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Control of root rot disease of sugar beet using certain antioxidants and fungicides

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Abstract

This study was carried out to investigate the effect of five chemical inducers *i.e.* salicylic acid, ascorbic acid, catechol, citric acid and potassium silicate and six fungicides *i.e.* Actamyl70%, Chlorothalonil 50%, Evito 48%, Shenzy 34%, Pyrus 40% and Fentobein 32.5% in order to control *Rhizoctonia solani* and *Macrophomina phaseolina* which infect sugar beet roots. The antioxidants, catechol and salicylic acid achieved the best disease control at all rates of application followed by citric acid and potassium silicate, respectively. Concerning fungicides, Shenzy 34% gave noticeable control in disease reduction followed by Evito 48% and Fentobein 32.5%, respectively. Usage of antioxidants as chemical inducers for enhancing plant resistance and capability of defying diseases is well recommended as fungicide alternatives due to their safe influence on human health. But, fungicides are still the most widespread used compounds in disease management strategies, based on their compliant application, reliable and efficient results than any other safer chemical or natural compound which controls the disease by reducing the losses, not by eradicating the disease in which fungicides can do successfully.

Keywords: sugar beet, *Rhizoctonia solani*, *Macrophomina phaseolina*, antioxidants, fungicides.

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1. Introduction

Sugar beet (*Beta vulgaris* L.), a specialized type of beet belonging to family Chenopodiaceae, it is an important part of the human diet, providing energy to maintain body temperature activity. Additionally, it is also widely used as a sweetener and preservative for foods. Beside sugar, it also provides valuable by products, *i.e.* beet tops and molasses being used as cattle feed. Root rot diseases are one of the major constraints in the profitable yield of sugar beet in the form of tonnage and sugar content. There are a number of soil borne fungal pathogens that are responsible for poor establishment and yield loss in sugar beet crop (Harveson & Rush, 2002; Kiewnick et al., 2001; Weiland & Sundsbak, 2000; Windels & Lamey, 1998). In Egypt, *Rhizoctonia solani* primarily causes root and crown rot disease on sugar beet. Also, *Macrophomina phaseolina* causes extensive damages in sugar beet. Chemical inducers act as alternative and safe trial for management of many diseases, especially those of vegetable crops (Abada et al., 2008). Plant resistance can be achieved by the use of antioxidants (Dov & llana, 1992**)**. Plant inducers may act on plants to induce high levels of systemic resistance to subsequent pathogen attack (Ward et al., 1991). Certain chemicals, such as salicylic acid and potassium salts induced systemic acquired resistance in plants against some plant pathogens (Lin et al., 2009). Growers typically use an integrated system including early planting, crop rotation, resistant varieties as well as fungicide applications to manage sugar beet root rot diseases. But, fungicides are still the most effective and dependable mean, achieving appreciable results in diseases reduction. The aim of the current study is to evaluate the severity of root rot disease of sugar beet crop and the efficacy of various chemical compounds *i.e.* antioxidants and fungicides and their proper doses in controlling root rot disease.

2. Materials and methods

This work was carried out in the Research Laboratory and Farm of Faculty of Agriculture, Al-Azhar University (Assiut Branch), Assiut, Egypt.

2.1 Isolation and identification of sugar beet root rot disease

Sugar beet root samples were collected from naturally infected sugar beet plants growing in different locations representing four governorates *i.e.* Assiut, Minia, Fayoum and Kafr-Elsheikh during 2017/2018 growing season. Plant roots were taken to the laboratory for the isolation and identification of the causal pathogens. Samples of roots were washed carefully with tap water to remove adhering soil particles, cut into small pieces of about 0.3 cm long, surface sterilized with dipping in 70% ethyl alcohol for 2 minutes and left to dry on sterilized filter paper, then transferred individually to Petri dishes, each containing about 20 ml potato dextrose agar (PDA) medium. Petri dishes were incubated at 25±2°C for 7 days and inspected for fungal growth. The developed fungal colonies were 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) and confirmed by

Agricultural Botany Department, Faculty of Agriculture, Al-Azhar University (Assiut Branch), Assiut, Egypt. The obtained isolates were maintained on PDA slants and kept in refrigerator at 5°C for further studies. The frequency of the isolated fungi was calculated separately for each of the collected samples. Stock cultures were routinely sub-cultured on fresh slants every month. The frequency of the isolated fungi from the infected roots was calculated according to the following formula:

$$\text{Fungal frequency \%} = \frac{\text{Number of isolates of each fungus}}{\text{Total number of all isolates}} \times 100$$

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. Sugar beet seeds of Farida, Lily and Pleno cultivars which selected as three of the most common cultivars grown in Egypt were sown in sterilized sand clay soil in plastic pots (50 cm in diameter).

2.3 Inoculum preparation

The fungal inocula of 22 fungal isolates representing 3 genera *i.e. Rhizoctonia solani, Fusarium* spp and *Macrophomina phaseolina* were grown in 250 ml glass 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 jar). The jars were autoclaved at 121°C for 30 minutes, left to cool, then inoculated and incubated at 25±2°C for 15 days to obtain sufficient growth of the fungi. Then, sterilized plastic pots in 5% formalin solution (50 cm in diameter) were filled with 5% formalin sterilized soil (10 Kg /pot). After that, the inoculum was mixed with the soil at the rate of 2% (w/w) of soil, then, pots were 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, which kept as control. The sugar beet seeds were washed with sterile distilled water to remove residual effect of seed dressing fungicides before sowing in plastic pots. Three seeds were planted in each pot, replicated three times for each tested fungus. The pots were irrigated and fertilized regularly under greenhouse conditions. Disease severity was recorded after 120 days from sowing. Disease severity index (DSI) was calculated according to Liu et al. (1995) as follows:

$$\text{DSI} = \frac{\Sigma d}{d\,\max \times n} \times 100$$

Whereas: d is the disease rating of each plant, d max is the maximum disease rating and n is the total number of plants examined in each replicate. The inspected plants were classified into seven categories according to Ruppel et al. (1979). The root rot rating scale was as follows: 0: Root surface clean with no visible lesions, 1= Superficial, scattered non-active lesion, 2= Shallow, dry rot canker on < 5% of root surface, 3= Deep dry rot cankers at crown or extensive lateral lesions affecting 6-25% root surface, 4= Extensive rot affecting 26-50% of root, which cracks and canker up to 5 mm deep, 5= > 50% of root blackened with rot extending into interior, 6= Entire root blackened except

extreme tip, 7= Root 100% rotted and foliage is dead.

2.4 Evaluation of antioxidant against sugar beet root rot disease

2.4.1 *In vitro* test

The inhibitory effect of five antioxidants on the mycelial growth of three fungal isolates *i.e. Rhizoctonia solani* (No. 16 and 22) and *Macrophomina phaseolina* (No. 11) which causing sugar beet root rot disease was estimated on PDA medium. Different rates of the tested antioxidants *i.e.* salicylic acid, ascorbic acid, citric acid, catechol and potassium silicate were individually added to conical flasks containing sterilized PDA medium before its solidification to obtain the concentrations of 20, 40, 60, 80 and 100 mM (Millimole), on the basis of molecular weight of each tested compound, then rotated gently to ensure equal distribution of antioxidant, poured in sterilized Petri plates (9 cm in diameter). Each Petri dish was contained with 20 ml of PDA medium, then individually inoculated in the center with 5 mm agar disc having active mycelial growth of the pathogenic fungi. Three replicate plates were used for each concentration. A set of antioxidant free PDA plates were inoculated by the tested pathogens to serve as control. All inoculated plates were incubated at 25±2°C for 7 days, when the fungal growth completely filled the antioxidant free plates, the inhibitory effects of the tested antioxidants were estimated by measuring the fungal growth in each treatment. The fungal growth inhibition percentage was calculated using the formula of Chapagain et al. (2007) as follows:

$$\text{Inhibition percentage} = \frac{\text{Growth in control- Growth in treatment}}{\text{Growth in control}} \times 100$$

2.4.2 *In vivo* test

The most effective antioxidants in the laboratory tests were tested to study their effectiveness under greenhouse conditions during 2018/2019 and 2019/2020 growing seasons. Farida cultivar sugar beet seeds, the highly tolerant to root rot disease were used in this study. Experiment was conducted using sterilized sand clay soil in pots (50 cm in diameter). Inocula of three fungal isolates *i.e. Rhizoctonia solani* (No. 16 and 22) and *Macrophomina phaseolina* (No. 11) were prepared by growing each fungus on autoclaved barley sand medium in 250 ml jars. Inoculated jars were incubated at 25±2°C for 15 days. The inoculum was thoroughly mixed with the sterilized soil at the rate 2% (w/w). The infested soil was watered every three days for one week before sowing the seeds. Three seeds were planted in each pot, artificially infested by any of the pathogenic fungi. In this respect, salicylic acid, citric acid, and catechol, the best antioxidants in suppressing the pathogenic fungi *in vitro* were used at the concentrations of 60, 80 and 100 mM. After one month from sowing, each tested compound was added as soil drench. Then, Farida cultivar plants in pots were sprayed every 15 days, four times, 45, 60, 75 and 90 days from sowing date. Infested soil without addition of chemical inducers served as control. Three replicates were used for each treatment, each replicate contained three plants. Disease assessment was recorded after 120 days from sowing date as previously described.

2.5 Evaluation of fungicides against sugar beet root rot disease

This study was carried out in both laboratory and under greenhouse conditions of Faculty of Agriculture, Al-Azhar University (Assiut Branch), Assiut, Egypt to evaluate the efficiency of six fungicides for controlling root rot disease of sugar beet caused by *M. phaseolina* and *R. solani* (No. 16 and 22 isolates). Used fungicides were as commercial products *i.e.* Actamyl 70% WP, Chlorothalonil 50% SC, Evito 48% SC, Pyrus 40% SC, Shenzy 34% SC and Fentobein 32.5% SC. The trade name, group name, chemical group, common name, formulation and mode of action of the fungicides were given in Table (1).

2.6.1 *In vitro* test

Both systemic and contact fungicides were used *In vitro* screening according to Dhingra & Sinclair (1985). The tested fungicides were suspended in sterile distilled water and added to PDA medium under aseptic conditions in conical flasks to obtain final concentrations of 5, 10, 25, 50, and 100 ppm. Medium without fungicides served as control. After solidification of the medium in Petri plates, 5 mm agar discs taken from the edge of 7 days old culture of each of the tested fungi were placed in the center of Petri plates. Three Petri plates were used for each concentration. The inoculated plates were incubated at 25±2°C and daily radial colony growth was checked till the upper surface in control treatment was fully covered with the mycelial growth of the fungus. The fungal growth inhibition percentage was calculated using the formula of Chapagain et al. (2007) as follows:

$$\text{Inhibition percentage} = \frac{\text{Growth in control- Growth in treatment}}{\text{Growth in control}} \times 100$$

Table 1: List of fungicides used against *M. phaseolina* and *R. solani*.

Trade name	Group name	Chemical group	Common name	Formula	Mode of action
Actamyl	Methyl Benzimidazole Carbamates	Thiophanates	Thiophanate methyl	70% WP	Systemic
Chlorothalonil	Chloronitriles (Phthalonitriles)		chlorothalonil	50% SC	Contact
Evito	Quinone Outside Inhibitors	Dihydro-dioxazines	Fluoxastrobin	48% SC	Systemic
Pyrus	Anilino-Pyrimidines	Anilino-pyrimidines	Pyrimethanil	40% SC	Contact
Shenzy	2,6-dinitro-anilines		Fluazinam	34% SC	Contact
Fentobein	Quinone Outside Inhibitors	Methoxy-acrylates	Azoxystrobin		Systemic
			Azoxystrobin	32.5% SC	
	DeMethylation Inhibitors	Triazoles	Difenoconazole		Systemic

2.6.2 *In vivo* test

The most effective fungicides in the laboratory were chosen and tested under greenhouse conditions during 2018/2019 and 2019/2020 growing seasons. The experiment was conducted using sterilized soil with formalin solution (5%). The inocula of the tested fungi were prepared by growing each fungus on autoclaved barley sand medium in 250 ml jars as previously mentioned. The inoculum was thoroughly mixed with sterilized soil at the rate 2% (w/w) and

the infested soil was watered every three days for one week before adding the fungicides and sowing the seeds. The three different fungicides applied were Evito 48%, Shenzy 34% and Fentobein 32.5% at 1, 2 and 3 g/Kg soil before sowing Farida cultivar seeds. The tested fungicides were mixed with infested soil and watered again to ensure complete distribution of the fungicides in the infested soil. Each pot was planted with 3 seeds. Three pot replicates were used for each treatment. The pots without any fungicides were served as control. The pots were irrigated and fertilized regularly. After 120 days of application, Plants were uprooted and disease severity was recorded as mentioned before.

2.7 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 sugar beet root rot disease

Different fungal isolates *i.e. Fusarium* spp., *Rhizoctonia solani* (Kuhn) and *Macrophomina phaseolina* (Tassi) Goid were isolated from rotted sugar beet roots collected from different locations of Assiut, Minia, Fayoum and Kafr-Elsheikh governorates, Egypt. Data in Table (2) indicated that different fungi varied from one location to another were able to infect sugar beet roots. *Fusarium* spp. was the most dominant fungal genus in all locations as its frequency was 68%. Also, *Rhizoctonia solani* reached about 18%, followed by *Macrophomina phaseolina* as reached it 14%.

Table 2: Source and frequency of fungi associated with diseased sugar beet roots during 2017/2018 growing season.

The isolated fungi	Source of isolates (governorate)	Occurrence	Frequency (%)
Fusarium spp.	Assiut, Fayoun, Minia and Kafr-Elsheikh	15	68
Rhizoctonia solani	Fayoum and Assiut	4	18
Macrophomina phaseolina	Assiut,Minia and Kafr-Elsheikh	3	14
Total		22	100

These results are in agreement with El-kazzaz et al. (1999) who reported that *Sclerotium rolfsii* and *Rhizoctonia solani* cause serious root rot diseases affecting sugar beet crop in Egypt. Perfect stage of *R. solani*, (*Thanatephorus cucumeris*) causes one of the most damaging sugar beet diseases, wherever sugar beet was grown. These fungi are considered common soil inhabitants (Windels et al., 1997). *Macrophomina phaseolina* survives in soil or host tissue for at least two years, microsclerotia are formed in sugar beet and other hosts such as common bean, cotton, maize, potato, sorghum, soybean, strawberry, sunflower and sweet potato (Su et al., 2001; Collins et al., 1991).

3.2 Pathogenicity tests

Twenty two fungal isolates were tested for their pathogenic capabilities on sugar beet plants (Farida cv.) under greenhouse conditions during 2017/2018 growing season. Data in Table (3) showed that all tested fungal isolates were able to infect sugar beet plants causing root rot disease. All the infected sugar beet plants showed typical symptoms of root rot disease. Also, all the tested isolates significantly increased root rot disease as compared with the control. *Rhizoctonia solani* (No. 22) gave the highest percentage of disease severity after 120 days from inoculation. Also, *R. solani* (No. 16) gave the same trend of disease severity after the same period, followed by *M. phaseolina* (No. 11) While, *Fusarium* sp. (No. 14) recorded the lowest disease severity after 120 days. In all cases, *R. solani* isolates caused the highest disease incidence of sugar beet roots. Pathogenicity tests proved that the obtained fungi were pathogenic to sugar beet (Farida cv.). At the same time, the most aggressive isolates were *R. solani* (No. 22), *R. solani* (No. 16) and *M. phaseolina* (No. 11), respectively. These results are in harmony with EL-Kazzaz et al. (2000), El-Kholi (2000) and Husseien, Manal (2005) who reported that the most important diseases affecting sugar beet production in Egypt are damping-off and root-rot caused by several pathogens, *i.e. R. solani, M. phaseolina, Sclerotium rolfsii and Fusarium spp.*

Table 3: Disease severity of sugar beet root rot disease caused by the most frequent fungi under greenhouse conditions during 2017/2018 growing season.

The tested fungi	Source (governorate)	Severity of infection after 120 days (%)
Fusarium sp. No. 1	Minia	54.05
Fusarium sp. No. 2	Minia	42.85
M. phaseolina No. 3	Minia	65.07
Fusarium sp. No. 4	Minia	34.91
R. solani No. 5	Kafr-Elsheikh	47.61
Fusarium sp. No. 6	Kafr-Elsheikh	30.15
Fusarium sp. No. 7	Kafr-Elsheikh	46.02
Fusarium sp. No. 8	Kafr-Elsheikh	23.8
Fusarium sp. No. 9	Kafr-Elsheikh	39.67
Fusarium sp. No. 10	Kafr-Elsheikh	38.09
M. phaseolina No. 11	Kafr-Elsheikh	71.42
Fusarium sp. No. 12	Assiut	22.21
Fusarium sp. No. 13	Assiut	47.61
Fusarium sp. No. 14	Assiut	17.45
Fusarium sp. No. 15	Assiut	23.8
R. solani No. 16	Assiut	73.01
M. phaseolina No. 17	Assiut	42.85
Fusarium sp. No. 18	Assiut	31.74
Fusarium sp. No. 19	Fayoum	33.33
Fusarium sp. No. 20	Fayoum	23.8
R. solani No. 21	Fayoum	39.67
R. solani No. 22	Fayoum	77.77
Control		0
LSD at 5 %		6.32

Several soil-borne fungi attack sugar beet causing a significant reduction of the production *viz., Rhizoctonia solni, R. crocorum, Aphanomyces cochlioides, M. phaseolina, Phoma betae, Pythium aphanidermatum* and *S. rolfsii* (Elwakil

et al., 2018).

3.3 Evaluation of different antioxidants against sugar beet root rot disease

3.3.1 *In vitro* test

Different concentrations of antioxidants *i.e.* catechol, salicylic acid, ascorbic acid, potassium silicate and citric acid were used to study their effectiveness on the linear growth of *R. solani* (No.16 and 22) and *M. phaseolina* (No. 11). Data presented in Table (4) clarified that catechol and salicylic acid completely suppressed the mycelial growth of the pathogenic fungi with all tested concentrations. At the same time, citric acid at 60, 80 and 100 mM completely inhibited the fungal growth of the tested fungi except, *M. phaseolina* and *R. solani* (No. 16), wherever inhibition percentage reached it 89.8 and 82.4 %, respectively. Also, potassium silicate completely suppressed the fungal growth at 80 and 100 mM. The same data exhibited that the increase in concentrations of ascorbic acid, potassium silicate and citric acid from 20 mM to 100 mM resulted in an obvious decrease in the mycelial growth of *R. solani* (No. 16 and 22) and *M. phaseolina* (No. 11). Such findings agree with those reported by Abdelaziz (2017) who mentioned that salicylic acid and catechol with tested concentrations were the most effective antioxidants in inhibiting the linear growth of *F. solani*, *R. solani* and *M. phaseolina*.

Table 4: Evaluation of different antioxidants on the linear growth of the pathogenic fungi.

Antioxidants (A)	Conc. (mM) (B)	Mycelial growth inhibition (%)		
		M. phaseolina	*R. solani* No. 16	*R. solani* No. 22
Salicylic acid	20	100	100	100
	40	100	100	100
	60	100	100	100
	80	100	100	100
	100	100	100	100
Citric acid	20	54.55	76.84	36.1
	40	88.88	77.77	72.22
	60	89.8	82.4	100
	80	100	100	100
	100	100	100	100
Ascorbic acid	20	28.69	0	0
	40	54.62	0	0
	60	87.03	33.33	34.25
	80	100	35.18	65.73
	100	100	64.8	100
Catechol	20	100	100	100
	40	100	100	100
	60	100	100	100
	80	100	100	100
	100	100	100	100
Potassium silicate	20	43.51	0	0
	40	49.99	12.03	0
	60	81.47	47.4	24.99
	80	85.18	100	100
	100	89.8	100	100
Control		0	0	0
LSD at 5%	A	0.82	2.7	0.78
	C	0.79	2.11	0.66
	A x C	1.94	5.18	1.62

While, thiourea followed by ascorbic acid and sodium benzoate reduced the mycelial growth only at high concentrations. Catechol and salicylic acid with all concentrations tested entirely suppressed the mycelial growth of *F. solani*, *F. oxysporum* and *R. solani*, potassium silicate with all concentrations tested strongly retarded the mycelial growth of *F. solani* and *F. oxysporum*, as well as entirely inhibited the linear growth of *R.solani*. In this respect, considerable reduction of the linear growth took place with citric acid and ascorbic acid at different concentrations (Kasem Aya, 2018).

3.3.2 *In vivo* test

The most effective antioxidants *in vitro* were chosen to study their effectiveness against the pathogenic fungi on Farida cv. plants in infested pots at the concentrations of 60, 80 and 100 mM under greenhouse conditions during 2018/2019 and 2019/2020 growing seasons. It was shown from Table (5) that all tested chemical inducers significantly reduced sugar beet root rot incidence caused by *R. solani* (No. 16 and 22) and *M. phaseolina* (No. 11) during 2018/2019 and 2019/2020 growing seasons. Root rot disease of sugar beet plants was decreased by using all tested concentrations and reached its minimum records at the highest concentration, 100 mM. The most effective treatment which minimized *R. solani* (No. 16) during 2019/2020 season was salicylic acid followed by catechol and citric acid, respectively. It is obvious from the same Table that all chemical inducers had significantly protected sugar beet plants against root rot pathogens as compared with control. Under greenhouse conditions, root rot diseases were controlled with the most selected antioxidants. Variability in the effect of antioxidants could be referred to differences in their activity or variation in the responses of the tested fungi. Control of root rot disease mainly depends on fungicides. However, intensive application of fungicides causes hazards to human health and environment.

Table 5: Effect of different antioxidants on controlling sugar beet root rot disease of Farida cv. under greenhouse conditions during 2018/2019 and 2019/2020 growing seasons.

Antioxidants (A)	Conc. (mM) (B)	Disease severity (%)					
		2018/2019			2019/2020		
		M. phaseolina	*R. solani* No. 16	*R. solani* No. 22	*M. phaseolina*	*R. solani* No. 16	*R. solani* No. 22
Salicylic acid	60	44.43	52.37	49.2	47.61	53.96	55.55
	80	23.8	28.56	26.98	22.21	31.74	23.8
	100	19.04	22.21	23.8	20.62	25.39	17.45
Citric acid	60	61.9	66.66	63.48	58.72	61.9	69.83
	80	49.2	52.37	53.96	47.61	46.02	50.79
	100	26.98	23.8	25.39	30.15	20.62	31.74
Catechol	60	34.91	41.26	39.67	42.85	36.5	44.43
	80	20.62	28.56	31.58	15.86	22.21	26.82
	100	11.1	9.52	14.28	12.69	12.69	7.93
Control		80.95	74.59	79.36	74.59	76.18	80.95
LSD at 5%	A	7.97	10.04	9.09	6.41	10.51	5.3
	C	2.47	3.5	4.38	3.82	3.63	2.82
	A x C	4.95	7	8.77	7.64	7.26	5.64

Therefore, alternative approaches for the control of plant diseases should be considered. Induction of resistance in plants to overcome pathogens infection is

a promising approach for controlling plant diseases. Exogenous or endogenous factor could substantially affect host physiology, lead to rapid and coordinated activation of defense-gene in plants, normally expressing susceptibility to pathogen infection (El-Mougy et al., 2004). This induced resistance to pathogens can be achieved by the application of various abiotic agents (chemical inducers) such as salicylic acid, potassium salts and sorbic acid (Mandal et al., 2009; Abdel-Monaim, 2010 and Akram & Anjum, 2011). It could be concluded that some chemical inducers may also have a direct antimicrobial effect and are involved of, gene expression, phytoalexin production and induction of systemic resistance. Data are in agreement with those reported by several researches when they used such compounds against several plant diseases caused by various pathogens (Abdelaziz, 2017; El-Samawaty & Galal, 2009; Galal & Abdou, 1996).

3.4 Comparative effectiveness of different fungicides against sugar beet root rot disease

3.4.1 *In vitro* test

The efficiency of certain fungicides against the tested fungi was screened *in vitro* in order to find out an effective chemical control method. It was clear from data presented in Table (6) that the increase in fungicides concentration had resulted an obvious increase in mycelial inhibition percentage of fungi. In this respect, all the tested fungicides significantly reduced the mycelial growth of the tested fungi. Shenzy fungicide was the most effective in reducing the mycelial growth of *M. phaseolina* with concentrations 5, 10, 25 and 50 ppm and completely suppressed the mycelial growth at 100 ppm. The same effect was obtained by using Evito and Chlorothalonil at 50 and 100 ppm, followed by Fentobein at the same concentrations. While, Actamyl was the least effective fungicide on the mycelial growth of *M. phaseolina.* Mycelial growth of *R. solani* (No. 16) was entirely inhibited with Actamyl and Shenzy each at 50 and 100 ppm. Whatever, Fentobein gave the same effect at 100 ppm, while Chlorothalonil and Pyrus fungicides were less effective in reducing and inhibiting mycelial growth. On the other hand, Shenzy, Evito and Fentobein fungicides each at 50 and 100 ppm significantly reduced mycelial growth of *R. solani* (No. 22) at 50 ppm as well as completely suppressed at 100 ppm. Pyrus and Chlorothalonil were the least effective fungicides on the mycelial inhibition of *R. solani* (isolate 22). It became clear that the linear growth of all pathogens was greatly retarded by increasing fungicide concentrations, which reached almost complete inhibition at 100 ppm. Shenzy, Fentobein and Evito with the highest concentration completely suppressed the three pathogenic fungi. The selective action of each fungicide and their concentrations on fungal growth might be due to the sensitivity of the different pathogens. Results are in agreement with the findings of Bhanumathi and Ravishankar (2007) who evaluated seven fungicides at 50, 100 and 150 ppm concentrations and found carbendazim (Bavistin, Sendo or Kema Z) most effective in inhibiting radial growth of *F. solani.*

Table 6: Effect of different concentrations of six fungicides on the mycelial growth of the tested pathogenic fungi *in vitro*.

Fungicide (A)	Conc. (ppm) (B)	Mycelial growth inhibition (%)		
		M. phaseolina	*R. solani* No. 16	*R. solani* No. 22
Actamyl 70% WP	5	9.25	0	0
	10	26.84	0	10.18
	25	34.25	67.58	74.99
	50	65.73	100	81.47
	100	78.69	100	89.8
Chlorothalonil 50% SC	5	45.36	0	8.33
	10	53.69	0	23.14
	25	67.58	7.4	40.73
	50	84.25	37.03	51.84
	100	100	54.62	57.4
Evito 48% SC	5	65.73	12.03	29.62
	10	77.77	38.88	66.66
	25	84.25	42.58	82.4
	50	86.1	47.22	87.95
	100	100	74.07	100
Shenzy 34% SC	5	81.47	60.18	57.4
	10	83.33	77.77	60.18
	25	87.95	87.03	71.29
	50	88.88	100	87.95
	100	100	100	100
Pyrus 40% SC	5	12.03	0	0
	10	22.22	0	10.18
	25	72.22	0	55.55
	50	84.25	51.84	73.14
	100	89.8	69.44	85.18
Fentobein 32.5% SC	5	71.29	72.22	74.07
	10	76.84	78.69	74.99
	25	81.47	80.55	79.62
	50	85.18	84.25	82.4
	100	90.73	100	100
Control		0	0	0
LSD at 5%	F	1.35	1.14	1.46
	C	1.09	1.03	1.06
	F x C	2.9	2.75	2.82

3.4.2 *In vivo* test

Application of the most effective fungicides was carried out to study the effect of fungicides on the development of sugar beet root rot caused by *M. phaseolina* and *R. solani* (isolates No. 16 and 22) under greenhouse conditions in pot experiments during 2018/2019 and 2019/2020 growing seasons. Data presented in Table (7) demonstrated that all fungicides significantly reduced the disease severity as compared with the control. In this respect, Shenzy fungicide exhibited distinct effect in reducing disease severity of both *R. solani* No. 16 and No. 22 at 3 gm/Kg soil compared with other treatment and with control during both 2018/2019 and 2019/2020 growing seasons, Evito fungicide came at the second order in the term of disease reduction. Meanwhile, Evito fungicide with the highest concentration 3 g /Kg soil achieved the best effect in reducing disease severity caused by *M. phaseolina* followed by Shenzy fungicide. Fentobein fungicide gave less effect in reducing disease severity although; it strongly reduced the three causal pathogens to a large degree as compared with the control. Data also revealed that the disease severity caused by all pathogens

was greatly reduced by increasing fungicide concentrations, which reached the highest reduction at 3 g /Kg soil for all tested fungicides.

Table 5: Comparative effectiveness of fungicidal treatments on sugar beet root rot disease under greenhouse conditions during 2018/2019 and 2019/2020 growing seasons.

Fungicide (A)	Conc. (g/Kg soil) (B)	Disease severity					
		2018/2019			2019/2020		
		M. phaseolina	*R. solani* 16	*R. solani* 22	*M. phaseolina*	*R. solani* 16	*R. solani* 22
Evito 48% SC	1	25.39	30.15	28.56	28.56	31.74	30.15
	2	15.86	20.63	17.45	14.28	22.21	22.21
	3	6.34	17.45	14.28	7.933	15.86	12.69
Shenzy 34% SC	1	20.62	28.56	26.98	26.98	31.74	28.32
	2	12.69	19.04	17.45	17.45	20.62	15.86
	3	7.93	9.52	12.69	9.52	11.1	7.93
Fentobein 32.5% SC	1	33.33	33.33	36.5	34.91	39.67	34.91
	2	19.04	23.8	25.39	17.45	26.97	25.39
	3	11.1	17.45	19.04	7.93	14.28	15.86
Control		80.95	74.59	79.36	74.59	76.18	80.95
LSD at 5%	A	5.3	9.67	8.39	5.69	9.67	8.31
	B	2.42	4.06	4.34	3.03	4.78	5.37
	A x B	4.85	ns	ns	6.06	9.56	Ns

Results are in agreement with Elwakil et al. (2018) who mentioned that several fungicides have been shown to be useful in reducing disease incidence including chlorothalonil, pencycuron, tebuconazole, azoxystrobin, trifloxystrobin and pyraclastrobin. As regard, azoxystrobin has provided the most consistent level of control in both inoculated and natural infection trials (Jacobsen et al., 2005; Kiewnick et al., 2001).

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