EFFECT OF SOME MEDICINAL PLANT EXTRACTS ON CONTROLLING CHOCOLATE SPOT 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 Devera, 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 (Eliott 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; zeidan et al., 1994 and Siropoulos et al., 1995).

Heweyda 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
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... 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 tricobata, Diplophyllum albicans and Sphaeria quinguefurarium caused the greatest inhibition (70%) of mycelial growth of Botrytis cinerea and Alternaria solani.

Ismail, (1998) found that the aqueous extracts of eucaluptus, salinina 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 greenhouse 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 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 pepperment on B. fabae in vitro.

The concentrations 25, 50 and 100% of thym and pepperment extracts were prepared by adding suitable amount of sterilized distilled water to the crude extracts of either thym or pepperment (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 pepperment on B. fabae spore germination:

Three concentrations of either thym or pepperment 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 $15 \times 10^6$ 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 $20^\circ$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 pepperment 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 $15 \times 10^6$ spores/ml and used immediately for artificial inoculation.

The extracts of thym or pepperment 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 pepperment on B. fabae in vitro:

a- Mycelial growth:

The antifungal properties of two aqueous plant extracts (thym and pepperment) against B. fabae were evaluated in vitro. Data in Table (1) showed that thym or pepperment 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 pepperment aqueous extracts as antifungal decreased with dilution. Data also showed that thym extract was more effective as antifungal against B. fabae than pepperment.
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.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Reduction in mycelial growth (%)</th>
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<tbody>
<tr>
<td></td>
<td>Concentration</td>
</tr>
<tr>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Thym</td>
<td>62.77</td>
</tr>
<tr>
<td>Peppermint</td>
<td>42.49</td>
</tr>
<tr>
<td>Control</td>
<td>0.0</td>
</tr>
</tbody>
</table>

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., (1996) and Zambonelli et al.,(1995) 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 thymol 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.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Spore germination (%)</th>
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<tbody>
<tr>
<td></td>
<td>Concentration</td>
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<tr>
<td></td>
<td>75</td>
</tr>
<tr>
<td>Thym</td>
<td>8.99</td>
</tr>
<tr>
<td>Peppermint</td>
<td>12.05</td>
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<tr>
<td>Control</td>
<td>74.29</td>
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</tbody>
</table>

L.S.D. at 5% for treatments (T) = 3.32
Conc (c) = 2.48
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.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Disease severity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration</td>
</tr>
<tr>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Thym</td>
<td>17.16</td>
</tr>
<tr>
<td>Peppermint</td>
<td>22.03</td>
</tr>
<tr>
<td>Control</td>
<td>68.94</td>
</tr>
</tbody>
</table>

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

These results are in agreement with those reported by Heweyid 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 roxilan 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 pear 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


تأثير مستخلصات بعض النباتات الطبية على مكافحة مرض التبقع الشكيولائي على الغوفة البلدي

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تم دراسة تأثير المستخلصات المائية لكل من الزعتر والثوم والدخنات الباليتي بثلاثة تركيزات على النسيم النباتي لداء التبقع الشكيولائي. وتم تكرير تجربة ثمانية مجموعات مبيدات احتمالية للنمل الحوريني. وتم قياس نسب التلوث ونسبة المرض المكتشفة. وتم قياس نسبة المرض الناجم عن التركيزات المختلفة. وتميز النتائج بانخفاض نسبة المرض في المجموعات التي تم تمعينها بالتركيزات المختلفة. وتم استخدام الطريقة المخبرية للمسح المتابعة للنمل الحوريني. وتم استخراج النتائج من خلال قياس نسبة المرض ونسبة التلوث ونسبة المرض المكتشفة. وتم استخدام النتائج كمقياس للمؤثرات المثيرة للمرض ونسبة المرض الناجمة عن التركيزات المختلفة. وتم استخدام النتائج كمقياس للمؤثرات المثيرة للمرض ونسبة المرض الناجمة عن التركيزات المختلفة.