Pertanika 6(1), 28–33 (1983)

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

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

RINGKASAN

Tumbuhan dan pengeluaran spora Colletotrichum dematium *var.* truncatum *telah dibuat dengan menggunakan media, suhu dan cahaya yang berlainan. Daripada tujuh media yang diuji, oat meal agar menghasilkan tumbesaran maisiliam yang terbaik. Tetapi pengeluaran spora yang terbaik hanya didapati pada Czapek Dox agar dan potato dextrose aga.r. Tumbesaran maisiliam yang terbaik diperolehi daripada 12 jam cahaya UV yang diselangi dengan 12 jam gelap dan di bawah cahaya yang berterusan. Pengeluaran spora yang terbanyak didapati di bawah 12 jam cahaya yang diselangi dengan 12 jam gelap Suhu optima untuk tumbesaran dan pengeluaran spora ialah 25°C. Kajian patogenisiti di rumah kaca menunjukkan yang cultivar 66D-16 adalah sangat peka pada* C. dematium *manakala cultivar 66F-4A adalah yang terlebih resistan. Kajian patogenisiti pada lenggai yang hijau menunjukkan 66D-16 adalah terlebih peka dan yang resistan ialah Palmetto. Kajian* in-vitro *untuk menguji racun kulat terhadap* C. dematium *menunjukkan yang Topsin M memberikan kawalan yang paling baik dengan* ED_{50} *bernilai 2.2 g/ml.*

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 most resistant. Inoculation studies on detached green pods indicated that 66D-16 was most susceptible and Palmetto most resistant. An* in vitro *efficacy test of five 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 subtropics, 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.

MATERIALS AND METHODS

Cultural characteristics

Growth of *C. dematium* (Pers. ex. Fr.) Grove var. *truncatum* (Schw.) Arx. isolated from soybean seeds was compared on seven media viz; potato dextrose agar (PDA), water agar (WA), bean pod agar (BPA), corn meal agar (CMA), Czapek-Dox agar (CDA), malt extract agar (MEA) and oat meal agar (OMA). All the media except OMA were prepared according to manufacturers (Difco) specifications. Oat meal agar was prepared by incorporating filtered extract of boiled oat meal with water agar. The plates were incubated for 10 days at room temperature (28 ± 1.5°C). Colony diameter of the test fungus was taken in mm. every two days. Two measurements at right angle

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 flourescent 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–(trichloromethylthiocylohex – 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, 10, 500 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 ED_{50} 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 BPA and PDA. 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 Na^+, K^+, Fe^{2+} and Mg^{2+} and anions such as N_3^-, SO_4^{2-} and PO_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

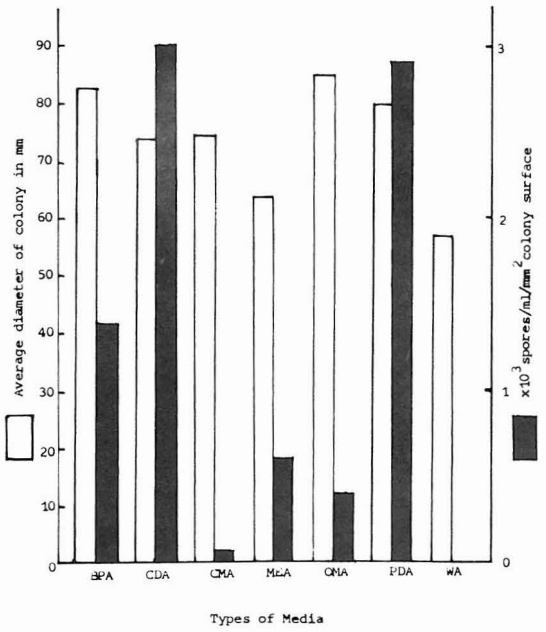

Fig. 1. Growth and sporulation of C. dematium *var.* truncatum *10 days after incubation at 25 ± 1.5°C on bean pod agar (PDA), Czapek Dox agar (CDA), corn meal agar (CMA), malt extract agar (MEAL), oat meal agar (OMA), potato dextrose agar (PDA) and water agar (WA).*

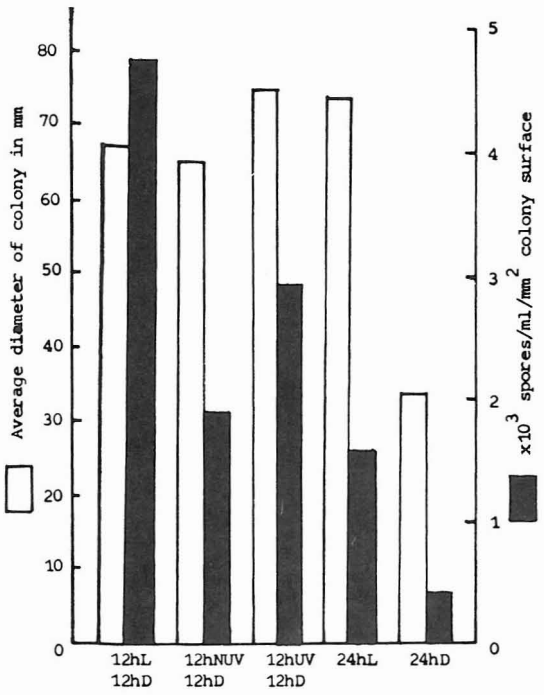

Fig. 2. Effect of light on the growth and sporulation of C. dematium *var.* truncatum *10 days after incubation at 20±2°C on PDA. Note 12hL = 12 hours light, 12hD = 12 hours darkness, 12hNUV = 12 hours near ultraviolet, 12hUV = 12 hours ultraviolet, 24hL = 24 hours light, 24hD = 24 hours darkness.*

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 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

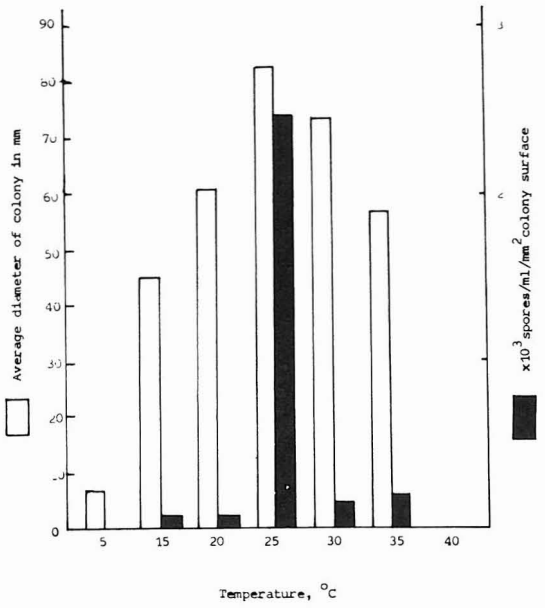

Fig. 3. Effect of temperature on the growth and sporulation of C. dematium *var.* truncatum *10 days after incubation on PDA.*

Table 1
Effect of temperature on conidial germination of *Colletotrichum dematium* var. *truncatum.*

Temperature °C	Percent germination (after 14 hours)*
5 ± 2	0.00 c
15	60.25 b
20 ± 2	77.00 a
25	65.00 b
30	62.00 b
35	2.25 c
40	0.00 c

*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.

temperature for spore germination, favourable relative humidity, virulence of the pathogen and cultivar susceptibility.

No symptoms of infection were observed on the green pods in the glass-house at two weeks after inoculation. However, studies on detached

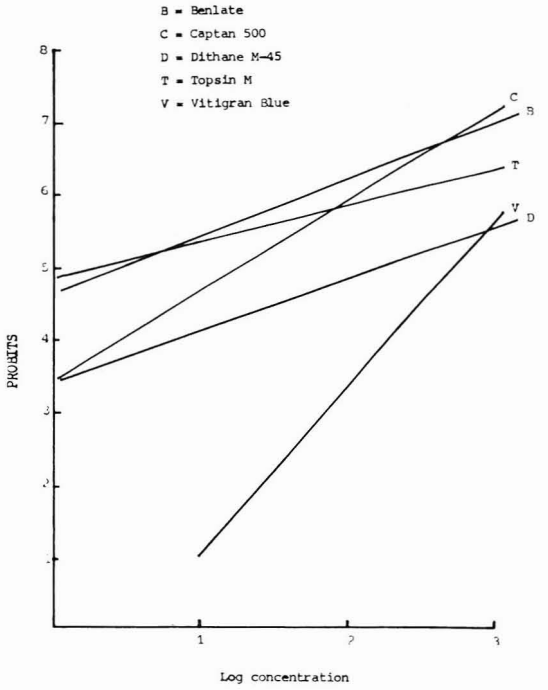

Fig. 4. Graphs of Probit versus Log concentration for determination of ED_{50} *values of each of the 5 funicides tested.*

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.

Cultivar	Palmetto		66D-16		66F-4A		66D-2	
Treatment	Control	Inoculated	Control	Inoculated	Control	Inoculated	Control	Inoculated
Disease severity index	0.0	2.3	0.0	4.0	0.0	2.0	0.0	3.5
Dry weight of shoot* (g/plant)	0.6175[a]	0.4994[a]	0.8697[a]	0.9016[a]	0.8477[a]	0.8499[a]	0.6808[a]	0.6770[a]
Dry weight of root* (g/plant)	0.3049[b]	0.2389[b]	0.3222[b]	0.3330[b]	0.3629[b]	0.3631[b]	0.2663[b]	0.2646[b]

*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 ED_{50} of 545.8 g/ml. Further *in vivo* studies are needed to test the effectiveness of fungicides against *C. dematium* var. *truncatum*.

ACKNOWLEDGEMENT

We wish to thank the Dean, Faculty of Agriculture, Universiti Pertanian Malaysia, for the facilities and permission to publish this paper. We are grateful to Miss Zuriyati Zainull Rashid for typing this manuscript.

REFERENCES

ANON, (1977a): Area of miscellaneous crops, Peninsular Malaysia. Ministry of Agriculture, Malaysia.

ANON, (1977b): *Malaysia annual statistics of external trade* (1977) Vol. II. Imports. Department of statistics. Malaysia.

CLEMONS, G.P. and SISLER, H.D. (1969): Formation of a fungitoxic derivative from Benlate. *Phytopathology* **59:** 705-706.

FINNEY, D.J. (1971): *Probit analysis.* (3rd ed.) Cambridge University Press.

LENNE' J.M. (1978): *Studies on the biology and taxonomy of Colletotrichum spp.* Ph. D. Thesis. Univ. of Melbourne.

LENNE' J.M. and SONODA, R.M. (1978): Occurrence of *Colletotrichum dematium* f. sp. *truncata* on *Stylosanthes* spp. *Plant Dis. Reptr.* **62:** 641-644.

MALHOTRA, R.K. and CHATARVEDI, C. (1974): Physiological studies on *Colletotrichum truncatum* causing pod blight diseases of soybean. *Friesia* **10:** 290-299.

NIK, W.Z. and LIM, T.K. (1980): Seed-borne pathogens of some selected grain legumes in Malaysia. *Proceedings of the legumes in the tropics.* Faculty of Agriculture. Universiti Pertanian Malaysia. pg. 259-266.

SINCLAIR, J.B. (1982): *Compendium of soybean diseases* (2nd edn.) American Phytopathological Society, Inc. St. Paul. Minnesota, U.S.A.

(Received 20 August 1982)