effectiveness of this form of extracts against some fungi (Bhardwaj, 2012) but much documents are available in favor of present findings from researchers who compared antifungal properties of chemically derived and aqueous extracts (Alizadeh Behbahani et al., 2013; Jat and Agalave, 2013; Moorthy et al., 2013; Ambikapathy et al., 2011; Ashraf et al., 2011).

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