Egypt. J. Exp. Biol. (Bot.), 5: 169 – 173 (2009)

RESEARCH ARTICLE

Hend A. Hamedo

CONTROL OF ROOT ROT DISEASE USING PLANT POWDER AND ESSENTIAL OIL FROM *ARTEMISIA MONOSPERMA*

ABSTRACT:

This study was carried out to determine the potential of using the plant powder and essential oil of *Artemisia monosperma* to control root rot disease caused by *Fusarium solani* and to investigate their influence on soil fungal flora population. The effect of the essential oil on dry weight and enzymes production of the fungus was investigated *in vitro*. *Psium sativum* seeds were treated with different concentrations of essential oil (0.25, 0.5, and 1.0%) before sowing, while the plant powder was mixed with the soil at 5, 10, 20 gms. The results obtained from the green house application of bioagent indicated that soaking seeds in different concentrations of essential oil from 0.25 to 1.0% (for 60 minutes) were significantly reduced the percentage of damping off and root rot when compared with control (pathogen only). *In vitro,* essential oil of *Artemisia monosperma* was found to be very toxic to the root-rot pathogens. The concentration of 1.0% was completely prevented the enzyme production, completely inhibited the dry weight of the fungus also significantly decreased the population of fungal flora in the soil as compared with control. The plant powder of *Artemisia monosperma*, when mixed with the soil, was significantly reduced the extent of root rot disease compared to the control. Untreated seeds grown in the infested soil (positive control) with *F. solani* showed higher percentage of infection. The plants grown under this treatment were significantly shorter than the corresponding figures of the other treatments. Soil mixed with plant powder or seeds treated with essential oil had a significant lower percentage of infection, significantly longer in height and better plant growth parameters.

KEY WORDS:

Artemisia monosperma, essential oil, plant powder, *Fusarium solani*, root rot disease.

CORRESPONDANCE:

Hend A. Hamedo
El Arish, Faculty of Education, Suez Canal University, Egypt

E-mail: hend_hamedo@hotmail.com

ARTICLE CODE:20.02.09

INTRODUCTION:

The fungal genus Fusarium causes several diseases on plants. Some species of Fusaria are responsible for vascular wilts, such as the crop-specific disease Fusarium wilt of melon (cantaloupe and muskmelon) caused by *Fusarium oxysporum* f. sp. *melonis*. Another Fusarium species, *Fusarium solani* f. sp. *cucurbitae*, causes a crown and foot rot of summer squash and pumpkin, and a fruit rot of pumpkin. Still other Fusarium species are responsible for preharvest and postharvest fruit rots of assorted cucurbits (Elad *et al.,* 1980).

Biological control of plant diseases especially soilborne plant pathogens has been subjected to extensive research in the last years. Aromatic plants are well documented as effective biological control agents of plant diseases caused by soilborne fungi (McLean *et al.,* 2004). Root-rot diseases caused by soil-borne fungi are the most important diseases of many crops. Several fungi were recorded as causal pathogens of root-rot and wilt diseases such as *Rhizoctonia solani, Fusarium solani* (Abou-zeid *et al*., 1997). Fusarium wilt disease caused by pathogenic forma specials of the soil inhabiting fungus *Fusarium oxysporum* can cause severe loses in a wide variety of crop plants (Larkin and Fravel, 1998).

Many fungicides have been found to be effective against many mould deterioration (Bankole, 1998). However, fungicides have various setbacks such as development of resistant strains in the treated fungi, environmental toxic residues and toxicity to consumers. Furthermore, fungicides are too expensive or not available at the appropriate time.

Natural plant extracts are of interest as a source of safer or more effective substitutes for synthetically produced antimicrobial agents and may provide an alternative way to prevent food or feed from fungal contamination (Thanaboripat, 2002 & 2003). Powders and extracts of various herbs, spices and essential oils have been reported to have antimicrobial activity.

Artemisia monosperma Delile is a wild herb growing in the Egyptian desert. It has

been shown to have some medicinal applications (Abu- Niaaj *et al.,* 1993) and antimicrobial activities (Hamedo, 2003). Many natural constituents have been identified in *A. monosperma,* some of them was proved to have an *in vivo* medicinal effect (Khafagy *et al.,* 1979).

α-Amylases are hydrolytic enzymes that are widespread in nature, being found in microorganisms and plants (Octávio *et al.,* 2000). Amylases are among the most important enzymes in present-day biotechnology. The amylase family is of great significance due to its wide area of potential application such as application in pharmaceutical and a clinical sector that requires high purity amylases (Pandey *et al.,* 2000). Thus, it is an important to develop economic processes for their purification to obtain pure enzymes with maximum specific activity.

Detailed studies on fungal enzymes production have largely been limited to a few species of fungi (AbouZeid, 1997). Nevertheless, Amirul *et al.* (1996) produced alpha-glucosidase, alpha-amylase and two forms of glucoamylase from *Aspergillus niger* grown on a liquid medium containing raw tapioca starch as the carbon source.

The present study was designed to evaluate *in vitro* the activity of plant powder and essential oil from *Artemisia monosperma* against root rot pathogen *Fusarium solani* growth and their effects on the enzyme productions of the fungus such as α-amylase and protease. Then study of the fungal population and determination the optimum concentration for controlling root-rot disease in *Psium sativum* plants, *in vivo* was carried out.

MATERIAL AND METHODS:

1. Collection of plant materials:

Upper parts (shoot system) of *Artemisia monosperma* was collected from El-Arish, Sinai. It was air-dried under the shade (25-29°C) until the plant became dry after 10 days. The dried plant was powdered in a grinder, and sieved with a 0.5 mm size mesh. Another quantity of the plant was extracted for the essential oil according to Baratta *et al*. (1998) using steam-distillation apparatus for 3 hours. The oils obtained were separately dried over anhydrous sodium sulphate and kept in the refrigerator at 4°C before use.

2. Media and growth conditions:

The test fungus *Fusarium solani* NRC (215) was obtained from NRC Microbial collection unit. The volatile oil was mixed with Tween-80 (0.05%) and diluted with distilled water to make a 1% stock solution. This was further diluted with distilled water to give concentrations of 0.25, 0.5, and 1.0 % v/v. To determine the effect of these concentrations of volatile oil on the dry weight and enzyme productions of the test fungus, 1 ml of each treatment concentration was added to 20 ml of the Czapeks Dox broth medium in 100 ml Erlenmeyer flask and inoculated with a 5mm disc of test fungi. The flasks containing medium with 1ml of Tween-80 (0.05%) in distilled water served as control. After 8 days dry weight of mycelia and enzyme production were determined. The entire experiment was repeated and the results are presented as means subjected to one-way ANOVA followed by separation of means at $P < 0.05$.

3. α-Amylases assay:

α- Amylase was assayed according the method adopted and described by Gabr *et al*. (1988). One enzyme unit (u/ml) is defined as the amount of enzyme which releases 1μ M glucose.

4. Protease assay:

Protease was assayed using Hammarsten casein as a substrate as described by De Marco and Carlos (2002). One unit of protease activity was defined as the amount of enzyme necessary to cause a change of one unit in the absorbance of the supernatant in 20 min.

5. Greenhouse experiment:

Pot experiments were carried out in the green house of the faculty of Science, Mansoura University to study the activity of plant powder and essential oil of *Artemisia monosperma* in suppressing the pathogenic fungus (*Fusarium solani)* the causal agent of wilt and / or root rot disease. Seeds of *Psium sativum* obtained from the Agricultural Research Centre in Giza were used. 10 Seeds (for every pot) were surface sterilized using 0.5 % w/v sodium hypochloride (NaOCl) for 5min and washed 3 times by sterilized distilled water, then soaked in the different concentrations of essential oil (0.25, 0.5, and 1.0% v/v) for 60 minutes (Hamed, 2001), then put in pots contained sterilized soil (500 gm) mixed with the pathogen. Another group of seeds were put in pots contain sterilized soil (500 gm) mixed with the pathogen and the plant powder (5, 10, and 20 gm). Third group of seeds were soaked in sterilized distilled water served as control. The effect of the powdered plant and essential oil of *Artemisia monosperma* on root rot and wilt disease was evaluated under green house conditions during seedling stage after 25 days from sowing.

Percentage of post- emergence damping off and root-rot were determined as:

% damping off =
(No. of died seedlings / pot / No. of survival plants / pot) × 100

% diseased plants =
(No. of infected plants with root-rot/pot / No. of survival plants / pot) × 100

6. Population of fungal flora in soil:

The effective concentration of essential oil was found to be 1.0%. The experimental pots

were repeated using unsterilized soil and treated with 3% wheat meal-sand medium (WSM) inoculated with the pathogen and seeds soaked in the effective oil concentration and soil mixed with 20 gm plant powder. Another three replicates of pots served as control (seeds were soaked in distilled water). Population of fungal flora in soil was estimated after four weeks from planting. Soil suspensions were prepared by mixing 10 gm of soil with 90 ml sterile water. The soil water was subjected to serial dilution (10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}) .Three replicate plates were prepared from each concentration. One mL of each sample was poured into sterilized plates and 20 mL sterilized medium (Czapek-Dox-Agar) previously prepared. The same steps were done to the control soil. The plates were incubated at 28°C and fungal count was estimated after 5 days and again after 8 days to count the slow growing fungal species. The isolated fungi were identified in the National Research Center, Microbial Culture Collection Unit (MCCU) according to their morphological characters (Barent, 1960). The results were statistical analyzed using Co. Stat. Progrm, Software. One way analysis was made and the treatments mean were compared by LSD at 1% probability (Snedecor and Cochrou, 1990).

RESULTS AND DISCUSSION:

The most interesting area of application for plant extracts and essential oils is the inhibition of growth and reduction in numbers of the more serious soilborne plant pathogens was implicated in root rot and wilt diseases of several plants (McLean *et al.,* 2004).

Results obtained from the *in vitro* part of this study showed that the essential oil and plant powder of *Artemisia monosperma* could be used effectively to control the plant pathogen. They achieved total control of the pathogen by growing fast and stopping further growth of the pathogen. Table 1 showed that the different concentrations of essential oil of *A. monosperma* had strong antimicrobial activities against *F. solani* which inhibited the fungus growth (dry weight) and the production of both α- amylase and protease enzymes. *F. solani* dry weight has decreased gradually with increasing essential oil concentrations until reached 1% concentration which was significantly decreased the enzymes productions and completely inhibited the fungus growth.

Table 1. Effect of essential oil of *Artemisia monosperma* on dry weight, α- amylase and protease enzymes produced by *F. solani*

Concentration		α.Amylase (u/mg)	Protease (u/mg)	Fungus dry weight(mg)
Control		193.5	87.0	0.931
Oil (%)	0.25	98.66[a]	44.9[a]	0.720[b]
	0.5	54.00[a]	42.0[b]	0.243[a]
	1.0	21.90[a]	17.5[a]	0.0

a means significant, b means nonsignificant at P< 0.05.

From the results in table 2, it is obvious that increasing the concentration of essential oil until 1 % reduced the percentage of damping-off and wilt disease as compared with the control (untreated seeds). Highly reduction in infection with pre-emergence damping off and root-rot disease of peas plants was observed also when treated the soil with 20 gm plant powder. The results in table 3 showed a significant decrease in fungi populations when the soil was treated with 20 gm of plant powder or the seeds were soaked in essential oil concentration of 1% after 4 weeks of planting.

Table 2. Effect of essential oil and plant powder of *Artemisia monosperma* on damping off and root rot ratio caused by *F. solani*

Concentration		Dumping off (%)	Root rot (%)
control		88.0	98.6
Oil (%)	0.25	50.0	38.9
	0.5	48.7	33.0
	1.0	25.8	15.0
Powder (gm)	5	42.8	38.0
	10	27.0	19.3
	20	15.0	10.0
L.S.D. 1%	-	5.3	7.8

Table 3. Fungal population percentage in soil for control and treated seeds with 1% essential oil and/or 20 gm plant powder of *Artemisia monosperma*

Isolates	Control (%)	Occurrence % (1% oil)	Occurrence % (20g powder)
Fusarium oxysporum	57.2	32.7	13.96
Fusarium solani	14.0	2.13	1.13
Aspergillus niger	55.0	38.8	11.51
Aspergillus flavus	23.9	20.3	3.62
Penicillium notatum	12.5	8.6	1.25
Other *Penicillium* sp	43.0	19.9	15.21
Rhizopus sp.	34.7	15.5	7.58
Alternaria dianthi	22.0	9.9	5.70
Rhizoctonia solani	11.9	8.5	2.38
Trichoderma viride	56.6	29.0	14.14
Cephalosporium sp.	10.6	3.9	1.4

Certain formulations of plant-derived extracts and oil were found to affect soil populations of many microorganisms and reduce disease development. Experimental conditions were optimized for survival of *F. solani* in soil and disease development in the greenhouse. Despite favorable conditions for the pathogen, formulations of *Artemisia monosperma* reduced pathogen populations that led to higher healthy plants compared with the pathogen- only control.

The essential oils of *Artemisia monosperma* contained high amounts of phenolic compounds, alcohols and ketones (Hamido, 2003). However, Gueldner *et al.*

(1985) mentioned that the essential oils containing phenols are the most producing inhibitory effect on microorganisms followed by other constituents. Dorman and Deans (2000) stated that, the inhibitory behavior of phenolic structure in the essential oils was due to the presence of hydroxyl group and the relative position of the hydroxyl group exerted an influence upon the component effectiveness.

Peas plants which survived in soil never attained the normal growth either in number of plants, height or fresh and dry weights (Table 4). The length of plants which survived in control soil were reduced to 26.05cm, while the length of plants which survived in seeds treated with 1% essential oil or soil treated with 20 gm plant powder were 34.05, 35.45cm respectively. Again *F. solani* isolate was the most aggressive fungus, which reduced the fresh and dry weights from 13.33, 3.22 gm at control to 22.84, 5.39 gm for seeds treated with 1% essential oil and 23.19, 5.46 gm in soil treated with plant powder. A similar trend was also observed with plant number. *F. solani* was not only the most aggressive pathogen but also the most frequently encountered fungus. For this reason, special attention was paid to *F. solani*. The pathogenic properties of *F. solani* in this study are in agreement with results obtained by Elad *et al.* (1980) who recorded that these fungi were capable of attacking a tremendous range of host plants causing seed decay, damping off, root rot and fruit decay.

Table 4. Morphological parameters of survival plants response to treatment with 1% essential oil or 20g plant powder

Treatments	Morphology data of survival plants			
	No. of survival plants	Main shoot length/ cm	Main fresh weight/g	Main dry weight/g
Control	3	26.05	13.33	3.22
1% Essential oil	8	34.05	22.84	5.39
20 g plant powder	9	35.45	23.19	5.46

The treatments using essential oil as seed dressing or plant powder as a fertilizer in the soil inoculated with pathogenic fungi greatly reduced the occurrence of *Fusarium solani.* The same result was also obtained by Aziz *et al.* (1997) who found that germination of conidia of root-rot fungi in bean rhizosphere soil was inhibited after soil or seed application with *Trichoderma*. The great reduction of the pathogen population densities in the rhizosphere soil could be a result of lower proliferation rate of the pathogen in the rhizosphere already colonized by the antagonist (Muhammad and Amusa, 2003).

CONCLUSION:

From the above results we found that the treatments of *Psium sativum* seeds with the essential oil or the addition of the plant powder to the soil control the root rot disease caused by *Fusarium solani* which can indicate by decreasing the number of diseased plants and lower of fungal dry weight and enzymes production.

REFERENCES:

AbouZeid AM. 1997. Production, purification and characterization of an extracellular alpha-amylase enzyme isolated from *Aspergillus flavus*. Microbios, 89(358): 55-66.

Abouzeid NM, El-Morsy GA, Hassanein AM. 1997. Major organisms causing root rot/ wilt and their relative importance on faba -bean, lentil and chickpea. Egypt. J. Agric. Res*., 25*: 529-542.

Abu-Niaaj L, Abu-Zarga M, Sabri S, Abdalla S. 1993. Isolation and biological effect of 7-O-methyleriodictyol, a flavonone isolated from *Artemisia monosperma* on rat isolated smooth muscles. Planta Med., 59: 42-45.

Amirul AA, Khoo SL, Nazalan MN, Razip Amirul AA, Khoo SL, Nazalan MN, Razip MS, Azizan MN. 1996. Purification and properties of two forms of glucoamylase from *Aspergillus niger*. Folia Microbiol. (Praha), 41(2):165-174.

Aziz NH, El-Fouly MZ, El-Essawy AA, Khalaf MA. 1997. Influence of bean seedling root exudates on the rhizosphere colonization by *Trichoderma lignorum* for the control of *R. solani*. Bot. Bull. Acad. Sci. Egypt, 38: 33-39.

Bankole SA. 1998. Postharvest fungal deterioration of melon seeds and its control. Ph.D Thesis, University of Ibadan, Ibadan, Nigeria. 219 p.

Baratta MT, Dorman HJ, Deans SG, Figueiredo AC, Barroso JG, Ruberto G. 1998. Antimicrobial and Antioxidant properties of some commercial essential oils. Flavour Frag. J., 13 : 235-244.

Barnet HL. 1960. Illustrated Genera of Imperfect Fungi 2nd Ed., Bulgess Publishing Company.

Dorman HJ, Deans SG. 2000. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. J. Appl. Microbiol. 88: 308-316.

De Marco JL, Carlos R. 2002. Characterization of a protease produced by a *Trichoderma harzianum* isolates which controls cocoa plant witches' broom disease. BMC Biochemistry, 3:3.

Elad T, Chet J, Katan J. 1980. *Trichoderma harzianum* a biocontrol effective against *Sclerotium rolfsii* and *Rhizoctonia solani*. Phytopathology, 70: 119-121.

Gabr MA, Mansour FA, Mohamed G. 1988. Some metabolic changes in *Streptomyces olivaceus* and *Streptomyces parvulus* with amylase and protease production as affected by nutrient requirements. III. Effect of inorganic phosphorus content. Egypt. J. Microbiol., 22(2): 263-284.

Gueldner RC, Wilson DM, Heidt A. 1985. Volatile compounds inhibiting *Aspergillus flavus.* J. Food Chem., 33: 441-443.

Hamed ER. 2001. The effect of secondary metabolites of some fungi on the growth of wilt and root-rot fungi. Ph. D. Thesis, Faculty of Agric. Cairo University.

Hamedo HA. 2003. Studies of antimicrobial activities of some essential oil- producing plants. Ph.D Thesis, Faculty of Science, Mansoura University, Mansoura, Egypt.

Khafagy SM, Metwally AM, El-Ghazoully MG. 1979. Isolation and characterization of a new keto–alcohol from *Artemisia monosperma*. Egypt. J. Pharm. Sci., 20: 115-120.

Larkin RR, Fravel DR. 1998. Efficiency of various fungal and bacterial biocontrol organisms for control of fusarium wilt of tomato. Plant Dis., 82: 1022-1028.

McLean KL, Dodd SL, Sleight BE, Hill RA, Stewart A. 2004. Comparison of the behavior of a transformed hygromycin resistant strain of *Trichoderma atoviride* with the wild-type strain. New Zeal. Plant Prot., 57: 72-76.

Muhammad S, Amusa NA. 2003. In-vitro inhibition of some seedling blight inducing pathogens by compost-inhabiting microbes. Afr. J. Biotechnol., 2(6): 161-164.

Octávio LF, Daniel JR, Francislete RM, Carlos B. Jr, Carlos PS, Maria FG. 2000. Activity of wheat α-amylase inhibitors towards burchid α-amylases and structural explanation of observed specificities. Eur. J. Biochem., 267: 2166-2173.

Pandey A, Nigam P, Soccol CR, Soccol VT, Singh D, Mohan R. 2000. Advances in microbial amylases (Review). Biotechnol. Appl. Biochem., 31: 135-152.

Snedecor GW, Cochrou WG. 1990. Statistical methods 8th Ed. Iowu state vniu pres Ames, Iowu, USA.

Thanaboripat D. 2002. Importance of aflatoxins. KMITL Sci. J., 2(1): 38-45.

Thanaboripat D. 2003. Mycotoxins: Occurrence and control in foods. In: The International Review of Food Science and Technology. IUFOST, pp. 131-133.

مقاومة مرض تعفن الجذور باستخدام الزيت العطري والنبات المطحون لنبات العادر

هند عبد الحميد محمد حميدو

قسم العلوم البيولوجية – كلية التربية بالعريش – جامعة قناة السويس

هدفت هذه الدراسة معرفة مدى قدرة الزيت العطري لنبات العادر وكذلك النبات المطحون علي مقاومة مرض تعفن الجذور والذي يحدث نتيجة الإصابة بفطرة الفيوزاريم سولاني. كما تم دراسة تأثيرها علي العدد الكلي للفطريات الموجودة في التربة . تم دراسة تأثير الزيت معمليا علي نمو الفطر بواسطة دراسة تأثيره علي الوزن الجاف للفطر وكذلك إنتاج إنزيمي الالفا اميليز والبروتييز. وقد وجد ان وجود الزيت يؤدي إلي تقليل ملحوظ في الوزن الجاف للفطر وانتاجه للإنزيمات المختبره . كما تم دراسة تأثير الزيت و النبات المطحون على نمو الفطر في التربة و تأثيره علي حدوث مرض تعفن الجذور لنبات البسلة ووجد أن نقع البذور في الزيت أو إضافة 10, 15 ,20 جم من النبات المطحون الي 500 جم من التربة يقلل نسبة حدوث المرض في النباتات المزروعة كما يعطى نباتات أكثر نضوجا بعد مرور 25 يوم من بداية الزراعة.

المحكمون:

أ.د. فتحي عواد منصور قسم النبات، علوم المنصورة

أ.د. يحي عبد الجليل محمود قسم النبات، علوم طنطا