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THE POTENTIAL ROLE OF SOME PLANT EXTRACTS OF ANTIFUNGAL PROPERTIES AGAINST THE EFFICACY OF *Trichoderma viride* FOR CONTROLLING GRAY MOLD OF ONION CAUSED BY *Botrytis cinerea*

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ABSTRACT

Botrytis cinerea Pers.; Fr., induces grey mold of onion worldwide. Fungicide applications are often used to control this pathogen. Natural plant extract do not show hazardous effects and likely to be used instead of the synthetic fungicides. In this study, antifungal activity of 5, 10, 20 and 40 µL of *Mentha longifolia*, *Origanum marjoram*, *Artemisia* sp and *Trichoderma* filtrate, respectively were applied in vitro to study their effect on the mycelial growth of *B. cinerea* using a poisoned food method. All treatments significantly presented their effect on the mycelia growth of the pathogen while the maximum inhibitory effect was shown when 40 µL of the extract was used. The inhibitory effect was affected by the amounts of the extract and the incubation time of the samples. Samples treatment of 40 µL concentration showed a maximum inhibition in the mycelia growth 5 days after incubation. This study shows that *Artemisia* sp has a potential to be used in the controlling management of *B. cinerea*. According to the available literature, this study could be the first to record the potential of *Mentha longifolia*, *Origanum marjoram*, *Artemisia* sp extracts and *Trichoderma* filtrate on the control of *Botrytis cinerea*, the causal agent of gray mold of onion in El-Beida, Libya.

Keywords: Onion, gray mold, antifungal activity, plant extracts, antagonistic fungi, Botrytis cinerea.

INTRODUCTION

Botrytis cinerea Pers.;Fr., is an aggressive pathogen attacks more than 200 plant species in the field (Coly-Smith, *et al*., 1980), greenhouse (Eden, *et al*., 1996; Sirjusingh, *et al*., 1996) and storage (Reyes, 1990; EL-Abd, 2002). The successive use of pesticides for controlling pest has caused many problems to the ecosystem. So far, a reduced pesticide usage is needed in the agricultural regime in the present era.

New strategies in the control of fungal contamination have been investigated. One of these is the use of biocontrol agents including bacteria (Ketterer, *et al*., 1992), yeasts (Droby, *et al*., 1997) , or antagonistic fungi (Eden, *et al*., 1996) , essential oils (Sun, *et al*., 2007), plant extracts (Soylu, *et al*., 2005). The presence of antifungal compounds, in higher plants, has long been recognized as important factors in disease resistance. Such compounds, being biodegradable and selective in their toxicity, are

considered valuable for controlling some plant diseases (Siva *et al*., 2008). In addition, plant extracts might have inhibitors to enzymes produced by the invading pathogens. The effects of different phenolic compounds found in many plants have inhibitory effects on the germination and growth of many fungal pathogens (Staub, 1991). Ramos *et al*. (2007) reported that components of Neem tree (*Azadirachta indica*) control *Crinipellis perniciosa* and *Phytophthora* species. *Ocimum* extracts which is used in traditional medicine contains active constituents of insecticidal, nematicidal, fungicidal and antimicrobial effect (Morales and Simon, 1996). Based on the above information, the objective of this study was carried out to investigate the effect of three plant extracts i.e.: Artemisia, Mint, Oregano and versus the antagonistic fungus *Trichoderma viride* on the growth of *B. cinerea* the causal agent of gray mold on onion bulbs in El-Beida, Libya.

MATERIALS AND METHODS

Fungal isolate:

B. cinerea was isolated from beneath the scales of the onion bulbs infected with gray mold and a pure culture was maintained on potato dextrose agar (PDA) medium for the further study.

Preparation of plant extracts:

Samples of plant leaves of *Mentha longifolia* L., *Origanum marjoram* L. and *Artemisia* sp L. were collected in a sterile polyethylene bags and transferred to the lab at the Department of Horticulture of the University for proper identification. Leaves were surface sterilized in 1% sodium hypochlorite solution for 3 min. and thoroughly rinsed in sterile distilled water. Thirty grams were mashed in a sterile mortar using a sterile pestle for 3 min. The resultant paste was soaked for 24hr in 100 µL of water, then filtered through sterile Whatman No. 1 filter paper. The resultant extract were filtered through 0.22 *µ*m sterile filter membranes and stored at 4◦C for further use.

Trichoderma metabolites:

Isolate of *Trichoderma* sp was grown on potato dextrose (PD) broth for 8 days, then filtered in Whatman No 1 papers and sterilized as shown above. The filtrates were used to study its effect on the growth of *B. cinerea* in vitro.

Linear growth test:

Poison plate method was followed, while different concentrations of plant extracts, and *Trichoderma* filtrate were obtained by amending 100 µL of molten PDA with 5, 10, 20 and 40 µL of the different extracts. The amended medium was dispensed into sterile Petri plates and allowed to solidify. Two perpendicular lines were drawn at the bottom of each plate to cross each other at the centre of the plate. Mycelia disk (o.5 mm diameter) cut from 7 day old fungus culture was placed in center of Petri-dishes. The petri plates were sealed with parafilm and incubated at 20±2^{o}C for 5 days. Control plates were treated with the same amount of sterile distilled water. The growth measured along the perpendicular lines for 3 and 5 days, and the mean growth values were obtained and then converted into the inhibition

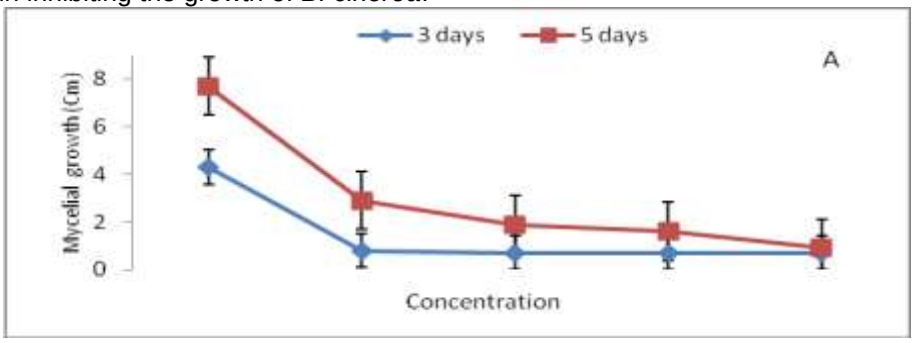
percentage of mycelial growth (MGI) in relation to the control treatment by using the formula, MGI (%) = [(dc – dt) / dc] × 100, where dc and dt represent mycelial growth diameter in control and treated Petri plates, respectively.

Statistical analysis:

Four replicates of each treatment were arranged according to Completely Randomized Design (CRD), The inhibition rate was measured as described above and subjected to analysis of variance using soft ware COSTAT. Comparison of means was performed by Duncan's multiple ranges test and least significant difference (LSD).

RESULTS

The antifungal activities of *Artemisia* sp, *M. longifolia* L., *O. marjoram* L., and *T. viride* filtrate were tested against *B. cinerea.* The results of antifungal effect of the extracts of the three plant species and *Trichoderma* filtrate are presented in Fig. 1 and Tables 1. The effects of different concentrations of the extracts on the mycelia growth of *B. cinerea* are shown in Fig. 1. Results of this study show that all the different concentrations of the extracts presented their inhibitory effects on the growth of *B. cinerea*. The potential of these extracts to inhibit the fungal growth was differed with the different extracts, and the concentration used. On the whole, *T. viride* was more effective in inhibiting the growth of *B. cinerea* than the other used plant extracts. Data in Table 1 show that the highest concentration tested in this study was (40 µL extract: 60 µL molten agar). All the tested extracts at the higher concentration inhibited the growth of *B. cinerea*. While, at low concentrations, differences began to show up among the different extracts. *Trichoderma* filtrate was highly effective against, *B. cinerea* as a complete inhibition of growth was observed. The inhibition occurred with *O. marjoram* was limited. The percentage of growth inhibition was varied from 36.6% at the fifth incubation day for *O. marjoram* and 40 up to 84.3% in case of *Artemisia* sp. The aqueous extract of *O. marjoram* was the least effective concentration in inhibiting the growth of *B. cinerea*.



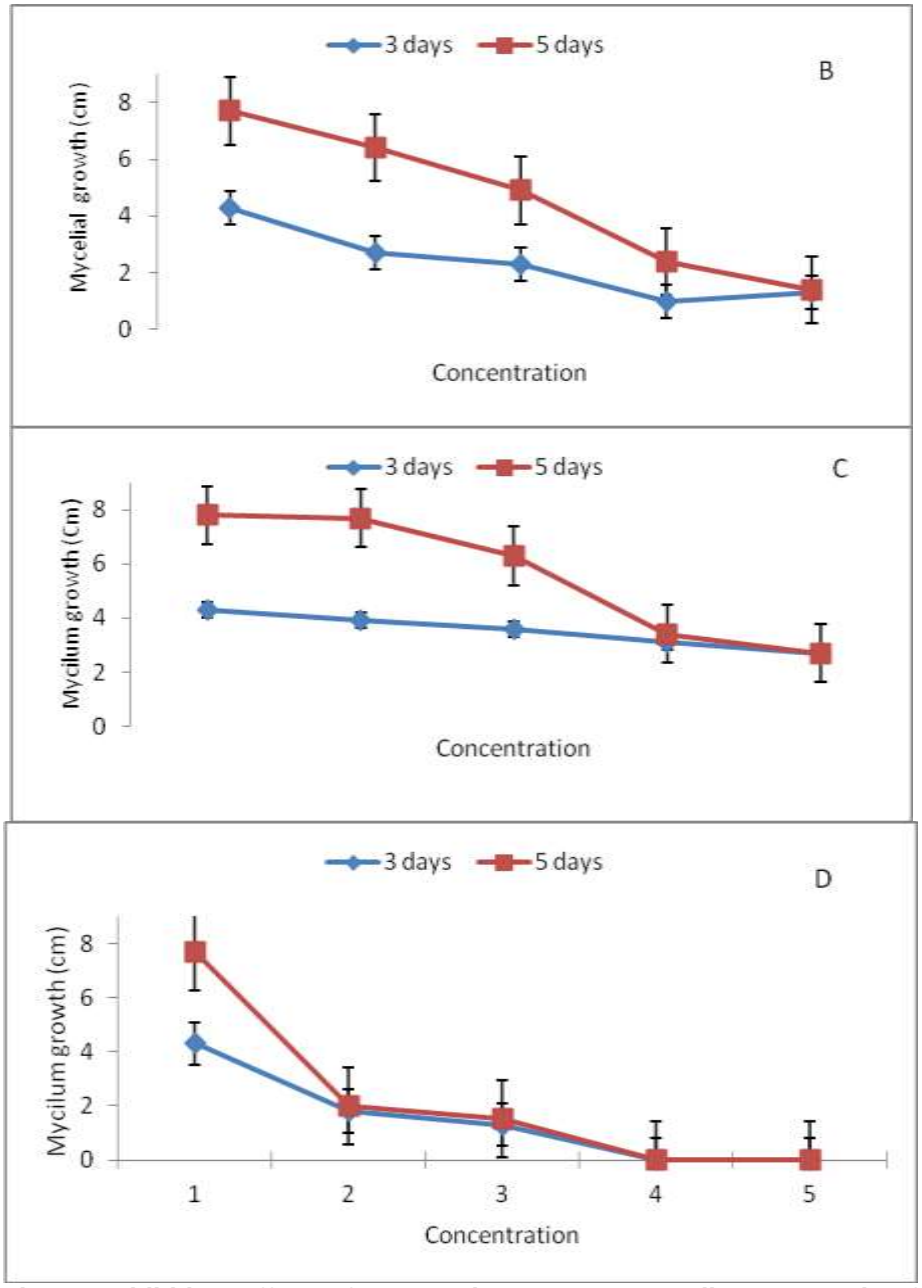


Fig. 1: Inhibition effect of some plant extracts on linear growth of *Botrytis cinerea Artemisia* sp, B. *M. longifolia* , C. *O. marjoram*, and D. *T. viride*).

Table 1. Percentage of growth inhibition of *Botrytis cinerea* treated by some plant extracts of antifungal properties.

Plant extracts	Days after incubation	Concentration (µL)					Mean
		0	5	10	20	40	
***Artemisia* sp**	**3**	0.00 b	81.4 a	84.3 a	84.3 a	86.2 a	67.2
	5	0.00 d	64.1 c	76.0 b	79.2 ab	84.3 a	60.7
Mentha longifolia	**3**	00.0 c	38.4 b	47.7 b	69.9 a	82.5 a	47.7
	5	00.0 c	17.6 bc	38.7 b	69.4 a	70.2 a	39.2
Origanum marjoram	**3**	00.0 d	8.5 cd	31.3 c	55.4 b	66.9 a	32.4
	5	00.0 d	4.4 d	18.0 bc	29.7 ab	36.6 a	17.7
Trichoderma viride	**3**	00.0 c	48.1 b	76.0 b	100 a	100 a	64.8
	5	00.0 c	41.0 b	87.0 a	100 a	100 a	65.6

The numbers are means of four replicates.
Means within a row followed by the same letter are not significantly (P < 0.05) different based on Dancans's multiple range test.

DISCUSSION

To develop environment-friendly alternatives methods for the control of fungal plant diseases, demand on the use of plant extracts has been increased. In this study, we investigated the antifungal activities of four plant extracts versus *Trichoderma* filtrates against *B. cinerea* in vitro using poison plate method. *Trichoderma* filtrate was more effective against *B. cinerea* than the other plant extracts, these results are in agreement with the results obtained by (Sarhan, 2006). *Trichoderma* spp produce inhibitory substances (antibiotics and toxins) such as Emodin, Gleotoxin, Pachybasin, Trichodermin and Trichodermol which retard the growth of the pathogen (Dossantos and Dhingra, 1982), while the release of some enzymes including B- 1,3-glucanase, Chitinase and Protease degrad the cell wall of the pathogen (Mehrotra, *et al*., 1997; Mathre, *et al*., 1999). It was also found that the plant extracts of *A. arborescens*, *M. longifolia* and *O. marjoram* were effective in reducing the fungal growth in vitro. However, all the extracts showed antifungal effect against *B. cinerea*. The antimicrobial effects of plant extracts depend on the kind of compound and its chemical components. More than twenty kinds of chemical compounds have been extracted from *M. longifolia*. The most important ones with antimicrobial activity are Menthol, Menthoforan, Menthon, Pipriton and Polgon. Additionally, it is also known that the compositions of the antimicrobial effect depends on plant species and regional condition as some research reported that there is a relationship between the composition of the extracts and the antimicrobial activity (Deans and Svoboda, 1990). Wilson *et al*. (1997) showed that 1,8-cineole, myrcene, α-pinene, and camphor are frequently found in different plant extracts and show that the essential oils found in *Artemisia* spp were associated with antifungal activities against *B. cinerea*. Similar antimicrobial activities of essential oils of *A. annua* from different countries have been previously reported against a variety of food-borne and human pathogens (Woerdenbag *et al*. 1993).On the other hand, *In vitro* studies on *B. cinerea* by using *O.*

majorana suggests that both the plant extract and essential oil can be used for the control of this fungus.

However, this is the first study dealing with the antifungal activities of *Artemisia* sp, *M. longifolia* and *O. marjoram* on *B. cinerea* the causal agent of grey mold and nick rot of onion in El-Beida, Libya.

REFERENCES

Coly-Smith, J.R., Verhoff, K. and Jarvis, W.R. (1980). The Biology of *Botrytis*. Academic Press, London.

Deans, S. and Svoboda, K. (1990). The antimicrobial properties of marjoram (*Oroganum majorana* L.) volatile oil. Flavour Fragr. J. 5:187-190.

Dossantos, A.F. and Dhingra, O.D. (1982). Pathogenicity of *Trichoderma* spp on *Sclerotinia sclerotiorum*. Canadian J. of Bot., 60: 472-475.

Droby, S., Wisniewski, M.E., Cohen, L. and Weiss, B. (1997). Influence of $CaCl_2$ on *Penicillium digitatum*, grapefruit peel tissue and biocontrol activity of *Pichia guilliermondii*. Phytopathology 87: 310-315.

Eden, M.A., Hill, R.A. and Stewart, A. (1996). Biological control of botrytis stem infection of greenhouse tomatoes. Plant Pathol. 45: 276-284.

EL-Abd, S.M. (2002). Studies on some fungal diseases infection crop in Egypt. Ph. D. Thesis Submitted to Univ. of Alexandria. Pp 204.

Ketterer, N., Fischer, B., Verhoeff, K. and Williamson, B. (1992). Biological control of *Botrytis cinerea* on grapevine by compost extract and their microorganisims in pure culture. Pages 179-186 in: Rec. Adv. In *Botrytis* Res., Proc. 10th Int. Symp. K. Verhoeff and N.E. Malathrakis, eds. Pudoc Scientific Publishers, Wageningen, Netherlands.

Mathre, D.F., Cook, R.J. and Callar, N.W. (1999). From discovery use: Traversing the world of commercializing biocontrol agent for plant disease control. Plant Dis., 83: 972-983.

Mehrotra, R.S., Aneja, K.R. and Aggarwal, A. (1997). Fungal control agents. In: Environmentally safe approaches to crop disease control. (Rechcigl, N.A. and Rechcigl, J.E.P. 111-137) CRC Press.

Morales, M.R. and Simon, J.E. (1996) New basil selection with compact Inflorescence for the ornamental market. In: J. Jamek (ed.), progress in new crops S. ASHS press. Alexandria, VA.

Ramos, A.R, Falcao, L.L., Barbosa, G.S, Marcellino, L.H. and Gander, E.S. (2007). Neem (*Azadirachta indica* a. Juss) components: Candidates for the control of *Crinipellis perniciosa* and *Phytophthora* spp. Microbiological Res. 162:238-243.

Reyes, A.A.(1990). Pathogenicity, growth and sporulation of *Mucor mucedo* and *Botrytis cinerea* in cold or CA storage. Hort. Science 25:549-552.

Sarhan, A.R.T. (2006). Interaction of Horse-mint leaf extract with fungal antagonistis on some legume seed-borne fungi. Arab J. Pl. Prot., 24: 118-124.

Sirjusingh, C., and Sutton, J.C. (1996). Effects of wetness duration and temperature on infection of geranium by Botrytis cinerea. Plant. Dis., 80: 160-165.

Siva, N., Ganesan, S., Banumathy, N. and Muthuchelian (2008). Antifungal Effect of Leaf Extract of Some Medicinal Plants Against *Fusarium oxysporum* Causing Wilt Disease of *Solanum melogena* L. *Ethnobotanical* Leaflets 12: 156-163.

Soylu, E.M., Yigitbas, H.F., Tok, S., Soylu1, S., Kurt1, Ö., Baysal, A. and Kaya, D.(2005). Chemical composition and antifungal activity of the essential oil of Artemisia annua L. against foliar and soil-borne fungal pathogens

Staub, T. (1991) Fungicide resistance: practical experience with antiresistance strategies and the role of integrated use. Annu. Rev. Phytopathol. 29, 421–442.

Sun, O.L., Gyung, J.C., Kyoung, S. J., He, K. L., Kwang, Y. C. and Jin Cheo,l K. (2007). Antifungal Activity of Five Plant Essential Oils as Fumigant Against Postharvest and Soilborne Plant Pathogenic Fungi. Plant Pathol. J. 23(2) : 97-102.

Wilson, C. L., Solar, J. M., el Ghaout, A., Wisniewski, M. E. (1997): Rapid evaluation of plant extracts and essential oils for antifungal activity against *Botrytis cinerea.* Plant Dis. 81, 204–210.

Woerdenbag, H. J., Bos, R., Salomons, M. C., Hendriks, H., Pras, N., Malingre, T. M. (1993): Volatile constituents of *Artemisia annua* L. (Asteraceae). Flav. Fragr. J. 8, 131–137.

التأثير التثبيطي لبعض المبيدات الحيوية ضد الفطر *Botrytis cineria*

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استهدفت هذه الدراسة تقييم فعالية التأثير التثبيطي لأربعة من المبيدات الحيوية والمتمثلة في مستخلصات أوراق البردقوش (*Origanum marjoram*)، النعناع (*Mentha longifolia*)، الشيح (*Artemisia* sp.) وراشح الفطر *Trichoderma viride* بتركيزات 0، 5، 10، 20 و 40% وباستخدام طريقة الغذاء المسموم ضد النمو القطري للفطر *Botrytis cinerea* المسبب لمرض العفن الرمادي في البصل. أوضحت النتائج أن جميع التركيزات المختبرة كانت ذات تأثير معنوي على النمو القطري لميسليوم الفطر، وان نسبة التثبيط اختلفت باختلاف التركيز ونوع المبيد المستخدم وزمن التحضين، حيث كانت نسبة التثبيط منخفضة عند التركيز 5% وازدادت بارتفاع التركيز إلى 40%. راشح الفطر المضاد كان أكثر فعالية في تثبيط النمو الميسليومي للفطر *B. cinerea* مقارنة بالمستخلصات النباتية، في حين اتضح أن لمستخلص أوراق الشيح فعالية اكبر في تثبيط النمو القطري للفطر الممرض مقارنة بمستخلصات أوراق البردقوش والنعناع.

كلمات مفتاحية: مضادات فطرية، مستخلصات نباتية، فطريات مضادة، *Botrytis cinerea*.

قام بتحكيم البحث

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