Journal of Herbal Drug
journal homepage: www.jhd.iaushk.ac.ir

In vitro anti-fungal activity of watercress (*Nasturtium officinale*) extract against *Fusarium solani*, the causal agent of potato dry rot

Jafar Nikan[1*], Hadi Khavari[2]

[1] *Plant Protection Research Department, Agricultural and Natural Resources Research Centre of Hamadan, Hamadan, Iran;*
[*]**E-mail***: JNikan@gmail.com***

[2] *University of Applied Science and Technology of Hamadan, Hamadan, Iran;*

ARTICLE INFO

Type: *Original Article*
Topic: *Plant Pathology*
Received *May 24th 2014*
Accepted *August 27th 2014*

Key words:

- ✓ *Potato Dry Rot*
- ✓ *Growth Inhibition*
- ✓ *Natural Fungicide*

ABSTRACT

Background & Aim: The aim of this study was to assess the antifungal effect of the watercress extract on the growth of *Fusarium solani*, the causal agent of potato dry rot.
Experimental: This research was done in a completely randomized design with three concentrations and four replicates on potato dextrose agar (PDA) culture medium.
Results: Results of this study indicated that all concentrations of alcoholic extract of watercress can significantly inhibit mycelia growth of *Fusarium solani* on PDA medium. Among different concentrations of watercress extract, the highest inhibition zone of the fungus (26.1 mm in diameter) was obtained at dose of 600 mg/ml. The rate of growth inhibition was concentration dependent. However, no significant difference in the rate of growth inhibition of the fungus by watercress extract was seen between the 500 mg/ml and 400 mg/ml or between 400 mg/ml and 300 mg/ml of the plant extract.
Recommended applications/industries: The overall results of this experiment indicated that the alcoholic extract of watercress can inhibit mycelia growth of *Fusarium solani* in a dose dependent manner. It seems therefore, that the watercress extract has the potential to be employed for manufacturing natural fungicidal compounds. It is recommended that the experiment to be tested on potato tubers (*in vivo* conditions).

1. Introduction

Potato (*Solanum tuberosum* L.) is as the fourth most important crop in the world (Anonymous, 2012). Dry rot caused by various species of *Fusarium* spp., is a post-harvest disease of significant importance in potato (Stevenson *et al*., 2001; Al-Mughrabi, 2010). Crop losses attributed to this disease has been estimated up to US$ 100 m per year (Rowe, 1993). Moreover, most species of *Fusarium* produce toxins that are dangerous to man and livestock (Marasas *et al*., 1984). *Fusarium solani* has been reported as the most pathogenic

Fusarium species causing potato dry rot in Hamadan province of Iran (Sharifi *et al*., 2009; Soheili-Moghadam and Hosseinzadeh, 2013).

Potato dry rot is commonly controlled by the proper handling of potato tubers to minimize the amount of wounds on tubers, proper storage to heal the wounds and chemical control of the pathogen. The fungicides thiabendazole and fludioxinil (Maxim-based products) have been recommended for seed treatment and for post-harvest use on tubers, respectively (Murdoch and Wood, 1972; Kirk and Wharton, 2008). Biological control of the disease has also been investigated and some bio-control agents been introduced (El-Kot, 2008; Gould *et al*., 2008). However, effective and efficient management of crop pests is generally achieved by the use of synthetic pesticides (Kiran *et al*., 2006). On the other hand, due to increased awareness about the risks associated with the use of pesticides including: the advent of pesticide resistant pests, ecological impact of pesticide application on the environment and particularly consumer concerns about pesticide residue in crops (Viana *et al*., 1996; Nikan and Morowati, 2013), much attention is being focused on the alternative methods of pest control. Natural plant extracts have been recommended as suitable alternative choices to synthetic chemicals (Suhr and Nielsen, 2003; Ownagh *et al*., 2010; Bahraminejad *et al*., 2010; Mangang and Chhetry, 2012; Jafarpour *et al*., 2013).

Babu et al. (2008) studied the effect of some plant extracts on the control of *Fusarium solani* f.sp. Melongenae *in vitro*. All the plant extracts showed significant reduction in the growth of the pathogen. Among the different extracts, 20% of *Azadirachta indica* was found most effective.

In another study, *Fusarium oxysporum* was treated with the various extracts of *Excoecaria agallocha* (different dilutions from different parts of the plant). It was observed that the 20% dilution of stem extract brought about 87.1 percent inhibition of spore germination (Deepa and Padmaja, 2013).

Dwivedi and Dwivedi (2012) studied antifungal activity of some plant extracts against *Fusarium solani* the causal agent of guava wilt. They found that the extract of Clove (*Eugenia caryophyllata*) was most effective against the fungus on 3rd, 5th and 7th day at 50% concentration and conquered the colony growth by 100% compared to control.

Shrestha and Tiwari (2009) assessed the antifungal activity of the crude extracts of six medicinal plants against *Fusarium solani*. The extracts of all the plant species were found to be effective in inhibiting the mycelia growth. The extract of *Allium sativum* completely inhibited the mycelia growth of the fungus at the concentration of 40% and the extracts of *Capsicum annuum* and *Phyllanthusem blica*inhibited the mycelia growth completely at the concentration of 100%.

It was revealed in a bioassay study that different concentrations of *Parthenium hysterophorus* was effective in inhibiting the growth of *Fusarium solani* FCBP-434, the most pathogenic strain of the fungus causing Fusarium wilt in potato (Shafique and Shafique, 2012).

Nasturtium officinale R.Br. (Watercress) belonging to the family Brassicaceae, is an important medicinal herb that grows naturally in flowing streams in most mountainous regions of the world including Iran. This plant has been used by the rural healers as nutritive, anti-inflammatory and antioxidant agent. It is also a vegetable that is consumed raw or cooked in salads, soups and other recipes in European and Turkish cuisine and also used to cure abdominal pain in traditional medicine (Ozen, 2009) and for treatment of diseases such as diabetes and bronchitis (Bahramikia *et al*., 2009). The extract of watercress contains secondary metabolites such as phenolic and flavonoid compounds (Mazandarani *et al*., 2013).

Asadi et al. (2012) investigated the effect of watercress extract on immunological and hematological parameters of rainbow trout (*Oncorhynchus mykiss*). The results indicated that oral administration of watercress extract might be useful to improve fish's immune system. The extract of watercress has shown nematicide effect on northern root-knot nematode (NRKN), *Meloidogyne hapla* (Zahradníková and Petříková, 2012). Freitas et al. (2013) found synergistic antibacterial activity effects between watercress extract and antibiotics, indicating the potential of watercress compounds as anti-microbial substances.

In this study the effect of alcoholic extract of watercress on inhibiting the mycelia growth of *F. solani*, the main causal agent of potato dry rot was investigated *in vitro*.

2. Materials and Methods

2.1. Plant materials

The medicinal plant used in the experiment was watercress (*Nasturtium officinale* R.Br.) collected from flowing streams in Lorestan province of Iran. The required amount of watercress was collected in autumn and after washing was air dried in the shade.

2.2. Preparation of plant extracts

The alcoholic plant extract of watercress was prepared using maceration method described by Durling et al. (2007). The extraction was done briefly as follows: 200 g dried watercress was ground and 1000 ml 70% ethanol was added to the resulting powder and mixed well. The mixture was stirred every day for two weeks. After 14 days, the resulting extract was clarified with filter paper. The clarified extract was then allowed to air dry at room temperature.

2.3. Pathogen

The pathogenic fungus was isolated from dry rot affected potato tubers gathered from potato fields in Hamadan province of Iran. The isolation was done by culturing the diseased potato flesh on potato dextrose agar (PDA) medium and subsequent purification of the fungus.

2.4. Preparation of spore suspension of the fungus

Two weeks old purified cultures of the *Fusarium solani* (when mycelia had produced spores) were used for preparing spore suspension. The spore suspension was prepared by adding some sterilized distilled water (~ 1 ml) to each petri dish containing the fungus culture to allow the spores to be suspended in the water. The spore suspension was then collected and the spore suspension was adjusted to 1×10^6 spore per milliliter.

2.5. Assessment of antifungal activity of the extract

Using the paper disc diffusion method (Rasooli *et al.*, 2006), the assessment was done by measuring the growth inhibition zone of the fungus on PDA medium. Different concentrations (300, 400, 500, and 600 mg/ml) of watercress extract were prepared by dissolving the required amounts of dried extract into one milliliter distilled water. To remove any microbial contamination, the extracts were filtrated by using two-micrometer filters (Millipore filter 2). Then some autoclaved filter paper discs (6 mm in diameter) were submerged in any of the prepared concentrations of the watercress extract or in sterilized distilled water (as negative control) and in 0.2 percent fungicide carbendazim+iprodion 52% WP solution (as positive control). The discs were allowed to be saturated with the solutions. The submerged paper discs were then air dried. Half a milliliter of spore suspension of *F. solani* (1×10^6 spore/ml) was transferred to PDA medium and was evenly spread on the surface of the medium. Then one paper disc loaded with each solution was placed in the center of *F. solani* inoculated plate.

The experiment was conducted as a completely randomized design with six treatments and four replicates. The treatments were five concentrations of the watercress extract, as mentioned above and two controls (sterilized water as negative and fungicide solution as positive control). The treated plates were then incubated at 26°C in the dark. The average diameter of the mycelia growth inhibition zone around the paper discs loaded with each treatment was measured 7 days post incubation (before the plates were completely covered with mycelia of the fungus). The growth inhibition percent was calculated using the formula:

IP = dc - dt/dc × 100, where IP was the growth inhibition percent, dc and dt were the diameter of growth inhibition zone in negative control and each of the other treatments, respectively.

2.6. Statistical analysis

The statistical analysis of the data and comparison of the means was done using the SAS software and the Duncan's multiple range test, respectively. Transformation of the data was done as needed.

3. Results and discussion

Statistical analysis indicated that the growth inhibition rates for *F. solani* due to treating with the various given treatments were significantly different (Table 1).

Table 1. Statistical analysis of the data of growth inhibition percent of *F. solani* by different concentrations of Watercress extract

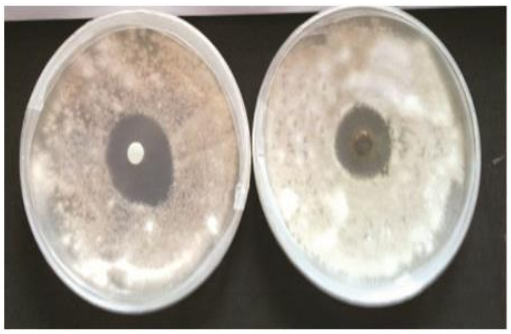
Sources of variance	Degrees of freedom	Sam of squares	Mean of squares	*F*-value
Treatment	5	62.316	12.46	347.7*
Error	18	0.645	0.36	8
Total	23	62.961		

* $P < .001$, CV: 4.63

Comparison of the means of growth inhibition rates resulting from various given treatments divided them into three groups (Table 2). The highest and the lowest growth inhibition zone were those of fungicide and Sterilized distilled water treatments, respectively. Among various concentrations of the watercress extract, the concentration 600 mg/ml inhibited the growth of *F. solani* more than the other concentrations. Moreover, the effect of the watercress extract on the growth inhibition of *F. solani* was dose dependent, that is: the higher the concentration the more inhibition growth of the fungus. However, no significant difference between the growth inhibition rates of *F. solani* was seen between the concentrations 500 mg/ml and 400 mg/ml or between the concentrations 400 mg/ml and 300 mg/ml of extract.

Negative Control treatment: Watercress treatment

Fig.1. *In vitro* growth Inhibition zone of *F. solani* on PDA medium resulting from treatment of the fungus with Watercress extract, negative control (left) and watercress extract (right).



Positive Control treatment: Watercress treatment 600mg/ml

Fig.2. *In vitro* growth Inhibition zone of *F. solani* On PDA medium resulting from treatment of the fungus with Watercress extract, negative control (left) and Watercress extract (right).

This report indicates that the extract of watercress has anti-pathogenic, in particular antifungal, activity, the fact that has been reported previously (Zahradníková and Petříková, 2012; Freitas *et al*., 2013). We found that the growth of *F. solani* can be inhibited by the application of the watercress extract as found Ataee-Azimi et al. (2006) by using the extract of *Sorghum bicolor* and Shrestha and Tiwari (2009) by using *Thymus* essence. This preliminary study proved that the watercress extract could be used as an alternative to for the control of *F. solani*, *in vitro*. However, it needs to be applied in virtual condition on the potato.

Table 2. Grouping the means of growth inhibition rates of *F. solani* by the different concentrations of Watercress extract using Duncan's multiple range test

Treatment	Mean of growth inhibition (percent)
Watercress extract 600mg/ml	26.2[b]
Watercress extract 500mg/ml	19.8[c]
Watercress extract 400mg/ml	17.6[cd]
Watercress extract 300mg/ml	16.5 [d]
Fungicide	32.7 [a]
Sterilized distilled water	0 [e]

*The means followed by different superscript letters are significantly different.

4. Conclusion

It was proved that the alcoholic extract of Watercress was capable of bringing about the desired antifungal effect in a concentration dependent manner.

5. References

Al-Mughrabi, K. I. 2010. Biological control of Fusarium dry and other potato tuber diseases using *Pseudomonas fluorescens* and *Enterobacter cloacae*. *Biological Control*., 53: 280-284.

Anonymous, 2012. FAOSTAT. http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor

Asadi, M.S., Mirvaghefei, A.R., Nematollahi, M.A., Banaee, M. and Ahmadi, K. 2012. Effects of watercress (*Nasturtium nasturtium*) extract on selected immunological parameters of rainbow trout

(Oncorhynchus mykiss). *Open Veterinary Journal*., 2: 32-39.

Ataee-Azimi, a., Delnavaz Hashemian, B. and Ghanaee, A.M. 2006. Effect of Watery, alcoholic and phenolic extracts of seed and leaf of *Sorghum bicolor* on *Fusarium solani* and *Fusarium poae*. *Journal of Medicinal Plants*., 21: 26-31.

Babu, J., Dar, M.A. and Kumar, V . 2008. Bioefficacy of plant extracts to control *Fusarium solani* f.Sp. Melongenae incitant of Brinjal Wilt. *Global Journal of Biotechnology & Biochemistry*., 3 (2): 56-59.

Bahramikia, S., Ardestani, A. and Yazdanparast, R. 2009. Protective effects of four Iranian, medicinal plants against free radical-mediated protein oxidation. *Food Chemistry*., 1159(1): 37-42.

Bahraminejad, S., MaerefatzadahKhamenah, M., Abbasi, S., ZareKhafari, A. 2010. In vitro screening of 27 plant species against phytopathogenic fungi. *Modern Technology in Agriculture*., 4(2): 1-11.

Deepa, M. and Padmaja, C.K. 2013. Effect of the extracts of *Excoecaria agallocha* on spore formation and budding in fungi. *Asian Journal of Plant Science and Research.*, (6): 14-19.

Durling, N.E., Catchpole,O. J., Grey, J.B., Weebby, R.F., Mitchell, K.A., Foo, L.Y., and Perry, N.B. 2007. Extraction of phenolics and essential oil from dried sage (*salvia officinalis*) using ethanol – water mixtures. *Food Chemistry.*, 101: 1417-1424.

Dwivedi, S.K. and Dwivedi, N. 2012. Antifungal activity of some plant extracts against guava wilt pathogen. *International Journal of Environmental Sciences*., 3(1): 412-420.

El-Kot, G.A.N. 2008.Biological Control of Black Scurf and Dry Rot of Potato. *Egyptian Journal of Phytopathology*., 36(1-2): 45-56.

Freitas, E., Aires, A., de Santos, R. EA, and Saavedra, M.J. 2013. Antibacterial activity and synergistic effect between watercress extracts, 2-phenylethyl isothiocyanate and antibiotics against 11 isolates of Escherichia coli from clinical and animal source. *Letters in Applied Microbiolgy*., 57(4): 266-73. doi: 10.1111/lam. 12105.

Gould, M., Nelson, L M., Waterer, D., and Hynes, R. K. 2008. Biocontrol of *Fusarium sambucinum*, dry rot of potato, by *Serratia plymuthica* 5-6. *Biocontrol Science and Technology*., 18(9/10): 1005-1016.

Jafarpour M., Golparvar, A.R., and Lotfi, A. 2013. Antibacterial activity of essential oils from *Thymus vulgaris*, *Trachyspermum ammi* and *Mentha aquatic* against *Erwinia carotovora* in vitro. *Journal of Herbal Drugs*., 4(3): 115-118.

Kiran, K., Linguraju, S. and Adiver, S. 2006. Effect of plant extract on *Sclerotiumrolfsii*, the incitant of stem rot of ground nut. *Journal of Mycology and Plant Pathology*., 36: 77-79

Kirk, W. and Wharton, P. 2008. Fusarium dry rot posing problems in potatoes. Michigan State University Extension, Department of Plant Pathology. http://msue.anr.msu.edu/news/fusarium dry rot posing problems in potatoes

Mangang, H.C. and chhetry, G.K.N. 2012. Antifungal Properties of certain Plant Extracts against *Rhizoctonia Solani* Causing Root Rot of French Bean In Organic Soil of Manipur International *Journal of Scientific and Research Publications*., 2(5): 1-4.

Marasas, W. F. O., Nelson, P. E. andToussoun, T. A. 1984. *Toxigenic Fusarium Species Identity and Mycotoxicology*. Pennsylvania State University Press 328pp.

Mazandarani, M., Momeji, A., ZarghamiMoghaddam, P. 2013. Evaluation of phytochemical and antioxidant activities from different parts of *Nasturtium officinale* R. Br. in Mazandaran. *Iranian Journal of Plant Physiology*., 3(2): 659-664.

Murdoch A.W. and Wood, R.K.S. 1972. Control of *Fusarium solani* rot of potato tubers with fungicides. *Annals of Applied Biology*., 75(1): 53–62.

Nikan, J. and Morowati, M. 2013. Investigation on pesticide residue levels in green-house grown cucumber and their influencing factors in Hamadan province, Iran. Final Report of the research project coded 04-83-16-89102, *Agricultural and natural resources Research Organization*., 44300,

Ownagh, A., Hasani, A., Mardani, K., and Ebrahimzadeh, S. 2010. Antifungal effects of thyme, agastache and satureja essential oils on *Aspergillus fumigatus*, *Aspergillus flavus* and *Fusarium solani*. *Veterinary Research Forum*., 1(2): 99 – 105.

Ozen, T. 2009. Investigation of antioxidant properties of *Nasturtium officinale* (Watercress) leaf extracts'.

Acta Poloniae Pharmaceutica Drug Research., 66 (2): 187-193.

Rasooli, I., Rezaei M. B., and Allameh, A. 2006. Growth inhibition and morphological alterations of *Aspergillus niger* by essential oils from *Thymus eriocalyx* and *Thymus x-porlock*. *Food Control*., 17: 359-364.

Rowe, R. C. 1993. *Potato Health Management*. APS Press, 178pp.

Shafique, Sh. and Shafique, S. 2012. Biological Control Potential of *Parthenium hysterophorus* against *Fusarium Solani* – A Cause of Fusarium Wilt in Potato. International Conference on Applied Life Sciences, 10-12 Sep., Turkey.

Sharifi, K., Zare, S., Zamanizadeh, H., and Arjmandian, A. 2009. Fusarium species causing dry rot of potatoes in Ardabil, Tehran and Hamedan Provinces. *Journal of Plant Pests and Diseases*., 76(2): 93-113.

Shrestha, A. K. and Tiwari, R.D. 2009. Antifungal activity of crude extracts of some medicinal plants against *Fusarium solani* (mart.) sacc. ECOPRINT 16: 75-78, Ecological Society (ECOS), Nepal.

Soheili-Moghadam, B. and Hosseinzadeh, A.M. 2013. Study of *Fusarium* Species causing dry rot of potatoes in Ardabil Province. *International journal of Agronomy and Plant Production*., 4(6): 1226-1233.

Stevenson, W.R., Loria, R., Franc, G.D. and Weingartner, P.D. 2001. *Compendium of Potato Diseases*. second ed. APS Press, St. Paul, Minnesota, USA, pp. 23–25.

Suhr, K.I. and Nielsen, P.V. 2003. Antifungal activity of essential oils evaluated by two different application techniques against rye bread spoilage fungi. *Journal of Applied Microbiology*., 94: 665–674.

Viana, E., Malto, J.C. and Font, G. 1996. Optimization of a Matrix Solid-Phase Dispension Method for the Analysis of Pesticide residues in Vegetables. *Journal of Chromatography*., 754: 437-444.

Zahradníková, H. and Petříková, K. 2012. Nematocide effects of watercress (Nasturtium officinale R. BR.) Acta Universitatis Agriculturaeet Silviculturae Mendelianae Brunensis., 233-236. http://dx.doi.org/10.11118/actaun201361010233.