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Research Article

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Antifungal effect of some plant extracts and their combination with Moncut fungicide on sugar beet root rot

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KEY WORDS

Sclerotium rolfsii, sugar beet root rot, plant extracts, Sucrose%, Phenolic compounds and Oxidative enzymes.

ABSTRACT

Sugar beet (*Beta vulgaris* L. Chenopodiaceae) has become the first Egyptian crop for sugar production because it contributed with 59.5% of total sugar production. *Sclerotium rolfsii* is a soil-borne plant pathogenic fungi incident of southern sclerotium rot in sugar beet. The aim of the present study was to investigate the effect of some plant extracts in different solvents as inhibitory control agents against the growth plant pathogen S. *rolfsii*. Five plant extracts; Roselle, pomegranate, rosemary, black pepper and chamomile were evaluated as inhibitors for *Sclerotium rolfsii* of sugar beet root rot under laboratory and greenhouse conditions during 2016/2017 growing season at Gemmeiza Agricultural Research Station, (ARC). The results indicated that all tested plant extracts caused remarkable growth inhibition of *S. rolfsii* compared with control. However, black pepper extract was the most effective against *S. rolfsii* (recorded 93.33% inhibition). On the other hand, Pomegranate extract in methanol solvent showed also high inhibition percentage (93.30%). All plant extracts mixed with Moncut fungicides demonstrated an antagonistic effect. The pomegranate and black pepper treatments showed the lowest disease severity compared with control. Furthermore, the pomegranate and black paper treatments recorded the highest root weight, root yield/plot, total soluble solids percentage and sucrose content. Also, pomegranate and black pepper treatments reflected the highest amount of total phenols, free phenols, and conjugated phenols. Additionally, the interaction of pomegranate and black pepper treatments with *S. rolfsii* pathogen, showed the highest activity of oxidative enzymes peroxidase and polyphenol oxidase. This study recommends the application of pomegranate and black pepper as natural alternative fungicides against *Sclerotium rolfsii* plant infection.

1. INTRODUCTION

The sugar considers as one of the most important food commodities in Egypt. It is one of the commodities relatively; cheap sources of energy, which impair the attention of Egyptian agricultural policy-makers. Sugar beet (*Beta vulgaris* L. Chenopodiaceae) has become the first crop for sugar production because it contributed 59.5% of sugar production in Egypt in the recent years (Ministry of Agriculture and land reclamation, Sugar Crops Council, annual report of crops sugary and sugar production in Egypt, 2019).

Sugar beet plants are often attack by several pathogens such as fungi, bacteria and viruses which cause great losses in yield **(Esfahani**, **2006, Abdalla *et al*., 2019 and Ghazy *et al*., 2021).** Soil-borne diseases can have a major impact on sugar beet during all stages of its development. In Egypt several investigations have been conducted on the pathogenic fungi to sugar beet **(Abada, 2003 and Abd Ellatif *et al*., 2019).** Root rot of Sugar beet is considered the most effective disease that affects yield and quality as well as its sugar production. The various fungal soil borne pathogens have a wide attributes for poor establishment and stand loss of sugar beet **(Kiewnick *et al*., 2001).** Also, the plant root yield and their sugar contents were significantly reduced and rotten with these pathogens infections **(Harveson and Rush, 2002).**

Scleritium rolfsii is one of the most important soil-borne as a damping-off fungus that has a wide host range of crops. This fungus was prevalent through tropical and subtropical cultivated areas. The production of sclerotia that have a long survival in soil, make *Scleritium rolfsii* control managements policy is so difficult. The damage of infection, comprising damping-off, root-rot and dry rot canker in adult plants **(Abada, 2003 and Abd Ellatif *et al*., 2019).**

The most distinctive effect of this pathogen is rotting of affected tissues that are directly attacked by the fungus. However, the mass of mycelium secretes oxalic acid as well as pectinolytic, cellulolytic, and some other enzymes which kill and disintegrates tissues before it could actually penetrates the host. Once the pathogen established in the plants, the fungus progress and generate both mycelium and sclerotia quite rapidly **(Agrios, 2005).** Long term of fungicides application caused hazard effects on human health and environmental pollution in addition to development of pathogen resistance cases. Therefore, it is more important to

produce an alternative natural product that are highly effective, economic, wide safety, and eco-friendly fungicides **(Mdee *et al*., 2009).** The application of plant extracts is more eco-friendly and effective to plant pathogens **(Latha *et al.*, 2009).** The use of biological control in plant disease became promising instead of chemicals for avoiding environmental pollution and hazardous effects. Recognizing the hazards effects of fungicides and pesticides to the environment in many countries today are considering biological control as the best alternative to chemical control of plant diseases **(Souto *et al.*, 2004).**

Some plant- extracts were evaluated against *Rhizoctonia solani* of sugar beet by (**Kishk *et al*., 2019),** were significantly effective in enhancing the seedling emergence.

Current study was aimed to select some effective plant extracts species, which might have antimicrobial activities against *Scleritium rolfsii* in vitro.

2. MATERIALS AND METHODS

The present work was carried out at Gemmeiza Agricultural Research Station, Agricultural Research Center (ARC) to control sclerotium root-rot of sugar beet caused by *Sclerotium rolfsii* under laboratory and greenhouse conditions during 2016/2017 growing season.

2.1. Isolation and identification of different causal organisms

Highly infected roots of sugar beet with characteristic symptoms of root rot disease, were cut into small pieces and then surface sterilized with 0.5% sodium hypochlorite solution for 3 min then rinsed in sterilized distilled water. Surface sterilized pieces were dried in sterile filter papers and transferred into water agar media and incubated at 25 ± 2˚C. Pure fungal cultures were preserved on potato dextrose agar (PDA) with hyphal tip technique (**Burgess *et al.*, 2008)** and identified according to key of **Barnett and Hunter (1998)**. Stock cultures of different pathogens were preserved on PDA slants at 4˚C for different studies.

2.2. Preparation of plant extracts

Extracts of five plant species were used in this study (Table 1). The flowers, fruits ped and seeds were air dried at room temperature and ground with blender into fine powder. Fifty grams of plant materials fine powder were soaked in 200 ml of the different solvents such as ethanol, methanol and acetone for active ingredient extraction. All samples were soaked for seven days and during soaking period the samples were shaken for 7 hours using an electrical shaker and filtrated through Wattman No. 1 filter papers. The obtained crude extract was

stored in glass vials and kept in refrigerator at 4˚C until use.

Table 1: Plant species that used as plant extracts in the present study.

English name	Scientific name	Family	Part used
Roselle	*Hibiscus sabdariffa* L.	Malvaceae	Flowers
Pomegranate	*Punica granatum* L.	Punicaceae	Fruits cortex
Black pepper	*Piper nigrum* L.	Piperaceae	Seeds
Rosemary	*Rosmarinus officinalis* L.	Lamiaceae	Leaves
Chamomile	*Matricaria chamomilla* L.	Asteraceae	Flowers

2.3. Preparation of *S. rolfsii* as inoculum and soil inoculation:

The inoculation method for sclerotium root-rot was done as follow; the mass culture of the pathogen was prepared through sand and rice husk with (2:1) inoculum technique. The mixture of sand and rice husk was autoclaved for 45 mins at 121ºC. Disks (5mm in diameter) from 7-days old culture of pure culture of S. *rolfsii* were used to inoculate 50 g of rice husk. The inoculated rice husk was incubated for 15 days at 28±°C **(Farooq *et al*., 2011)**. After 15 days, the colonized rice husk was air dried and used as inoculum in field soil at the rate of 100 g/m^2 10 days before sowing **(Islam *et al*., 2007).**

2.4. Synergistic interaction of Moncut fungicide mixed with plant extracts of different solvents against *S. rolfsii*

The fungicide, Moncut was used at the same concentrations for comparison with extracts under study. Four replicate plates were used, and extract-free PDA acted as control. The regression lines were drawn on probit-log paper and the median inhibition concentration (IC_{50}) was detected.

Observations were taken after the *S. rolfsii* reached full growth in the control plate. The radial mycelial growth of the *S. rolfsii* and inhibition percent (I %) over control was calculated by using the suggested formula as adopted by **Topps and Wain, (1957)** as follows:

$$I\ \% = (A - B) / A \times 100$$

Where: I % =Inhibition percentage.

A = Mean diameter of growth in the control. B = Mean diameter of growth in treatment, the mean lethal concentration ((IC_{50}) and slopes were estimated by probit analysis **(Finny, 1971).**

Mixture of Moncut fungicide with other plant extracts in different solvents were tested against *S. rolfsii* by using the radial growth. The mixtures were applied at the rate of 1:1and 1:3. Four concentrations (0.01, 0.1, 1.0, 10.0 ppm) from each mixture were used (**Kishk *et al*., 2019)**. The antifungal tests were evaluated as described above with three

replicates. The mean lethal concentration (observed IC_{50}) and slopes were estimated by probit analysis **(Finny, 1971).**

The expected median lethal concentration (Expected IC_{50}) of fungicide mixtures was calculated by the next equation which described by **(Gisi *et al*., 1985).**

Expected IC_{50} = (a + b) / {(a / IC_{50} a) + (b / IC_{50} b)}.

In this equation, the expected IC_{50} of the mixture, which is the harmonic mean of the IC_{50} observed for fungicide (a) and (b) acting separately, (a) and (b) are the relative proportions of fungicide and fungicide (b) in the mixture, respectively.

The synergism ratio (SR) of fungicide mixtures was calculated by dividing the (IC_{50}) expected IC_{50} by the observed IC_{50} values, based on **Gisi, (1996).**

Synergism Ratio (SR) = expected IC_{50} / observed IC_{50}

The value of SR greater than 1 indicates a positive synergistic effect (synergism), while less than 1 indicates a negative synergistic effect (antagonism). SR equal to one indicates an additive effect **(levy *et al*., 1986)**

Seeds of sugar beet Panther variety were soaked in plant extracts for overnight and seeds were immediately sown. Plant extracts with different solvents were diluted by tap water to the required concentration (1000 to 6000 ppm).

2.5. Greenhouse experiments

Greenhouse experiments were conducted under artificial inoculation with *S. rolfsii* at Gemmeiza Agricultural Research Station, Agricultural Research Center (ARC), Garbiha governorate, Egypt, during 2016/2017 growing season. Sugar-beet seeds were surface sterilized by immersing in 0.5% sodium hypochlorite solution for 2 minutes, washed several times with sterile distilled water and left to dry. Normal cultural practices were carried out as recommended for conventional sugar beet planting according to instructions of Egyptian Ministry of Agriculture. Treated seeds were sown in microplot (two- 2 m rows in a randomized complete block design with three replications for each particular treatment. The microplots were of two rows 2 m long. The spacing was 0.8 m between rows and 0.25 m within rows.

2.5. Disease assessment:

Disease severity was calculated for the rotted roots according to the scale of 1-10 grades **(Grainger, 1949)**. The disease severity percent (% D.S.) was estimated using the following formula:

$$D.S.\ \% = \frac{\sum (\text{Number of infected plants x numerical grads})}{\text{Total number of the tested plants x higher degree in the category}} \times 100$$

In addition, yield / plot Kg and analysis of the total soluble solids (TSS) and sucrose content were estimated.

TSS and sucrose contents were estimated in beet roots at harvest time. T.S.S. was estimated in fresh roots using the hand refrectometer according to **Mc Ginnis (1982).** The sucrose content was determined according to **Anonymous (1990)** by the aid of succarometer.

2.6. Analysis of treated leaves for phenolic and some oxidative enzyme compounds

Samples of leaves of each treatment were collected from all replicates in order to determine the concentration phenolic compounds and oxidative enzymes activity.

a) Phenolic compounds were colorimetrically determined using the folin-ciocatteu (phosphotungstic-phosphomolybdic acid) reagent at 650 nm according to **Snell and Snell, (1953).** The results were expressed as milligram per gram fresh weight of plant sample (mg/g fresh weight).

b) Oxidative enzymes activity

1-Peroxidase activity was spectro-photometrically determined by measuring the oxidation of pyrogallolin the presence of H_2O_2 at wave length at 425 nm, according to **Allam and Hollis (1972).**

2- Polyphenol oxidase activity was determined using spectrophotometer by measuring the absorption at 495 nm, as described by **Ismail *et al*., (1995).**

2.7. Statistical analysis

The obtained data as percentage were transformed using arcsine transformation to achieve normality and the transformed data sets were subjected to analysis of variance (ANOVA) using statistical program Genestat 5 version and the mean of treatments were compared with least significant differences (LSD) according to **Gomez and Gomez (1984).**

3. RESULTS

Five plant extracts were evaluated for their effects on root infected fungus pathogen of sugar beet *S. rolfsii* Table 1**.** This experiment was carried out *in vitro* and under greenhouse conditions. Three solvents (acetone, methanol and ethanol) were used for extract preparation.

3.1. Laboratory experiments

Data presented in Table 2 and Figure 1 indicate that, all used plant extracts inhibited the growth of *S. rolfsii* compared with control, in general the extracts differed in their efficiency to reduce the fungal growth, where the effects were increased gradually by increasing the concentration from 1000 to 6000 ppm.

Data present in Table 2 and Figure 1 indicate that the all tested plant extracts reduced the liner growth and caused marked inhibition compared with control. The results shown that black

pepper is the most effective extract against *S. rolfsii*, at the tested concentrations.

The black pepper extract was the most effective one against *S. rolfsii* which recorded 93.33% inhibition starting from 3000 to 6000 ppm followed by Chamomile showed 93.33 % starting from 5000 to 6000 ppm and Rosemary showed 93.33% at 6000 ppm then Roselle extracts which showed inhibition percentages of 90.00% at 5000 ppm. Pomegranate extract showed the highest inhibition percentage 93.3% from 3000 to 6000 ppm with methanol solvent. Methanol was the proper solvent especially with the most effective plant extracts, pomegranate and black pepper.

Table 2: Inhibition percentage of *S. rolfsii* growth under the effect of different solvents extracts of some plant species.

Treatments	solvents	Concentrations (ppm)					
		1000	2000	3000	4000	5000	6000
Control		0	0	0	0	0	0
Pomegranate	Acetone	5.20	7.80	24.07	19.27	76.27	69.27
	Methanol	41.83	38.90	93.30	93.30	93.30	93.30
	Ethanol	8.90	19.63	32.93	48.17	55.57	64.80
Rosemary	Acetone	85.57	93.30	10.73	10.00	0.00	93.30
	Methanol	44.83	38.90	32.20	30.73	25.57	31.47
	Ethanol	8.90	25.93	32.93	36.30	52.20	58.53
Roselle	Acetone	26.30	31.83	69.63	54.43	90.00	82.57
	Methanol	87.80	93.30	93.30	93.30	54.80	42.97
	Ethanol	25.53	35.93	49.63	55.57	59.63	79.27
Chamomile	Acetone	89.63	93.30	35.20	45.93	93.30	93.30
	Methanol	50.37	41.83	80.00	74.83	25.57	59.27
	Ethanol	26.30	30.73	41.10	47.80	51.47	64.43
Black pepper	Acetone	62.93	65.57	93.30	93.30	93.30	93.30
	Methanol	69.63	92.20	93.30	93.30	93.30	93.30
	Ethanol	52.60	61.10	68.90	69.27	72.20	77.07
L.S.D. at 5%	Acetone	Treatments: 0.975 - Concentrations: 0.975					
	Methanol	Treatments: 1.076 - Concentrations: 1.076					
	Ethanol	Treatments: 1.186 - Concentrations: 1.186					

Where: I %: Inhibition percentage % = percent reduction in culture growth compared to growth measure in control plates, I. % = (control-treatment/control) x 100

Control, plates diameters measures started when the complete growth of fungi reaches to maximum growth (9 cm).

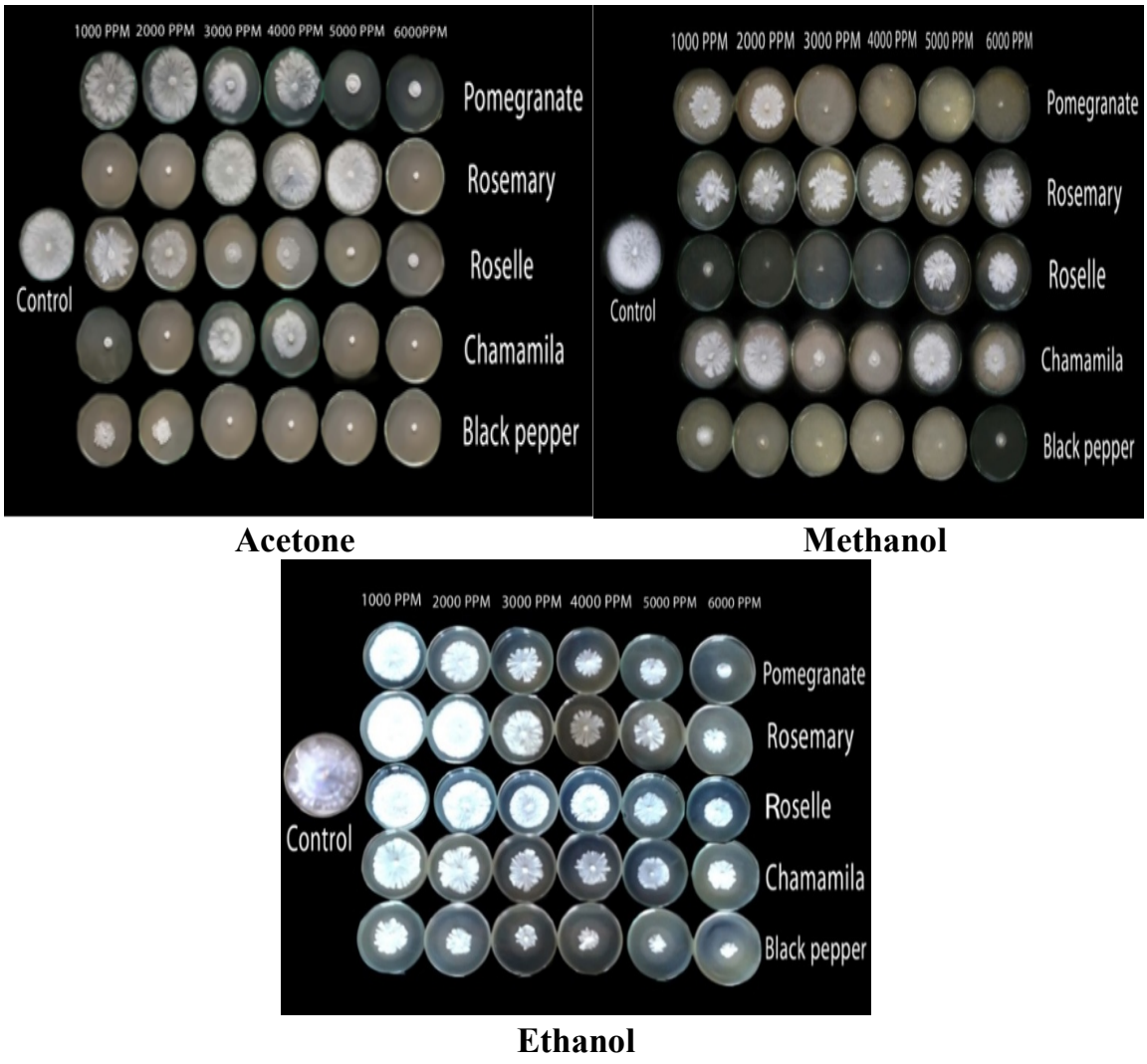


Fig. 1: Influence of different concentrations of acetone, Methanol and ethanol extracts of plant species on linear growth of *S. rolfsii*.

The effect of Moncut fungicide mixed with plant extracts at the rate 1:1 was listed in **Table 3** and **Fig. 2**. All mixtures demonstrated an antagonistic effect i.e. Moncut mixed with Roselle, Pomegranate, Rosemary, Black pepper and Chamomile with synergistic ratio (SR) values 0.058, 0.055, 0.052, 0.036 and 0.033 respectively. Data also revealed that, the observed values of IC_{50} for these mixtures were lesser than those of expected one. In the case 3:1 the mixture of Moncut with tested plant extract of all combinations indicated an antagonistic effect hence the observed values of IC_{50} for these mixtures were lesser than 1.00 except, for Chamomile (1.081).

Table 3. Effects of the interaction of Moncut fungicide mixed with some acetone plant-extracts at 1:1 and 3:1 concentration, against *S. rolfsii*.

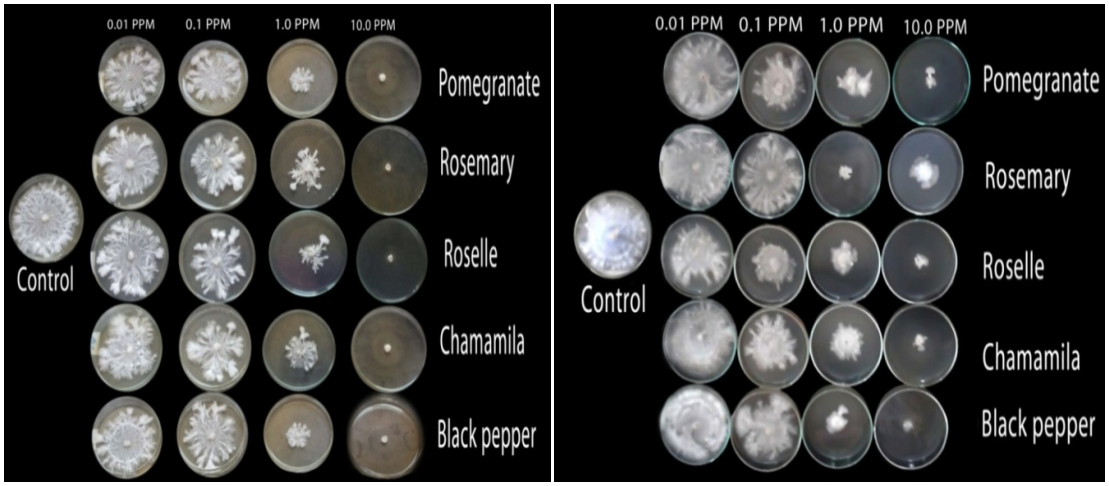
Concentrations Treatments	Mixture ratio	Inhibition percentage %				Observed IC_{50}	Expected IC_{50}	Synergistic ratio	Biological response
		0.01 ppm	0.1 ppm	1.0 ppm	10.0 ppm				
Control		**0**	**0**	**0**	**0**	-	-	-	-
Moncut		**35.00**	**75.00**	**91.00**	**93.00**	-	-	-	-
Pomegranate	**plant extract : Moncut (1:1)**	**6.67**	**22.60**	**65.17**	**93.30**	**0.413**	**0.023**	**0.055**	**Antagonistic**
Rosemary		**11.83**	**26.33**	**54.47**	**93.30**	**0.439**	**0.023**	**0.052**	**Antagonistic**
Roselle		**6.70**	**23.30**	**66.30**	**93.30**	**0.396**	**0.023**	**0.058**	**Antagonistic**
Chamamila		**2.93**	**20.73**	**48.17**	**93.30**	**0.706**	**0.023**	**0.033**	**Antagonistic**
Black pepper		**3.67**	**9.27**	**61.50**	**93.30**	**0.633**	**0.023**	**0.036**	**Antagonistic**
Pomegranate	**plant extract : Moncu t(3:1)**	**1.83**	**24.43**	**62.20**	**80.37**	**0.695**	**0.045**	**0.065**	**Antagonistic**
Rosemary		**1.10**	**7.80**	**64.07**	**82.20**	**0.950**	**0.046**	**0.048**	**Antagonistic**
Roselle		**4.03**	**26.30**	**57.80**	**80.00**	**0.722**	**0.045**	**0.063**	**Antagonistic**
Chamamila		**0.00**	**24.80**	**51.83**	**73.70**	**1.081**	**0.046**	**0.042**	**Antagonistic**
Black pepper		**0.00**	**10.00**	**62.57**	**82.20**	**0.948**	**0.046**	**0.048**	**Antagonistic**

L.S.D. at 5% for I. %. at (1:1) : Treatments: 2.650 Concentrations: 2.164

L.S.D. at 5% for I. %. at (3:1): Treatments: 1.035 Concentrations: 0.845

Where I. %: Inhibition percentage = percent reduction in culture growth compared to growth measure in control plates.

$$I.\ \% = (control\text{-}treatment/control) \times 100$$



Plant extract : Moncut (1:1) **Plant extract : Moncut (3:1)**

Fig. 2: In vitro evaluation of moncut fungicide and different plant species with aceton extract at the rate of plant extract: Moncut (1:1) and plant extract: Moncut (3:1) with four concentrations (0.01, 0.1, 1.0, 10.0 ppm) concentrations, on linear growth of *S. rolfsii.*

In case of Moncut fungicide mixed with methanol extract at the ratio (1:1) and (3:1) **(Table 4** and **Figure 3).** All mixtures demonstrated antagonistic effect i.e. Roselle, Black pepper, Chamomile, Rosemary and pomegranate with (SR) values 0.065, 0.031, 0.029, 0.022 and 0.020 respectively.

Table 4. Effects of the interaction of Moncut fungicide mixed with methanol extract of some plants at 1:1 and 3:1 concentrations, against *S. rolfsii* (the casual organism of sugar beet root rot).

Treatments	Mixture ratio	Inhibition percentage I.%				Observed IC50	Expected IC50	Synergistic ratio	Biological response
		0.01	0.1	1.0	10.0				
Control		0	0	0	0	-	-	-	-
Moncut		35.00	75.00	91.00	93.00	-	-	-	-
Pomegranate	plant extract : Moncut (1:1)	3.33	4.43	37.77	93.30	1.190	0.023	0.020	Antagonistic
Rosemary		0.00	2.60	49.63	93.30	1.070	0.023	0.022	Antagonistic
Roselle		10.73	12.23	74.83	93.30	0.350	0.023	0.065	Antagonistic
Chamamila		11.47	10.37	46.70	90.73	0.796	0.023	0.029	Antagonistic
Black pepper		7.80	3.33	50.73	93.30	0.745	0.023	0.031	Antagonistic
Pomegranate	plant extract : Moncut (3:1)	24.43	31.83	61.47	93.30	0.226	0.041	0.180	Antagonistic
Rosemary		1.47	55.20	72.60	93.30	0.157	0.039	0.245	Antagonistic
Roselle		2.20	18.90	62.93	85.93	0.663	0.045	0.068	Antagonistic
Chamamila		1.83	15.93	57.03	93.30	0.656	0.045	0.069	Antagonistic
Black pepper		1.47	17.80	73.33	93.30	0.453	0.044	0.097	Antagonistic

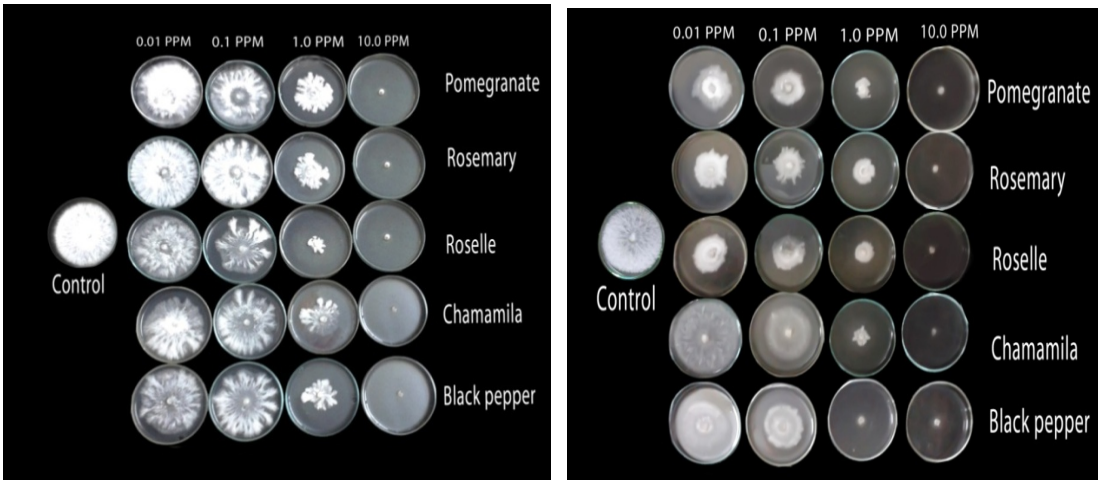
L.S.D. at 5% for I. % at (1:1): Treatments: 2.338 Concentrations: 1.909

L.S.D. at 5% for I. % at (3:1): Treatments: 1.251 Concentrations: 1.021

Where

I. %: Inhibition percentage % = percent reduction in culture growth compared to growth measure in control plates. I. % = (control-treatment/control) x 100

Control, plates diameters measures started when the complete growth of fungi reaches to maximum growth (9 cm).



Plant extract : Moncut (1:1) **Plant extract : Moncut (3:1)**

Fig. 3. In vitro evaluation of Moncut fungicide and different plant species with methanol extract at the rate of plant extract: Moncut (1:1) and plant extract: Moncut (3:1) with four concentrations (0.01, 0.1, 1.0, 10.0 ppm) concentrations, on linear growth of *S. rolfsii.*

The mixtures of Moncut with ethanol extract of the tested plant at 1:1 are listed in Table 5 and Figure 4. All mixtures demonstrated an antagonistic effect i.e. Moncut mixed with Black pepper, pomegranate, Rosemary, Chamomile and Roselle with synergistic ratio (SR) values 0.072, 0.043, 0.038, 0.033 and 0.032 respectively. The observed values of IC_{50} for these mixtures were lesser than those of expected one.

In the case of mixture 3:1 the results show that, Moncut and plant extract demonstrated an antagonistic effect i.e., Moncut mixed with Roselle, Rosemary, Pomegranate , Black pepper and Chamomile with (SR) 0.089, 0.790, 0.660, 0.262 and 0.130 respectively . The observed values of IC_{50} for mixtures were lesser than one (1.00).

Table 5. Effects of the interaction of Moncut fungicide mixed with ethanol extract of some plant-extracts at 1:1 and 3:1 concentrations, against *S. rolfsii*

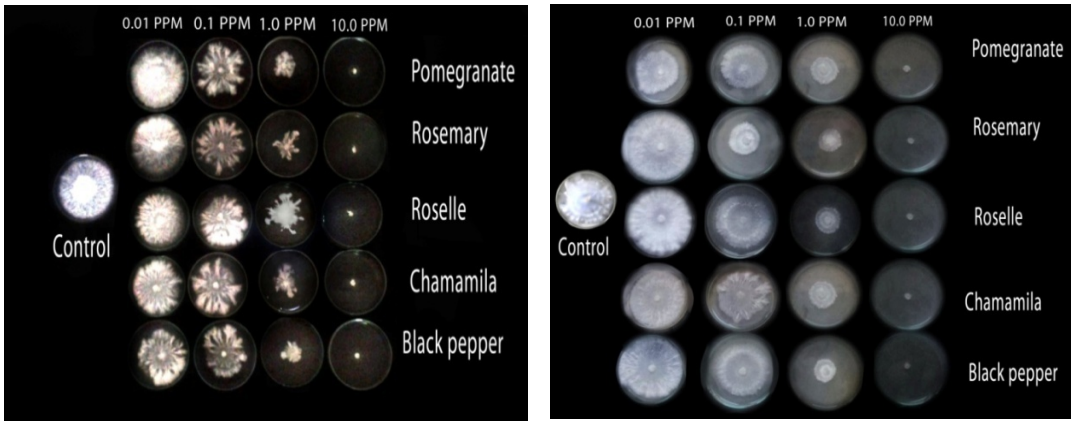
Treatments	Mixture ratio	Inhibition percentage I.%				Observed IC_{50}	Expected IC_{50}	Synergistic ratio	Biological response
		0.01	0.1	1.0	10.0				
Control		0	0	0	0	-	-	-	-
Moncut		35.00	75.00	91.00	93.00	-	-	-	-
Pomegranate	**plant extract : Moncut (1:1)**	5.57	11.47	64.43	93.30	0.534	0.023	0.043	Antagonistic
Rosemary		7.43	7.70	54.43	93.30	0.616	0.023	0.038	Antagonistic
Roselle		14.80	13.00	44.07	93.30	0.735	0.023	0.032	Antagonistic
Chamamila		8.90	10.00	50.37	93.30	0.712	0.023	0.033	Antagonistic
Black pepper		14.10	18.90	70.37	93.30	0.317	0.023	0.072	Antagonistic
Pomegranate	**plant extract : Moncut (3:1)**	43.70	52.20	76.70	93.30	0.036	0.024	0.660	Antagonistic
Rosemary		51.47	53.33	64.07	93.30	0.024	0.019	0.790	Antagonistic
Roselle		54.10	52.23	74.43	93.30	0.017	0.016	0.890	Antagonistic
Chamamila		0.00	28.87	77.40	93.30	0.314	0.043	0.130	Antagonistic
Black pepper		12.23	38.90	91.10	93.30	0.145	0.038	0.262	Antagonistic

L.S.D. at 5% for I. % at (1:1): Treatments: 1.827 Concentrations: 1.492
L.S.D. at 5% for I. % at (3:1): Treatments: 2.215 Concentrations: 1.809

Where

I. %: Inhibition percentage % = percentage of reduction in culture growth compared to growth measure in control plates. I. % = (control-treatment/control) x 100

Control, plates diameters measures started when the complete growth of fungi reaches to maximum growth (9 cm).



Plant extract : Moncut (1:1) **Plant extract : Moncut (3:1)**

Fig. 4. In vitro evaluation of Moncut fungicide and different ethanol plant extracts at the rate of 1:1 and 3:1 concentrations, on linear growth of *S. rolfsii.*

3.2. Greenhouse experiments:

The used plant extracts were evaluated for their effect on sugar beet infected with *S. rolfsii* in greenhouse condition.

Data present in Table 6 revealed that, the total mean regardless of all treatments showed that pomegranate and black pepper showed the lowest disease severity in this respect (1.445 and 3.556 %) respectively compared with control (8.433%).

Concerning the estimated TSS% and sucrose content in beet roots under study, it was observed that, black pepper extract recorded the highest percentage of TSS and sucrose content (19.569 and 13.551 %) respectively, compared with control pathogen. Generally, TSS and sucrose content were found to be increased significantly in the treated and healthy roots compared with infected roots.

As regards to root weight tabulated in Table 6, there is improvements in all treatments concerning root weight in comparing with infected control. Generally, pomegranate plant extract gave the highest increase in root weight (2.272 Kg).

The results of root yield / plot as an end product of interaction for all treatments revealed that total mean of pomegranate and black pepper was recorded the highest yield/plot (17.594 and 16.306) kg/plot compared with control with pathogen (14.167) kg/plot, (Table 6).

Table 6: Effect of different plant extracts treatments on disease severity (%), total soluble solid (TSS %), sucrose content (%), root weight and yield / plot of sugar beet as affected by *S. rolfsii*

Treatment	Disease severity %	Total soluble solid (TSS %)	Sucrose content %	Root weight (Kg)	Yield / plot (Kg)
Black pepper (6000 ppm)	**3.556**	**19.569**	**13.551**	**2.165**	**16.306**
Pomegranate (6000 ppm)	**1.445**	**19.440**	**12.451**	**2.272**	**17.594**
Control healthy	**0.000**	**16.385**	**11.173**	**2.158**	**16.168**
Control with pathogen	**8.433**	**16.012**	**9.630**	**1.445**	**14.167**
LSD 5%	**0.350**	**0.882**	**1.115**	**0.143**	**0.619**

3.3. Effect of different plant extracts on phenolic compounds and some antioxidative enzymes of sugar beet at pre-harvest stage

Total phenols, free phenols and conjugated phenols were estimated, and results are tabulated in (Table 7). Data revealed that, pomegranate exhibited the highest amount of total phenols (14.878 mg/g fresh weight) followed by black pepper (14.458 mg/g fresh weight) compared with infected control (10.636 mg/g fresh weight). Furthermore, treatment with pomegranate indicated high amount of free phenols (8.510 mg/g fresh weight) compared with infected control (6.908 mg/g fresh weight). Also, treatment with pomegranate and black pepper caused production of high amount of conjugated phenols (6.369 and 6.223 mg/g fresh weight) while infected control showed the lowest amount of conjugated phenols (3.728 mg/g fresh weight).

The increase in the activity of peroxidase and polyphenoloxidase within sugar beet as a result of plant extract treatments were found to be the highest in comparison with the control (Table 7)**.** Plant extract of black pepper and pomegranate recorded the highest peroxidase activity 0.811 and 0.792 (min/gm fresh weight) respectively, while polyphenoloxidase recorded (0.359 and 0.293 min/gm fresh weight) respectively compared with infected control (0.140 min/gm fresh weight).

Table 7: Effect of different plant extracts on phenolic compound and antioxidative enzymes of sugar beet leaves as affected by *S. rolfsii* infected soil

Treatment	Total phenols mg/g	Free phenols mg/g	Conjugated phenols mg/g	Peroxidase (min/gm fresh weight)	Polyphenol oxidase (min/gm fresh weight)
Black pepper (6000 ppm)	**14.458**	**8.236**	**6.223**	**0.811**	**0.359**
Pomegranate (6000 ppm)	**14.878**	**8.510**	**6.369**	**0.792**	**0.293**
Control healthy	**11.746**	**6.900**	**4.846**	**0.728**	**0.142**
Control with pathogen	**10.636**	**6.908**	**3.728**	**0.682**	**0.140**
LSD 5%	**0.454**	**0.614**	**0.736**	**0.051**	**0.059**

4. DISCUSSIONS

Many plant extracts have been reported to have efficacy against *S. rolfsii* under laboratory condition (**EL-Shahawy, 2002 and Okemo *et al.*, 2003).** It was observed that, the plant extract of black pepper used as acetone extract was the most inhibitory and

effective extract against *S. rolfsii.* While, the mixture of moncut fungicide with plant extracts demonstrated antagonistic effect. These results are almost consistent with those obtained by **EL-Shahawy (2002) and Sana *et al.*, (2016).** They found that methanolic leaf extract of black pepper showed the highest inhibition of mycelium growth of *S. rolfsii*. However, many researchers observed that, the mixture of Moncut and plant extract demonstrated an antagonistic effect and this result could be attributed to the presence of minor component such as loliolide which has antifungal activity as reported by **EL-Sayed (2007) and Shukla** and **Dwived (2012).** These results are consistent with those obtained by **EL-Shahawy (2002) and EL-Sayed (2007)** who reported that black pepper (*P. nigrum*) has the potential to reduce the growth of *S. rolfsii* fungus in the lab. Black pepper is well known to contain some chemicals having fungicidal effect. Volatile oil and crystalline alkaloids piperine are one of the active ingredients found in the pepper fruit that enhance the fungicidal properties.

Gouda (2001) studied the effect of some plant- extracts against damping-off and root-rots disease of sugar beet caused by *S. rolfsii, R. solani, F. oxysporum* and *M. phaseolina*. He found that, piper *nigram* and *punico granatum* gave strong inhibitory effect of against growth of *S. rolfsii* followed by vitavax-thiram fungicide.

Under greenhouse condition, the tested plant extracts revealed that plant extract of pomegranate and black pepper showed the lowest disease severity compared with control. These results are almost in accordance with many workers **(Gouda, 2001, EL-Sayed, 2007 and Derbalah *et al.,* 2012)** who reported that the plant extracts improve resistance of root rot disease on sugar beet. Also, these extracts significantly improved plant stand indicating their potentially to reduce the post emergence damping-off caused by several pathogen. These results are supported with those obtained by **Ouf *et al*., (1994)** who stated that root rot was decreased and stand of healthy plant was obviously improved due to this application. Pomegranate and black pepper were found to be highly effective in decreasing beet rot compared with the other evaluated extracts. Chemical constituents of black pepper were previously identified, and it was found that piperine, piperidine and piperettine are the most common components responsible for the antifungal and antibacterial effect of the extracts derived from this plant species.

Plant extracts of pomegranate and black pepper were recorded the highest yield /plot. These results are supported

by the results obtained by **Gouda, (2001), EL-Sayed, (2007) and Abdallah *et al.*, (2009)** who found that all seed treatment with selected plant extracts, were significantly increased the yield parameter.

Concerning phenolic compounds, plant extracts pomegranate and black pepper scored the highest amount of total, free and conjugated phenols in this respect. Similar trend was observed regarding to free and conjugated phenols. These results are in agreement with those found by **Setty *et al.*, (2001) and EL-Sayed (2010).** They reported that phenols contents were higher in resistant maize and /or sorghum genotypes compared to susceptible genotype of *P-sorghi*. Also, **Chowdhury, (2002), Reddy and Sireesha, (2014), EL-Sayed and El-sherbeni, (2017) and Abou-habal, (2018)** reported that using plant extract for controlling Sclerotium root rot or Cercospora leaf spot disease on sugar beet was increased the total, free and conjugated phenols.

Phenols (total and ortho-dihydric, OD phenols) are components that induce resistance in plants against pathogens. Phenol is oxidized to highly toxic OD phenol by enzymatic action (polyphenol oxidase) and its concentration is highly correlated with disease susceptibility as observed in grapes anthracnose **(Vidhyasekaran, 1973)**. According to **Matern and Kneusal (1988)**, the first step involves the rapid accumulation of phenols at the infection site, which acts as mobilized defense system that can be translocated by plants, and converted enzymatically into defensive substance at the site of the attack.

The activities of oxidizing enzymes of the infected roots were studied throughout the present study. Unlike the findings of other investigators **(Karthikeyan *et al.*, 2006 and EL-Sayed 2007)** it was found that the infection decreased the production of peroxidase and esterase in roots. This is may be due to that these enzymes prevail and oxidizing and almost disappear due to early decay of the tissues, particularly in infected roots that accelerate the senescence if compared with the healthy tissues. Treating beet seeds with plant extract of Infection of plants with *Penicillium nigrum* caused substantial increase in the activity of peroxidase and esterase in the produced roots. These results are supported with the finding of other investigators dealing with sugar beet rots or other crop disease **(Metwally, 2004 and Karthikayan *et al.*, 2006).** Actually, these enzymes as reported by **Bi and Zhang, (1993)** play a role in oxidizing phenols to quinines that control disease. **Hammerschmidt and Kuc, (1982**) reported that the resistance phenomena such as lignin production or

lignifications may be due to enhancing of peroxidase activity in plants.

5. CONCLUSION

The tested plant extracts can be considered as natural source of fungicidal material which are potentially useful for the control of *S. rolfsii* in sugar beet crop. Antifungal activity was confirmed in all plant species assayed, despite some variation in their efficacy against root rot disease. In vivo, results under greenhouse conditions confirmed that the plant extracts used in this study can be used as a viable and safe alternative for controlling *S. rolfsii*. The obtained results recommended that pomegranate and black pepper extracts exhibited the lowest disease severity. Therefore, we recommended the application of pomegranate and black pepper as natural alternative fungicides which are eco-friendly, economical and safety as fungicide and are easily variable in local environment.

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التأثير المضاد للفطريات لبعض المستخلصات النباتية ومخلوطها مع مبيد المونكت ضد عفن جذور بنجر السكر

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يعتبرمحصول بنجرالسكر من اهم المحاصيل لإنتاج السكرفى مصرحيث يساهم بنسبة 59.5٪ من إنتاج السكرالكلى. كما يعتبر مرض عفن جذور بنجرالسكر والمتسبب عن فطر *S. rolfsii* من اخطر الأمراض التي تصيب المجموع الجذرى. تهدف هذه الدراسة الى تحديد المستخلصات النباتية الأكثرفعالية مع مذيبات مختلفة لتثبيط نمو الفطر. حيث تم تقييم خمسة مستخلصات نباتية وهى الكركديه، الرومان،الفلفل الأسود ، الروزمارى والبابونج لمقاومة عفن جذور بنجر السكر تحت ظروف المعمل والصوبة الزراعية خلال موسم 2017/2016 في محطة البحوث الزراعية بالجميزة - مركز البحوث الزراعية. وقد اظهرت النتائج أن جميع المستخلصات النباتية المختبرة لها تثبيط ملحوظ لنمو الفطرمقارنة بالكنترول. كما أظهرت النتائج أن الفلفل الأسود هو المستخلص الأكثر فعالية ضد *S. rolfsii*. حيث سجل تثبيط 93.33٪ بالإضافة إلى ذلك أظهرمستخلص الرمان أعلى نسبة تثبيط نمو 93.3٪ بمذيب الميثانول. وأظهرت جميع مخاليط المستخلصات مع مبيد المونكت تأثيرتضاد . وقد سجلت المعاملة بمستخلص الرمان والفلفل الاسود اقل شدة للمرض مقارنة بالكنترول بالإضافة إلى ذلك سجلت مستخلصات الرمان والفلفل الأسود أعلى وزن للجذر ومحصول الجذور/ قطعة التجريبية ، والمواد الصلبة الذائبة ومحتوى السكروز .كما اظهرت مستخلصات الرمان والفلفل الأسود أعلى كمية من الفينولات الكلية والفينولات الحرة والفينولات المرتبطة ، وكذلك أعلى نشاط لبعض إنزيمات الاكسدة (peroxidase ،. polyphenol oxidase). ولذلك تعتبر تلك المستخلصات النباتية مصادر فعالة وبديلة للمبيدات الفطرية والتي لها تأثيرات مثبطة على الكائنات الحية الدقيقة لخصائصها الفعالة فى مقاومة المسببات المرضية وامنه على البيئة.