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Role of *Purpureocillium lilacinum* Cultural Filtrate in Controlling Onion White Rot

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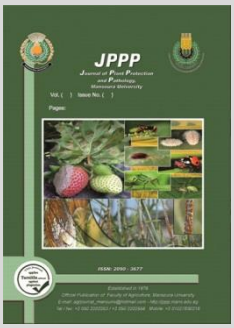

ABSTRACT

Onion white rot is one of the most destructive diseases threat onion production in Egypt. The present study assessed the effect of *Purpureocillium lilacinum* culture filtrates *in vitro* and *in vivo* conditions on *Stromatinia cepivora* at 75%concentration. Bioagent culture filtrates inhibited the mycelial growth, sclerotial formation and myceliogenic germination of *S. cepivora* by 82.59, 100 and 93.33%, respectively. The culture filtrates at 75% increased cell membrane permeability of *S. cepivora* compared to the control. The *in vitro* analysis revealed that 92.5% of the sclerotia were lost its activity in the soil when treated with filtrates and significantly decreased the disease incidence and severity by 95.10 and 98.30%, respectively under greenhouse conditions. However, soil previously infested with *S. cepivora* sclerotia and handled continuously with bioagent culture filtrate for 6 months before onion cultivation undoubtedly decreased the disease incidence and severity. During 6 months after field culture filtrates treatment, more than 80 and 60% of the sclerotia failed to emergence in trial I and II plots experiment, respectively. After one-year culture filtrate treatment in consecutive onion field crop cultivation, filtrates were more efficient than control in decreasing white rot incidence attendant with low inoculum density trial field (77.67sclerotia/kg of soil). Consequently, a decrease in white rot incidence resulting promotion to vegetative parameters of culture filtrate treated onion plants in pots and increase growth and bulb yield productivity in field. Decreasing disease incidence and severity was associated with increase activities of antioxidant enzymes polyphenol oxidase, peroxidase and chitinase by application of culture filtrates.

Keywords: Onion white rot, *Stromatinia cepivora*, *Purpureocillium lilacinum*, culture filtrates, antioxidant-related enzymes.

INTRODUCTION

Onion crop (*Allium cepa* L.) is the most important vegetable crop and most widely cultivated for local consumption and exportation in Egypt. Onion is widely cultivated for its medicial and nutritional characterizations. Approximately 81517 hectares were cultivated in 2018 producing 2.96 million tonnes (FAO, 2018). Onion white rot caused by *Stromatinia cepivora* (Berk.) Whetzel. (teleomorph of *Sclerotium cepivorum* Berk.), is one of the most prevalent, harmful and destructive disease worldwide (Ulacio-Osorio *et al.*, 2006 and Elshahawy *et al*., 2017a). *Stromatinia cepivora* is a necrotrophic ascomycete fungus, which especially infects *Allium* crops. The anamorph *Sclerotium cepivorum*, was first recorded as white rot of *Allium* crops in Egypt in 1922 (Elshahawy *et al*., 2017b). Since that, white rot attacks have been ascent gradually and at current days the disease incidence can reach 80% in some *Allium* fields causing yield catastrophe (Pinto *et al.*, 2000 and Elshahawy *et al*., 2018a). White rot pathogen survives in the soil in the absence of *Allium* crops as a dormant small, round and poppy-seed-sized black sclerotia on plant debris for more than 20 years (Entwistle, 1990). *Stromatinia cepivora* boost their producible sclerotia with thick-hard wall during the infection of onion crop, which helps sclerotia to remain dormant yet viable in the soil for many years. Thus, *Allium* crop may not be able to grow there for many years (Coley-Smith *et al.*, 1987). *Allium*-specific root exudates prompt sclerotia to germinate, specifying that the host range restricted to *Allium* species (Amin *et al.*, 2014). According to the pathogen properties control of white rot disease is difficult (Crowe *et al.*, 1993). White rot severity is commonly associated on sclerotial population in soil at seedling cultivation (Crowe *et al*., 1980). White rot disease management is very difficult and need a multi-strategy to put the disease under control (Ulacio-Osorio *et al.*, 2006). Hitherto, no complete strategy has been yet described to completely eradicate the pathogen from the soil. Hence, growers still depending on chemical control to eradicate the pathogen (Fullerton and Stewart, 1991; Fullerton, *et al*., 1995; Melero-Vara, *et al*., 2000). Different previous safety strategies have been applied for controlling white rot including biological and physical measurements such as soil fumigation (Entwistle, 1990), soil solarization (Melero-Vara, *et al*., 2000), the compost of *Allium* crops waste (Elshahawy *et al.*, 2019a), crop rotation with non-*Allium* crops (Banks and Edgington, 1989), plant extracts (Montes-Belmont and Prados-Ligero, 2006), biological control agents and /or bioagents culture filtrates (Clarkson *et al*., 2006; Dilbo *et al*., 2015; Elshahawy *et al.*, 2017 a,b; Elshahawy *et al.*, 2018 a,b and Elshahawy *et al.*, 2019b).

One of the auspicious strategies to decrease or avoid the excessive usage of chemical fungicides in onion production harmonizes to handle the microbial culture

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filtrates. Notwithstanding, more studies on safety and toxicity should be achieved on these filtrates before use.

Purpureocillium lilacinum fungus has been principally utilized as a bioagent against plant parasitic nematodes (Siddiqui and Mahmood, 1996). Additionally, *P. lilacinum* was used to control the oilseed rape and bean white mould diseases (Yang *et al.*, 2015 and Elsherbiny *et al*., 2019). Moreover, the secondary metabolites of *P. lilacinum* is reported to include bioactive properties against plant pathogens. These metabolites including antibiotic like leucinostatins (Arai *et al*., 1973) and extracellular enzymes like esterase, acid phosphatase, leucine arylamidase, and esterase-lipase (Giné and Sorribas, 2017).

At present, little attempts has been cited in the literature on the probability of utilizing *P. lilacinum* to control plant pathogenic fungi. Moreover, no former studies are accessible on the utilize of *P. lilacinum* to control white rot in onions. Therefore, the objective of this study was to estimate the impact of *P. lilacinum* culture filtrates in the absence of onion crop on the sclerotial viability as well as sclerotia population produced by *S. cepivora*. In addition, the incidence and the severity of onion white rot under greenhouse and field conditions, onion plant growth parameters as affected by application of culture filtrates and the role of some remarkable oxidative enzymes included in the systemic resistance responses to understand the defence mechanisms in onion plants.

MATERIALS AND METHODS

1. Laboratory experiments

Isolation of *Stromatinia cepivora*

Mycelium or sclerotia of *Stromatinia cepivora* were isolated from infected onion plants according to the methods of Clarkson *et al.* (2002). Samples were collected from different white rot naturally infested fields of two locations at Abo Hamad and Belbis, El-Sharqia Governorate, Egypt.

Pathogenicity test

Ten isolates of *S. cepivora* obtained during isolation were subjected to pathogenicity test using susceptible onion cultivar "Giza 6, red" seedlings. The test was carried out in pots under greenhouse conditions according to the method reported by El-Sheshtawi *et al.* (2009). Isolate coded as Scep7 proved to be the most virulent isolate was selected as the main isolate throughout the present study towards onion transplants.

Dual culture test

Identified culture of *Purpureocillium lilacinum* AUMC 10620 was obtained from Assiut University Mycological Centre (AUMC), Egypt. *Purpureocillium lilacinum* was evaluated for antagonism for *S. cepivora* by using dual culture technique described by Elsherbiny *et al*. (2019).

Preparation purified culture filtrate of *Purpureocillium lilacinum*

The mycelial discs of 7 d-old growing cultures of *P. lilacinum* were inoculated into 0.5 litter conical flasks, each contained 200 ml of potato dextrose broth (PDB) medium. The cultures were incubated in the dark for 30 days without shaking at 22 °C. Then fungal growth was gently removed, and filtrates were centrifuged at 4 °C for 30 min at 12000×g. The purified culture filtrate was then sterilized by filtration through a syringe filter (0.22 µm pore size) and stored in sterile bottles at 4 °C until use (Elsherbiny *et al*., 2019).

Impact of *P. lilacinum* culture filtrates on mycelial growth and sclerotial formation of *S. cepivora*

The sterilized fungal filtrate was considered as 100% concentration. Different concentrations of previously prepared culture filtrates of *P. lilacinum* viz., 25, 50 and 75 were prepared by adding different volumes of this filtrate to melted PDA medium then (PDA) poured into sterile Petri dishes (90 mm in diameter). The culture filtrates free medium was used as a control. After solidification, mycelial discs (5 mm in diameter) from 5 days old cultures of *S. cepivora* (Scep7 isolate) were planted in the prepared PDA dishes. The dishes were incubated for 4 days at 20 °C. Five replicate plates were used for each concentration. Mycelial growth reduction percentage was calculated according the following formula:

$$\text{Reduction of mycelial growth (\%)} = \frac{(\text{mycelial growth in control} - \text{mycelial growth in treatment})}{\text{mycelial growth in control}} \times 100$$

After 15 days, the sclerotial formation inhibition was calculated as follows:

$$\text{Reduction of sclerotial formation (\%)} = \frac{(\text{number of sclerotia in treatment})}{(\text{number of sclerotia in control})} \text{ X } 100$$

Impact of culture filtrates on *S. cepivora* myceliogenic sclerotial germination

The effect of culture filtrates of *P. lilacinum* on *S. cepivora* myceliogenic sclerotial germination was carried out using sclerotia formed on PDA medium after 30 days of incubation at 20 °C (Elsherbiny *et al*., 2019). Inhibition of myceliogenic germination was estimated according to the following formula:

$$\text{Inhibition of myceliogenic germination} = \frac{(\text{number of myceliogenically germinated sclerotia in treatment})}{(\text{number of myceliogenically germinated sclerotia in control})} \text{ X } 100$$

Impact of culture filtrates on *Stromatinia cepivora* cell membrane permeability

Stromatinia cepivora cell membrane permeability as affected by *P. lilacinum* culture filtrate at 75% was carried out using the method described by Elsherbiny *et al*. (2019). The relative conductivity in each treatment as indicator of cell membrane permeability for mycelia was determined as follows:

$$\text{Relative conductivity (\%)} = \frac{\text{conductivity}}{\text{final conductivity}} x100$$

The experiment was repeated twice with five replicates for each treatment.

Impact of culture filtrates on sclerotial viability

Production of sclerotia

Five mm mycelial discs obtained from 5 days old *S. cepivora* culture on PDA medium were used to inoculate apparently onion bulbs (Giza red cv.) at their basals (Coley Smith, 1985). Inoculated onion bulbs were stored in sterilized moist sand plastic containers (40 x 40 x 20 cm) at 18 ± 2 °C. The formated sclerotia on the stored onion bulbs were gathered after 6 weeks and maintained (Gerbrandy, 1992).

***In vitro* experiment**

Sandy-loam soil was wetted to 25% of the field holding capacity and mixed with 100 ml 75% concentration

of *P. lilacinum* culture filtrate and left in glass jar (2 L volume). Three mesh bags containing sclerotia-sand mix (10 sclerotia/g sand) were buried in the middle of the jar. Soil without culture filtrate additions containing mesh bags of sclerotia was served as control. After 4 months of incubation at 18 ± 2 °C, the sclerotia were picked up from soil and counted under a stereo microscope. The retrieved sclerotia were surface sterilized using 2% NaOCl for 1 min, then washed 3 times in sterile distilled water and dried using sterilized Whatman No. 1. The dried sclerotia were sown individually onto PDA medium droplets in Petri plates and incubated for 7 days in dark at 18 ± 2°C. The sclerotial viability percentage, related to the previously sclerotial buried number, was evaluated according to Ritchie *et al.* (2013). Five replicates were designed for each treatment, and the experiment was repeated twice.

2. Greenhouse experiments

Impact of culture filtrates on sclerotial viability

The experiment was carried out on November first 2016, under greenhouse conditions at a temperature range 10 to 20 °C. Sixteen kg unsterile sandy-loam soil in absence of *S. cepivora* sclerotia were filled into polystyrene containers (50 x 50 x 30 cm). Metcalf *et al.* (2004). Soil was then amended with 5% (v/w) with 75% of *P. lilacinum* culture filtrate, then moisten reached to 25% and preserved throughout the course of the experiment. Soil without culture filtrate was used as a control. To determine the influence of culture filtrate on sclerotia viability, five nylon mesh bags including 100 sclerotia were buried in each polystyrene container at a depth of 10 cm. Five polystyrene containers were used for each treatment. Nylon mesh bags including the sclerotia were picked up from soil 180 days after application and the sclerotial viability percentage, corresponding to the previously sclerotia number buried, was calculated as mentioned before.

Impact of culture filtrates on white rot incidence, severity and yield production

Recent soil which used to determine the sclerotial viability was used after removing the nylon mesh bags to evaluate the white rot incidence. Soil in polystyrene containers were transferred to sterilized pots (30 cm in diameter containing 16 kg soil). Soil was infested with *S. cepivora* sclerotia according to the method described by Abd El-Moity (1976), then soil wetted to 25% of water holding capacity and maintained under greenhouse conditions. Onion seedlings (five seedlings cv. Giza red, 50- days old/ pot) were sown on November 1st, 2017. Five pots were used as replicates even for the control ones. After 100 days from sowing date number of infected onions and infection percentage as well as the disease severity, were evaluated. White rot infection percentage was calculated according to the formula: white rot incidence = No. of infected plants/No. of total plants × 100 (Brix and Zinkernagel, 1992). The individual infected plants were rated for disease severity and calculated according to Zewide *et al.* (2007). The onion bulbs fresh weight in each pot was also calculated after onion maturity as g/pot.

3. Field experiments

Selection of the experiment locations

Two districts at El-Sharqia Governorate, Abo Hamad and Belbis, in which different fields have a well-established record history of white rot disease incidence were selected. The *S. cepivora* population density (sclerotia number) of white rot disease was preliminarily determined according to the procedure of Utkhede and Rahe (1979). Belbis location was defined by a rate of 77.67 sclerotia/kg soil. While, Abo Hamad district, was characterized by a rate of 627.33 sclerotia/kg soil. To avert the dormancy of sclerotia, the two chosen fields did not cultivate with onion crops for two years before initiation this experiment. The experiment was designed from November 2017 to May 2019 and included two stages. The first stage (November 1st, 2017- May 1st, 2018) to evaluate the efficiency of culture filtrate application in the absence of onion crop on the frequency and sclerotial viability of *S. cepivora*. The second stage (November 1, 2018- May 1, 2019) to estimate the efficiency of culture filtrate application on white rot disease incidence, onion growth parameters and yield production.

Culture filtrate application

In each location, the investigation plot area was divided into two blocks each of 5.0 x 55.0 m with 5.0 m margins in between. Each block was divided into six plots, each of 5.0 x 5.0 m plot areas and separated by a 5-m border. Randomized block designs with six replicates were designed for each treatment. At the beginning of the experiment, field water holding capacity was adjusted to be 25% using irrigated water. Culture filtrate of *P. lilacinum* (75%) was utilized at a rate of 100 L/feddan (1.04 L/ plot) three times: 1/11/2017, 1/1/2018 and 1/3/2018. At the time of field application, the amount of 1.04 L culture filtrate per plot was applied to soil as drenching suspension. Plots drenched only with water was used as a control. Soil in each replicate of the first application and before sowing at 1/11/2017 was tilled to a depth of 15 cm to boost culture filtrate permeation pursued by flood irrigation. Soil was irrigated as needed (Crowe and Hall, 1980). Attentively soil was not allowed to shift from replicate to another.

Impact of culture filtrate on sclerotial viability

The sclerotial viability of *S. cepivora* was evaluated from each replicate of each field directly before applying the culture filtrate three times every two months of the last application. Thus, soil samples were evaluated four times: 31/10/2017, 31/12/2017, 28/2/2018 and 30/4/2018. In the second time, soil samples were evaluated only before onion sowing date (31/10/2018). The number of viable sclerotia was determined according to Utkhede and Rahe (1979). The obtained sclerotia were gathered from recent technique and were then sterilized in sodium hypochlorite (2%) for 2 min, rinsed twice in sterile distilled water then dried. The disinfested sclerotia were subsequently implanted on Petri plates containing PDA medium and incubated at 18 ± 2 °C for two weeks. Plates were inspected at x10 using stereo microscope. The inoculum densities were considered as the number of viable sclerotia per kg soil.

Impact of culture filtrates on white rot incidence

Field plots were prepared for sowing onion seedling on November 1st, 2018. Soil plots were hand spaded and tilled. Six replicate plots were used for each culture filtrate as well as untreated controls. Plot area was 3.0 x 3.5 m and each plot consisted of 6 rows (3.0 in length and 50 cm in width). Fifty days old, onion (cv. Giza red) seedlings were transplanted in each row at a spacing of 15 cm x 15 cm up reaching to maturity under flood irrigation, fertilization and

pest management practices were followed. White rot disease incidence was evaluated periodically during the 2018/2019 growing season depended on the external symptoms appeared on infected onion. Infected onion bulbs (%) as well as the reduction (%) was determined according to Elshahawy *et al.* (2017b).

Impact of culture filtrates on plant growth parameters and bulb yield

Onion growth parameters involving plant height (cm), number of leaves/plant and plant biomass (g), were calculated after 120 days of transplanting. At onion maturation period (180 days from transplanting), onion bulbs were collected and weighed (kg/plot).

Impact of culture filtrates on defense-related enzyme activity

The effect of tested *P. lilacinum* culture filtrate preparations amended to the soil on the activities of the defense enzymes of peroxidase, polyphenol oxidase and chitinase of onion plants grown under field conditions were estimated 60 days after sowing. Procedure described by Elshahawy *et al.* (2017a) was followed to extract the enzymes. Peroxidase activity was estimated as described by Lee (1973) and polyphenol oxidase enzyme activity as described by Bashan *et al.* (1985). Chitinase enzyme activity was preformed using the method described by Monreal and Reese (1969).

4. Statistical analysis

Data were subjected into SPSS software version 14.0 and analyzed statistically by the analysis of variance test (ANOVA) and the means were compared by Duncan's multiple range test at $P< 0.05$ according to Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Dual culture test

The results of dual culture test showed that the fungus *P. lilacinum* has high suppression potency against *Stromatinia cepivora*. No physical touch between *P. lilacinum* and *S. cepivora* was detected during incubation for seven days at 22 °C (Fig. 1). In addition, *P. lilacinum* produced an inhibition zone (IZ) by 8.0 mm in front of *S. cepivora* growth, causing reduction in mycelial growth of *S. cepivora* reached 71.23% (Fig. 1). When agar discs from the fungus-free interaction zone picked up and transferred on PDA cultures of *S. cepivora*, the mycelial growth was obstructed (Fig. 1). On the other hand, when transferring uninoculated control agar discs in plates inoculated with *S. cepivora* mycelium simply overgrew them (Fig. 1). It is worthy to mention that *P. lilacinum* has been demonstrated recently as an effective bioagent towards plant-parasitic nematodes threats, specially against the root-knot nematodes (Askary and Martinelli, 2015). The present study affords new insights about using *P. lilacinum* as a biocontrol agent for controlling onion white rot.

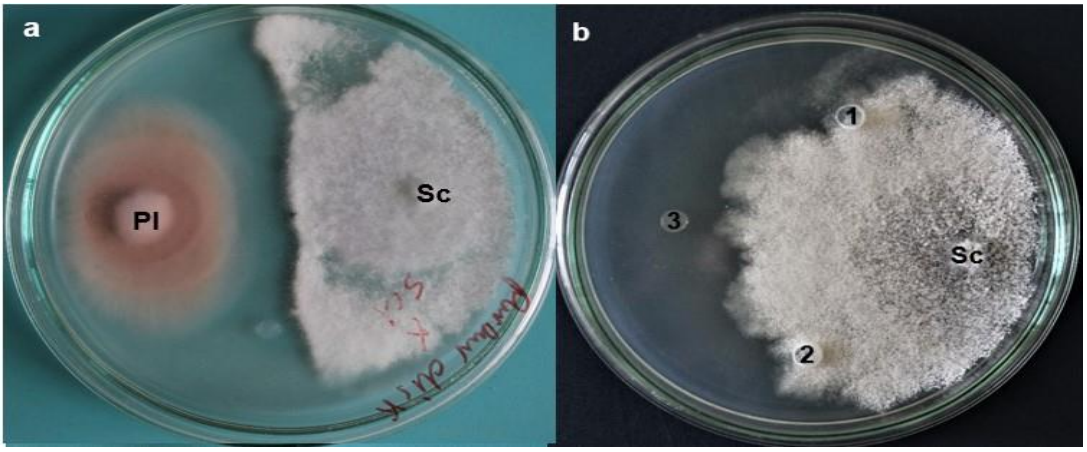

Fig. 1. Dual culture test of *P. lilacinum* (Pl) vs. *S. cepivora* (Sc) after seven days incubation at 22 °C in darkness (a). While in (b) *S. cepivora* mycelial growth in the presence of an agar disk (3) taken from the fungus-free interaction zone, no effect is observed with the agar disks (1 and 2) taken from noninoculated PDA medium dishes.

Effect of culture filtrates on mycelial growth and sclerotial formation of *S. cepivora*

P. lilacinum at the three culture filtrate concentrations significantly reduced the mycelial growth of *S. cepivora* (Table 1). The concentration (75%) of culture filtrate reduced 82.59% of fungal growth, while 74.81% reduction in fungal growth was recorded by using 50% concentration of culture filtrate (Table 1). Furthermore, all tested concentrations of *P. lilacinum* culture filtrate significantly reduced the sclerotia produced by causal pathogen (Table 1). The reduction in sclerotial formation was ranged from 42.72 to 100% according to culture filtrate concentration test (Table 1). Obtained results are in accordance with those registered by Yang *et al.* (2015) and by Elsherbiny *et al.* (2019).

Table 1. Impact of different concentration of *P. lilacinum* culture filtrates on the mycelial growth, sclerotial formation and myceliogenic germination of *Stromatinia cepivora*.

Concentration (%)	Mycelial growth reduction (%)	Sclerotial formation reduction (%)	Myceliogenic sclerotial germination (%)
0	0.0 a*	0.0 a	0.0 a
25	64.07 b	42.72 b	63.33 b
50	74.81 c	70.80 c	86.67 c
75	82.59 d	100.00 d	93.33 d

*** Results represent the means of three replicates. Different letters in the same column are significantly different according to Duncan test (P<0.05).**

Impact of culture filtrates on *S. cepivora* myceliogenic sclerotial germination

The target concentration of *P. lilacinum* culture filtrates exhibited significant depression of myceliogenic germination of *S. cepivora* sclerotia in a dose-dependent pattern (Table 1). It was also obvious from data that the myceliogenic germinated from sclerotia formed on PDA supplemented with different concentration of *P. lilacinum* culture filtrate was significantly inhibited.The highest inhibition of myceliogenic sclerotia germination was obtained from sclerotia formed on PDA supplemented with 75% culture filtrate of *P. lilacinum*, being 93.33%.This might be due to the accumulation of phospholipids and sterol formation in the membrane of *S. cepivora* as well as lipid peroxidation in mycelia which prevent sclerotial development (Lucini *et al*., 2006). Effect of *P. lilacinum* have been reported by Elsherbiny *et al.* (2019) to be a bio-agent against *Sclerotinia sclerotiorum* pathogen and the present data are in accordance with their obtained data. Also, Elsherbiny *et al.* (2019) reported that *P. lilacinum* culture filtrates inhibited the myceliogenic sclerotial germination by 93.5%. This proposes that culture filtrates of *P. lilacinum* may contain lytic enzymes, antibiotics or peptides. They added that the malondialdehyde (MDA) concentration is a sign of cell membrane damage and they observed the MDA accumulation was raised in *S. sclerotiorum* mycelia after treated with culture filtrates of *P. lilacinum* at 75% compared with control.

Impact of culture filtrate on cell membrane permeability

P. lilacinum culture filtrates significantly increased he relative electrical conductivity over time. Conductivity is an index of cell membrane permeability. The treated mycelia of *S. cepivora* with culture filtrate at 75% concentration recorded high value of relative conductivity compared with control over 180 min (Fig. 2). This might be the first case to estimate the effect of *P. lilacinum* culture filtrates on *S. cepivora* cell membrane permeability. The obtained results showed significant increases in cell membrane permeability of *S. cepivora* after remediation with the culture filtrate of *P. lilacinum* at the concentration of 75%. This might be attributed to the increase of the intracellular plasma leakage that causing acute damage in the cell membrane structure of *S. cepivora*. These results are in accordance with the results obtained when the same culture filtrate of *P. lilacinum* used to control *Sclerotinia sclerotiorum* mycelia (Elsherbiny *et al.*, 2019).

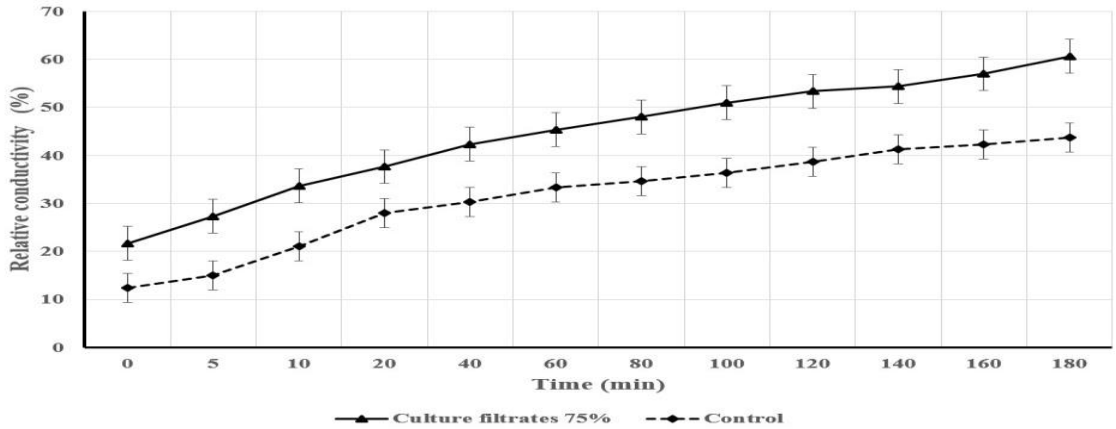

Fig. 2. Relative conductivity of *Stromatinia cepivora* mycelia as affected by *P. lilacinum* culture filtrate treatment at the concentration of 75% (Results represent the means of two experiments).

Impact of culture filtrates on sclerotial viability

Gradual decrease in sclerotial viability was observed according to prolonged exposure to the culture filtrates under laboratory, greenhouse and field conditions.

1. *In vitro* experiments:

Exposed *S. cepivora* sclerotia to *P. lilacinum* culture filtrates resulted in decreasing the sclerotial viability after 180 days of exposure (Table 2).

Table 2. Percentage recovery of *Stromatinia cepivora* sclerotia and their viability as affected by *P. lilacinum* culture filtrate (75%) after 180 days incubation*, in vitro*.

Treatment	Sclerotial recovery (%)	Sclerotial viability (%)*
Non-amended control	90.33 a**	87.67 a
Culture filtrate (75%)	7.67 b	7.50 b

*** Percentages of sclerotial germination were calculated and compared to non-treated control at zero time. Germination of sclerotia at zero time was 100%.**

**** Means within a column followed by the same letter are not significantly different by Duncan multiple range test at P < 0.05.**

After 180 days, 87.67 % of sclerotia had emerged in glass jars non amended with culture filtrates (control) compared with jars treated with culture filtrates (75%) which recorded 7.50% of the sclerotial viability. The decline in sclerotial viability of *S. cepivora* by culture filtrate of *P. lilacinum* selected in the present study exposes that *P. lilacinum* may be created some diffusible substances which constrain the sclerotial germination in the media.

2. Greenhouse experiments

After 6 months, of soil frontage with culture filtrates of *P. lilacinum* at the rate of 5% depressed the recovery of viable sclerotia compared with untreated control (Table 3). At the end of experiment, the survival of viable sclerotia was decreased to 8.67% of culture filtrates treatment compared with 87.33% in untreated soil. The results presented in this study show a proportion of the degraded sclerotia are in accordance with those obtained by Shalaby *et al.* (2013) who recorded that the sclerotial germination percentage of *S. cepivora* was affected by soaking in culture filtrates of *Bacillus subtilis* isolates.

They explained that bacterial filtrates may have lytic enzymes or antibiotics, as reported by recent studies (Stein, 2005 and Gupta *et al*., 2006). Similarly, other studies concluded that glucanase, protease and chitinase secreted by bacterial isolates degrade the role of pathogen cell wall, thus causing a fundamental harm (Mclean and Stewart, 2000 and Clarkson *et al*., 2002). In addition, antibiotics produced by bioagents into filtrates also influence sclerotial germination as well as growth of soil-borne pathogens (Clarkson *et al*., 2002).

White rot disease incidence

After 6 months of soil handled with different concentration of culture filtrates, it was obvious that the white rot incidence was reduced to be 4.90% in case of soil amended with 75% culture filtrate concentration compared with the untreated soil, being 100% (Fig. 3). This treatment led to obtain the least percentage of disease severity (1.70%) comparing with 96.70% for the untreated soil (Fig. 3). Reduction in percentage of infection of onion plants attacked by *S. cepivora* might be referred to the high cumulative of culture filtrates into the root area, before sowing and throughout growing season. Similar demonstration was explained by Kay and Stewart (1994), Shalaby *et al.* (2013) and Elshahawy *et al.* (2017b). Moreover, under greenhouse conditions, *P. lilacinum* culture filtrates have been reported to reduce *Sclerotinia sclerotiorum* disease severity by 83.3% (Elsherbiny *et al*., 2019). The mode of action of *P. lilacinum* culture filtrates referred to antibiotics leucinostatins produced in media by *P. lilacinum*, this antibiotic named paecilotoxins. Different studies have described that leucinostatins have a highly antifungal properties against different plant pathogens (Sharma *et al*., 2016; Wang *et al*., 2016 and Elsherbiny *et al*., 2019).

It was observed that application of *P. lilacinum* culture filtrate of 75% concentration enhanced onion cv. Giza red bulb yield in greenhouse trials. The culture filtrate treatment was more effective than in case of control one where the average of bulb yield of onion in treated soil reached 195.33 g/pot. Whereas, it was 45 g in the untreated soil (Table 3).

Table 3. Effect of culture filtrate (75%) on white rot inoculum density, disease incidence, disease severity and yield (g/pot) in artificially infested soil with *Stromatinia cepivora* sclerotia (6 months incubation), under greenhouse conditions.

Treatment	inoculum density at planting[(a)]	Disease incidence (%)	Reduction (%)	Disease severity (%)	Reduction (%)	Yield (g/pot)
Control	87.33 a*	100.00 a	0	96.70 a	3.30	45.00 a
Culture filtrate	8.67 b	4.90 b	95.10	1.70 b	98.30	195.33 b

[(a)]The number of germinating sclerotia was counted 6 months after application compared with the number of germinating sclerotia before application. Germination of sclerotia at zero time was 100%. *Results represent the means of two experiments. Different letters indicate significant differences according to Duncan test (P < 0.05).

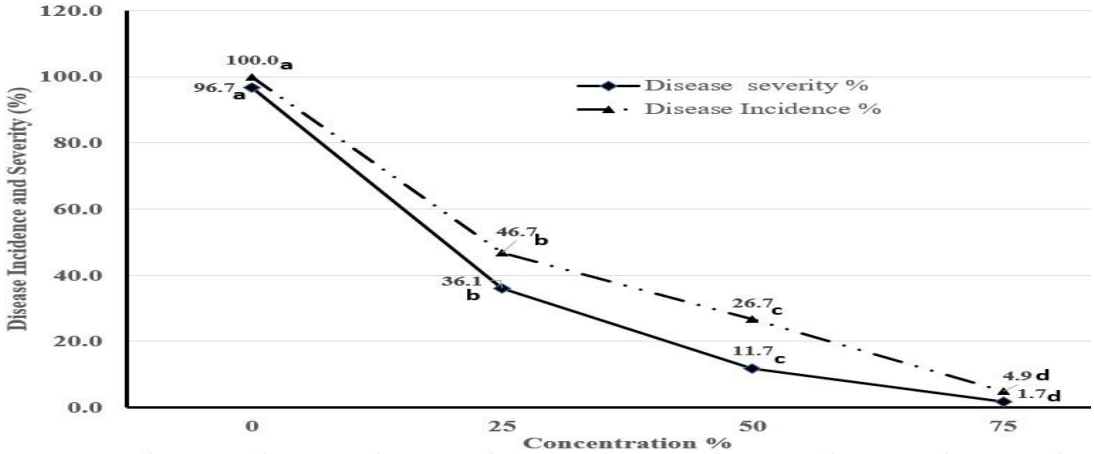

Fig. 3. White rot disease parameters (Disease incidence and severity percentage) due to effect of *Purpureocillium lilacinum* culture filtrates at different concentrations to artificially infested soil with *Stromatinia cepivora* (six months before cultivating cv. Giza red) under greenhouse conditions. Different letters indicate significant differences according to Duncan test (P < 0.05).

3. Field experiments

The tested culture filtrates significantly reduced the sclerotial germination of *S. cepivora* compared with the untreated soil (Table 4). Results obtained revealed that tested culture filtrate influenced the viable of sclerotia in the trials with low inoculum population (trial I). The initial population of viable sclerotia in the trial I of soil before culture application recorded 77.67 sclerotia /kg soil. Subsequently, over the 6 months, the density of viable sclerotia descended progressively. Moreover, after 6 months the reduction percentage recorded 82.84% for culture filtrate treatment. *S. cepivora* sclerotia in the trials with high inoculum population (trial II, 627.33 sclerotia/kg soil) showed also a progressive decrease. Nevertheless, the sclerotia retrieving at each investigating date was greater than that registered during the same investigating date under trial with low inoculum density. After 6 months of initial application in trial II, the reduction percentage recorded 60.57 (Table 4). An additive decrease in the sclerotial population viability was recorded at transplanting date of onion. The viability of sclerotia under field condition depends on many complementary aspects such as initial number of sclerotia, soil type, previous crops, soil inhabitant microflora and environmental conditions, but

how and to what degree they affect the sclerotial viability is not well understood. The present results are consistent with those obtained by Elshahawy *et al*. (2017b), who announced that the sclerotial germination percentage of *S. cepivora* isolate (Sc2) was affected by soaking in bioagent isolates culture filtrates. Furthermore, they suggested that the cultural filtrates of bioagents might have extracellular lytic enzymes or antibiotics. Further study purified and characterized the extracellular β-1, 3-glucanase produced by the bioagent *Chaetomium globosum* (Ahammed *et al*., 2012). Other studies declared the importance of cell wall degrading enzymes as well as toxins which secreted by bioagents in culture filtrates and its affecting role in germination of pathogen sclerotia (Pachenari and Dix, 1980 and Tweddell *et al*., 1994).

Table 4. Numbers of viable sclerotia of *Stromatinia cepivora* in one kilogram of soil sampled immediately before culture filtrate addition (the first sampling date in each trial).

Treatment	Viable sclerotia/kg soil				Reduction % after 6 months
	Sampling date (month)				
	Zero time	2	4	6	
Trial I (77.67 sclerotia/kg soil)					
Control	81.33 a*	78.67 a	73.33 a	69.67 a	10.30 a
Culture filtrate	79.76 a	56.33 b	45.33 b	13.33 b	82.84 b
Trial II (627.33 sclerotia/kg soil)					
Control	654.50 a	637.33 a	619.33 a	607.67 a	3.13 a
Culture filtrate	644.33 a	507.33 b	327.67 b	247.33 b	60.57 b

***Results represent the means for the two experiments. Different letters indicate significant differences according to Duncan test (P < 0.05).**

Application of culture filtrate in field trial has provided similar effect in controlling onion white rot caused by *S. cepivora*. Results of white rot incidence under field conditions followed the same trends in greenhouse, but rot incidence was correlated to the inoculum potential. The average of white rot incidence among onion plants in trial I, containing 77.67 sclerotia/kg of soil, significantly decreased than those in trial II which including density 627.33 sclerotia/kg of soil. Thus, culture filtrate was more efficient in decreasing white rot incidence in the trial with low inoculum density than in that with high inoculum one. In trial I, culture filtrates recorded the least white rot incidence (4.33%) compared with 42.76% in the control soil (Table 5). However, in treated trial II, soil recorded 55.33% of disease incidence compared with 87.33% in the control. The culture filtrates treatment in both trials reduced white rot disease incidence by 89.67 and 36.64 %, respectively. Results were in agreement with the earlier findings by (Kay and Stewart, 1994 and Elshahawy *et al*., 2017b). Several mechanisms might utilize by biocontrol agents including nutrients competition, parasitism, secretion of cell-wall degradation enzymes and/or production of antifungal compounds (Hoitink and Boehm, 1999).

However, in spite of effectiveness of culture filtrates treatment to decrease sclerotial density in field trial II including 627.33 sclerotia/kg, the white rot incidence in subsequent onion crops was very high. This might be due to high accumulation of sclerotial population at planting. The average densities of viable sclerotia of *S. cepivora* in the soil at date of planting, were 57.33 viable sclerotia/kg soil in the field including 627.33 sclerotia/kg (Table 5). Similarly, previous studies proposed direct relation between inoculum density and final white rot disease incidence, where a high inoculum density caused high percentage of mortality plants (Crowe *et al.*, 1980 and Entwistle, 1990). The present results of this study were also in agreement with those reported by Entwistle (1990), who concluded that accumulation of pathogen populations are present and are needed for infection.

Table 5. Impact of *P. lilacinum* culture filtrate (75%) on white rot incidence (as percent of dead plants) in the absence of onion plants during the previous season under field conditions.

Treatment	Viable sclerotia kg/soil[(a)]			Onion white rot	
	Before application	At planting	Reduction (%)	Infected plants (%)	Reduction (%)
Trial I (77.67 sclerotia/kg soil)					
Control	81.33 a*	60.67 a		42.76	-
Culture filtrate	79.76 a	7.33 b	90.81	4.33	89.67
Trial II (627.33 sclerotia/kg soil)					
Control	654.50 a	533.67 a		87.33	-
Culture filtrate	644.33 a	57.33 b	91.10	55.33	36.64

***Results represent the means for the two experiments. Different letters indicate significant differences according to Duncan test (P < 0.05).**

The culture filtrate applied in the absence of onion plants affected the plant growth parameters of onion cv. Giza red in two field trials (Table 6) and growth improvement was correlated to the inoculum density. The average growth parameters of onion plants in soil containing 77.67 sclerotia/kg were significantly greater than in soil containing 627.33 sclerotia/kg of soil. Generally, culture filtrate was more effective in enhancing onion growth parameters in the trial of low inoculum density resulting an average increase of plant height by 68.33%, average number of leaves/plants by 82.19% and average of plant biomass by 81.05% over the control treatment (Table 6) and compared with trial II with high inoculum density.

Culture filtrates application in the absence of onion crop and on onion blub yield at both trials reveal the same trend, the bulb yield kg/plot was greater in trial I than in trial II (Table 7). Results in Table (7) also detected that culture filtrate applied to soil had clarified effects in comparison with the untreated one (control). Plots in the low inoculum density trial increased the average bulb yield of onion per plot by 76.61%.

Plant health and bulb yield were certainly affected by *P. lilacinum* culture filtrate. Culture filtrate significantly enhanced plant growth parameters i.e., plant height, number of leaves/plant and plant biomass in field trials including 77.67 sclerotia/kg. Culture filtrates significantly boosted onion yields compared to the non-amended control in the two field trials. These results are in concordance with the pervious findings of Davis *et al.* (2007) and Elshahawy *et al.* (2019b). This was also supported by Harman *et al.* (2004) who found that after colonization of bioagent, *Trichoderma* spp. of root surfaces and penetration the root epidermis, the bioagent produce or release varieties of compounds that induce localised or systemic acquired resistance. Therefore, plants become guaranteed from the pathogenic fungus attacks by signalizing induction of self-resistance in plants treated with the bioagent isolates.

Table 6. Impact of *P. lilacinum* culture filtrate on plant growth parameters in the absence of onion plants during the previous season (2017-2018).

Treatment	Onion plants growth parameters					
	Plant height (cm)	Increase (%)	Number of leaves/plant	Increase (%)	Plant biomass (g)	Increase (%)
Trial I (77.67 sclerotia/kg soil)						
Control	47.33 b*		5.67 b		70.33 b	
Culture filtrate	79.67 a	68.33	10.33 a	82.19	127.33 a	81.05
Trial II (627.33 sclerotia/kg soil)						
Control	39.67 b		5.33 b		59.67 b	
Culture filtrate	57.67 a	45.37	8.33 a	56.29	88.67 a	48.60

*** Means within a column followed by the same letter are not significantly different by Duncan multiple range test at P < 0.05.**

Table 7. Effects of *P. lilacinum* culture filtrate on onion bulb yield (2018/2019 season) in the absence of onion plants during the previous season (2017/2018 season).

Treatment	Onion bulb yield (kg/plot)			
	Trial I (77.67 sclerotia / kg soil)	Increase (%)	Trial II (627.33 sclerotia / kg soil)	Increase (%)
Control	21.33 b		9.33 b	
Culture filtrate	37.67 a	76.61	15.67 a	67.95

*** Means within a column followed by the same letter are not significantly different by Duncan multiple range test at P < 0.05.**

Defence enzyme activities

The present study supplies clue for the first record that *P. lilacinum* culture filtrates induce systemic acquired resistance (SAR) of onion plants through improving the defence-related enzyme activities.

The oxidative enzymes (PPO and POX) have an important role to eliminate the fungal activity by oxidation the phenolic compounds to oxidised toxic products called quinones, which are harmful to fungi. Several reactions catalyse by peroxidase activity might produce materials that improve plant cell walls. These reactions consist of integration of the phenolics into cell walls through lignification and suberisation processes. Contrarily, chitinase enzymes play roles in plant defence versus fungal attacks by degrading their cell wall. Significantly increasing amount of chitinase in plant cell play a big role to enhance the resistant towards pathogenic fungi where chitin is the major constructive compound in pathogenic cell wall. Results showed that improving the resistance to white rot disease in treated onion plants with *P. lilacinum* filtrates at the concentration of 75% correlated with PPO, POX and CHI enhancement as compared to the control. Results in Table (8) reveal that the culture filtrate application was pronounced in induction of defence enzymes in onion plants compared to the control treatment. Culture filtrate application activated polyphenol oxidase enzyme by 174.57% over the control treatment. Moreover, the culture filtrates increased peroxidase and chitinase enzyme activities by 167.40 and 229.16%, respectively over the control (Table 8). Obtained results are in harmony with those obtained by Sharma *et al.* (2012), Shalaby *et al.* (2013) and Elshahawy *et al.* (2017a).

Table 8. Impact of *P. lilacinum* culture filtrate on peroxidase, polyphenol oxidase and chitinase activities of onion plants grown in soil naturally infested with *Stromatinia cepivora*.

Treatment	Enzyme activities					
	Polyphenol oxidase (PPO)		Peroxidase (POX)		Chitinase (CHI)	
	activity	Increase (%)	activity	Increase (%)	activity	Increase (%)
Control	0.173	-	0.227	-	0.878	-
Culture filtrate	0.475	174.57	0.607	167.40	2.890	229.16

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دور الراشح الفطري لفطر بيربورسليم ليلكنيوم في مقاومة العفن الأبيض في البصل

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يعتبر مرض العفن الأبيض في البصل أحد أهم الأمراض التي تهدد انتاج البصل في مصر. قيمت الدراسة تأثير الراشح الفطري لبيربورسليم ليلكنيوم بتركيز ٧٥% على فطر سترومتينيا سيبيفورا تحت الظروف المعملية والحقلية. أثبتت دراسة التأثير التثبيطي للراشح الفطري على النمو الميسليومي، معدل تكوين الأجسام الحجرية بالأضافة لتأثيره على معدل انبات الأجسام الحجرية تسجيل نسب ٨٢,٥٩، ١٠٠ و٩٣,٣٣ % على التوالي. أدت المعاملة بالراشح الفطري بتركيز ٧٥% الى زيادة معدل نفاذية غشاء خلايا فطر سترومتينيا سيبيفورا مقارنة مع الكنترول. أثبتت الأختبارات المعملية كفاءة الراشح الفطري بفقد حيوية الأجسام الحجرية للفطر الممرض (٩٢,٥ %) عند معاملتها بتركيز ٧٥%، علاوة على خفض نسبة المرض وشدته تحت ظروف الصوبة بمعدلات سجلت ٩٥,١ و٩٨,٣% على التوالي. أدت العدوي الصناعية المسبقة للتربة بالأجسام الحجرية للفطر الممرض ثم معاملتها لمدة ستة أشهر متواصلة قبل زراعة البصل بالراشح الفطري بتركيز ٧٥% الى خفض نسبة وشدة الأصابة بالمرض. خلال المدة الزمنية السابقة (ستة أشهر) وبفحص التربة المعاملة بالراشح الفطرية ثبت فشل الأجسام الحجرية في الأنبات في كلا من حقلي التجارب الأول والثاني بنسبة ٨٠ و٦٠% على التوالي. بعد مرور عام وبالاستمرار بزراعة البصل حقليا أثبتت المعاملة بالراشح الفطري كفاءة بالمقارنة بالكنترول حيث أدى الى خفض نسبة حدوث المرض خاصة في حقل التجارب الأول ذو الكثافة المنخفضة للأجسام الحجرية للفطر الممرض (٧٧,٦٧ جسم حجري/كجم تربة). أدى كل ما سبق من نتائج خاصة بالمعاملات الى تحسين الصفات المحصولية للأبصال المعاملة بالراشح الفطري في الأصص بالأضافة الى زيادة معدلات نمو الأبصال وزيادة إنتاجية الأبصال بالحقل. علاوة على ذلك ارتبط انخفاض نسبة حدوث المرض وشدته مع زيادة لإنزيمات مضادات الأكسدة لكل من البولي فينول اكسيديز، البيركسيديز والكيتينيز بعد المعاملة بالراشح الفطري.