International Journal of Agricultural Sciences ISSN: 2167-0447 Vol. 5 (5), pp. 849-853, July, 2015. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

The effectiveness of wood vinegar in controlling *Rhizoctonia solani* and *Sclerotinia sclerotiorum* in green house-cucumber

Mahin Saberi[1], Abolfazl Sarpeleh[2], Hassan Askary*[2] and Flora Rafiei[1]

[1]Agriculture college, Islamic Azad University, Ashtian branch, Ashtian, Iran
[2]Iranian Research Institute of Plant Protection, Tehran, Iran.

Accepted 24 October, 2013

Wood waste recycling in addition to reducing environmental hazards can replace chemical pesticides for organic farming purposes. In this study, the inhibitory effects of non-volatile and volatile components of wood vinegar were evaluated on the mycelial growth of *Rhizoctonia solani* and *Sclerotinia sclerotiorum* as well as on the control of root and crown rot disease of green house-cucumber (*Cucumis sativus* L.) induced by *R. solani*. To study the effects of wood vinegar, fresh mycelial disks of *R. solani* and *S. sclerotiorum* were placed on Petri dishes containing artificial media and different concentrations of wood vinegar (0.75, 0.5, 0.37, 0.25, 0.125, 0.05, 0.025 and 0%). Both volatile and non-volatile components of wood vinegar inhibited significantly the mycelial growth of both pathogens (α =0.05). Three concentrations of wood vinegar (0.125%, 0.25% and 0.50%) inhibited significantly the mycelial growth of *R. solani* were used to control the associated disease in cucumber plants inoculated with this pathogen. Diseases severity was significantly reduced in all concentrations ($\alpha_{=}$ 0.05) compared to untreated control plants. Wood vinegar reduced the pathogenicity rate of the pathogen up to 87% compared with the untreated control.

Key words: Pathogen, soil-borne, fungistatic, natural products, growth stimulator, pyroligneous acid.

INTRODUCTION

Rhizoctonia solani is one of the most important fungal pathogens induces root, crown and stem rot disease in a variety of crops such as cucumber (Sneh et al., 1991; Strashnov, 1985; Tu et al., 1996). *Sclerotinia sclerotiorum* is the causal agent of rot in many vegetable crops including cucumber, can cause severe early infections that results in high yield losses of greater than 50% in the crop fields and green houses (Jagger, 1920; Kohn, 1979; Cho et al., 1997; Kim et al., 1999). In an intensive survey on six cucurbitaceous crops namely nettedmelon, cucumber, pumpkin, summer squash, watermelon and oriental melon, disease incidence by *S. sclerotiorum* was recorded as high as 30-70% (Kim et al. 1999).

In general, several chemical, biological and cultural methods are being used to control these pathogens (Li et al. 2006). Different methods have been used to control *R. solani.* Chemical fungicides are often used when losses from *R. solani* are substantial (Brewer and Larkin 2005). However, current cultural and chemical controls are not completely effective and *Rhizoctonia* disease remains a persistent problem.

Biological control is an efficient and environmentally friendly way to prevent damping-off disease. Many microbial species such as *Trichoderma atroviride* (Reithner et al., 2007) *T. harzianum* (Hadar et al., 1979), *Pseudomonas fluorescens* (Nagarajkumar et al., 2004) and *Bacillus subtilis* (Asaka and Shoda 1996) have been shown to effectively control *R. solani*. Fluorescent pseudomonads are one group of rhizospheric bacteria that have been described as biological control agents and showed great promise with respect to protecting plant roots by reducing the incidence of fungal-induced diseases (De La Fuents et al., 2004; Andersen et al., 2003).

Wood vinegar (WV) also called as Pyroligneous acid is a brown transparent liquid that is produced by the condensation of the smoke from the process of producing

*Corresponding author. E-mail:askary2@gmail.com

charcoal (Anonim, 2001). The synonyms for WV include pyrolysis oil, pyrolysis liquid, wood liquid, liquid smoke, liquid wood, bio-oil, bio-crude oil and wood distillate (Zulkarami et al., 2011). Major groups of compounds in WV includes: hydroxy aldehydes, hydroxy ketones, sugars, carboxylic acid and phenolic acid (Fengel and Wegener, 1983; Guillen and Manzanos, 2002).

It is a completely natural product that is inexpensive and without any destructive or adverse environmental effects on living organisms (Yatagai et al., 2002). It has been shown to inhibit several fungal plant pathogens (Yodthong et al., 2008).

Wood Vinegar obtained from the apricot tree, has restricted the mycelial growth of *Plasmopara viticola, Verticillium dahliae, Phytophthora capsici* and *Fusarium graminearum* (Qiaozhi et al., 2009). Application of WV at a 1:32 dilution completely restricted the mycelila growth of *Alternaria mali* (causing agent of apple *alternaria* blight) where it was as efficient as polyoxin-B fungicide at 2 mg/ml (Jung, 2007). Wood Vinegar extracted from *Cryptomeria japonica*, showed effective antifungal properties on *Ralstonica solanacearum, Phytophthora capsici* and *Pythium splendens* (Hwang et al., 2005). Velmurugan et al. (2009), observed that WV obtained from bamboo, reduced significantly the growth of *Ophiostoma* spp. the causal agent of wood rot in the forest trees. In the present study, inhibitory effects of WV in controlling plant pathogens were examined on *R.solani* and *S.sclerotiorum* in vitro and in situ conditions.

METHODS

Fungal Isolates and Wood Vinegar Preparation

R.solani and *S.sclerotiorum* was isolated from the infected cucumber collected from greenhouses at Varamin and Garmsar Cities, Iran. Wood vinegar prepared from citrus wood and obtained from Iranian research institute of plant protection. It mainly includes acetic acid, methanol, acetone, phenol and tar with a pH of 3.4. Wood Vinegar was initially infiltrated using Wattman filter paper No. 1 (Maidston, England) and then sterilized using 0.22 µm filter (Sartorious AG, Gottingen, Germany) before assessment for antifungal activity.

The Effect of Non-volatile and Volatile Components of WV on *Sclerotinia sclerotiorum* and *Rhizoctonia solani*

Sterile solution of WV was mixed with 20 ml melted potato dextrose agar medium to give concentrations of 0.025, 0.05, 0.125, 0.25, 0.37 and 0.75 (V/V%). A 3-mm disk of agar with *R.solani* and *S.sclerotiorum* hyphae was cut from a 4-day old culture and placed on Petri dishes containing the the respectively concentrations of WV. The cultures were incubated at 25±2 ˚C for 10 days.

The inhibitory effect of volatile components of WV was examined using bipartite Petri plates where a 3-mm mycelial disk of either pathogen was placed in one side of the Petri dish containing 15 ml of PDA culture medium and different concentrations of WV were added to the opposite side. The plates were then sealed with Parafilm and kept at 25±2 ˚C, up to 10 days.

In both experiments, colony diameter was measured and growth suppression calculated relative to the untreated controls. Four replicates (Petri dishes) were considered for each concentration.

The Effect of WV on the Control of Root and Crown Rot Disease

Cucumber seeds CV Sultan were sown in seedling tray containing coco peat and Perlite (1:3 ratios). At one-leaf growth stage, seedlings were transplanted to pots containing sterile soil which included 20% rotted leaf soil, 15% perlite, 30% rotted animal manure, 20% sand and 15% virgin soil. Plants were maintained in a greenhouse at 22- 24°C under 12/12h light/dark conditions. Four days after transplanting, cucumber plants were inoculated with two plugs (5 mm in diameter) of actively growing mycelium of *R. solani* placed close to the main root. Control plants were treated with sterile agar plugs. The test was performed in a completely randomized design with 5 treatments (Table 5) and 5 replications.

Three concentrations of WV, including 0.125, 0.25 and 0.5% which showed significant inhibitory effects on mycelia growth of *R. solani*, were drenched into the soil one day after inoculation and two weeks interval until 45 days post-inoculation. The pots were kept in greenhouse and the plants were checked for disease symptoms every second day. When 60% of inoculated plants showed disease symptoms, the efficacy of WV was evaluated considering the incidence and severity of the disease. Disease severity index (DSI) was determined based on a scoring system introduced by Mathew and Gupta (1996) as 0: Healthy plant, 1: 1-10% necrotic roots, 2: 21-40% necrotic roots, 3: 41-60% necrotic roots, 4: 61-80 necrotic roots and 5: 81-100% necrotic roots.

Statistical Analysis

All obtained data subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS institute) program (version 9.2). Comparison of means was analyzed by Duncan's multiple range test and differences were considered significant when $P<05$.

RESULTS AND DISCUSSION

The Effect of Non-volatile and Volatile Components of WV on the Growth of *S. sclerotiorum*

Non-volatile phase of WV decreased significantly the growth of the fungus at 0.37, 0.50 and 0.75 concentration

Table 1. The effect of non-volatile phase of Wood vinegar on the mycelia growth of *S. sclerotiorum.*

Concentration of WV (%)	Colony diameter (mm)	Anti-fungal activity (%)
0 (Control)	78	-
0.025	75	3.84d
0.05	74	5.12d
0.125	56	28.20cd
0.25	47	39.74c
0.37	7	91.02b
0.5	6	92.30b
0.75	0	100a

C.V. =14%
Means with similar letters are not significantly different based on Duncan's Multiple Range Test (α=0.05).

Table 2. The effect of Wood vinegar volatiles on *S. sclerotiorum* colony in the laboratory.

Concentration of WV (%)	Colony diameter (mm)	Anti-fungal activity (%)
0 (Control)	80	-
0.025	80	0c
0.05	80	0c
0.125	80	0c
0.25	80	0c
0.37	80	0c
0.5	67	16.25b
0.75	59	26.25a

C.V. = 3 %
Means with similar letters are not significantly different based on Duncan's Multiple Range Test (α=0.05).

of WV (V/V%) (Table1). The mycelial growth was completely suppressed at 75% concentration of WV. Volatile phase of WV at 0.50 and 0.75 concentrations (V/V%) caused a significant inhibition in the mycelia growth of *S. sclerotiorum* where the fungus colony was measured 67 and 59 mm in diameter respectively (Table 2).

The Effect of Non-volatile and Volatile Components of WV on the Growth of *R. solani*

The mycelia growth of *R. solani* was significantly restricted in the presence of WV (Table 3). No mycelia growth of the fungus was observed at 0.75, 0.5 and 0.37 concentration of WV (V/V%).

Volatile metabolites of WV caused a decrease in the mycelia growth of *R. solani* (Table 4). There was a positive correlation between WV concentration and growth of fungal colony where the maximum anti-fungal activity was observed for 0.75 concentration of WV (V/V%).

The Effect of WV on Rhizoctonia Root and Crown Rot Disease

Green house studies showed that concentrations of 0.125, 0.25 and 0.5 (V/V%) of WV lead to the control of root and crown rot of greenhouse cucumbers. Analysis of

Table 3. The effect of Wood vinegar non-volatile phase on *R.solani* colony in the laboratory.

Concentration of WV (%)	Colony diameter (mm)	Anti-fungal activity (%)
0 (Control)	90	-
0.025	90	0d
0.05	81	10.00d
0.125	56	37.77c
0.25	7	92.22b
0.37	0	100a
0.5	0	100a
0.75	0	100a

C.V. =3%
Means with similar letters are not significantly different based on Duncan's Multiple Range Test (α=0.05).

Table 4. The effect of Wood vinegar volatiles on *R.solani* colony in the laboratory.

Concentration of WV (%)	Colony diameter (mm)	Anti-fungal activity (%)
0 (Control)	80	-
0.025	80	0d
0.05	80	0d
0.125	71	11.25d
0.25	59	26.25c
0.37	57	28.75c
0.5	48	40.00b
0.75	42	47.50a

C.V. = 3 %
Means with similar letters are not significantly different based on Duncan's Multiple Range Test (α=0.05).

variances showed that disease severity was significantly reduced in all concentrations used ($\alpha_{=}$ 0.05). The maximum control was observed in inoculated cucumber plants treated with 0.25% of WV (Table 6).

The anti-fungal features of WV have been shown in different plant pathogens (Kadota and Niimi, 2004; Qiaozhi et al., 2009). WV decreased the growth of *Penicillium griseofulvum* on the PDA culture medium (Baimark et al., 2008). Yodthong et al. (2008), proposed that the antifungal properties of WV may be due to an interaction between acetic acid and phenolic compounds present within the compound, while Yatagai, (2004) suggests that the antifungal effects of WV may be due to phenolic and creosol compounds.

Yashimoto, (1994) in his studies, on the mode of action of WV, proved that its constitutive chemicals act as hormones and positively influence the soil and the pathogen at suitable concentrations. WV contains 15 macro and micro elements such of calcium, cadmium, chromium, copper, iron, potassium, manganese, aluminum, sodium, zinc, arsenic, molybdenum, phosphorus, lead and bromine (Zulkarami et al. 2011) which most of these elements are involved in vital activities of the plant such as photosynthesis (Loo et al.

Table 5. Treatments in efficacy trial of Wood vinegar against *R.solani* in the greenhouse on cucumber seedlings.

1- Non-inoculated plants (Negative control)*
2- Inoculated plants with *R.solani* (Positive control)
3- Inoculated plants with *R.solani* + 0.125% WV
4- Inoculated plants with *R.solani* + 0.25% WV
5- Inoculated plants with *R.solani* + 0.50% WV

***** Inoculated with PDA blocks without *R. solani.*

Table 6. Mean comparison of disease severity index of cucumber (g) treated with WV in greenhouse conditions.

Treatment	Disease severity
Non-inoculated plants	o^a
Inoculated plants+0.125%WV	8^c
Inoculated plants+0.25%WV	4^b
inoculated plants+0.5%WV	20^d
inoculated plants (Ctrl+)	80^e

Means with similar letters are not significantly different based on Duncan's Multiple Range Test (α=0.05).

2007). The simultaneous presence of acetic acid with calcium and iron cations forms a complex through reaction of acetic acid with these elements, in which the ionic bonds replaces the covalent bonding. As a result, the precipitation of iron as well as the leaching of other beneficial elements is prevented in the soil (Taiz and Zieger, 2006).

CONCLUSIONS

The study showed that volatile and non-volatile metabolites of WV inhibited the mycelia growth of *R. solani* and *S. sclerotiorum* and this substance decreased the crown and root rot of cucumber plants induced by *R. solani*.

ACKNOWLEDGMENT

We would like to thank Dr. Mehran Ghazavi for his kind support in this project. We also thank Mr. Ali Mohammadipour for his technical assistance. We would like to thank Iranian Research Institute of Plant Protection for the financial support of this research.

REFERENCES

Andersen JB, Koch B, Nielsen TH, Sørensen D, Hansen M, Nybroe O, Christophersen C, Sørensen J, Molin S, Givskov M (2003). Surface motility in *Pseudomonas* sp. DSS73 is required for efficient biological containment of the root-pathogenic microfungi *Rhizoctonia solani* and *Pythium ultimum*. Microbiol. 149: 37–46.

Anonim (2001). Wood vinegar. Forest Energy Forum No. 9, FAO of United Nations.

Asaka O, Shoda M (1996). Biocontrol of Rhizoctonia solani damping-off of tomato with Bacillus subtilis RB14. Appl Environ Microb. 62:4081–5.

Brewer MT, Larkin RP (2005). Efficacy of 14. Elad ,Y., I. Chet P. Boyle and Y. Henis, 1983. several potential biocontrol organisms against Parasitism of *Trichoderma* spp. On *Rhizoctonia* Rhizoctonia solani on

potato. Crop Protection, *solani* and *Sclerotium rolfsii*. Scanning electron. 24: 939-950.
Cho WD, Kim WG, Jee HJ, Choi HS, Lee SD, Choi YC (1997). Compendium of Vegetable Diseases with Color Plates. Suwon, Korea, National Institute of Agricultural Science and Technology. p. 447.
De La Fuente L, Tomashow L, Weller D, Bajsa N, Quagliotto L, Chernin L, Arias A (2004). *Pseudomonas fluorescens* UP61 isolated from birds foot trefoil rhizosphere produces multiple antibiotics extracts and exerts a broad spectrum of biocontrol activity. Eur. J. Plant Pathol. 110: 671–681
Fengel D, Wegener G (1983). Wood: Chemistry, Ultrastructure, Reactions. Walter de Gruyter, Berlin, Germany.
Guillen MD, Manzanos MJ (2002). Study of the volatile composition of an aqueous oak smoke preparation. Food Chem. 79: 283-292.
Hadar Y, Chet I, Henis Y (1979). Biological control of *Rhizoctonia solani* damping-off with wheat bran culture of *Trichoderma harzianum*. Phytopath. 69: 64–8.
Hwang Y, Matsushita Y, Sugamoto K, Matsui T (2005). Antimicrobial effect of the wood vinegar from *Crytomeria japonica* sapwood on plant pathogenic microorganisms. J. Microbiol. Biotechn. 15(5): 1106-1109.
Jagger IC (1920). *Sclerotinia minor*, n. sp., the cause of a decay of lettuce celery, and other crops. J. Agric. Res. 20: 331-334.
Jung KH (2007). Growth inhibition effect of pyroligneous acid on pathogenic fungus, *Alternaria mali*, the agent of Alternaria blotch of apple. Biotechnol. Bioproc. Eng. 12: 318-322.
Kadota M, Niimi Y (2004). Effects of charcoal with pyroligneous acid and barnyard manure on bedding plants. Sci. Hortic. 101(3): 327-332.
Kim HS, Sneller CH, Diers BW (1999). Evaluation of soybean cultivars for resistance to Sclerotinia stem rot in field environments Crop Sci. 39: 64-68.
Kohn LM (1979). Delimitation of the economically important plant pathogenic *Sclerotinia* species. Phytopath. 69: 881-890.
Li M-S, LI S-D, Miao Z-Q, Guo R-J, ZhaoZ-Y (2006). Biofumigation for management of soilborne plant diseases. Chinese J. Biol. Control. 22: 296-302.
Loo AY, Jain K, Darah I (2007). Antioxidant and radical scavenging activities of the pyroligneous acid from a mangrove plant, Rhizophora apiculata. Food Chem. 104: 300-307.
Mathew KA, Gupta SK (1996). Studies on web blight of French bean caused by *Rhizoctonia solani* and its management. Indian J. Mycol. Pl. Pathol. 26: 171-177.
Nagarajkumar M, Bhaskaran R, Velazhahan R (2004). Involvement of secondary metabolites and extracellular lytic enzymes produced by *Pseudomonas fluorescens* in inhibition of *Rhizoctonia solani*, the rice sheath blight pathogen. Microbiol Res. 159: 73–81.
Qiaozhi M, Zhong Z, XiHan M (2009). Preparation, toxicity and components analysis of apricot branch wood vinegar. J. Northwest Agr & For Uni. Nat. Sci. 37(10): 91-96.
Reithner B, Schuhmacher R, Stoppacher N, Pucher M, Brunner K, Zeilinger S (2007). Signaling via the *Trichoderma atroviride* mitogen-activated protein kinase Tmk1 differentially affects mycoparasitism and plant protection. Fungal Genet. Biol. 44: 1123–33
SAS Institute Inc (2002). SAS/STAT User's Guide. Version 9.1, Cary, North Carolina, SAS Institute Inc.
Sneh B, Burpee LL, Ogoshi A (1991). Identification of *Rhizoctonia* species. St, Paul Minnesota, USA, APS Press.
Strashnov Y, Elad Y, Sivan A, Rudich Y, Chet I (1985). Control of *Rhizoctonia solani* fruit rot of tomatoes by Trichoderma harzianum Rifai. Crop Prot. 4: 359–64.
Taiz L, Zeiger E (2006). Plant Physiology. 4 th ed. Sinauer Associates, Inc. Publishers, Massachusetts. p. 676
Tu CC, Hsieh TF, Chang YC (1996). Vegetable diseases incited by *Rhizoctonia* spp. In: SNEH B; JABAJI-HARE S; NEATE S; DIJST G. (eds). *Rhizoctonia species: taxonomy, molecular biology, ecology, pathology and disease control*. Dordrecht: Kluwer. p. 369-377.
Velmurugan N, Chun SS, Han SS, Lee YS (2009). Characterization of Chikusaku-eki and Mokusaku-eki and its inhibitory effect on sapstaining fungal growth in laboratory scale. Int. J. Environ. Sci. Te 6(1): 13-22.
Yashimoto T (1994). Present status of wood vinegar studies in Japan for agricultural usage. In: Toward enhanced and sustainable agricultural productivity in the 2000's: Breeding Research and Biotechnology. Proceedings of The 7th International Congress of the Society for the Advancement of Breeding Researches in Asia and Oceania (SABRAO) Vol 3, pp. 811-820.
Yatagai M (2004). Production and utilization of wood carbonization. Makalah pada seminar Penggalakan Pengembangan Arang dan Cuka kayu, 27 Juli 2007, Bogor, Indonesia.
Yatagai M, Nishimoto M, Hori K, Ohira T, Shibata A (2002). Termiticidal activity of wood vinegar, its components and their homologues. J. Wood Sci. 48: 338-342.
Yodthong B, Jirasak T, Nuethip D, Yaowalak S, Nuanchai K (2008). Utilization of wood vinegars as sustainable coagulating and antifungal agents in the production of natural rubber sheets. J. Env. Sci. Tech. 1(4):157-163.
Zulkarami B, Ashrafuzzaman M, Husni MO, Razi Ismail M (2011). Effect of pyroligneous acid on growth, yield and quality improvement of rockmelon in soilless culture. Aust. J. Crop Sci. 5(12): 1508-1514.