Research Article

Botanicals to Control Post-harvest Decay of Potato (*Solanum tuberosum* L.) Tubers in Odisha, India

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ABSTRACT

Petroleum ether and methonolic leaf extracts from eight different plant species such as *Eucalyptus globulus* Labill, *Haldinia cordifolia* (Roxb.) Rids/ dale, *Justicia adhatoda* L., *Lawsonia inermis* L., *Murraya paniculata* Jack., *Pithecellobium dulce* (Roxb.) Benth., *Pongamia pinnata* (L.) Panigrahi and *Tamarindus indica* L. were tested for their fungitoxic potential against fungi causing post-harvest storage rot of potato namely *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporum*, *Geotrichum candidum*, *Mucor* sp. and *Penicillium* sp., under *in-vitro* condition. Previously, these fungi were isolated from the rotten potato tubers collected from different market places of some districts of Odisha, India. The efficacy of botanicals was compared with two commercial fungicides such as Blitox-50 and Dhanustin. The result revealed that the plant extracts were more effective against the six isolated plant pathogenic fungi as compared to the test commercial fungicides. It was observed that the petroleum ether extract of *Murraya paniculata* was most effective (60.52 ± 2.64 %) against *A. flavus*, petroleum ether extract of *Eucalyptus globulus* (53.56 ± 2.82 %) against *A. niger*, methanolic extract of *Halidinia cordifolia* (66.39 ± 4.57 %) against *F. oxysporum*, methanolic extract of *Justicia adhathoda* (82.42 ± 2.13 %) against *G. candidum*, petroleum ether extract of *Justicia adhathoda* (87.5 ± 2.04 %) against *Mucor* sp., petroleum ether extract of *Pongamia pinnata* (72.24 ± 1.73 %) against *Penicillium* sp.

Keywords: Post-harvest storage rots, fungi, plant extracts, antifungal activity, commercial fungicides.

INTRODUCTION

After poverty, the world's third most pressing problem is food scarcity [1] which is the major important problem of several countries.[2] According to a report of Kana *et al*., (2012),[3] around 1 billion people are challenging by severe hunger in these food scarcity nations of which 10% actually die from hunger-related complications. The reason of this problem is inadequate agricultural storage and preservation from microbes-induced spoilages. About 10 to 40% of worldwide losses of agricultural productions occur by post-harvest diseases. The amount of loss in developing countries is more than developed countries.[4] Potatoes are one of the major constituents of daily diet in Odisha.[5] Traditionally, synthetic fungicides[6] has been used to control the post-harvest diseases, but their excessive use complemented with high costs, residues in plants, and development of resistance which has left a negative effect on human health and the environment.[7] Consumers dislike the use of chemical preservatives in their food which is associated with public health risk. This influences the removal of these chemicals from food and adoption of more natural preservation. Recent reports on the use of plant extract as bio-fungicides have opened a new approach to control plant diseases. These plant extract are safe, non-toxic to man, but effective against plant pathogens.[8] Plant extracts have been successfully used to control a number of plant diseases.[9]

The present study was carried out to evaluate the *in-vitro* antifungal activity of some medicinal plants against those fungi which are responsible for post-harvest storage rots of potato (*Solanum tuberosum* L.) tubers in Odisha (India). The significance of the present work lies with the management of post-harvest diseases by employing botanicals which can be recommended for use as natural fungicides in the state of Odisha.

MATERIALS AND METHODOLOGY

Collection and identification of plant material

The leaves of *Eucalyptus globulus* Labill (Myrtaceae), *Haldinia cordifolia* (Roxb.) Rids/ dale, *Justicia adhatoda* L. (Acanthaceae), *Lawsonia inermis* L. (Lythraceae), *Murraya paniculata* Jack. (Rutaceae), *Pithecellobium dulce* (Roxb.) Benth. (Mimosaceae), *Pongamia pinnata* (L.) Panigrahi (Papilionaceae/ Fabaceae) and *Tamarindus indica* L. (Caesalpiniaceae) were collected from the "Chandaka reserve forest" area near Bhubaneswar, Odisha in the month of March, 2015. Identification of the voucher specimen was done by available literature.[10] The voucher specimens were deposited in the herbarium of Post Graduate Department of Botany, Utkal University, Vani Vihar, and Bhubaneswar. The leaves were collected in bulk amount, washed in running tap water, dried under shade and made to coarse powder form.

Processing of plant material and preparation of extract

The collected leaves were shade dried and ground to form coarse powder and had been successively extracted with the solvents (petroleum ether and methanol) by Soxhlet apparatus and the extract was recovered under

reduced pressure in a rotatory evaporator.[11] The extracts were kept in desiccators for further use.

In-vitro evaluation of antifungal efficacy of plant extracts

For the evaluation of antifungal effect of petroleum ether and methanolic leaf extracts, the leaf extracts were diluted with dimethyl sulfoxide (DMSO) @ 20mg/ml. Then antifungal activities of those extracts were carried out based on the method of Satish *et al*. (2007)[12] with modification. For sample treatment, 1 ml of diluted plant extracts plus 19 ml of Potato Dextrode Agar (PDA) were poured into each petri plate, mixed thoroughly and allowed to solidify. The concentration of plant extract on PDA medium was 1mg/ml. PDA medium without the plant extracts served as control. Disc of 0.5 cm culture of the test fungi was placed at the centre of the petri plate based on poison food technique (both sample and control) and incubated at 28 ± 1 ºC inside incubator for respective days of their growth of 8 cm diameter on petriplates such as *A. flavus* was incubated for 7 days, *A. niger* for 8 days, *F. oxysporum* for 10 days, *G. candidum* for 11 days, *Mucor* sp. for 5 days and *Penicillium* sp. for 15 days. The efficacy of each plant extracts were evaluated by measuring fungal radial growth (cm) using ruler. The antifungal activity in terms of percentage inhibition was calculated by using formula as mentioned below:

$$\% \text{ Inhibition} = (X-Y)/X \times 100$$

Where X= average increase in mycelial growth in control, Y= average increase in mycelial growth in treatment.[12]

Comparision of the efficacy of selected fungicides and plant extracts

A separate experiment was conducted in order to compare the relative efficacy of four fungicides, viz., Dhanustin and Blitox-50 and 16 extracts (petroleum ether and methanolic extracts) of eight selected plants. The concentration of fungicides was 0.05 mg/ml. The required quantity of chemicals (0.5 %) and plant extracts (5%) were incorporated after sterilization of PDA medium, and were inoculated by six test fungal isolates in four replicates and incubated for respective days of their growth as described earlier. At the end of incubation period, the colony diameter of each fungichemical/ plant extract combination was measured, transformed to percentage of mycelial inhibition as per the method described earlier.

RESULTS

In-vitro antifungal activity of botanicals and commercial fungicides

In-vitro antifungal activity of eight medicinal plants against six plant pathogenic fungi revealed that almost all the test plants were effective to inhibit the mycelial growth of the test fungi under study. The percentage of mycelial growth inhibition ranged from 19.33 ± 1.69 % to 87.5 ± 2.04 %. The petroleum ether leaf extract of *Murraya paniculata* was most effective (60.52 ± 2.64 %) to inhibit the mycelial growth of *A. flavus*. The petroleum ether extract of *Eucalyptus globulus* was found to be more effective against *A. niger* than other test extracts of the botanicals used. It controls the mycelia growth of *A. niger* by 53.56 ± 2.82 %. The mycelial growth of *F. oxysporum* was more inhibited by methanolic extract of *Haldinia cordifolia* (66.39 ± 4.57 %) leaves. All the test plant's extract were seen to be quite good effective (61.91 ± 1.89 % to 82.42 ± 2.13 %) against the mycelial growth of *G. candidum*. The inhibition of mycelial growth of *Mucor* sp. was ranged from 47.39 ± 1.9 % (petroleum ether extract of *Pithecellobium dulce*) to 87.5 ± 2.04 (petroleum ether extract of *Justicia adhatoda*). The mycelial growth of *Penicillium* sp. was severely inhibited by petroleum ether extract of *Pongamia pinnata* (72.24 ± 1.73 %) (Table 1 and Figure 1-6). Two fungicides were tested against the six isolated plant pathogenic fungi such as Blitox-50 and Dhanustin. It can be revealed from Table 1 that Dhanustin is more effective against test fungi as compared to Blitox-50. It completely inhibited the growth of *A. flavus* (100 %), while *A. niger* by (81.41 ± 1.23 %), *F. oxysporum* by (86.7 ± 1.2 %), *Geotrichum candium* (31.41 ± 1.23 %), *Penicillium* sp. by (68.58 ± 1.31 %) followed but against *Mucor* sp. Blitox-50 was found to be more effective than Dhanustin. It inhibited the growth of *Mucor* sp. by 24.8 ± 1.88 % (Table 1 and Figure 7).

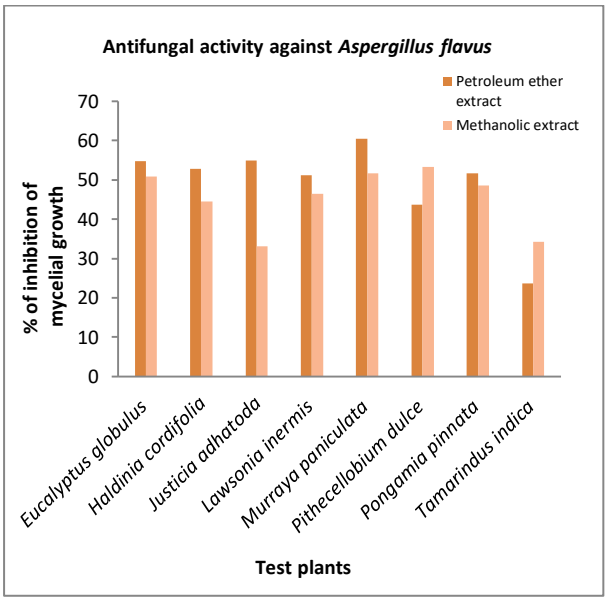

Figure 1: *In-vitro* antifungal activity of test plants against *Aspergillus flavus*

Comparative efficacy of botanicals and commercial fungicides

The comparative result of Plant extracts and test fungicides (Table 1) revealed that plant extracts were more efficient in controlling the mycelial growth of all the isolated fungus under study. For inhibiting the mycelial growth of *A. flavus*, *A. niger* and *F. oxysporum*, Dhanustin was more effective than the test botanicals but Blitox-50 was found to be less effective as compared to all test botanicals. But for the inhibition of mycelial growth of *G. candidum* and *Mucor* sp. and *Penicillium* sp. the plant extracts were found to be more effective than the test fungicides.

Table 1: *In-vitro* antifungal activity of botanicals and commercial fungicides

Test Plants		Percentage of Inhibition of Mycelial Growth					
		Af	An	Fo	Gc	M sp.	P sp.
Eucalyptus globulus	A	54.76 ± 2.24	53.56 ± 2.82	35.83 ± 2.01	81.66 ± 2.35	85.25 ± 2.5	62.08 ± 2.12
	B	50.91 ± 2.23	40.91 ± 5.91	54.41 ± 3.13	72.33 ± 1.69	83.33 ± 1.69	43.75 ± 2.96
Haldinia cordifolia	A	52.83 ± 4.08	46.83 ± 2.37	64.78 ± 3	74.66 ± 2.86	86.83 ± 2.89	64.06 ± 0.74
	B	44.5 ± 3.24	35.77 ± 2.04	66.39 ± 4.57	67.41 ± 3.36	82.12 ± 4.21	57.9 ± 1.56
Justicia adhatoda	A	54.91 ± 2.56	42.41 ± 1.16	43.91 ± 3.26	71.61 ± 2.65	87.5 ± 2.04	40.29 ± 2.1
	B	33.08 ± 1.53	36.5 ± 3.34	63.16 ± 2.09	82.42 ± 2.13	73.14 ± 2.35	36.12 ± 2.3
Lawsonia inermis	A	51.16 ± 4.58	52.77 ± 3.56	34.2 ± 3.16	75.45 ± 1.12	62 ± 1.63	57.87 ± 2.18
	B	46.5 ± 1.77	47.76 ± 4.81	48.37 ± 1.5	72.77 ± 1.98	75.32 ± 1.25	44.29 ± 2.45
Murraya paniculata	A	60.52 ± 2.64	42.83 ± 1.64	43.42 ± 2.62	62.83 ± 2.46	72.75 ± 2.6	35.13 ± 2.3
	B	51.62 ± 2.38	32.33 ± 2.05	39.71 ± 1.77	66.68 ± 1.94	70	54.24 ± 2.39
Pithecellobium dulce	A	43.61 ± 3.47	19.33 ± 1.69	34.73 ± 3.15	61.91 ± 1.89	47.39 ± 1.9	34.99 ± 1.63
	B	53.35 ± 2.45	41.66 ± 1.69	41.94 ± 4.37	74.98 ± 0.84	62.16 ± 1.64	45.91 ± 2.05
Pongamia pinnata	A	51.72 ± 2.77	43.96 ± 2.9	50.66 ± 4.1	76.83 ± 2.89	84.66 ± 2.04	72.24 ± 1.73
	B	48.55 ± 1.27	52.71 ± 4.22	44.25 ± 2.87	67.58 ± 1.82	85.33 ± 1.24	53.77 ± 3.08
Tamarindus indica	A	23.66 ± 2.62	23.63 ± 1.23	43.73 ± 3.04	72.76 ± 2.36	83.34 ± 2.57	43.08 ± 3.51
	B	34.26 ± 3.22	24.43 ± 3.41	47.91 ± 2.12	75.74 ± 3.68	53.88 ± 3.22	53.89 ± 2.83
Dhanustin		100	81.41 ± 1.23	86.7 ± 1.2	31.41 ± 1.23	11.83 ± 0.84	68.58 ± 1.31
Blitox-50		21.74 ± 1.33	17.66 ± 1.05	0	17.09 ± 2.37	24.8 ± 1.88	12.5 ± 1.22

Results expressed as mean ± S.D. of three determinations

A = Petroleum ether extract, B = Methanol extract, *Af* = *Aspergillus flavus*, *An* = *Aspergillus niger*, *Fo* = *Fusarium oxysporum*, *Gc* = *Geotrichum candidum*, *M* sp. = *Mucor* sp., *P* sp. = *Penicillium* sp.

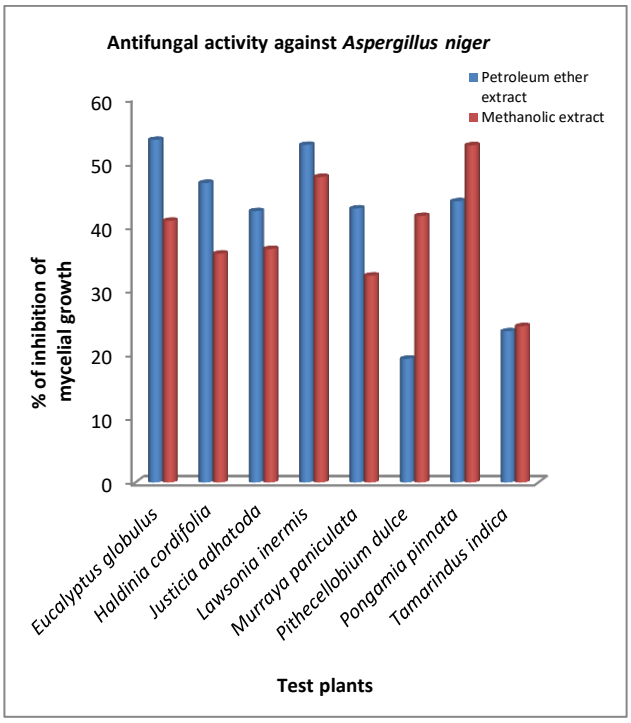

Figure 2: *In-vitro* antifungal activity of test plants against *Aspergillus niger*

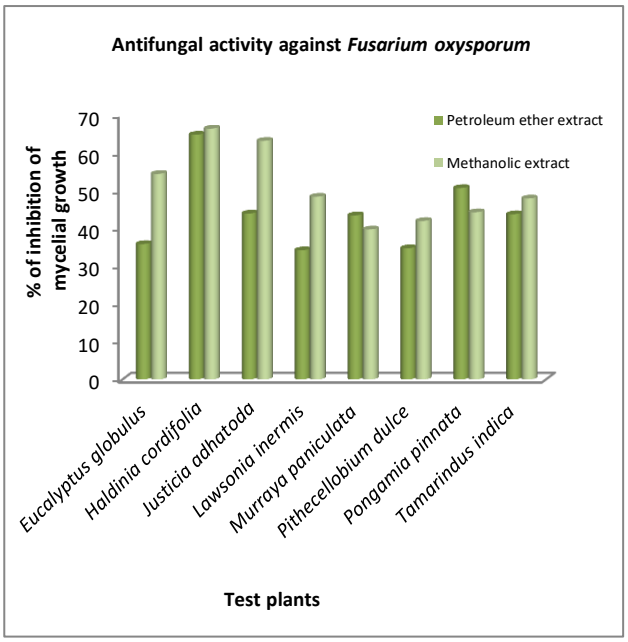

Figure 3: *In-vitro* antifungal activity of test plants against *Fusarium oxysporum*

DISCUSSION

Fungitoxicity of some plant extracts tested during the present investigation were also tested earlier by other workers. The list includes *Eucaluptus globules*,[13]

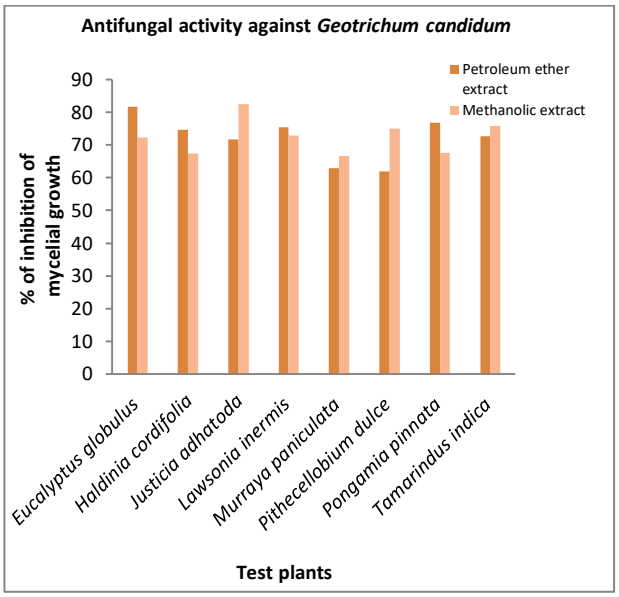

Figure 4: *In-vitro* antifungal activity of test plants against *Geotrichum candidum.*

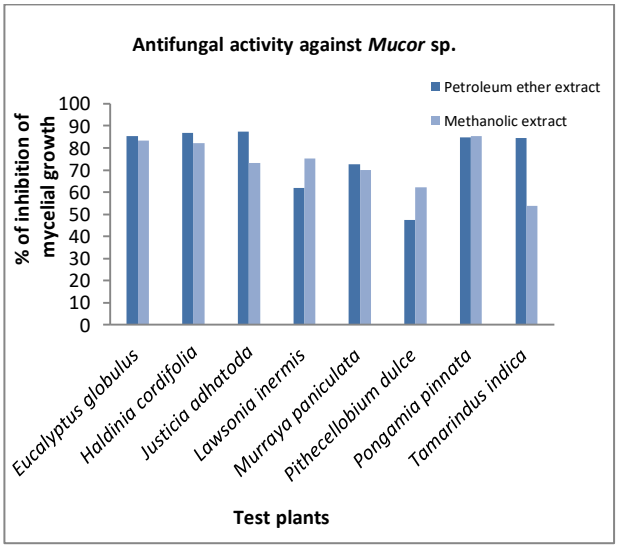

Figure 5: *In-vitro* antifungal activity of test plants against *Mucor* sp.

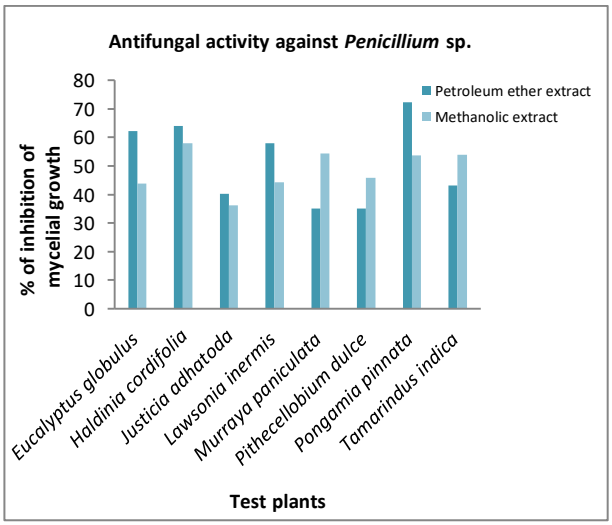

Figure 6: *In-vitro* antifungal activity of test plants against *Penicillium* sp.

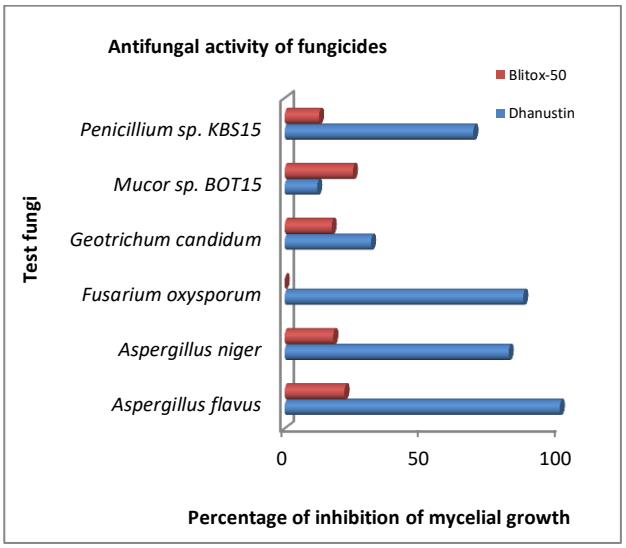

Figure 7: *In-vitro* antifungal activity of two commercial fungicides against the six test fungi**.**

Justicia adhatoda against *Fusarium oxysporum*,[14] *Lawsonia inermis* against *Fusarium oxysporum*,[15] *Aspergillus flavus*,[16] *Pongamia pinnata* against *Aspergillus flavus*; *Aspergillus niger*,[17] *Pithecellobium dulce* against *Fusarium oxysporum*,[18] *Aspergillus niger*; *Aspergillus flavus*;[19] *Tamarindus indica* against *A. niger*; *A. flavus*; *Fusarium oxysporum* [20] and *Penicillium* sp.[21]

CONCLUSION

In this study, the effects of botanicals against fungi causing storage rot of potatoes were evaluated *in-vitro*, and results indicated that leaf extracts of all plants had inhibitory activity against the test fungi which may be due to presence of some compounds in the plant extracts. However, the extract is chemical compounds and their controlling mechanism to the fungi storage rots need to be further elucidated. Since leaves of those plants are available easily and being devoid of phytotoxic effects, the botanicals can be recommended to be used as biofungicides for controlling post-harvest diseases of vegetables in general and storage tuber of potato in particular under the agroclimatic conditions of Odisha.

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