Studies of *Colletotrichum dematium* f. sp. *truncatum* on soybean

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Key words: *Colletotrichum dematium* f. sp. *truncatum*; anthracnose soybean; *Glycine max*.

SUMMARY

Growth and sporulation studies of *Colletotrichum dematium* var. *truncatum* were conducted using different media, temperatures and light regimes. Of the seven media employed, oat meal agar supported the best mycelial growth, with sporulation best on Czapek-Dox agar and potato dextrose agar. Greater mycelial growth was obtained under 12 hours alternating ultraviolet light and continuous light than in darkness. Sporulation was highest under 12 hours alternating light treatment. The optimum temperature for growth and sporulation was 25°C while germination was optimum at 20°C. Pathogenicity studies in the glasshouse showed that seedlings of cultivar 66D-16 were most susceptible while cultivar 66F-4A was most resistant. T. Palmetto was more resistant. An in vitro test of fungicides against the fungus showed that Topsin M was the most toxic with an ED$_{50}$ of 2.2 μg/ml.

INTRODUCTION

In Malaysia, soybean cultivation is still in its infancy. The area under cultivation in 1977 was 36 hectares (sole crop), although, Malaysia imported soybean and soybean products in 1977 valued at M$72,766.28.00 (Anon., 1977b). One drawback to soybean cultivation is the wide occurrence of pests and diseases. Over 100 pathogens have been reported to attack soybean in the USA (Sinclair, 1982). In the tropics and sub tropics, one of the serious diseases of soybean is anthracnose caused by *Colletotrichum dematium* (Pers. ex Fr.) Grove var. *truncatum* (Schw.) Arx. In Malaysia, Nik et al., (1980), showed that seeds of most soybean cultivars were infested with the anthracnose pathogen.
to one another were taken per plate. The same plates were used for spore counts. Effect of seven temperatures on growth and sporulation were done as follows: 5 ± 2°C (refrigerator), 15°C (incubator), 20 ± 2°C (culture room), 25°C (incubator), 30°C (incubator), 35°C (incubator) and 40°C (incubator).

Effect of light on growth and sporulation was conducted under the following light regimes: a) 12 hours fluorescent light alternated with 12 hours darkness, b) continuous light, c) continuous darkness, d) 12 hours near ultra violet light (NUV) with 12 hours darkness, e) 12 hours ultra violet light (UV) with 12 hours darkness.

Spore germination studies were conducted using the cellophane agar method (Lenne, 1978). Cellophane pieces (1.5 cm square) were taken from the wrapper of Whatman No. 1 filter paper and soaked in distilled water for 20 minutes before autoclaving. Four pieces of the sterilized cellophane were placed on water agar plates. One drop of spore suspension of the fungus was placed on each cellophane piece. Plates were incubated under the temperatures previously listed. Germination counts were made after 14 hours by counting 100 spores in each microscopic field. A spore was considered germinated when the germ tube length exceeded the breadth of the spore.

Pathogenicity studies The pathogenic potential of the fungus was determined on two-week-old plants and on detached and intact green pods. Four soybean cultivars were used: 66D-16, 66D-2, 66F-4A and Palmetto. A completely randomised design with five replications per treatment was employed. Two-week-old plants were sprayed with a spore suspension using a "Shandon Laboratory Spray Gun". The control plants were sprayed with a sterile distilled water. Disease ratings were made two weeks after spraying by using the following scale (Lenne and Sonoda, 1978): 1 = no lesion, 2 = 1–3 lesions per seedling, 3 = 4–6 lesions per seedling, 4 = more than 6 lesions per seedling, 5 = abundant lesions and defoliation, 6 = abundant lesions, defoliation and seedling death. The disease severity index (DSI) was computed as follows:

\[
DSI = \frac{\text{Sum of numerical ratings}}{\text{total number of seedlings assessed}}
\]

The dry weights of the roots and shoot of each plant were taken after oven-drying the plants for three days at 80°C.

Green pods of the four soybean cultivars were sprayed using the spore suspension as previously stated. The pods were left to maturity and the seed harvested for reisolation of C. dematium var. truncatum. Inoculation was also performed in the laboratory on detached green pods of each cultivar.

Efficacy of five fungicides against C. dematium var. truncatum

Five fungicides viz., Benlate 50 W.P. (R) (Methyl-1 (butylcarbamoyl) 2 benzimidazole carbamate), Dithane M-45 (R) 50 W.P. (Zinc manganese ethylene-bisdithiocarbamate), Captan 500(R) –50 W.P. (N-(trichloromethylthio)glycolohex - 4 - ene - 1, 2 - dicarboximide); Topsin M(R) 1, 2 – di – (3-methoxy carbomyl – 2 – thiouredo) benzene and Vitigran Blue(R) – 60 W.P. (copper oxychloride) were tested for in vitro – efficacy against the test fungus. Concentrations of each fungicide were 10, 50, 100 and 1000 µg/ml active ingredient, added aseptically in molten PDA. Five mm diameter PDA agar plugs of the test fungus taken from a week old PDA culture then were placed in the centre of each plate. Four replications were done for each concentration. The plates were incubated at room temperature (28 ± 1.5°C) for 10 days and colony diameter recorded in mm, every two days. The \( t_{DS0} \) values were determined by Probit analysis (Finney, 1971).

RESULTS AND DISCUSSION

Growth and sporulation of C. dematium var. truncatum on different media

The rate of mycelial growth varied among the media tested. OMA supported the best growth followed by PDA and BPA. Poorer growth was obtained on MEA and CMA due to the inadequate or unsuitable carbon source present in the media. Malhotra and Chaturvedi (1974) reported that sucrose was the best carbon source for the growth of the fungus. Malt-extract agar possesses high maltose content but little sucrose. In the case of sporulation, PDA and CDA gave significantly (\( P = 0.05 \)) higher number of spores per ml spore suspension compared to BPA, MEA, OMA and CMA (Fig. 1). Although CDA was not the best medium for mycelial growth, its high sucrose content coupled with the presence of metallic ions such as \( \text{Na}^{+} \), \( \text{K}^{+} \), \( \text{Fe}^{2+} \) and \( \text{Mg}^{2+} \) and anions such as \( \text{N}_{3}^{-} \), \( \text{SO}_{4}^{2-} \) and \( \text{PO}_{4}^{3-} \) could have accounted for the high sporulation. PDA has a high sucrose content which favours sporulation.

Effect of light on growth and sporulation

Good mycelial growth was obtained under alternating 12 hours of UV light and continuous
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Fig. 1. Growth and sporulation of C. dematium var. truncatum 10 days after incubation at 25 ± 1.5°C on bean pod agar (PDA), Czapek Dox agar (CDA), corn meal agar (CMA), malt extract agar (MEAL), oat meal agar (OMA), potato dextrose agar (PDA) and water agar (WA).

light, with poor growth under continuous darkness (Fig. 2). Sporulation was highest under the alternating 12 hours light followed by alternating 12 hours UV light. Good sporulation also was obtained under alternating 12 hours of NUV continuous light. Poor sporulation was obtained under continuous darkness.

Effect of temperature on growth and sporulation of C. dematium var. truncatum
Mycelial growth was good between 15°C and 35°C (Fig. 3). There was little growth at 5°C and no growth occurred at 40°C. The ability of the fungus to grow at 5°C could aggravate the seriousness of C. dematium f. sp. truncatum as a seedborne pathogen of soybeans. Sporulation occurred at all temperatures except 40°C. Sporulation was greatest at 25°C and was significantly (P = 0.01) higher than at the other temperatures. No significant difference was obtained among the other temperature treatments. Setae were produced at the temperature treatments of 15, 20 and 25°C but not at 5, 30, 35 and 40°C.

Effect of light on the growth and sporulation of C. dematium var. truncatum
Fig. 2. Effect of light on the growth and sporulation of C. dematium var. truncatum 10 days after incubation at 20±2°C on PDA. Note 12hL = 12 hours light, 12hD = 12 hours darkness, 12hNUV = 12 hours near ultraviolet, 12hUV = 12 hours ultraviolet, 24hL = 24 hours light, 24hD = 24 hours darkness.

Effect of temperature on germination
More than 60% germination occurred between the temperatures of 15 and 30°C (Table 1). The highest germination was recorded at 20°C. Spores of C. dematium var. truncatum did not germinate at 5°C and 40°C. Most conidia produced two germ-tubes, usually from opposite ends of the conidium.

Pathogenicity of C. dematium var. truncatum on soybean seedlings and soybean pods
Cultivar 66D-16 had the highest disease severity index followed by 66D-2, Palmetto and 66F-4A in descending order (Table 2). There was no significant difference between the dry weight of roots and shoots of the inoculated plants and the control. This could be due to the less severe infection obtained. The infection process is controlled by many factors such as
Fig. 3. Effect of temperature on the growth and sporulation of *C. dematium* var. *truncatum* 10 days after incubation on PDA.

![Graph showing effect of temperature on growth and sporulation](image)

**Table 1**

Effect of temperature on conidial germination of *Colletotrichum dematium* var. *truncatum*.

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>Percent germination (after 14 hours)*</th>
</tr>
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<tbody>
<tr>
<td>5 ± 2</td>
<td>0.00 c</td>
</tr>
<tr>
<td>15</td>
<td>60.25 b</td>
</tr>
<tr>
<td>20 ± 2</td>
<td>77.00 a</td>
</tr>
<tr>
<td>25</td>
<td>65.00 b</td>
</tr>
<tr>
<td>30</td>
<td>62.00 b</td>
</tr>
<tr>
<td>35</td>
<td>2.25 c</td>
</tr>
<tr>
<td>40</td>
<td>0.00 c</td>
</tr>
</tbody>
</table>

*Values are means of four counts of 400 conidia and values followed by similar letters denote no significant differences at *P* = 0.05 as determined by Duncan New Multiple Range Test.

No symptoms of infection were observed on the green pods in the glass-house at two weeks after inoculation. However, studies on detached green pods showed that the inoculated pods exhibited brown, water-soaked lesions which were covered with grey spore masses borne in dark acervuli. Cultivar 66D-16 was most susceptible followed by 66D-2, 66F-4A with Palmetto the least susceptible. The lack of infection in the glass-house could be due to wide fluctuations in temperatures during the period of study which ranged from 22°C at night to 40°C at noon. This may have affected spore germination and infection because no mycelial growth, sporulation and spore germination occurs at 40°C.

**Efficacy of selected chemicals.**

Among the five chemicals tested Topsin M had the lowest *ED*$_{50}$ value, followed by Benlate, Captan 500, Dithane M-45 and Vitigran Blue in ascending order (Fig. 4). The *ED*$_{50}$ of Topsin M and Benlate were 2.2 and 2.5 g/ml respectively. Topsin M (a thiophanate compound) and Benlate (a benzimidazole compound) break down in water into methyl benzimidazole – 2-yl carbamate (Clemons and Sisler, 1969). Captan 500 (a dicarboximide compound) gave fairly good response with an *ED*$_{50}$ of 19.5 g/ml. Dithane M-45, of the dithiocarbamate group had an *ED*$_{50}$ of 173.8 g/ml, and Vitigran Blue (Copper oxy-
Table 2
Pathogenicity tests on 2-week-old soybean seedlings.

<table>
<thead>
<tr>
<th>Cultivar Treatment</th>
<th>Palmetto</th>
<th>66D-16</th>
<th>66F-4A</th>
<th>66D-2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Inoculated</td>
<td>Control</td>
<td>Inoculated</td>
</tr>
<tr>
<td>Disease severity index</td>
<td>0.0</td>
<td>2.3</td>
<td>0.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Dry weight of shoot* (g/plant)</td>
<td>0.6175&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.4994&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.8697&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.9016&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dry weight of root* (g/plant)</td>
<td>0.3049&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.2389&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.3222&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.3330&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Values for each cultivar followed by similar letters in the same row are not significantly different by t-test.
chloride) the least effective with an FID$_{50}$ of 545.8 g/ml. Further in vivo studies are needed to test the effectiveness of fungicides against $C.$ dematium var. truncatum.

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REFERENCES


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