

EFFECT OF SOME MEDICINAL PLANT EXTRACTS ON CONTROLLING CHOCOLATE SPOTE DISEASE OF FABA BEAN

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ABSTRACT

Crude extracts of thym plants (*Thymus vulgaris* L.) and peppermint plants (*Mentha piperita* L.) were evaluated as antifungal on the mycelial growth, spore germination and disease severity of *Botrytis fabae* Sard, the causal pathogen of chocolate spot disease of faba bean *in vitro* and *in vivo*. Three concentrations 25, 50 and 100% were used from the tested plant extracts. Thym plant extracts were more effective than peppermint on inhibiting mycelial growth and spore germination. The efficiency of the tested extracts as antifungal were decreased with dilution. The same trend was found *in vivo*. Thym plant extract was more effective than that of peppermint plant extracts on reducing disease severity percentages on faba bean. Increasing concentrations of the tested extracts caused significant reduction in disease severity.

Keywords: chocolate spot disease, plant extracts.

INTRODUCTION

Chocolate spot disease causing great losses in faba bean yield and serious damage to the crop especially in the Northern parts of Delta, Egypt under low temperature and highly humidity (Mahmoud, 1985 and Abou- Zeid and Mohamed 1987). The significance of chocolate spot disease is related to its severity and the timing of infection. The highest infection with chocolate spot disease can cause 50% yield reduction of faba bean if infection occurs early in the growing season (Mansfield and Deverall, 1974 and Harrison, 1980).

Chemical control is the most widely used means of controlling chocolate spot disease in faba bean and led to many problems, according to the interaction of their residue with biological systems in the environment (Elliott and Whittington, 1980, Mamluk *et al.*, 1989 and Abou-Zaid *et al.*, 1990).

The modern trends were directed to study the effect of various natural substances against some pathogens caused plant disease. The medicinal and aromatic plants as antifungal were also documented (Saksena and Tripathi, 1987; Agha, 1992; zedan *et al.*, 1994 and Sivropoulou *et al.*, 1995).

Heweidy *et al.*, 1997 tested the aqueous extracts of garlic and henna against *Botrytis fabae* Sard, the causal pathogen of chocolate spot disease on faba bean. *In vitro* test showed that both extracts had positive effect on reducing mycelial growth and spore germination. The *in vivo* test gave the same results.

The essential oils of *Thymus vulgaris* and *T. capitatus* have fungicidal activity due to thymol oil against soil borne fungi, *Rhizoctonia solani*, *Pythium*

ultinum and *Fusarium solani* and also post harvest phytopathogens, *Botrytis cinerea* and *Penicillium italicum* (Arras *et al.*, 1995; zambonelli *et al.*, 1996 and Eloff, 1998).

Mekuria *et al.*, 1998 studied the antifungal nature of ethanolic extracts from 18 bryophytes species *in vitro*. Bioassays indicated that extracts from *Bazzania trilobata*, *Diplophyllum albicans* and *Sphanum quinquefarium* caused the greatest inhibition (? 50%) of mycelial growth of *Botrytis cinerea* and *Alternaria solani*.

Ismail, (1998) found that the aqueous extracts of eucalyptus, salinin and acacia plants as soil drench decreased the percentage of damping-off and root rot diseases of tomato seedlings.

This study was carried out to evaluate the effect of thym and peppermint plant extracts on the mycelial growth, spore germination of *Botrytis fabae in vitro* and disease severity on faba bean plants under green house conditions .

MATERIALS AND METHODS

A virulent isolate of *Botrytis fabae* previously isolated from faba bean leaves was obtained from Legume Dis. Res. Dept. Plant Pathol. Inst. ARC. Giza. Fungal culture was grown on PDA medium for further studies.

1- Plant materials:

Extracts of two medicinal aromatic plants Thym (*Thymus vulgaris* L.) and peppermint (*Mentha piperita* L.) were tested for their antifungal activity.. The tested plants were provided by the Department of Aromatic plants (ARC) Alexandria.

2- Preparation of crude extracts:

Samples of 50 gm of air dried plants were ground and soaked in 100 ml sterilized distilled water for 24 hrs. the extracts were filtered through tow layers of sterilized cheese cloth. The supernatant were centrifuged at 4000 rpm for 20 min. The yielded extracts were sterilized through centered glass (G4) to be used in further studies.

3-Effect of crude extracts of thym and peppermint on *B. fabae in vitro*.

The concentrations 25, 50 and 100% of thym and peppermint extracts were prepared by adding suitable amount of sterilized distilled water to the crude extracts of either thym or peppermint (v/v). Two ml of each tested concentration were added to PDA medium in Petri dishes before solidification. Inoculation was done with fungal discs, 5 mm in diameter obtained from *B. fabae* 7 days old culture. Four replicates were used for each tested concentration.

Another group of PDA plates free from plant extracts, inoculated with the fungus as check treatment. All plates were incubated at 20 °C for 7 days. Linear growth was recorded. The percentages of reduction in the mycelial

growth were calculated. The obtained data were statistically analyzed, according to Snedecor and Cochran, (1967).

4- Effect of crude extracts of thym and peppermint on *B. fabae* spore germination:

Three concentrations of either thym or peppermint plant extract (25, 50 and 75 %) were used to test their antifungal effect on spore germination of *B. fabae*. Spore suspension was adjusted with the haemocytometer to contain 15×10^4 spores/ml from 12 days old *B. fabae* cultures. A suitable amount of the spore suspension was pipetted on 4 replicates of germination. A suitable amount of each tested concentration was separately added. Sterilized distilled water was used in the control slides. The slides were incubated at 20 °C for 12 hrs. spore germination was determined.

5- Green house experiment:

Seeds of faba bean Gize 843 cv. were sown in pots, 25 cm in diameter, each planted with 5 seeds. The growing plants 60 days old, grown plants were sprayed with either thym or peppermint plant extract. *B. fabae* spores obtained from 12 days old culture grown on PDA medium. Spores were separated using a brush with sterilized distilled water and counted by a haemocytometer then adjusted to 15×10^4 spores/ml and used immediately for artificial inoculation.

The extracts of thym or peppermint at three concentrations (25, 50 and 100%) were separately spread on faba bean plants a day before inoculation with spore suspension of *B. fabae*. Another set of 4 pots was only inoculated with the spore suspension and served as check treatment. All treated plants were covered with plastic bags for 24 hrs. to maintain suitable humidity around the plants, then kept under green house conditions. All plants were examined after 7 days, disease severity was determined according to Horsfall and Heuberger, (1942).

Data were statistically analyzed as Randomized Complete Block Design suggested by Snedecor and Cochran (1967). Least significant difference (L.S.D. at 5% probability) was used to compare between treatment averages.

RESULTS AND DISCUSSION

1- Effect of aqueous extracts of thym and peppermint on *B. fabae* in vitro:

a- Mycelial growth:

The antifungal properties of two aqueous plant extracts (thym and peppermint) against *B. fabae* were evaluated *in vitro*. Data in Table (1) showed that thym or peppermint extracts exhibited a variable degree of antifungal activity against *B. fabae*. The reduction in the mycelial growth ranged from 62.77% to 12.21%. The efficiency of thym and peppermint aqueous extracts as antifungal decreased with dilution. Data also showed that thym extract was more effective as antifungal against *B. fabae* than peppermint.

Effect of thym extracts may be attributed to the antifungal activity of the natural components, i.e. thymol (Muller-Riebau *et al.*,1995) gallic acid (Cowan, 1999) and phenolic alcohol, polyphenols and flavones (Vokou *et al.*,1984). At the same time, efficiency of peppermint extracts may be due to its chemical compounds, menthol and terpenoid (Hammer *et al.*,1999).

Table (1): Effect of different concentrations of either thym or peppermint plant extract mycelial growth of *B. fabae*.

Treatment	Reduction in mycelial growth (%)		
	Concentration		
	100	50	25
Thym	62.77	24.07	18.51
Peppermint	42.49	18.31	12.21
Control	0.0		

L.S.D. at 5% for treatments (T) : 2.89
 Conc. (c) : 2.36
 TxC : 4.09

b- Spore germination:

Data in Table (2) showed significant reduction in spore germination of *B. fabae* attributed to the inhibitory effect of the concentrations of thym and peppermint plant extracts. The lowest percentage of spore germination was obtained at 75% of thym (8.99%). The efficiency of thym and peppermint aqueous extracts as antifungal decreased with dilution. All the tested concentrations significantly reduced spore germination of *B. fabae* compared with the control. Muller- Riebau *et al.*, (1995) and Zambonelli *et al.*,(1996) found that the essential oil of thym had strong antifungal activity against soil borne fungi. This fungicidal activity due to thymol compound. Antonov *et al.*,(1995) tested the effects of 21 plant extracts on conidium germination and germ tube growth of *B. cinerea*. The most effective extract was thym oil which completely inhibited conidial germination and germ tube growth at the lowest concentration (0.1%).

Table (2): Effect of three concentrations of either thym or peppermint plant extracts on spore germination of *B. fabae*.

Treatment	Spore germination (%)		
	Concentration		
	75	50	25
Thym	8.99	38.11	40.03
Peppermint	12.05	24.39	43.22
Control	74.29		

L.S.D. at 5% for treatments (T) : 3.32
 Conc. (c) : 2.46
 TxC : 4.71

2- Greenhouse experiment:

Data in Table (3) showed that all treatments with the three tested concentrations of thym and peppermint plant extracts reduced disease severity compared with control 66.94%. Increasing concentration of thym or peppermint led to decrease the percentage of disease severity and differences between infested control and all treatments were significant.

Table (3): Effect of thym and peppermint aqueous extracts on severity of chocolate leaf spot disease of faba bean under greenhouse conditions.

Treatment	Disease severity (%)		
	Concentration		
	100	50	25
Thym	17.16	27.27	31.46
Peppermint	22.03	24.08	33.9
Control	66.94		

L.S.D. at 5% for treatments (T) : 2.24
 Conc. (c) : 1.82
 TxC : 3.16

These results are in agreement with that reported by Heweidy *et al.*, (1997). They found that the aqueous extracts of garlic and henna reduced disease severity of chocolate spot disease on faba bean plants but the fungicide ronilan was more effective than them. Hassanein and Eldoksch, (1997) reported that thym, peppermint and caraway oils showed high antimicrobial activity against *Agrobacterium tumefaciens*, *Pseudomonas solanacearum* and *Erwinia carotovora* *in vitro* and *in vivo*. Arras *et al.* (1995) found that thym essential oil inhibited the growth of postharvest pathogens *B. cinerea* and *Penicillium italicum* on peel and orange fruits.

Generally, it could be concluded that natural extracts of different aromatic and medicinal plants exhibited antifungal activity against faba bean chocolate spot disease. However, further experiments are needed in this field.

REFERENCES

- Abou- Zeid, N.M. and H.A. Mohamed (1987). Factors affecting severity of *Botrytis fabae* on faba beans in Egypt. Proc. 5th. Cong. Egypt. Phytopathol. Soc. Giza., 13-26.
- Abou- Zeid, N.M. M.S. Moustafa; A.M. Hassanien and I. EZ-Eldin (1990). Control of chocolate spot disease of faba bean and the effect of fungicides on the behavior of the causal fungus. Agric. Res. Rev. 68 (3): 411-421.
- Agha, M.S. (1992). Studies on antifungal agents in certain ornamental medicinal and aromatic plants with special reference to control seedlings damping-off disease of sesame. Egypt .J. Appl. Sci., 7 (1): 104-114.
- Antonov, A.; A. Stewart and M.Walter (1995). Inhibition of conidium germination and mycelial growth of *Botrytis cinerea* by natural products. Proceedings 48th New Zealand plant Protection Conference.
- Arras, G.; M. Agabbio; A. Piga and G.D. Hallewin (1995). Fungicide effect of volatile compounds of *Thymus capitatus*. Acta Horticulture No (379): 593-600 (Rev. Pl. Path. 75 (3), 1463, 1996).

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- Cowan, M.M. (1999). Plant products as antimicrobial agents. *Clin. Microbial. Rev.*, 12 (4): 564-582.
- Elliot, J.E.M. and W.J. Whittington (1980). The control of chocolate spot (*Botrytis fabae*) infection of field beans (*Vicia faba* L.) by the fungicides Benlate, Bavistin, cercobin and BAS 552 F. *J. Agric. Sci.*, 94: 461-464.
- Eloff, J.N. (1998). Which extractant should be used for the screening and isolation of antimicrobials components from plants. *J. Ethnopharmacology*, 50: 1-8.
- Hammer, K.A.; C.F. Carson and T.V. Riley (1999). Antimicrobial activity of essential oils and other plant extracts. *J. App. Microbio.*, 86: 985-990.
- Harrison, J.G. 1980. Effect of environmental factors on growth of lesions on field bean leaves infected by *Botrytis fabae*. *Ann. Appl. Biol.*, 95: 53-61.
- Hassanein, M. Ferial and H.A. El-Doksch (1997). Antibacterial action of carvone and some plant extracts on certain phytopathogenic bacteria and pathogenicity of *Agrobacterium tumefaciens*. *Alex. J. Agric. Res.*, 42 (1): 127-136.
- Heweidy, M.A.; Mahmoud, A.F. Fatma; Essmat, E.A. Nadia and El-Shami, A.M.Mona (1997). The use of crude extracts of garlic cloves and henna leaves as a biological control against chocolate spot disease of faba bean. 8th Congress of Egyptian Phytopathol. Soc., Cairo, 161-171.
- Horsfall, J.G. and J.W. Heuberger (1942). Measuring magnitude system for measuring plant disease. *Phytopathol.*, 32: 226-232.
- Ismail, A.E.A. (1998). Control of Soil-borne fungi causing root rot and wilt of tomato plants by some plant extracts. *J. Agric. Sci. Mansoura Univ.*, 23: 1081-1091.
- Mahmoud, R.A. 1985. Studies on leaf spot of faba bean. M.Sc. Thesis. Faculty of Agric. Kafr El-Sheikh, Tanta University pp 67.
- Mamluk, O.F.; M.P. Hawre; K.M.Makkouk and S.B. Haounik (1989). Occurrence, losses and control of important cereal and food legume disease in west Asia and North Africa. *Tropical Agriculture Research Series*. 22: 131-140 (*Rev. Pl. Path.* 70 (10), 6832, 1991).
- Mansfield, J.W. and D.C. Deverall (1974). Fungal development and lesion formation in leaves of *Vicia faba* during infection by *Botrytis cinerea* and *B. fabae* *Ann. Appl. Biol.*, 76: 77-89.
- Mekuria, T; P. Blaeser; U.Steiner and H.W. Dehne (1998). Bryophytes as a new source of antifungal substances in crop protection. In *Modern International Reinhardsbrunn. Symposium. Friedrichroda Thuringia, Germany*, 483-490 (*Rev. Path.* 6696, 78 (10), 1999).
- Muller-Riebau, F.;B. Berger, and O. Yegen (1995). Chemical composition and fungitoxic properties to phytopathogenic fungi of essential oils of selected aromatic plants growing wild in Turkey. *J. Agric. Food. Chem.*, 43 (8): 2262-2266.

- Saksena, N. and H. S.Tripathi (1987). Antifungal substance in the essential oil of anise (*Pimpinell anisum* L.). *Agric. & Biol. Chim.*, 51 (7): 1991-1993.
- Sendecor, G.W. and W.G. Cochran (1967). *Statistical Methods*. Oxford and J.B.H. Publishing co. 6th edition.
- Sivropoulou, A.; S. Kokkini; T. Lanaras and M. Arsenakis (1995). Antimicrobial activity of ment essential oils. *J. Agric. Food chem.*, 43 (9): 2384-2388.
- Vokou, D.; N. S. Margaris and J.M. Lynch (1984). Effects of volatile oils from aromatic shrubs on soil microorganisms. *Soil Biol. Biochem.*, 16: 509-513.
- Zambonelli, A.; Z.D. Aulerio; A. Bianchi and A. Albasinin (1996). Effects of essential oils on phytopathogenic fungi *in vitro*. *Journal of phytopathology* 144 (9/10) 491-494. (*Rev. Pl. Path.* 76 (6), 4275, 1997).
- Zedan, A.M.; A.M. EL-Toony and N. G.H. Awad (1994). A comparative study on antifungal activity of certain plant extracts, essential oils and fungicides on tomato wilt pathogens. *AL-Azhar. J. Agric. Res.*, 20: 217-236.

تأثير مستخلصات بعض النباتات الطبية في مكافحة مرض التبقع الشيكولاتي على الفول البلدي

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تم دراسة تأثير المستخلص المائي لكل من الزعتر والنعناع الفلفلي بثلاثة تركيزات على النمو الميسليومي وانبات الجراثيم للفطر بوترايس فابي المسبب لمرض التبقع الشيكولاتي للفول البلدي وكذلك شدة الإصابة بالمرض. وقد وجد من النتائج المتحصل عليها ان التركيزات الثلاثة من الزعتر والنعناع الفلفلي كان لها تأثير معنوي في تقليل النمو الميسليومي وانبات الجراثيم للفطر المختبر وذلك في الاختبارات المعملية. كما وجد ايضا ان المستخلص المائي للزعتر كان ذات تأثير افضل من المستخلص المائي للنعناع الفلفلي.

واظهرت دراسة تأثير تلك المستخلصات في الصوبة لمكافحة المرض على نباتات الفول نفس النتائج المتحصل عليها معمليا فقد قلت تركيزات المستخلصات المائية المستخدمة لكل من الزعتر والنعناع الفلفلي من شدة إصابة نباتات الفول بمرض التبقع الشيكولاتي. كما ادت زيادة التركيزات المستخدمة من مستخلصات النباتات المختبرة إلى تقليل شدة الإصابة بالمرض بدرجة معنوية.