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An *in vitro* Study on the Fungicidal Effects of Percidin 535® (Per Acetic Acid 15%) Against Phytopathogenic Fungi

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Abstract: In order to evaluate fungicidal effects of Percidin 535® (Per acetic acid 15%) against some phytopathogenic fungi; six concentration of Percidin 535® including 10, 40, 160, 630, 2500 and 10000 ppm mixed with PDA media were evaluated against *Fusarium oxysporum*, *Rhizoctonia solani* and *Phytophtora citrophthora* radial growth. Antifungal activity was determined in terms of growth inhibitory concentration for 50% growth inhibitory (EC_{50}) and inhibition percentage of some dosages was obtained. Also fungicide effect of Percidin 535® was compared with Captan (Ortocide®) in same concentrations of 1000 and 2000 ppm in PDA media. Compound transport by root to aerial parts was evaluated by adding to soil of soybean plants in pots and culturing some parts of leaves and shoots in equal size on PDA media against mycelia plug disc of *Fusarium oxysporum*. Percidin 535® showed a significantly high effect against linear growth of tested fungi. At 10000 ppm Percidin 535® gave a complete inhibition on mycelia growth of tested species. EC_{50} for *Fusarium oxysporum*, *Rhizoctonia solani* and *Phytophtora citrophthora* were counted 1381, 887.91 and 947.65 ppm, respectively. Percidin 535® was effective than Captan (Ortocide®) in mycelia growth inhibition of *Fusarium oxysporum*. The results of compound transport indicate that PAA is able to move in soybean plant from root to aerial parts.

Key words: Per acetic acid, percidin 535®, fungicide, *Fusarium oxysporum*, *Rhizoctonia solani*, *Phytophtora citrophthora*

INTRODUCTION

Per acetic acid (PAA), the peroxide of acetic acid, is one of the most important organic peroxides with wide spectrum of antimicrobial activity (Abd-Alla *et al*., 2006). Per acetic acid is a mixture of acetic acid (CH_3COOH) and hydrogen peroxide (H_2O_2) in an aqueous solution. It is a very strong oxidizing agent with no foaming capability and has stronger oxidation potential than chlorine or chlorine dioxide, innocuous and lack harmful decomposition products and infinite water solubility (Bundgaard-Nielsen and Nielsen, 1996). PAA's primarily used in food processing and handling, is as a sanitizer for food contact surfaces and as a disinfectant for fruit, vegetable, meat and eggs (Evans, 2000) also used to treat bulbs, seed treatment to inactive fungal or other types of plant disease (Bundgaard-Nielsen and Nielsen, 1996; Mari *et al*., 1999, 2004; Hopkins *et al*., 2003; Abd-Alla *et al*., 2006). In addition, it has been widely used in medical and hygiene fields and disinfection of waste water (Zhao *et al*., 2008). There is a growing to develop alternative chemicals recognizing as safe, less harmful to human health and the environment, for controlling phytopathogenic fungi. Identification of such compounds with wide biological activity and more environmentally friendly is critical for mankind as it aids in the search for chemical structures that should help in control and inhibition of the growth phytopathogenic fungi and bacteria without chemicals problem. The use of chemical fungicides has residual harmful effect to the man which causes dangerous disease. Moreover, some fungal isolates developed significant resistance to use fungicides.

The objectives of this study were to evaluate the efficacy of PAA as a fungicide for inhibitory mycelia growth of *Fusarium oxysporum*, *Rhizoctonia solani* and

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Phytophtora citrophthora. Also fungicide effect of PAA was compared with Captan (Ortocide®) in same concentrations as well as compound transport ability from root to aerial parts was evaluated.

MATERIALS AND METHODS

Experiment was carried out at Biology laboratory of Department of Plant protection, Faculty of Horticulture and Plant protection, University College of Agriculture and Natural resources, University of Tehran, Iran at 2006.

In order to evaluate fungicidal effects of Percidin 535® (Per Acetic Acid 15%) against phytopathogenic fungi; six concentrations of Percidin 535® including; 10, 40, 160, 630, 2500 and 10000 ml L^{-1} PDA medium were tested against linear growth of the *Fusarium oxysporum*, *Rhizoctonia solani* and *Phytophhtora citrophthora*. A 5 mm diameter mycelia disc with a No. 3 cork borer were picked up from the edge of the *Fusarium oxysporum*, *Rhizoctonia solani* and *Phytophhtora citrophthora* 7 days-old culture and placed on the center of Petri-dishes containing PDA consisting tested concentrations of Percidin 535®. In control growth plates, there are no concentration of Percidin 535® exist. Petri dishes were sealed by wrapping them with plastic film and incubated at 25±1°C for 7 days or until the control plates reached full growth. The experiment was conducted with four replications for each treatment. Radial growths of the pathogens were recorded each day and the average diameter was calculated. The average mean growth of the measurements was applied to determine the EC_{50} values (concentration causing 50% inhibition of mycelia growth on control media). EC_{50} values were calculated from the data subjected to probit analysis (Statistical software SPSS 7.0 Inc Chicago, IL). Also percent inhibition of average growth mycelia in relation to growth of the controls was calculated by using Abotte's formula (Edington *et al*., 1971):

$$I = (C-T)/C \times 100$$

where, I is percentage inhibition of radial mycelia growth, C is radial growth measurement of the pathogen in control and T is radial growth of the pathogen in treated plates.

For evaluation of fungicidal effects of Percidin 535® in compared with Captan (Ortocide®), two common used concentrations of Captan (Ortocide®) on some phytopathogenic fungi (1000 and 2000 mg L^{-1} Ortocide®) were evaluated in same concentrations of Percidin 535® against *Fusarium oxysporum* by culturing a 5 mm in diameter mycelia plug on affected medium. The experiment was conducted with 4 replications for each treatment and in control plates there were no contents of Ortocide® or Percidin 535® were exist. The inhibition percentages were calculated by using mentioned formula. In order to evaluate Percidin 535® transport by root to aerial parts of plants, 2000 ml L^{-1} in double distilled water of Percidin 535® was added to soil of soybean plants that were planted one month ago in pots. Four plots per each treatment were prepared. Some parts of leaves and shoots in equal size on PDA media after 48 h were cultured against same distance towards mycelia plug disc of *Fusarium oxysporum*. In control plants, double distilled water with no contents of Percidin 535® was used. The inhibition zone of *Fusarium oxysporum* growth around the cultured parts of soybean was measured in each plate and compared with control.

RESULTS AND DISCUSSION

The global drive for sustainable agricultural systems involves optimizing resources to satisfy human needs and at the same time maintaining the quality of the environment and sustaining natural resource. These important aspects make PAA a very interesting chemical in controlling plant disease reducing environmental contamination and human health risks.

PAA was patented in 1950 to treat fruits and vegetables to reduced spoilage from bacteria and fungi destined for processing. Nowadays there is a long history of experimental field use as a fungicide/bactericide; efficacy has only recently established (Abd-Alla *et al*., 2006).

The primary mode of action is oxidation. PAA disinfects by oxidizing of the outer cell membrane of vegetative bacterial cell, endospore, yeast and spores. The mechanism of oxidation is the transfer of electrons, therefore the stronger the oxidizer, the faster electrons are transferred to microorganism and the faster the microorganism is inactivated or killed. PAA also inactivates enzymes that are responsible for discoloration and degradation, such as proxidase in the browning of potatoes (Greenspan and Margulies, 1950). It is demonstrated that PAA has a higher oxidation potential than chlorine sanitizers but less than ozone.

Results in Table 1 indicated that all concentrations of tested PAA against linear growth of the pathogenic fungi have inhibitor effect on mycelia growth. Their effects increased with increasing the PAA content. Also PAA at 10000 ppm gave a complete reduction in all tested fungi linear growth and transferring of these cultured disks into

Table 1: Effect of per acetic acid on preventing linear growth of phytopathogenic fungi (% linear growth inhibition)

	Per acetic acid concentration (ppm)					
Fungus	10	40	160	630	2500	10000
Fusarium oxysporum	6.90*	10.60	13.10	23.03	86.14	100
Rhizoctonia solani	13.85	16.29	19.46	38.20	97.19	100
Phytophtora citrophthora	6.30	12.35	14.34	35.65	98.00	100

*Values are means of four replicates

Table 2: EC_{50} (µl L^{-1}) values of Percidin 535® on phytopathogenic fungi

Fungus	EC_{50} (µl L^{-1})
Fusarium oxysporum	1381 (758.9-3022.9)*
Rhizoctonia solani	887.9 (396.7-2827)
Phytophtora citrophthora	947.7 (472.4-3500.7)

*Numbers in parentheses indicate 95% confidence limits determined by probit analysis

PDA plates did not showed fungal growth after 7 days that confirming fungicidal activity of PAA. The antifungal activity of PAA was confirmed also against some other phytopathogenic fungi (Mari *et al*., 1999, 2004; Abd-Alla *et al*., 2006).

EC_{50} were recorded 1381, 887.9 and 947.7 ppm Percidin 535® for *Fusarium oxysporum*, *Rhizoctonia solani* and *Phytophtora citrophthora*, respectively. The most fungicidal activity was observed against the *Rhizoctonia solani* (Table 2). The some different observed in antifungal activity of PAA may suggest susceptibility of various phytopathogenic fungi against oxidation potential of PAA.

Percidin 535® was effective than Ortocide ® and obtained 98.86, 99.42% reduction in fungal growth at 1000 and 2000 mg L^{-1} respectively while they were 95.46 and 97.88% about Ortocide® at the same concentrations, if these treatments compared with control treatments. Percidin 535® transported by root to aerial parts of soybean plants.

It is noted that PAA has excellent antifungal and sporocidel activities (Alasri *et al*., 1993; Mari *et al*., 1999; Hopkins *et al*., 2003) suggesting the possibility of using PAA as a substitute of fungicides with desirable properties in controlling post harvest disease during handling, transportation and storage (Mari *et al*., 1999, 2004; Abd-Alla *et al*., 2006), seed treatment (Hopkins *et al*., 2003) and other type of plant disease (Hanks and Linfield, 1999). limited data are available on the control of phytopathogenic fungi in field conditions by PAA, so more studies are needed to test another important phytopathogenic fungi and further study are also needed to test it phytotoxicity towards host plants.

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