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# THE USE OF TOXIN FOR THE SCREENING OF BLACK-PEPPER FOR FOOT ROT RESISTANCE

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## Ringkasan

Kajian inokulasi *Phytophthora palmivora* (Butl.) Butl. yang telah dijelaskan ke atas 2 jenis *Piper spp.* dan 5 *Piper nigrum* L. menunjukkan bahawa inokulasi toxin tidak berbeza dengan inokulasi kulat. Dengan itu teknik yang mula-mula adalah lebih sesuai untuk menggantikan inokulasi kulat di dalam menguji ketahanan *Piper* terhadap penyakit busuk akar. Kelebihan-kelebihan menggunakan toxin daripada inokulasi kulat telah dibincangkan.

Kajian ini juga menunjukkan bahawa ada terdapat 2 kumpulan *Piper* yang sungguh berbeza: kumpulan tahan-penyakit yang mengandungi *P. colubrinum* dan *P. sarmentosum* dan kumpulan peka yang mengandungi 5 jenis *P. nigrum* iaitu Kuching, Bangka, Djambi, Uthirancotta dan Belantung.


## Introduction

Foot rot caused by *Phytophthora palmivora* (Butl.) Butl. is the most serious disease of black-pepper (*Piper nigrum* L.) in Malaysia as well as in other countries where the crop in commercially grown (Alconero *et al.*, 1971; Leather, 1967; Nambier *et al.*, 1965; Raj and Jose, 1966). The disease is characterized by rapid wilting and defoliation leading subsequently to the death of the plant. The pathogen normally infects the plant through the collar or the underground parts of the stem and roots. The healthy tissues are usually clearly demarcated from the diseased tissues by an advancing black margin of infection. Direct infection through the leaves may sometimes occur. This often takes the form of dark brown lesions with characteristic fimbriate margin.

Recent attempts to control the disease are geared mainly towards the breeding for resistant cultivars. A sound breeding programme for disease resistance requires the existence of a reliable, easy and inexpensive method of screening the planting materials. In relation to this, a study was conducted recently to compare the relative susceptibility of the various *Piper* spp. and cultivars of *P. nigrum* using 2 different techniques of inoculation:

(I) Toxin inoculation

(II) Fungal inoculation

## Materials and Methods

(I) *Toxin inoculation*

A glucose-asparagine liquid medium (Lilly and Barnett, 1951) incorporated with mineral supplements to enhance toxin production (Brian *et al.*, 1961) was used in the present study for the production of the fungal toxin. The hydrogen-ion concentration of the medium before autoclaving was adjusted to pH 5.6 by the use of 0.1 N aqueous sodium hydroxide. After autoclaving, the pH level dropped slightly to 5.4. Fifty ml of this medium contained in a 100-ml Erlenmyer flask were inoculated with small pieces of fungal mycelium obtained from the growing edge of young *P. palmivora* cultures. The flasks were then incubated at 25 ± 2°C under normal laboratory conditions. Cell-free culture filtrate containing the toxin was obtained from 4-week-old liquid cultures by initially filtering the cultures through 2 layers of tissue paper (Kleenex) and later through micropore filters (Sartorius, pore size 0.2 u).

For inoculation, 8-month-old seedlings of the following *P. nigrum* cultivars namely Bangka, Belantung, Djambi, Kuching and Uthirancotta, seedlings of *P. colubrinum* and cuttings of *P. sarmentosum* were used as test plants. The plants were carefully removed from the pots and the adhering soil particles on the roots washed off under a slow stream of running

tap water. The roots and collars of 6 plants from each *Piper* species or cultivar were then immersed in 100 ml of the prepared toxin solution for 48 hours after which the plants were removed, washed and replanted. In the control, cultivar Kuching was used as test plants. The seedlings were allowed to stand in the liquid medium without the toxin and later replanted.

(II) *Fungal inoculation*

Tapioca extract agar was used as the medium for the growth of the fungus. The medium was prepared by boiling 200 gm tapioca chips *(Manihot utilissima L.)* in distilled water for 30 minutes and filtering off the residue to retain the supernatant suspension. The suspension was then adjusted to pH 5.6 using 0.1 N aqueous sodium hydroxide and solidified with 15 gm agar per litre. Seven-day-old cultures of the fungus growing in Petri-dishes were blended in a Waring blender with sterile distilled water. The inoculum level was standardized by blending 8 culture plates to make a final mycelial suspension of 1 litre. For inoculation, 25 ml of the mycelial suspension were drenched around the collar and roots of each test plant, care being taken not to damage the roots of the plant. Immediately after inoculation, the plants were placed in humid chambers for 48 hours. For the control, sterile tapioca extract agar suspension was used.

The number of plants from each *Piper* sp. and *P. nigrum* cultivar used for toxin and fungal inoculations was 6 and 9 respectively. Recordings on the number of plants killed in relation to the total number of plants inoculated were made 14 days after inoculation.

## Results

Symptoms of foot rot were observed in plants killed by toxin and fungal inoculations. The plants wilted 10—14 days after treatment. Closer examination showed that the lesions developed on the roots and collars of these plants were dark brown in colour and the cortical tissues along these lesions sloughed off readily. However, in the case of plants killed by toxin, a visible secondary colonization by saprophytic fungi such as *Penicillium* and *Fusarium* invariably set in on the lesions; whereas plants killed by fungal inoculation remained free of colonization within the same period.

The 2 techniques of inoculation were tested using the Chi-Square. Results on the number of plants killed in relation to the total number of plants inoculated and the computed values of Chi-Square are given in Table 1.

TABLE 1. A comparison on the toxin and fungal inoculation techniques

| *Piper* spp. | No. of plants killed / No. of plants inoculated | | $\chi^2$ (1 df) |
|---|---|---|---|
| | Toxin | Mycelium | |
| *P. nigrum* var. | | | |
| Kuching | 6/6 | 7/9 | 0.2163 |
| Bangka | 5/6 | 6/9 | 0.0142 |
| Djambi | 5/6 | 5/9 | 0.3125 |
| Belantung | 3/6 | 5/9 | 0.0010 |
| Uthirancotta | 5/6 | 5/9 | 0.3125 |
| *P. colubrinum* | 0/6 | 0/9 | 0 |
| *P. sarmentosum* | 1/6 | 0/9 | 1.1161 |
| Control (Kuching) | 0/6 | 0/9 | 0 |
| | | Total | 1.9726 |

The computed Chi-Square with 8 degrees of freedom was 1.97 and this was between the probability of 0.98 and 0.95. Each individual Chi-Square was also computed using Yates correction for Continuity. Results showed that there was no evidence of any difference between the 2 techniques of inoculation.

The relative susceptibility of the various *Piper* spp. and cultivars of *P. nigrum* based on the combined results of toxin and fungal inoculations was tested using the t-test. The test was made using the standard error of the difference between 2 proportions as given by the formula:

$$\sqrt{\frac{\hat{p}_i(1-\hat{p}_i)}{n_1} + \frac{\hat{p}_j(1-\hat{p}_j)}{n_2}}$$

where $\hat{p}_i$ and $\hat{p}_j$ refer to the probabilities of dead plants. The results are given in Table 2.

TABLE 2. *Piper* spp. and *Piper nigrum* cultivars ranked in the order of susceptibility based on the combined results of toxin and fungal inoculations

| *Piper* spp. | Estimated proportion of dead plants, $\hat{p}$ |
|---|---|
| *P. colubrinum* | 0.07 |
| *P. sarmentosum* | 0.00 |
| *P. nigrum var.* | |
| Belantung | 0.53 |
| Djambi | 0.67 |
| Uthirancotta | 0.67 |
| Bangka | 0.73 |
| Kuching | 0.87 |

*Piper* spp. and *P. nigrum* cultivars covered by the bars showed the difference between probabilities were not significant at the 5% level.

A similar test on the relative susceptibility based on toxin inoculation alone was also carried out and the results are given in Table 3.

TABLE 3. *Piper* spp. and *Piper nigrum* cultivars ranked in the order of susceptibility based on toxin inoculation alone

| *Piper* spp. | Estimated proportion of dead plants, p |
|---|---|
| *P. colubrinum* | 0.00 |
| *P. sarmentosum* | 0.17 |
| *P. nigrum var.* | |
| Belantung | 0.50 |
| Bangka | 0.83 |
| Djambi | 0.83 |
| Uthirancotta | 0.83 |
| Kuching | 1.00 |

*Piper* spp. and *P. nigrum* cultivars covered by the bars showed the difference between probabilities were not significant at the 5% level.

## Discussion

Results on the relative susceptibility of the 2 *Piper* spp. and the 5 cultivars of *P. nigrum* using the 2 different inoculation techniques indicated that toxin inoculation was comparable to fungal inoculation. Toxin inoculation could therefore be recommended in place of fungal inoculation in the screening of *Piper* spp. and *P. nigrum* cultivars for foot rot resistance. This is the first report of toxin studies involving *P. palmivora* on *Piper* spp.

The use of toxin in the screening for disease resistance in plants has been reported (Wheeler and Luke, 1955; Steiner and Byther, 1971). Advantages of using toxins over the usual fungal inoculations are many. Toxins are easier to prepare than fungal inocula. They can be easily handled and are not subjected to environmental influences. Most important of all, the possible changes in the virulence of the fungus in culture are eliminated as large quantities of toxins can be made and stored over long periods of time.

From the susceptibility figures, 2 distinct groups of *Piper* spp. are apparent: the resistant group consisting of *P. colubrinum* and *P. sarmentosum* and the susceptible group consisting of the 5 cultivars of *P. nigrum*. Ranking of the results obtained by toxin inoculation and fungal inoculation indicated that it corresponded closely with the results obtained in Sarawak (Holliday and Mowat, 1963). The study further confirmed the relative resistance of Belantung and the high susceptibility of Kuching.

## Summarry

Inoculation study of *Phytophthora palmivora* (Butl.) Butl. on 2 *Piper* spp. and 5 cultivars of *Piper nigrum* indicated that toxin inoculation was comparable to fungal inoculation. The former technique could therefore be recommended in place of fungal inoculation in the screening of *Piper* for foot rot resistance. The advantages of using toxins over fungal inoculations were discussed.

The study further showed that 2 distinct groups of *Piper* spp. are present: the resistant group consisting of *P. colubrinum* and *P. sarmentosum* and the susceptible group consisting of the 5 cultivars of *P. nigrum* namely Kuching, Bangka, Djambi, Uthirancotta and Belantung.

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