ANTIFUNGAL AND KINETIC ACTIVITIES OF SOME PLANT EXTRACTS, COMPOST TEA AND FUNGICIDE RHIZOLEX-T IN CONTROLLING WHITE ROT DISEASE OF ONION PLANTS

Ghoniem, K. E.; M. E. Shalaby*; M. A. El-Diehi
Agric. Botany Dept., Faculty of Agriculture, Kafrelsheikh Univ., Egypt. Plant Pathology & Agric. Microbiology*

ABSTRACT

Antifungal activities of acetone extracts of garlic, ginger and black pepper as well as tea of the composted rice straw in comparison with a chemical fungicide of Rhizolex-T against *Sclerotium cepivorum* Berk, the causal agent of onion white rot disease were tested. Mycelium and germination of sclerotia formed by the causal was prevented completely due to use 211.00 ppm of black pepper extract and 2.00 g L^{-1} of Rhizolex-T compared with the effect of garlic, ginger and compost tea. Modeling of the *in vitro* data indicated remarkable compatibility with the kinetic parameters of Monod's equation with great regression (R^2-degrees) ranged from 96.9 to 99.9 %. Due to their efficacy, applicability of the pronounced concentrations of the tested control agents was also *in vivo* tested. Superiority of black pepper and Rhizolex-T was confirmed in pots with lowest disease incidence of 8.33 % each as well as to 6.26 and 7.29 % of disease severity, respectively. Under field conditions, disease incidence and severity were sharp declined to 2.78 and 1.39 % by Rhizolex-T as well as to 5.55 and 2.78 % by black pepper extract, respectively in comparison with control. These data were also linked with the enzyme activities of peroxidase (PO) and polyphenol oxidase (PPO). Results showed remarked induction of both enzymes due to garlic and black pepper extracts in addition to Rhizolex-T, indicating enhancement of the systemic acquired resistant (SAR) against *S. cepivorum*. According to these treatments, chlorophyll, root and foliage lengths, foliar, bulb dry matter and then bulb productivity were also enhanced in comparison with control.

Keywords: Plant extracts, compost tea, Rhizolex-T, *Sclerotium cepivorum*, defensive enzymes.

INTRODUCTION

Onion (*Allium* cepa L.) white rot is considered one of the most serious world wide spread diseases affecting onion crops causing massive reduction of bulb yield to uneconomic level. *Sclerotium cepivorum* is its causal fungus, which has been spread widely at both northern and southern governorates in Egypt (Shaheen *et al.*, 2011). In the last few decades, application of effective and environmentally safe tools alternative to chemical fungicides were required. So, utilization of plant-source control agents, including plant extracts and tea of the composted rice straw were suggested in the presented study.

Carcia and Lawas (1990) stated that garlic and black pepper were the most effective plant extracts against *Rhizoctonia solani* and *Sclerotium* sp. Ismail and Ahmed (2000) found that garlic and onion aqueous crude extracts showed toxic effects on the mycelial growth of *S. rolfsii, R. solani, Macrophomina phaseolina, Fusarium moniliforme* and *F. oxysporum* f. sp *vasinfectum*. Dawar *et al.* (2008) found that ethanol extract of such spices was more effective in the control of root rot pathogens as compared to their aqueous extract. On the other hand, tea of the composted plant wastes is safe substance to destroy and remove sclerotia of *S. cepivorum* and *S. rolfsii* (Coventry *et al.*, 2002). Shalaby and El-Kot (2009) used certain composted plant wastes for controlling *S. cepivorum* successfully under laboratory conditions. Antagonistic effect of the composted rice straw tea was confirmed by Noble and Coventry (2005) against *Pythium ultimum, R. solani, Phytophthora* spp, *F. oxysporum, Verticillium dahlia* and against *Cephalosporium maydis* under disease nursery conditions by Shalaby *et al.* (2011). Whereas, synthetic fungicides are still the superior strategy effectively applicable against the causal pathogenic isolates of different plant diseases. Zein *et al.* (1990) compared the effect of Rizolex-T, Vitavax-captan, Monceren, Kocide and Mancoper against *R. solani, F. solani, S. bataticola, F. oxysporum* and *Alternaria solani*. As well as, Vitavax, Basitac, Rhizolex-T and Baytan were evaluated by Henriquez and Montealegre (1992) against *S. rolfsii*. Satyanarayana and Begum (1996) indicated that seed treatments with Rhizolex-T, Vitavax, Bavistin and Captan significantly reduced severity of maize late wilt disease caused by *C. maydis*.

Kinetic models could be used successfully to describe the relationship between pathogen and its control agents (inhibitors). Barr and Aust (1994) indicated that the enzymatic system of white rot fungi was induced by nutrient limitation. It often follows Michaelis-Menton kinetic parameters. Therefore, Ks (constant affinity of the substrate) values of the enzymes must be considered. They reported also that the fungi are able to degrade nutrient sources, such as sawdust, wood chips, surplus grains, and agricultural wastes, which could be used by white rot fungi. For inhibitory kinetic principles, Minaeva *et al.* (2008) described degree of antagonistic activity by the constant of inhibition (Ki) and residual rate of fungal growth. Applicability of different control agents are not only for inducing systemic acquired resistant (SAR), and then disease control, but also for enhancing growth and yield parameters (El-Kafrawy and Radwan 2008). The presented work aimed to use plant-source agents, which to be environmentally safe and effective for controlling onion white rot disease in comparison with the chemical strategy.

MATERIALS AND METHODS

This study was carried out at Agricultural Botany Dept., Faculty of Agriculture, Kafrelsheikh University as well as within fields of El-Gharbia and Kafr El-Sheikh governorates during 2010 and 2011 seasons.

Media:

A preliminary potato dextrose agar (PDA) medium (1 L; pH 6.5-6.8) contained 200 g potato, 20 g dextrose and 15 g agar (Tsao, 1970) was used for cultivation of the pathogenic fungus. The enrichment barley grains (BG) culture medium (glass bottles of 500 ml capacity containing 100 g of barley grains and 50 ml water) was also used to carry inocula of the pathogen as described by Abd El-Moity (1976). Both media were autoclaved at 121°C for 30 min. before use.

Pathogen:

Samples of onion fields in El-Gharbia and Kafr El-Sheikh Governorates showed typical white rot symptoms were collected and divided into small pieces to isolate the causal agent. Using 0.25% sodium hypochlorite solution, pieces were surface sterilized for 4 minutes, washed thoroughly with sterile distilled water, blotted between sterile filter papers and plated on PDA medium. Inoculated plates were incubated at 18-20 °C for 10-15 days and examined daily for observing growth of the causal mycelium. Formation of distinguishable sclerotia was also noticed and the cultures were purified using the hyphal tip technique based on the methods of Booth (1977). Pure cultures were maintained on PDA slants and kept in a refrigerator at 4 °C as stock cultures.

Eight isolates were subjected to test their pathogenicity using onion cultivar (Giza-20) in pots under out-door conditions. Pots (30 cm in diameter) were sterilized by immersing in 5 % formalin solution for 15 minutes and left till its complete evaporation (Hanafi, 1989). Clay soils were washed several times by 0.1 N HCl solutions and further washed with distilled water before filling them in the pots. According to the methods of Abd El-Moity (1976), inocula of the eight isolates were prepared using sterilized BG medium. After sterilization, bottles were inoculated with 5 mm diameter discs of the fungal isolates using 10 days old cultures and incubated at 18-20 °C for 25 days. Inoculum of each isolate was mixed thoroughly with the sterilized clay soils at rate of 2 % w/w. Each pot was filled with 5 kg of infested clay soils. Pots filled only with sterile soils acted as control. All pots were watered two weeks before planting to achieve very well growth and distribution of the pathogen. Four healthy onion transplants (60 days old) were sown in each pot. Four pots of each isolate were used as replicates. 100 days after transplanting, plants were uprooted and pathogenicity was determind according to Abd El-Moity, 1976 and Shatla *et al.*, 1980). According to Walker (1952) and Alexopoulos and Mims (1979), cultural, microscopic and phytopathological properties, the tested eight isolates were found to belong to *Sclerotium cepvorum.* Based on its massive pathogenicity, only one isolate of *S. cepivorum* coded as Sc_2 was selected for the further studies.

Antagonists:

For *in vitro* suppressing *S. cepivorum*, certain plant extracts and tea of the composted rice straw were suggested in comparison with Rhizolex-T fungicide commonly used at onion cultivations.

a) Acetone plant extracts:

Three plant species belonging to three families were used throughout these experiments. Samples were purchased from local market. English, scientific, family, arabic name as well as the used part of the plants tested in this study are shown in Table (1).

Table (1): English, scientific, family, arabic name as well as the used part of the plants tested in this study.

No.	English name	Scientific name	Family name	Arabic name	Parts used
1	Garlic	*Allium sativum* L.	Liliaceae	ثوم	cloves
2	Ginger	*Zingiber officinale* Rosc.	Zingibaraceae	زنجبيل	Rhizomes
3	Black pepper	*Piper nigrum* L.	Piperaceae	فلفل أسود	Dry fruits

The tested plant materials were air dried at room temperature, ground using blender into fine powder. Batches of 100 g from the powdered plant materials were macerated in 500 ml of acetone for five days. During the maceration periods the samples were shaken for 5 hours using an electric shaker. Extracts were filtered using cotton and filter paper (wathman No 10/10), dried over anhydrous sodium sulfate and evaporated to dryness by rotary evaporator. The residues were weighed, dissolved in acetone and kept at 4 °C to prepare the required concentrations (Ismail and Ahmed, 2000).

To estimate their antagonistic activities against *S. cepivorum*, *in vitro* trials were carried out using five concentrations of each plant extract. Calculated as stock solution, 20150 ppm of garlic, 12150 ppm of ginger and 10550 ppm of black pepper were resulted in acetone extract processes. Out of the stock solution, five successive volumes were used as treatments. These volumes were calculated based on the crude solutions and they found to be 0.92, 1.85, 3.70, 36.98 and 46.23 ppm of garlic and 7.29, 14.58, 24.30, 48.60 and 72.90 ppm of ginger in addition to 52.75, 105.50, 211.00, 422.00 and 633.00 ppm of black pepper. The required concentrations were obtained by adding sterilized certain appropriate amount of stock extract to flasks containing integral portions of autoclaved PDA medium cooled to about 45°C by using membrane filter syringe 0.2 µm. Petri-dishes (9 cm in diameter, 15 mL $dish^{-1}$) were inoculated centrally with agar discs (5 mm) bearing mycelium of 7-days old cultures of *S. cepivorum*, then incubated at 18-20°C. These trials were represented by three replicates of each treatment. The non-amended Petri-dishes of plant extracts were served as control. Radial growth was daily observed and measured till a time of full growth in the control treatment. However, percentage of inhibition (I %) was calculated according to the formula suggested by Topps and Wain (1957):

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

Where:-

I % = Percentage of inhibition.
A = Mean diameter growth in the control.
B = Mean diameter growth in a given treatment.

b) Tea of the composted rice straw:

As a beneficial plant wastes required being recycled, composting of rice straw is the most suitable processes to obtain products be applicable at many agricultural areas. Of these outputs, compost tea is produced by helping bentonite, rock phosphate and some amendments. Bentonite is a type of clay believed to be formed from decomposed ash containing several minerals and characterized by its water holding ability. Rock phosphate is an organic matter contains plant available phosphorous, calcium, silica and a broad range of trace elements. However, rice straw was chopped, constructed in successive layers and collected in heap form. Each layer of the chopped rice straw was incorporated with equal portion of farmyard manure, bentonite, rock phosphate, urea and elemental sulfur at rates of 10, 15, 10, 2.5 and 1%, respectively and received suitable water. Turning process was done every 30 days with keeping the moisture within the range of 40-60 % along the composting process.

After the first turning, fungal inoculant's of *Trichoderma viridi* (750 mL ton^{-1}), as cellulose decomposer, was spread on the compost heap to accelerate the decomposition rate. At maturity stage (three months), heap was also inoculated with *Azotobacter chroococcum*, *Azosperillum brasilense* and *Paenibacillus polymexa* (400 g solid carrier ton^{-1} each). The additive bacteria belong to the well-known plant growth promoting rhizobacteria (PGPR) having antagonistic potency against different phytopathogens. These bacteria were grown individually on nutrient broth media and mixed with solid carrier consists of vermiculite and peat-moss at ratio of 3: 1 w/w, respectively. Fifteen days after the bacterial inoculation, one kilogram of the maturated compost was immersed in 10 L water and filtered to obtain tea compost. Some chemical and biological properties of the prepared compost tea were described in Table (2) according to Badawi (2003).

To test its antagonistic effect against *S. cepivorum*, successive concentrations of compost tea (0.2, 0.4, 0.6, 8.0 and 10.0 %) were *in vitro* used. The required concentrations were obtained by adding sterilized certain appropriate amount of the crude compost tea to flasks containing integral parts of autoclaved PDA medium cooled to about 45°C by using membrane filter syringe 0.2 µm. Petri-dishes (9 cm containing 15 mL medium) were inoculated centrally with 5 mm agar discs bearing mycelium of 7-days old cultures of *S. cepivorum*, then incubated at 18-20 °C. These trials were represented by three replicates of each treatment. The non-amended Petri-dishes of compost tea were acted as check control. Linear growth was daily observed and measured till a time of full growth in the control treatment. However, percentage of inhibition (I %) was calculated for each concentration according to identical formula of Topps and Wain (1957), which previously applied with the plant extracts.

Table (2): Some chemical and biological characters of the composted rice straw tea.

Property	value
Acidity (pH)	7.28
EC (ds m^{-1} at 25 °C)	4.63
Organic carbon (%)	21.35
Total nitrogen (%)	1.52
C/N ratio	14.05
Calcium (mg L^{-1})	22.00
Manganese (mg L^{-1})	105.00
Magnesium (mg L^{-1})	4.90
Zinc (mg L^{-1})	44.20
Copper (mg L^{-1})	12.70
Total count of bacteria (CFU mL^{-1})	8.7×10^7
Total count of fungi (CFU mL^{-1})	1.3×10^8

c) Chemical fungicide:

A certain chemical fungicide commonly used in disease control programs of soil-borne phytopathogens of different crops under field conditions were also tested against *S.*

cepivorum (Sc_2). in this study, Rhizolex-T {O-(2, 6-dichloro-p-tolyl) O, O-dimethyl phosphorothioate (IUPAC) 20 % + Tetramethylthiuram disulfide; bis (dimethylthiocarbamoyl) disulfide 30 %} was tested. It is known as Tolclofos-methyl 20 % WP + Thiram 30 % WP and 3 g L^{-1} is its recommended dose. To investigate its effect, different concentrations of the fungicide (0.5, 1.0, 2.0, 3.0 and 5.0 g L^{-1}) were tested. A known weight of Rhizolex-T was first dissolved into 10 ml distilled water before adding into flasks containing 90 ml of still warm sterilized PDA medium under aseptic conditions using membrane filter syringe of 0.2 µm. After thorough handling shaking, medium was poured in Petri-dishes (9 cm in diameter, 15 ml $dish^{-1}$). As replicates, three dishes of each treatment were inoculated centrally with 5 mm agar discs bearing mycelium of 7-days old cultures of *S. cepivorum*, then incubated at 18-20 °C. PDA-medium free from fungicide served as check treatment. Growth was daily observed and the maximum linear growth was measured at the time of full growth in the control treatment. Net growth data of each concentration were calculated and percentages of inhibition (I %) was tabulated according to similar formula suggested by Topps and Wain (1957).

Modeling:

To describe the relationship between the maximum inhibition percentage of the mycelium and concentration of the tested antagonists on a limitation growth, Monod (1949) proposed the following equation:

$$\mu = \mu_{max} * C_S / (K_S + C_S)$$

Where: μ = Specific growth rate

μ_{max} = Maximum specific growth rate.

C_S = Concentration of the used control agents.

K_S = Constant affinity of the control agents.

This equation was derived from the well-known Michaelis-Menten equation of enzyme kinetics and is often used to express of substrate conversion kinetics. Therefore, Monod-equation was modified with replace I % and $I\ \%_{max}$ instead of μ and μ_{max}, respectively to arise the following modified form:

$$I\ \% = I\ \%_{max} * C_S / (K_S + C_S)$$

Where: I % = inhibition percentage

$I\ \%_{max}$ = maximum inhibition percentage.

To establish a kinetic model valid for describing the relationship between *S. cepivorum* and the tested control agents, the experimental data of I % versus concentrations of the control agents with the modified Monod-equation were fitted. For this purpose, the nonlinear least squares fitting routine of MicroCal's ORIGIN® software package was used and regression degree (R^2) of each agent was achieved. Based on the regression data and their slope values, concentration inhibited 50 % of the fungal growth (IC_{50}) could be plotted (Finny, 1971).

Antagonistic effect on germination of sclerotia:

Due to their widespread and importance in the life cycle of *S. cepivorum*, germination of sclerotia was also investigated. Based on the antagonistic results against growth of mycelium, the most effective dose of each tested agents was suggested against germination of sclerotia. Filtrates of 1.72, 0.60, 2.00 and 10.00 mL of garlic, ginger, black pepper extracts and compost tea were respectively added to flasks containing 98.28, 99.40, 98.00 and 90.00 ml of warm sterilized PDA medium using 0.2 µm membrane filter syringe. So, 36.98, 72.90, 211.00 ppm and 10.00 % of garlic, ginger, black pepper and compost tea, were achieved respectively as required doses. For Rhizolex-T, 0.2 g was well dissolved to 10 mL concentrated solution using sterile distilled water using magnetic stirrer. It was added and mixed very well with 90 ml sterilized still warm PDA medium in conical flask using 0.2 µm membrane filter syringe to obtain 2.0 g L^{-1} as required dose.

Sclerotia of 30-days old cultures of *S. cepivorum* were collected from edges of Petri-dishes and then soaked in test tubes containing the tested doses for 12 h at room temperature. At end of the dipping period, sclerotia were washed by sterile distilled water and 10 sclerotia from each treatment were individually transferred under aseptic condition to Petri-dishes containing a thin layer of PDA medium. Water soaked sclerotia were used for control treatment. Three replicates (dishes) were used for each treatment. Petri-dishes-bearing sclerotia were incubated at 18-20°C for 6 days and percentages of germinating sclerotia were accordingly determined.

Experimental preparations and treatments:

Under infested pot and open field conditions, performance of the tested antagonists was also investigated. As effective doses, antagonistic solutions of 36.98, 72.90, 211.00 ppm and 10.00 % of garlic, ginger, black pepper and compost tea, were also *in vivo* used respectively. Giza 20 onion transplants (60-days old) were immersed for 12 h at room temperature in each of the antagonistic control agents. One month after transplanting, an additive dose (5 mL each) was added around plants in both pots and open field trials. Sixty days after transplanting, defensive enzyme activities of both polyphenol oxidase (PPO) and peroxidase (PO) in addition to certain plant growth and yield parameters were determined.

Pot experiments:

Under outdoor conditions, pots (30 cm in diameter) were filed with infested 8 kg clay soil (field capacity, FC 41 %, pH 6.4). Soil was infested with the pathogenic isolate (Sc_2) of *S. cepivorum* using enrichment BG-medium (Abd El-Moity, 1976). Bottles containing BG-medium were inoculated with 5 mm diameter agar discs bearing mycelium of the pathogen (10 days old cultures) and incubated at 18-20 °C for 25 days. Inoculum was mixed thoroughly with the clay soil at rate of 2 % w/w. Infested pots containing undipping transplants were acted as control. All pots were watered two weeks before planting to achieve very well growth and distribution of the pathogen. Four healthy onion transplants (60 days old) were sown in each pot. Four pots of each isolate were used as replicates. Agricultural practices were applied as recommended.

Open field experiments:

A natural infested field (EC 41 %, pH 6.4) with *S. cepivorum* located at Negrig village, El-Gharbia Governorate was used for controlling white rot disease of onion plants. Plots of 1.5 m long and 75 cm wide (as area of 1.125 m^2) each were planted with 30 onion transplants immersed in the tested control agents. Each treatment was represented by 3 plots. Transplants dipped in distilled water represented the control treatment. Irrigation and fertilization were conducted as generally recommended for onion production regime.

Determinations:

a) Disease index parameters:

100 days after planting in both pots and open field trials, plants were uprooted and examined for disease index parameters. Percentages of white rot disease incidence (Di %) were calculated based on the formula suggested by Crowe *et al.* (1994) as follows:

$$\text{Disease incidence (Di \%)} = \frac{\text{No of infected plants}}{\text{No of total plants}} \times 100$$

As well as, percentages of disease severity (Ds %) were estimated based on the 0-100 scale suggested by Abd El-Moity (1976) and Shatla *et al.* (1980) as follows:

0 = Healthy plants;

25 = Slight severe (yellowing of the leaves, reduced root system);

50 = Moderate severe (yellowing and die-back of leaves, root system badly decayed);

75 = Severe (complete yellowing of the plant, die-back of the leaves, semi watery soft rot of scales and roots);

100 = Highly severe (completely dead plants, extensive decayed bulbs and roots).

b) Enzyme activities:

Effect of the studied antagonists on activities of some defense-responsible enzymes of both polyphenol oxidase (PPO) and peroxidase (PO) were estimated 60 days after planting in the shoots. Therefore, crude enzyme extract was prepared according to the methods described by Maxwell and Bateman, (1967). Where, tube-leaf samples were cleaned, weighted and triturated in a ceramic mortar in the presence of 0.1 M sodium phosphate as buffer solution (pH 7.1). Samples of one gram fresh weight in 2 mL buffer solution were filtrated through cheese cloth and centrifuged at 3000 rpm at 6 °C for 20 min.

Enzyme activity of PPO was assayed according to the colorimetric procedures adopted by Matta and Dimond (1963). The reaction mixture contained 1.0 mL of enzyme extract, 1.0 ml 0.2 M sodium phosphate buffer at pH 7.0, 1.0 mL of 10^{-3} M catechol ($C_6H_4(OH)_2$) and 3.0 ml distilled water to brought a final volume of 6.0 mL. Using UV-VIS spectrephotometer

(Jenway, 6105 UV-VIS), absorbance was measured at optical density (OD) of 495 nm and the optical densities were recorded at 0, 30, 60, 90 and 120 seconds intervals. Enzyme activities of polyphenol oxidase were expressed as changes in the optical density s^{-1} g^{-1} fresh weight. Treatment contained all chemical reagents except the enzyme extract served as blank, which used to reset or calibrate the device.

Enzyme activity of PO was assayed according to the methods of Srivastava (1987), by measuring the oxidation of pyrogallol to pyrogallin in presence of H_2O_2. A combination consists of 0.5 ml of 0.1 M sodium phosphate, 0.3 mL enzyme extract, 0.3 mL of 0.05 M pyrogallol (C_6 H_3 COH_3), 0.1 mL of 10 % H_2 O_2 (v/v) were diluted to brought 3 mL final volume using distilled water. At 425 nm, absorbance of the samples was measured and optical densities (OD) were recorded after 0, 30, 60, 90 and 120 s intervals using spectrephotometer (Jenway 6105 UV-VIS). Reading of OD s^{-1} g^{-1} fresh weight was used as activity indication. Treatment contained all chemical reagents except the enzyme extract served as blank, which used to reset or calibrate the device.

c) Plant growth and yield parameters:

Chlorophyll contents (a and b) of plant leaves 60 days after planting were estimated according to Moran (1982). A known area (one disk 0.5 cm in diameter) equal 0.1963 cm^2 of leaves was taken and extracted their chlorophyll contents by immersing in 5 mL of N, N-dimethylformamide in the dark. Absorbance was measured at 647 and 664 nm using spectrephotometer (Jenway 6105 UV-VIS). Readings were used to calculate chlorophyll a, b and total chlorophyll per µg mL^{-1} based on the following equations:

$$\text{Chl. a} = 12.46\,(A664) - 2.49\,(A647)\ \mu g\ mL^{-1}$$

$$\text{Chl. b} = -\,5.6\,(A664) + 23.26\,(A647)\ \mu g\ ml^{-1}$$

So, chlorophyll contents in relative to the leaf area per µg $(cm^2)^{-1}$ were recalculated. Additionally, root and foliage lengths (cm) were also measured. To determinate dry matter, weighted fresh samples of both bulbs and leaves were separately dried at 60 °C for a time showed constant weight ranged from 4 to 7 days. Specimens were let to cool before further weighted. Difference between the two weights per gram was recorded as moisture and percentage of the dry matter was resulted. As well as, yield productivity of onion bulbs were weighted as kg.

Statistical analysis:

A complete randomized block was applied for trials of this study. Data were subjected to statistical analysis of variance by ANOVA test in SPSS, 11 software statistical packages (Gomez and Gomez, 1984). Duncan's multiple range tests were performed for comparing means (Duncan, 1955).

RESULTS AND DISCUSSION

***In vitro* antagonistic activities:**

a) Radial growth:

Antifungal impacts using different doses of three acetone plant extracts (garlic, ginger and black pepper) and the composted rice straw tea against radial growth of the most pathogenic isolate (Sc_2) of S. *cepivorum* were tested **(Table 3).** Data showed highest inhibition percentages due to use higher concentrations of the tested control agents. Radial growth was complete inhibited using concentration of black pepper extract up to 211.00 ppm and it continued constant even after increasing doses till 633.00 ppm. It indicates that increase of black pepper dose more than 211.00 ppm is not required and then becomes un-economic. Similar behavior was noticed by the fungicide up to 2.00 g L^{-1} of Rhizolex-T. Radial growth was inhibited for about 97.65 and 79.61due to use 36.98 and 72.90 ppm of both garlic and ginger extracts, respectively. On the other hand, mycelium was moderate reduced (54.51 %) at the higher dose (10 %) of compost tea, indicating dose increase is required to obtain more inhibition.

Superiority of black pepper extract might be due to produce toxins and antifungal substances which make the fungal cell decayed. Katzer (1998) and Vaughan and Geissler (1998) reported that black pepper has two main components included volatile oils and alkaloids such as piperine. It contains about 0.6 – 2.6 % essential oil that gives the aromatic flavour, which is dominated by monoterpene hydrocarbons. Black pepper fruits were the most effective extracts group inhibited growth fungi. Fungicidal activities of the ethanol extracts of black pepper, garlic and ginger were also *in vitro* achieved against *Fusarium solani*, *Rhizoctonia solani* and *Macrophomina phaseolina* (Dawar *et al.*, 2008).

Table (3): Antagonistic activities of some plant-source control agents and Rhizolex-T against radial growth of *S. cepivorum*.

Treatment	Concentration (ppm, %*, g L^{-1}**)	Net diameter of radial growth (cm)	Inhibition (%)
Control	0.00	8.50	0.00
Garlic extract	0.92	8.50	0.00
	1.85	8.50	0.00
	3.70	8.50	0.00
	36.98	0.20	97.65
	46.23	0.19	97.25
Ginger extract	7.29	5.47	35.69
	14.58	4.93	41.96
	24.30	3.70	56.47
	48.60	3.23	61.96
	72.90	1.73	79.61
Black pepper extract	52.75	1.33	84.31
	105.50	0.93	89.02
	211.00	0.00	100.00
	422.00	0.00	100.00
	633.00	0.00	100.00
Composted rice straw tea *	0.20	8.50	0.00
	0.40	8.50	0.00
	0.60	8.50	0.00
	8.00	4.63	45.49
	10.00	3.87	54.51
Rhizolex-T **	0.50	0.60	92.94
	1.00	0.37	95.69
	2.00	0.00	100.00
	3.00	0.00	100.00
	5.00	0.00	100.00

They found also that ethanol extract of spices was more effective in the control of root rot pathogens as compared to aqueous extract. Antifungal action of such extracts involves cytoplasm granulation, cytoplasmic membrane rupture and inactivation and/or inhibition of intercellular and extracellular enzymes. Brull and Coote (1999) and Cowan (1999) indicated that the lytic enzymes excreted from such extracts caused breakage of ß-1,3 glycan, ß-1,6 glycan and chitin polymers of the fungal cell wall. Degradation of cell wall and damage of cytoplasmic membrane were the clearest disorders on the fungal cell of *S. sclerotiorum*, *R. solani* and *M. phaseolina* due to effect of volatile oils and enzymes of garlic extract (Atta-Alla *et al.*, 2003). Antifungal activity of ginger extract was also explained via production of essential oils containing terpenoids and phenylpropanoids (Katzer, 1998 and Dhirendra-Mishra and Mishra, 1990). For Rhizolex-T, disorder and striking changes in the cell wall of hyphae, phialides and conidiophores were found by Abd El-Ghany and Tayel (2009). These alterations in the wall were not detected with the untreated hyphae. Disorganization of the cytoplasm was also recorded. Additionally, vacuoles were completely disappeared under influence of the fungicides (Amer and El-Shenawy, 2003).

Kone *et al.* (2010) attributed effect of compost tea to the physical and chemical properties of its nutrients. This may improve the nutritional status of plants, be directly toxic to the pathogen, and/or induce systemic resistance to the pathogen. A hypothesis stated that compost tea seems to act as a bio-control of pathogens by favoring the growth of beneficial bacteria. Our results were also in agreement with Morsy and El-Korany (2007), they found weak suppression rate of checking sunflower disease ranged only between 47.6 and 51.3 % with rice straw composted tea.

To describe the relationship between percentages of inhibition and concentration of the tested control agents (catabolic behavior), enzymatic kinetic model of Monod (1949) was proposed. Accordingly, concentration causes 50 % growth inhibition (IC_{50}) of the pathogen was obtained. For this purpose, nonlinear least squares fitting routine of MicroCal's ORIGIN® software package was used. The fitted functions of the plant-source agents with the experimental data of mycelium inhibition were plotted in Fig. (1).

It showed remarked compatibility of the experimental data with the fitted data of Monod equation with great regression (R^2-degrees) ranged from 96.9 to 99.9 %. As well as, tendency of Rhizolex-T and black pepper curves seem to be more sharply, indicating more efficacy with less concentration. Accordingly, efficiency of ginger, compost tea and garlic were also respectively followed. Kinetic data of Monod-parameters (I_{max}, Ks) and IC_{50}-concentrations of the tested antagonists were tabulated in Table (4). It indicated the lower Ks (curve tendency) the more efficient antagonistic agent. It indicated that Rhizolex-T and black pepper extract were the most effective (the least Ks-values of 0.04 and 0.12, respectively) in comparison with the other treatments.

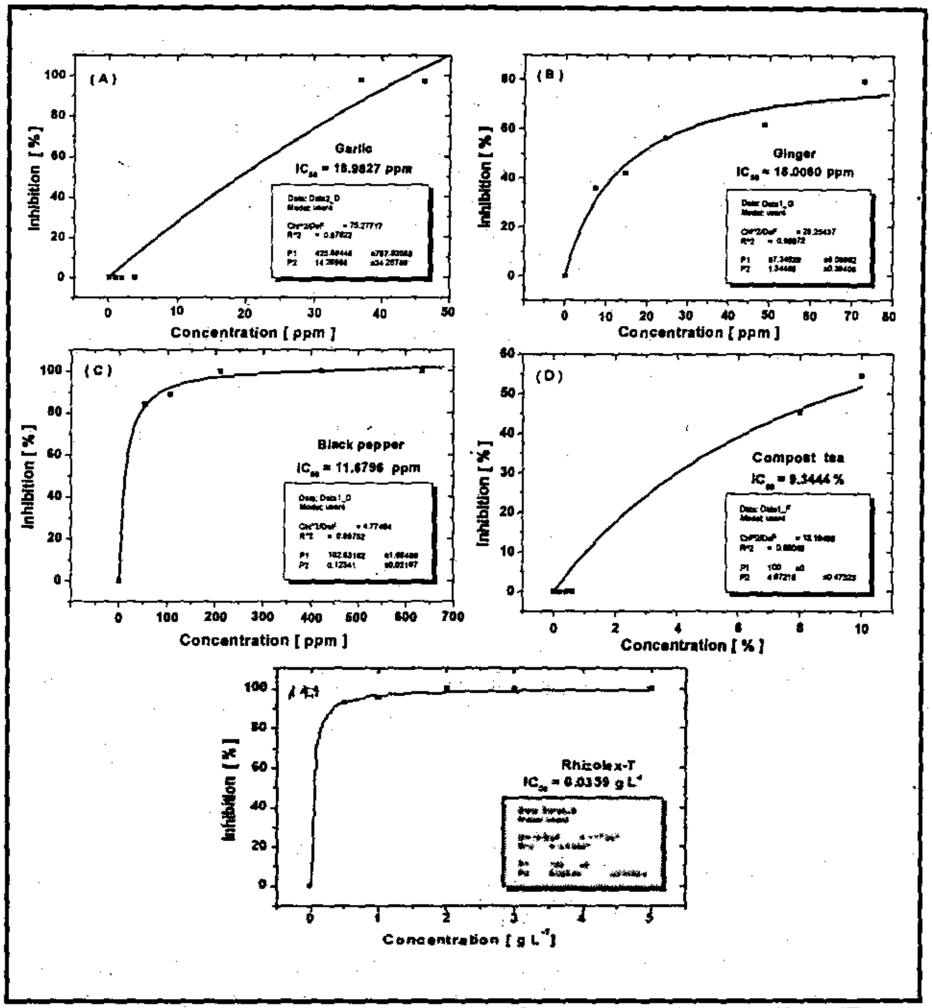

Fig. (1): Modeling describing effects of (A): garlic, (B): ginger, (C): black pepper extracts, (D): compost tea and (E): Rhizolex-T against *S. cepivorum*. Symbol refers to the experimental data and line refers to fitted data with Monod's equation.

Similar explanation was confirmed by Barr and Aust (1994) with the white rot causing fungus. They found that the metabolism of chemicals by bacteria and fungi involves mostly enzymatic conversions; pollutant degradation often follows Michaelis-Menton-type kinetics, which represented the enzymatic form of Monod kinetics. Therefore, Ks values of various destructive enzymes with respect to the pollutant must be considered. So, higher enzyme sensitivity of the pathogen toward acetone black pepper extracts in addition to Rhizolex-T and Topsin-M, with greatest antagonistic effects than the other agents was obtained. Nesci *et al.* (2003) explained that they may inhibit the functions of several enzymes by the oxidized compounds or/and by more nonspecific interactions with the proteins. The reminder control agents as substrates for pathogen showed relative consumption of the substrates leading to less antagonistic activities. Based on regulation of the enzyme activity explained by Schlegel (1992), the cells may be containing sensitive system to adjust activity of these enzymes, in addition to regulation of its levels.

Table (4): Kinetic parameters of Monod-equation recorded by the tested antagonists against *S. cepivorum*.

Treatment	I_{max} (%)	ppm, %*, g L^{-1}**	
		Ks	IC_{50}
Garlic	100.00 ± 6.45	14.27 ± 34.26	18.98
Ginger	87.35 ± 8.07	1.34 ± 0.39	18.01
Black pepper	102.00 ± 1.66	0.12 ± 0.02	11.68
Compost tea	100.00 ± 00	4.67 ± 0.47*	9.34*
Rhizolex-T	100.00 ± 00	0.04 ± 0.005**	0.04**

Where: I_{max}= Maximum percentage of inhibition, Ks = Constant affinity and IC_{50}= Concentration inhibits 50 % of growth.

b) Germination of sclerotia:

Table (5) showed that germination of sclerotia was totally prevented due to soak in either Rhizolex-T or black pepper extract. Efficiency of ginger, compost tea and garlic were also respectively followed. Damaged effect preventing germination of sclerotia totally might due to strong suppression of their enzymatic regulations by rhizolex-T. It was in agree with Nesci *et al.* (2003), who stated that fungicides inhibit functions of several enzymes by the oxidized compounds or/and by more nonspecific interactions with the proteins.

Table (5): Germination percentages of *S. cepivorum* sclerotia soaked for 12 h in filtrates of the most antagonistic doses of the tested control agents.

Treatment	Concentration	Rank	Germination of sclerotia (%)
Control	0.00	5	93.33 c
Garlic	36.98 ppm	4	83.33 c
Ginger	72.90 ppm	2	13.33 ab
Black pepper	211.00 ppm	1	00.00 a
Compost tea	10.00 %	3	43.33 b
Rhizolex-T	2.00 $g\ L^{-1}$	1	00.00 a

The number in the same column means followed by the same letter are not significantly different according to DMRT at 0.05 level.

In vivo antagonistic activities:

In pots and in natural infested field trials, effect of the plant-source antagonists and Rhizolex-T were also tested using seedling dip method. Disease index parameters (incidence and severity) were estimated in both cultivation types. Under open field conditions, enzyme activities of polyphenol oxidase (PPO) and peroxidase (PO), growth and yield parameters were therefore determined.

Disease index parameters:

Applicability of the tested control agents of onion white rot disease in both pots and open field was determined (Table 6). It indicates similar superiority *in vitro* recorded of both Rhizolex-T and black pepper extract for disease controlling. Both treatments reduced disease incidence in pots strongly to 8.33 % each compared with 95.83 % for control. As well as, disease severity was reduced to 6.25 and 7.29 % by black pepper and rhizolex-T, respectively. Although their superiority was also obtained under open field conditions, Rhizolex-T was more aggressive than black pepper against the pathogen. It indicates that rhizolex-T is more adapted under field conditions. So, disease incidence and severity were reduced to 2.78 and 1.39 % by Rhizolex-T, as well as to 5.55 and 2.78 % by black pepper, respectively.

Table (6): Effect of the tested control agents on disease index parameters of onion white rot in pots and natural infested field trials.

Treatment	Disease index parameters %			
	Pots trials		Field trials	
	Disease incidence	Disease severity	Disease incidence	Disease severity
Control (untreated)	95.83 e	90.63 e	80.55 d	73.15 e
Garlic	25.00 bc	19.79 cd	19.45 abc	17.36 d
Ginger	16.67 ab	13.54 abc	11.11 abc	7.64 abc
Black pepper	8.33 a	6.25 a	5.55 ab	2.78 ab
Compost tea	29.17 bcd	18.75 bcd	22.22 bc	14.58 cd
Rhizolex-T	8.33 a	7.29 a	2.78 a	1.39 a

The number in the same column means followed by the same letter are not significantly different according to DMRT at 0.05 level.

Results indicated that black pepper extract was the most efficient plant source creating superiority or at least close to the same antagonistic efficacy played by Rhizolex-T for suppressing incidence and severity of onion white rot disease under pot and field conditions. This result was in full agreement with the findings of Alice and Rao (1987) and Ismail and Ahmed (2000). They found that black pepper extract was the most effective among of 31 extracts against *Drechslera oryzae* and *R. solani*, respectively. Due to use Rhizolex-T, massive antifungal effect were recorded against *Phytophthora* root rot of soybean (Amer and El-Shenawy, 2003), *R. solani* on cotton (Goulart, 2002), *R. solani*, *F. solani*, *F. oxysporum* and *S. rolfesii* causing root rot disease of Lupine plants (Ali *et al.*, 2009).

Enzyme activities:

Enzyme activities of both PPO and PO seem to be constant during the measuremnt time courses. Therefore, averages data were blotted in Fig. (2). It illustrated that all treatments were pronounced in comparison with control of both enzymes. As well as, lower activation levels of PPO in comparison with PO were noticed. It showed also that garlic followed by black pepper extracts induced the highest activities of PPO, indicating induction of the systemic aquired resistant compared with Rhizolex-T or others. For PO, ginger, Rhizolex-T, black pepper and garlic were the most treatments induced its activity, respectively. It is in full agreement with the findings of Goncagul and Ayaz (2010). They found, in addition to more than 200 components, that garlic is having massive enzyme amount of peroxidase, alliinase and miracynase. Data indicated varied induction of the systemic acquired resistant (SAR) suggested phenomenon by Hatcher (1995) against a broad spectrum of phytopathogens. It was supported also by Scalbert (1991), who found that the more highly oxidized phenols are the more inhibitory effect to the pathogen. Higher induction of PPO was observed in tea plants treated with *Pseudomonas fluorescens* lead to accumulation of higher phenolic compounds, which may play an important role in defense mechanism in plants against pathogen (Sivakumar and Sharma, 2003). These consequences are in agreement also with the findings of Saravanakumar *et al.* (2007), who found remarkable increase in PPO and PO activities in tea plants treated with *P. fluorescens* in comparison with the untreated plants.

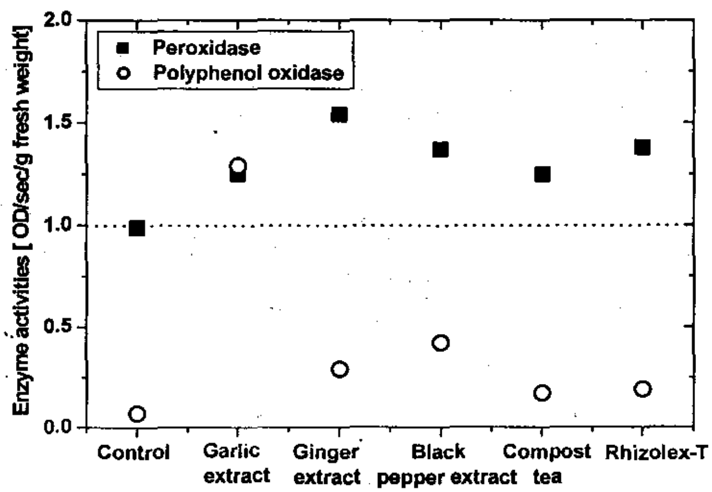

Fig. (2): Enzyme activities of both PPO and PO of onion plants under natural infested field conditions.

Plant growth and yield parameters:

Data presented in Table (7) proved that the tested growth and yield parameters were developed due to treatments. Role played by black pepper extract as competitive antagonist to

Rhizolex-T in controlling white rot disease was also obtained to promote onion plant growth. Due to use black pepper extract and Rhizolex-T, significant enhancement in the vegetative growth parameters was obtained. It might due to induce formation of some substances in the plants, by which onion plants become strong under pathogenic conditions. It was in agreement with the findings of Amaresh and Bhatt (1998). They stated that a large viability of the nutrients and essential components required to photosynthesis that formed in the host plants to prevent the harmful effect of the pathogen. Regarding bulb productivity, superiority of both Rhizolex-T and black pepper extract (3.02 and 2.83 kg plot^{-1}, respectively) was extended. This may be due to enhance physiological, biochemical and defensive enzyme activities by them which positively reflected on increase bulb yield of onion plants (El-Safty *et al.*, 2001 and El-Deeb *et al.*, 2002). However, utilization of black pepper extract represented more competitive antagonist to the chemical fungicide for controlling white rot disease and enhance growth and productivity of onion plants. Harman *et al.* (2004) reported that the natural control agents of the plant diseases have a great capability to enhance growth and yield parameters of the plants.

Table (7): Effect of the tested control agents on certain plant growth and yield parameters of onion plants (Giza 20 cv.) grown at a natural infested field with *S. cepivorum*.

Treatment	Chlorophyll (µg (cm^2)$^{-1}$)		Length (cm)		Dry matter (%)		Bulb yield (kg plot^{-1})
	Chl. a	Chl. b	Root	Foliage	Foliage	Bulb	
Control (untreated)	33.91 a	7.68 a	8.23 a	38.43 a	7.12 a	9.09 a	1.45 a
Garlic	50.35 b	29.25 b	10.00 ab	45.33 ab	8.16 ab	9.84 a	2.65 b
Ginger	54.14 b	35.94 bc	12.67 b	46.00 ab	8.44 ab	10.46 ab	2.05 ab
Black pepper	63.69 bc	41.95 c	11.80 ab	51.00 bc	6.99 a	10.18 ab	2.83 bc
Compost tea	44.71 ab	25.94 ab	10.30 ab	48.60 b	7.78 a	10.77 ab	2.47 ab
Rhizolex-T	72.79 c	40.34 c	10.37 ab	49.33 bc	8.41ab	10.73 ab	3.02 c

The number in the same column means followed by the same letter are not significantly different according to DMRT at 0.05 level.

CONCLUSION

Although chemical fungicides are still the major mean to control plant diseases under field conditions, there are effective more safe substances must be taken in the consideration. Black pepper acetone extract is one of them. The presented work suggests its applicability, not only as a promising antagonist to *S. cepivorum* with or instead of the chemical fungicide, but also as plant growth promoter.

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الملخص العربى

النشاط الإبادى الفطرى والديناميكى لبعض المستخلصات النباتية وشاى الكمبوست والمبيد الفطرى ريزوليكس - ت فى مقاومة مرض العفن الأبيض فى نباتات البصل

كمال السيد غنيم , مصطفى السيد شلبى* , محمد عبد الحميد الديهى
قسم النبات الزراعى – كلية الزراعة – جامعة كفر الشيخ – مصر
أمراض النبات & ميكروبيولوجيا زراعية *

تم إختبار النشاط الإبادى الفطرى لمستخلصات نباتات الثوم والزنجبيل والفلفل الأسود بالأسيتون وكذلك شاى الكمبوست لقش الأرز مقارنة بالريزوليكس – ت كأحد المبيدات الكيميائية ضد فطر *Sclerotium cepivorum* المسبب لمرض العفن الأبيض فى نباتات البصل. فقد منع نمو الميسيليوم وإنبات الأجسام الحجرية المتكونة بواسطة المسبب المرضى كلية نتيجة المعاملة بـ ٢١١ جزء فى المليون من مستخلص الفلفل الأسود و تركيز ٢ % من مبيد الريزوليكس –ت على التوالى, مقارنة بتأثير الثوم والزنجبيل وشاى الكمبوست. وقد دلت نمزجة نتائج المعمل على توافق ملحوظ مع المقاييس الديناميكية لمعادلة مونود بدرجات تطابق كبيرة (R^2-degrees) وصلت إلى مدى بين ٩٦,٩ و ٩٩,٩ % . نتيجة لكفاءتها , فقد تم إختبار إمكانية تطبيق التركيزات الفعالة لعوامل المقاومة المختبرة تحت الظروف الطبيعية. حيث تم تأكيد سيادة كل من مستخلص الفلفل الأسود والريزوليكس – ت فى الأصص بأقل نسبة لحدوث المرض بلغت ٨,٣٣ % لكل منها و بـ ٦,٢٦ و ٧,٢٩ % شدة مرضية لهما على الترتيب. أما تحت ظروف الحقل, فقد إنخفضت بشكل حاد نسبة حدوث المرض وكذا شدة المرض إلى ٢,٧٨ و ١,٣٩ % نتيجة إستخدام ريزوليكس - ت وإلى ٥,٥٥ و ٢,٧٨ % بواسطة مستخلص الفلفل الأسود, على الترتيب مقارنة بالكنترول. وقد أرتبطت هذه النتائج بالنشاط الإنزيمى لكل من البيروكسيديز والبوليفينول أوكسيديز. كما أوضحت النتائج أن هناك إستحثاث ملحوظ لنشاط كلا من الإنزيمين بسبب إستخدام مستخلص الثوم ومستخلص الفلفل الأسود بالإضافة إلى الريزوليكس – ت, مما يدل على تحسن المقاومة الجهازية المستحثة (SAR) نحو *S. cepivorum*. وتبعا لهذه المعاملات, فقد تحسنت مقاييس الكلوروفيل وأطوال الجذور والأوراق والمادة الجافة لكل من المجموع الخضرى والأبصال ومن ثم إنتاجية الأبصال مقارنة بالكنترول.