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SHORT COMMUNICATION

Antifungal Properties of *Elephantopus scaber* L. (Asteraceae) Against Crop Pathogenic Fungi

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ABSTRACT

Harmful effect of synthetic fungicides towards environment and other organisms have become important issues nowadays. Research on the plant product to be developed as an alternative for synthetic fungicides has attracted interest of many scientists. The aim of this study is to determine the effect of antimicrobial properties of *Elephantopus scaber* L. from the family Asteraceae against six crop pathogenic fungi viz., *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk, *Fusarium oxysporum* Schltdl., *Pyricularia oryzae* Cavara, *Fusarium solani* (Mart.) Sacc., *Pestalotiopsis* sp., and *Colletotrichum* sp. Three different concentrations of *E. scaber* crude extract- 1%, 5% and 15%, were used in fungal growth inhibition test. At 15% (w/v) concentration, the mycelia growth of *Colletotrichum* sp., *F. solani*, and *P. oryzae* were significantly retarded. It indicates that the extract of *E. scaber* could potentially be used as a biofungicide.

Keywords: *Elephantopus scaber*, biofungicides, crop pathogenic fungi

Losses in crops production due to plant diseases is an important issue. Common pathogens in plants are fungi because they are the most widespread (Alrajhi, 2013). The usage synthetic fungicides such as Thiram and Chlorothalonil are required to reduce the damage on crops (Adaskaveg *et al*., 2012; El-Khateeb *et al*., 2013). However, the excessive usage of synthetic fungicides have led to a series of problems: (1) fungi have developed resistance or tolerance to the synthetic fungicides (Luo *et al*., 2010; Matsumura & Sanada-Morimura, 2010), and (2) risks of high-level of toxic residue in the products that are harmful to human and environment (Kim *et al*., 2013). Organic compounds that are safe for human and environment are needed as an alternative to synthetic fungicides to control pathogenic fungi.

Plant is a good source for harvesting organic compounds that may be utilized as an alternative to synthetic fungicides because plant produces phytochemicals which involve in defence against predation or infection (Javale & Sabnis, 2010). These phytochemicals are biodegradable and non-toxic (Soylu *et al*., 2006; Satish *et al*., 2007).

One example of plant species which has the potential to be exploited is *Elephantopus scaber* L. *E. scaber* is a medicinal plant which is known for its medicinal properties (Kamalakannan *et al*., 2012). Various kinds of solvents such as ethanol and acetone had been used before to extract the chemical constituents in the *E. scaber* (Ho *et al*., 2009). The main chemical constituents in *E. scaber* include sesquiterpene lactones, phenolic acids, flavonoids, triterpenoids, steroids, essential oil, salt and minerals (Wang *et al*., 2004; Ahmad *et al*., 2009; Ali *et al*., 2010; Kabeer & Prathapan, 2014; Wu *et al*., 2014). It has been reported this plant possess phytochemical compounds with antimicrobial activity against crop pathogenic fungi such as *Aspergilus niger* Tiegh. and *A. flavus* Link (Kamalakannan *et al*., 2012). This study aims to test the methanolic crude extract from *E. scaber* in inhibiting the growth of pathogenic fungi isolated from pepper.

Young plants of *E. scaber* were collected from housing area in Tarat, Serian division, and planted at UNIMAS greenhouse. Soil beds were prepared with soil mixture of top soils, organic soils and sands at the ratio of 3:2:1. Nitrogen – Phosphorus – Potassium (15:15:15)

compound fertilizer were applied once per month.

Healthy leaves were harvested from mature plants. Leaf samples were washed with tap water and rinsed with distilled water to remove contaminants. After patted dry with tissue paper, the leaves were air dried for five days. The air dried leaves were grounded into powder form using Panasonic branded blender. Fifty gram of grounded sample was immersed in 500 mL of 80% methanol (v/v) for 24 hours in a beaker sealed with aluminium foil at room temperature. The extracts obtained were then filtered by using Whatman No. 3 filter paper. The filtered extracts were poured onto pre-weighed watch glasses and let evaporated for a week to obtain crude extract of methanol. The air dried crude extract were weighed again. There was approximately 7 g of crude extract obtained (yield ~ 8%).

The inhibition test was adapted from Cockerill *et al.* (2012). A total of 30 g of methanol crude extract was then dissolved with 200 mL of distilled water to obtain 15% concentration (w/v) of extract and further diluted into 5% and 1% concentration. To prepare the media, 30 ml of the different concentrations; 1%, 5% and 15% concentration of crude extract were mixed into 470 ml potato dextrose agar (PDA), respectively. The crop pathogenic fungi tested were *Pestalotiopsis* sp., *G. cingulata, P. oryzae, Colletotrichum* sp., *F. solani* and *F. oxysporum* (provided by Agricultural Research Centre, Sarawak). Plug of pathogenic fungi with a diameter of 5 mm obtained from fresh culture (~10 days old) was placed at the centre of the Potato Dextrose Agar (PDA) media containing the extract. The colony diameter of the pathogenic fungi was recorded daily in the afternoon (4 pm to 6 pm). The growth rate was estimated by dividing the total colony diameter growth over the total number of days required by the colony in control plates to reach full plate (Table 1). For each pathogenic fungus, five replicates for each concentration of crude extract were tested. In addition, five replicates of control culture containing distilled water without crude extract were prepared. The data collected were analysed by using SPSS software and the test used was Mann Whitney U.

The growth rate of all the tested crop pathogenic fungi on the 1% and 5% concentration of crude extract had no significant different compared to the control plates (Table 1). Interestingly, at 15% concentration, *Colletotrichum* sp., *F. solani* and *P. oryzae* showed significantly slower growth rate compared to their growth on control plates (Table 1), *Colletotrichum* sp. was affected the most (Figure 1) as its growth rate was slower by approximately 0.3 cm/day, which is 9 days slower compared to its growth on control medium. There is a probability that there is/are compound(s) present in the crude extract of *E. scaber* which can retard the growth of *F. solani*, *Colletotrichum* sp. and *P. oryzae*. The crude

Table 1. Growth rate (cm/day) of the crop pathogenic fungi cultured on different concentrations of crude extract from *E. scaber*.

Crop Pathogenic Fungi	Growth rate (cm/day)				Standard deviation	N
	Control #	1%	5%	15%		
Colletotrichum sp.	0.74 (11)	0.74	0.72	0.42**	0.14	20
Fusarium solani	0.72 (11)	0.72	0.72	0.45*	0.71	20
Pyricularia oryzae	0.44 (19)	0.40	0.43	0.37*	0.04	20
Glomerella cingulata	0.74 (11)	0.73	0.69	0.57	0.11	20
Fusarium oxysporum	0.73 (11)	0.68	0.68	0.63	0.07	16
Pestalotiopsis sp.	0.58 (14)	0.55	0.46	0.43	0.16	20

Note: The analysis only compare the growth rate of fungi on the control medium and the respective treated medium. * $p < 0.05$; ** $p < 0.01$; N = number of samples; # the number in brackets indicates the total number of days required by the culture to reach full plate.

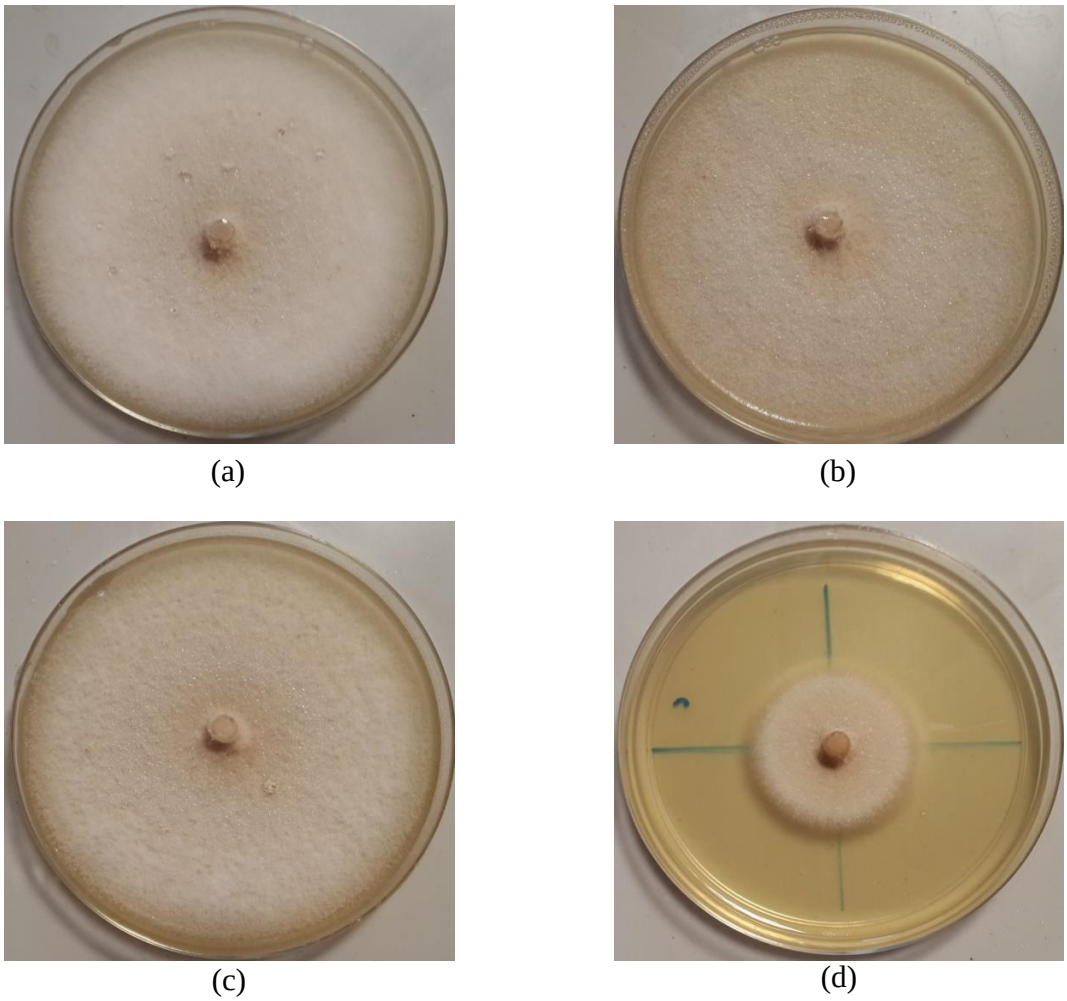

Figure 1. The growth of *Colletotrichum* sp. on the last day of observation on different concentrations of *E. scaber* crude extract. (a) control, (b) 1% concentration, (c) 5% concentration, and (d) 15% concentration.

extract from *E. scaber* need to be further purified to identify the candidate compounds which may have antimicrobial properties against those six pathogenic fungi; *G. cingulata, F. oxysporum, F. solani, P. oryzae*, *Pestalotiopsis* sp., and *Colletotrichum* sp..

Previous phytochemical analysis of leaf samples from *E. scaber* showed that saponins, stigmasterol, lupeol, deoxyelephantopin, luteolin and alkaloids are present in the methanolic extract (Ahmad *et al*., 2009; Kamalakannan *et al*., 2012; Das & Bandyopadhyay, 2015). Saponins, stigmasterol, lupeol, luteolin and alkaloids isolated from other plant species were known to have antimicrobial effects (Lee *et al*., 2010; Saratha *et al*., 2011; gowdu Viswanathan *et al*., 2012; Maatalah *et al*., 2012; Okusa *et al*., 2014; Edilu *et al*., 2015). They are probably good candidates to be the compound(s) responsible for retarding the growth of *Colletotrichum* sp., *F. solani* and *P. oryzae.*

In conclusion, methanol crude extract were successfully obtained from *E. scaber* plant. The antimicrobial properties of *E. scaber* were shown through the retardation of mycelia growth of three crop pathogenic fungi namely *P. oryzae*, *F. solani*, and *Colletotrichum* sp. The other three crop pathogenic fungi, *F. oxysporum, G. cingulata* and *Pestalotiopsis* sp. did not significantly show any retardation of the mycelia growth. As the extract of *E. scaber* plant showed inhibitory effect on the growth of the selected plant pathogenic fungi, further study can be done to utilize this plant as a source for biofungicide.

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