



Antifungal activity of selected plant extracts against *Curvularia* sp. infecting local purple sweet potato (*Ipomoea batatas*)

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ABSTRACT

Aims: The present study was designed to evaluate *in vitro* antifungal activity of plant extracts against *Curvularia* sp., a causative agent of leaf blotch in local purple sweet potato crops.

Methodology and results: The plants were selected on the basis of commonly used traditional remedies. Various dilutions, 1/2, 1/4, 1/6, 1/8 and 1/10 of black pepper, garden croton, garlic, tobacco and turmeric extracts were used for screening. The lesion characteristics on purple sweet potato leaves were collected from plots in MARDI Bachok. The "poisoning agar technique method" was used to get the antifungal activity. The results of antifungal activities were reported in terms of inhibition of mycelial growth of the test fungus. Out of five types of plant extracts used, only garlic and tobacco showed significantly high antifungal activity against the test pathogen based on poisoned food technique. Garlic extract showed complete inhibition (100%) at 1/2 dilution and more than 94% growth inhibition at concentrations as low as 1/10 dilution after seven days of incubation. However, black pepper and turmeric extracts showed moderate inhibition (20-70%) whereas, no inhibition was recorded in 1/8 and 1/10 dilution of garden croton extract.

Conclusion, significance and impact of study: Our findings suggested that garlic extract is the most potential antifungal agent against *Curvularia* sp. and can be used as bio-fungicide thus would reduce the dependency on synthetic fungicides by farmers.

Keywords: Antifungal, purple sweet potato, *Curvularia* sp., plant extract

INTRODUCTION

Purple sweet potato is one of the major tuber crops grown on bris sandy soil (BRIS) and is cultivated mostly in rural areas for local food products. Fungal pathogens cause a moderate threat to purple sweet potato leaves that sometimes require the use of chemicals fungicide. The chemical can be harmful or toxic to human. *Curvularia* sp., which is common in soil, is one of the facultative pathogens of purple sweet potato leaf disease. Most *Curvularia* sp. is found in tropical regions, though a few are found in temperate.

There is a large demand for new and safer fungicide to be used in food production and agriculture. Recently, there has been a growing trend to evaluate the antifungal activity of the plant extracts, due to reduced resistance of pathogens, negative effect of chemical fungicide, safety concern and cost of food product. Black pepper (*Piper nigrum*), garden croton (*Codiaeum variegatum*), garlic (*Allium sativum*), tobacco (*Nicotiana tabacum*) and turmeric (*Curcuma longa*) were used in the experiment where the selection was based on previous report on preservative actions towards pathogen of these local

ornamental plants. Turmeric has an active ingredient called "curcumin" reported to have antimicrobial effects on fungi, bacteria and protozoa (Mioranza *et al.*, 2017). "Piperine" is an active ingredient contained in black pepper and can act as an antimicrobial in fungi (Mioranza *et al.*, 2017). It was also reported that the garden croton (*C. variegatum* Linn.) able to control *Alternaria alternate* and *Fusarium oxysporum*. The garden croton is an example of the most common plant in the experiment that has not been fully explored. Naidu (1988) carried out the studies about thirty years ago on antifungal effect of aqueous crude extract of young and mature leaves of garden croton for their antifungal effect on *A. alternate* and *F. oxysporum* and reported that young leaves are being more active against *A. alternata* whereas old leaves more active against *F. oxysporum*.

Garlic exhibited the strongest antibacterial activity against bacterial wilt of tomato *in vitro* and *in vivo* followed by *Datura* sp. and then *Nerium* sp. (Lopes and Boiteuk, 1994; Abo-Elyousr and Asran, 2009). The antimicrobial effects of tobacco leaves using ethanol and water showed

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that the extract can inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* (Okorundu *et al.*, 2015).

Therefore, the present study was an attempt to evaluate the antifungal activity of five local ornamental plant extract *viz.* black pepper dried seed or referred as peppercorns, garden croton leaves, garlic bulb, tobacco leaves and turmeric rhizome against *Curvularia* leaf blotch pathogen of sweet potato.

MATERIALS AND METHODS

Collection and isolation of *Curvularia* sp. isolates

Purple sweet potato leaves with lesions characteristic of blotch were collected from plots in MARDI Bachok, Kelantan (Figure 1). These lesions are same as per literature review described by Lopes and Boiteuk (1994). To obtain isolates, a small piece of dried tissue (5 × 5 mm) was excised from the lesion and cultured on water agar (WA). After incubation overnight at 25 °C, grown mycelium were transferred aseptically to potato dextrose agar (PDA) and incubated at 25 °C in the dark for two weeks. From the taxonomic classification, the cultures were observed in the laboratory to determine the pathogen as a *Curvularia* sp. All characteristic that observed were similar with *Curvularia* sp. described by Lopes and Boiteuk (1994).



Figure 1: Symptoms of *Curvularia* leaf blotch on purple sweet potato.

Antifungal activity

The percentage of mycelial growth inhibition was determined using agar “poisoning agar technique method” (Rahman *et al.*, 2009) with modification. Three-week-old culture of *Curvularia* sp. was plugged out using cork borer and put onto five different types of “poisoning agar” containing plant extract of black pepper, garden croton, garlic, tobacco and turmeric. Plates were prepared by adding plant extract to the PDA based on the ratio of 1/2, 1/4, 1/6, 1/8 and 1/10. A 10 mm mycelia plug of *Curvularia* sp. was cultivated at the centre of the plate, and incubated at 25 °C. The negative control plate was prepared in the same way using the sterile distilled water instead of the test solution extract. Fungal growth inhibition was done by measuring the largest diameter of the colony after several

days, until the negative control plates were completely full. Three replicate plates were used per/treatment. Control, non-extract-amended PDA plates were included as reference of fungal colony growth.

The effective concentration of diluted plant extract that inhibited 50% and more of the fungal colony growth was estimated by using following formula (Vincent, 1947):

$$I = \frac{C - T}{C} \times 100$$

Where,

I = percent inhibition

C = colony diameter in control

T = colony diameter in treatment

The data was recorded in triplicates and conclusion was drawn on the basis of analysis of means.

RESULTS AND DISCUSSION

The results of five different dilutions of five selected plant extracts are given in Table 1 and graphical representation of antifungal activity of *Curvularia* sp. of purple sweet potato is shown in Figure 2. Among the different plants whose extracts were found to be effective, garlic bulbs showed maximum potential. Garlic bulbs completely inhibited the growth of *Curvularia* sp. at a concentration of two-fold dilution (100%) and recorded to inhibit more than 94% even at ten-fold dilution.

Garlic or *A. sativum* is well-known to control diseases especially against human pathogens (Samuel *et al.*, 2000) and also against plant pathogens (Sindhan *et al.*, 1999). The growth of *Curvularia* sp. was inhibited with black pepper and tobacco extracts where the growth percentages were 52-70% and 74-88% respectively. The minimum inhibitory concentration (MIC) study showed that all dilutions of three plants extract namely, garlic, black pepper and tobacco inhibited more than 50% while the remaining two inhibited less than 50% except for two- and four-fold dilution of turmeric extract. No inhibition was recorded at eight-fold and ten-fold dilution of garden croton.

High inhibitory effect of black pepper also was found in a report by Shiva Rani *et al.* (2013) which indicates that the growth of *F. oxysporum* and *A. alternate* were only 17 and 19 mm colony of diameter, respectively. The *in vitro* application of tobacco extract showed complete inhibition of growth at 60% concentration and more than 50% inhibition even at 20% concentration against *Aspergillus viridae* and *Penicillium digitatum* (Suleiman, 2011) which is similar to the result of this experiment. The result of turmeric inhibition is in agreement with findings reported in research by Jayaprakasha *et al.* (2001) where rhizome extract of turmeric showed toxicity towards *Aspergillus flavus* and *Trichoderma viride* only at higher concentrations. But the inhibitory effect of turmeric extract was contrast to the findings by Saha *et al.* (2005) where turmeric aqueous extracts was found to be very effective

against *Curvularia eragrostidis* with a record of 98.2% of inhibition. Different chemical compounds particularly in different species of plant and also the method of extraction can be responsible for the differences in antifungal activity.

It is evident from this work that garlic followed by tobacco and black pepper extract is efficient to control the sweet potato from *Curvularia* sp. leaf blotch infestation. The plant extracts have high potential to be developed as bio-control agents for sweet potato disease caused by *Curvularia* sp.

Table 1: Effect of poisoning agar on the growth of *Curvularia* sp. frequently found on purple sweet potato leaves blotch (data shown are percentage value of growth inhibition on PDA).

Fungi sp./Dilution		<i>Curvularia</i> sp.
Plant extract	Black pepper 1/2	54
	Black pepper 1/4	52
	Black pepper 1/6	65
	Black pepper 1/8	70
	Black pepper 1/10	67
	Garden croton 1/2	27
	Garden croton 1/4	6
	Garden croton 1/6	6
	Garden croton 1/8	-
	Garden croton 1/10	-
	Garlic 1/2	100
	Garlic 1/4	99
	Garlic 1/6	99
	Garlic 1/8	96
	Garlic 1/10	94
	Tobacco 1/2	88
	Tobacco 1/4	84
	Tobacco 1/6	83
	Tobacco 1/8	79
	Tobacco 1/10	74
	Tumeric 1/2	70
	Tumeric 1/4	56
	Tumeric 1/6	37
	Tumeric 1/8	34
	Tumeric 1/10	28

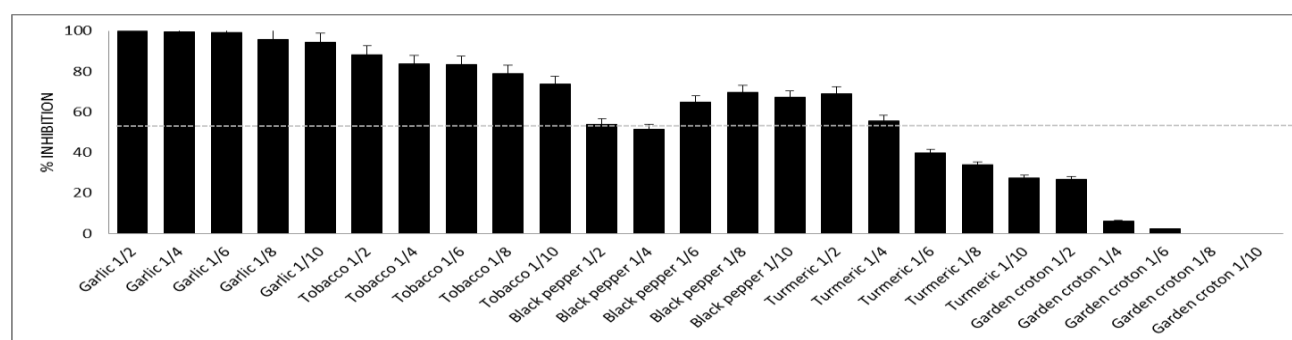


Figure 2: Antifungal activity comparison of several dilution of plant extract against *Curvularia* sp. by the poisoning agar assay. Data given are mean of triplicate.

CONCLUSION

The antifungal potential of garlic, tobacco, black pepper, turmeric, and garden croton offers a broader fungicidal spectrum against tested fungi. The study indicated that garlic has strong antifungal activity followed by tobacco. Further research on purification and formulation may be needed to confirm the true value of antifungal activity of the extracts comparable to standard antifungal.

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