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Effective and Cheap Methods to Control *Sclerotium cepivorum* Through Using Clorox or Sulfur Powder And/or Calcium Oxide

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ABSTRACT

Clorox, carrot roots and *Eucalyptus* leaves and fruits water extracts were tested against *S. cepivorum* growth inhibition on Potato Dextrose Agar (PDA) growth medium. *In vitro* study indicated that Clorox completely inhibited the linear growth of the pathogen, even in a concentration of 1 ppm. Carrot extract inhibited the pathogen growth with inhibition percentage reached from 11 to 20% and from 3 to 8% after 7 and 10 days, respectively (75% w/v). *Eucalyptus* leaves and fruits water extract gave the greatest reduction in the linear growth of the fungus have scored a linear growth value of 2.4±0.5 and 2.3±0.3 cm, respectively compared to the control which gave 8.3±0.28 cm after seven days of inoculation. Furthermore, the field experiments indicated complete inhibition for onion white rot infection with *S. cepivorium* by using either Clorox or sulfur powder and/or Calcium oxide (lime stone).

Key words: *S. cepivorium*, clorox, carrot, *Eucalyptus*, onion white rot

INTRODUCTION

White rot disease on onion (*Allium cepa* L.) is one of the most serious fungal diseases of onion cultivations in many regions of the world. It is a devastating disease caused by a longstanding pathogen *Sclerotium cepivorum* Berk (Crowe *et al*., 1979). Its infection propagules remain active in soil for several years (sclerotia) (Abd-Al-Moity, 1984; Utkhede and Rahe, 1983; Satour *et al*., 1989).

Many approaches have been used to control white rot disease on onion, extending from using fungicides to attempts to producing engineered resistant onion lines. From the early to mid 1980s, the dicarboximide fungicides, iprodione and vinclozolin, were commonly used for the control of white rot on onion (*Allium cepa* L.). However, the presence of high levels of white rot disease in the area where the dicarboximide fungicides were used indicated that these fungicides were losing their effectiveness (Fullerton and Stewart, 1991) due to their enhanced degradation in the soil (Walker, 1987). On the other hand, a related dicarboximide, procymidone was found to be highly effective (Fullerton and Stewart, 1991) because of its relative stability in the soil (Fullertona *et al*., 1995). Fullerton *et al*. (1995) used fungicides of different chemical groups (tebuconazole and triadimenol) to reduce both the risk of fungicide resistance and the selection pressure for fungicide-degrading microorganisms in the soil. They obtained a high level of protection in this experiment. The mode of action of dicarboximides is still not certain. However, Yamaguchi and Fujimura (2005) suggested that they interfere with the osmotic signal transduction pathway consisting of histidine kinase and MAP kinase cascades, causing mutations in the histidine kinase genes (Yamaguchi and Fujimura, 2005; Dry *et al*., 2004; Yoshimi *et al*., 2003; Oshima *et al*., 2002).

The problem with post planting fungicide application may be overcome with the use of fumigation (application of fungicides via irrigation) using a compound with known activity against white rot e.g., methyl bromide or Metam sodium. Methyl bromide is effective, but it will not totally eradicate the fungus and it may not be economical. Metam sodium reduces sclerotia levels but its performance in the control of white rot is sporadic and therefore is less reliable than methyl bromide.

Many efforts have previously been made to detect resistance to *Sclerotium cepivorum* in edible species of the genus *Allium* in field and greenhouse tests (Utkhede and Rahe, 1978; Van Deer Meer *et al*., 1983). Although, several hundred onion lines have now been tested, there is still disagreement about whether or not significant differences in resistance exist in *A. cepa*, although, some reports indicate little or no resistance (Semb *et al*., 1978; Coley-Smith and Ester, 1983). Others have demonstrated substantial differences (Utkhede and Rahe, 1987; Van Deer Meer *et al*., 1983). Most of the varieties of *A. cepa* appear to be highly susceptible (Rahe, 1986) and no genotype with a high level of resistance had yet been found within this species.

Because of the durability and inaccessibility of the sclerotia in the soil, the effect of fungicides, soil sterilants and crop rotations have a limited impact. The absence of truly resistant crop cultivars for *Allium* white rot and the desire to reduce fungicide inputs to avoid soil and environment contamination has therefore led to research on alternative and more sustainable control strategies. These have focused on the use of biological control agents to eliminate sclerotia of *S. cepivorum*. The *Trichoderma* spp. (*T. harzianum* and *T. viride*) degraded *Sclerotium cepivorum* sclerotia and reduced white rot on onions in pots in the glasshouse and in the field (Clarkson *et al*., 2002, 2004).

The use of composted plant wastes to control *Allium* white rot has been developed by many workers (Ismail *et al*., 1991; Coventry *et al*., 2002; Smolinska, 2000). Onion compost, *Eucalyptus* leaf amendments and cruciferous plant residues (*Brassica juncea*, *Brassica napus* cv. Bolk and *B. napus* cv. Gorczonshi) incorporated into the soil, resulted in efficient, disease control and effectively reduced the viability of fungal propagules and significantly decreased the number of *S. cepivorum* sclerotia. This suppression effect was suggested to be due to release phenolic compounds, which stimulate the antagonistic microorganisms.

Genetic modified onion germplasm with enhanced disease resistance is still in juvenil stage, though it is developed with fast paces. Two genes with the potential to improve *Allium* white rot resistance in onion have been identified: The oxalate oxidase (oxo) gene, to be produced by *S. cepivorum* during hyphal penetration and a synthetic magainin (mgd) gene, which produces antimicrobial peptides which may be capable of destroying *S. cepivorum* hyphae during infection (Eady *et al*., 2000). Producing cultivars segregate these genes may be achieved in the foreseen future. Until achieving this task, the laboratories engaged in controlling white rot disease are racing against time to reduce the devasting effect of *S. cepivorium* on onion.

The main goal of this study was to evaluate the efficacy of Clorox to control the white rot disease on onion. This compound is cheap, environmental friendly and more effective than the previous approaches.

MATERIALS AND METHODS

Pathogen isolation: Infected root parts obtained from infected onion bulbs that were surface sterilized by immersion in 1% sodium hypochloride for 3 min, followed by three washes in sterile distilled water. Small portions from the area between healthy and diseased tissues were cut and

transferred onto Potato Dextrose Agar (PDA) Petri plates. Inoculated plates were incubated at 20°C and the fungal growth was examined daily. Developed colonies of the sclerotia were identified to be *Sclerotium cepivorum* according to Metcalf *et al.* (1997). *S. cepivorum* was purified using hyphal tip technique. Disks were taken from each culture (3 slants from each isolate) and kept on PDA slants until further use. *S. cepivorum* was purified and stored on PDA slopes at 4°C for subsequent work.

***In vitro* evaluation of the efficacy of some plant extracts and Clorox on the growth of *S. cepivorum* the causal agent of white rot disease:** The effect of plant carrot root extract, leave and fruit extracts of *Eucalyptus* and Clorox on mycelia growth of *S. cepivorum* were evaluated *in vitro*. The efficacy of the extracts was measured as the mean percent inhibition of mycelial growth.

One hundred grams of carrot roots, leaves and fruits of *Eucalyptus* were rinsed under tap water and blended with 1000 mL of distilled water. The extracts were filtered through coarse filter papers and the filtrates were stored in dark bottles (50 mL) in a refrigerator until use. The stored filtrates were sterilized by filtering through Millipore membranes (0.45 μm) and then were mixed with PDA at 48°C to concentrations of 25, 50 and 75% (v/w). On the other, hand 1 ppm were prepared from Clorox and added to PDA medium. PDA medium free of either plant extracts or Clorox were used as a control.

Evaluation of mycelial growth: The amended media with the used extracts and Clorox were dissiminated into 9 cm plates (10 mL per plate). One (5 mm) mycelial disc was cut from 7 day old colony and seeded in the centre of each plate and incubated at 20°C. The experiment was replicated six times. The colony diameter was measured daily until day seven and then left in incubation, until mycelial growth in the control treatment covered the surface of all cultures. Inhibition of growth was calculated in relation to the growth in the control, according to the equation proposed by Sztejnberg *et al.* (1963):

$$\%\text{Inhibition} = \frac{\text{1-Diameter of treated colony}}{\text{Diameter of control colony}} \times 100$$

Field experiments: Three strategic methods has been tried in order to evaluate its efficiency in inhibiting *S. cepivorium* persistence in contaminated fields as following:

- In field experiment, the farm is ploughed and the upper surface soil is thoroughly mixed. Then, the farm was watered. During farm watering, chlorine was mixed with the watered water in a constant manner to equally disseminate in farm (20 L Clorox/Faddan)
- After the farm has been ploughed the upper surface soil is thoroughly mixed with either Sulfur powder (one package: 10 kg/Fadden) or Calcium oxide (lime stone) with a dose equal to 3-4 package: 90-120 kg/Fadden) just before the farm watering

RESULTS

In vitro evaluation of the efficacy of some plant extracts and Clorox on the growth of *S. cepivorum*. The causal agent of white rot disease. The water extracts of carrot roots and *Eucalyptus* leaves and fruits were evaluated against *S. cepivorum* growth in PDA growth media.

The suppression effect of carrot roots extract on the linear growth of *S. cepivorum* was less than those of other treatments (Table 1 and Fig. 1a). The inhibition percentages ranged between 11 to

Table 1: Evaluation of the efficacy of some plant extracts of linear growth and %inhibition

Treatment	Concentration		Linear growth (cm) 7 days	%Inhibition	Linear growth (cm) 10 days	%Inhibition
Control	(w/v) (%)	0	8.3 ±0.28	-	9.0 00	-
Carrot root extract		25	6.7 ±0.61	20	8.3 ±0.28	8
		50	7.3 ±0.17	13	8.3 ±0.28	8
		75	7.4 ±0.17	11	8.8 ±0.28	3
Eucalyptus leaf extract		25	3.4 ±0.17	40	8.8±0.28	3
		50	2.9 ±0.12	66	4.8 ±0.53	47
		75	2.4 ±0.15	72	4.3 ±0.15	53
Eucalyptus fruit extract		25	6.3 ±0.3	25	8.000	12
		50	3.1 ±0.1	63	7.000	23
		75	2.3 ±0.3	73	6.2 ±0.29	32
Clorox	ppm	1	0	100	0	100

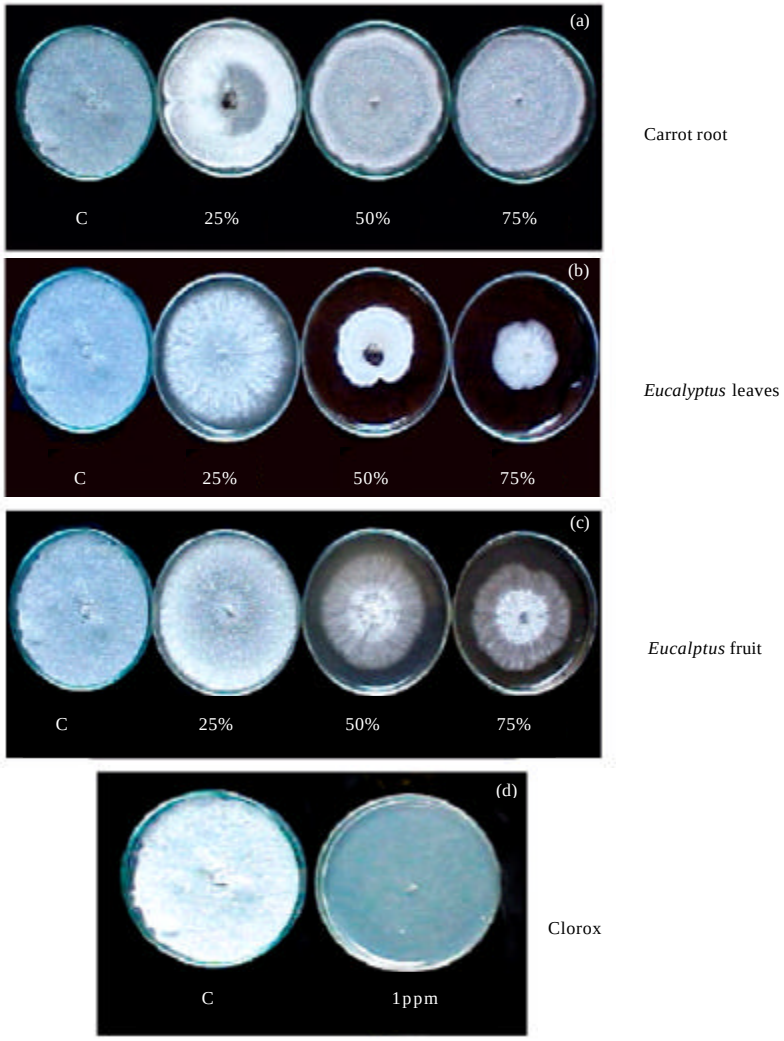

Fig. 1: *In vitro* evaluation of plant extracts and clorox on the growth medium of *S. cepivorum*

20% and 3 to 8% after 7 days and 10 days of inoculations, respectively. The suppression effect decreased with the increase of the incubation time, as in other treatments. The more interesting thing is that low concentration of the carrot root extract was more effective than the highest one. The extracts of *Eucalyptus* leaves and fruits reduced the fungus linear growth (Table 1 and Fig. 1b and c). However, the reduction in the linear growth of the fungus on growth media is proportional with the concentration of the added extractions. The highest concentration (75% w/v) of the water extraction of the leaves and fruits of *Eucalyptus* gave the greatest reduction in the linear growth of the fungus *in vitro,* 2.4±0.5 and 2.3±0.3 cm compared to the control which gave 8.3±0.28 cm after seven days of inoculation. Generally, it can be noticed that the pathogen commenced to build a resistant to the antagonistic effect of *Eucalyptus* leaves and water extracts after 10 days of inoculation. Further, the risk of resistant was relatively higher against *Eucalyptus* fruit extracts after ten days of inoculation (Table 1 and Fig. 1b and c).

In vitro study, Clorox completely inhibited the linear growth of the pathogen, even in a concentration of 1 ppm (Fig. 1d). Also, the field experiments indicated complete control for onion white rot infection with *S. cepivorium*.

In field experiment, examining the soil samples from the fields pre ploughed and watered with chlorine in a constant manner to equally disseminate in farm for the presence of *S. cepivorium* indicated no recovery for the pathogen. Furthermore, treating the infected soil with clorox leads to complete inhibition for either the pathogen vegetative growth or sclerotia. Also, treating the ploughed soil with Sulfur powder (one package/Fadden), or calcium oxide (Lime stone) with a dose equal to 3-4 package/Fadden) just before the farm watering leads to null recovery for the pathogen.

DISCUSSION

Controlling the white rot disease on onion caused by *S. cepivorum* is on the top agenda of in many Agriculture departments in the world. Many controlling methods have been used to mange white rot disease on onion. The method, which eradicates the disease has not yet been reached. In this piece of work we adopt a new approach to deal with this harmful pathogen. The water extracts of carrot roots, *Eucalyptus* leaves, *Eucalyptus* fruits and the Clorox were used to control the growth of the pathogen *in vitro* and *in vivo*.

The consistently decrease of sclerotial populations of *S. cepivorum* during the season in plots of organic cropped with carrots (Banks and Edgington, 1989) stimulated using the water extract of carrot roots to study its effectiveness on *S. cepivorum* linear growth *in vitro*. The carrot roots extract showed a positive limited effect. The positive effect was attributed to the presence of an antimicrobial activity in carrot roots extract (Babic *et al*., 1994). However, the limited effect detected in the present study was interpreted by losing of antimicrobial activity during pigments purification. However, relative high inhibition effect of low concentration of carrot roots extract compared to high one suggested that high concentration may contain some compounds which mask the antimicrobial activity of carrot roots extract. The effective inhibition of the purified carrot roots extract (Babic *et al*., 1994) supports this conclusion.

The inhibitory effect of plant extracts of *Eucalyptus* leaves and fruits on the *S. cepivorum* sclerotial production might be attributed to the presence of some antifungal ingredients. Singh and Dwivedi (1987) recorded fungi toxic activity of the oils of *Eucalyptus globules* against the sclerotia production of *Sclerotium rolfsii*. Salama *et al.* (1988) identified three phenolic compounds in *Eucalyptus* leaf extracts. Inhibitory effects of phenolic compounds, such as salicylic and galic acid extract from leaves of *E. rostrata* on several fungi were studied (Salama *et al*., 1989). Soil

amendment with *Eucalyptus* leaves completely inhibited sclerotial germination of *S. cepivorum* (Salama *et al*., 1988). Ismail *et al*. (1989) found three phenolic compounds (citronellal, cineole and limonene) in the acetone and water extracts of *E. rostrata* leaves, which might be responsible for the inhibition of fungal growth. These compounds, being biodegradable and selective in their toxicity are considered valuable for controlling some plant diseases (Singh and Dwivedi, 1987).

The Clorox totally eradicates the growth of the seclerotia *in vitro*. It has been used for applications, such as the deactivation of pathogens in drinking water, swimming pool water and wastewater, for the disinfection of household areas and for textile bleaching, for more than two hundred years. The active ingredient in colorox is the chlorine, which is one of the most widely used disinfectants. Chlorine can be easily applied, measures and controlled. We anticipate that chlorine inhibited and destroyed both the vegetative mycelium and sclerotia of *S. cepivorum* in a similar way to its killing of other pathogens as bacteria and viruses by breaking the chemical bonds in their molecules. Chlorine compound can exchange atoms with other compounds such as enzymes in fungi or bacteria and/or other cells. When enzymes come in contact with chlorine, one or more of the hydrogen atoms in the molecule are replaced by chlorine. This causes the entire molecule to change shape or fall apart. When enzymes do not function properly, a cell will die. This chemical is potentially useful for the control of onion white rot and its effect will be evaluated under field conditions.

In field experiments when Clorox (chlorine) is added to water, underchloric acids formed. Depending on the pH value, underchloric acid partly expires to hypochlorite ions. Underchloric acid (HOCl, which is electrically neutral) and hypochlorite ions (OCl-, electrically negative) will form free chlorine when bound together. This results in disinfection. Both substances have very distinctive behaviour. Underchloric acid is more reactive and is a stronger disinfectant than hypochlorite. Underchloric acid is split into hydrochloric acid (HCl) and atom air Oxygen (O). The oxygen atom is a powerful disinfectant. The disinfecting properties of chlorine in water are based on the oxidising power of the free oxygen atoms and on chlorine substitution reactions.

The cell wall of pathogenic microorganisms is negatively charged by nature. As such, it can be penetrated by the neutral underchloric acid, rather than by the negatively charged hypochlorite ion. Underchloric acid can penetrate slime layers, cell walls and protective layers of microorganisms and effectively kills pathogens as a result the microorganisms will either die or suffer from reproductive failure. Therefore, we believe that underchloric acid is the effective element in the process of *S. cepivorum* sclerotia destroying especially in field applications.

The results of this study are promising and might be applicable for controlling *S. cepivorum* white rot infection on onion. Considering the inhibitory effect of *Eucalyptus* extract on the mycelia growth of *S. cepivorum*, the addition of leaves or fruit extracts of *Eucalyptus* to soil might reduce the incidence of white rot in onion growing areas contaminated with *S. cepivorum* sclerotia. Also, Using either Clorox or Sulfur powder and/or Calcium oxide at field experiments proved to be a very efficient agents for onion white rot control.

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