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Screening of different botanicals extract on two polyphagous postharvest pathogens from mango and banana

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Abstract
Aqueous extract of eight different locally available botanicals namely *Eucalyptus globulus*, *Piper betel*, *Ocimum sanctum*, *Annona raticulata*, *Mikania micrantha*, *Asteracantha longifolia*, *Aegle marmelos* and *Murraya paniculata* have been screened to evaluate their antifungal activity against two polyphagous postharvest pathogens *Thielaviopsis paradoxa* and *Curvularia lunata* from mango and banana. The results showed that all the botanicals extract significantly inhibit the mycelial growth as compared to control under *in vitro*. *Murraya paniculata* and *Eucalyptus globulus* showed best results as they inhibit maximum mean radial growth of *T. paradoxa* 92.70% and 73.86%, *C. lunata* 60.67% and 72.08% respectively. Sporulation of both the fungi reduced by these extract compared to control. The outcome of the study might be useful for alternative way to manage the postharvest disease caused by these pathogens.

Keywords: Botanicals extract, antifungal, polyphagous, growth inhibition, postharvest

Introduction
Mango and banana are two important tropical fruits. As natural systems they are infected by various diseases both in preharvest and postharvest stages. As compared to other plant parasites, fungi cause the greatest impact with regard to diseases and crop losses. Numbers of fungi and bacteria causes serious loss in postharvest and it is a serious problem for producer, distributor as well as consumer. Various methods have been adopted to reduce the postharvest losses out of which chemical methods shows the best but it cannot denied that there is no residual effect on the fruits and vegetables. So to avoid the chemical residual other alternate methods are now given more attention. Botanical extract of several higher plant have the antifungal effect which can be effectively utilize to manage the postharvest diseases of perishables. Natural products may be a viable solution to the problems caused by the synthetic pesticides and many researchers are trying to identify the effective natural products to replace the synthetic pesticides (Kim *et al*., 2005) [19].The exploitation of natural plant products to control decay and prolong storage life of perishables has received more and more attention (Kamlesh Mathur *et al.*, 2007; Archana Singh *et al.*, 2008; Babu *et al.*, 2008; Jeeva Ram and Thakore, 2009; Chandra and Mahesh, 2013) [18, 5, 7, 17, 12]. Plant extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trails (Okigbo and Ogbonnaya, 2006; Shariff *et al*., 2006; Bouamama *et al*., 2006; Ergene *et al*. 2006; Kiran and Raveesha, 2006; Mohana and Raveesha, 2006) [27, 28, 10, 15, 20, 25]. Singh *et al*. 1993 [30] recorded that aqeous extract of neem, tulsi and castor effectively control the disease development in banana. Strong antifungal activity was reported from the plant species like *Aegle marmelos*, *Croton roxburghii*, *Artobotrytis hexapentalous*, *Physalis peruviana* (Singh and Tripathi, 1993) [29].

Screening of plant products for its effective antifungal activity against the pathogen is essentially required to minimize the use of fungicides and to consider as one of the components in the integrated disease management. The plant based pesticides are cheap, locally available, non-toxic, and easily biodegradable. It was believed to be worthwhile to screen the antifungal effects of locally available flora (Bhardwaj, 2012) [9]. The objective of present study is to determine the antifungal activity of aqueous extract of leaves of some commonly available botanicals against *Thielaviopsis paradoxa* and *Curvularia lunata* from mango and banana.

Percent reduction of radial mycelial growth = $\frac{C-T}{C}$ X 100

Where C = the radial mycelia growth in control
T = the radial mycelia growth in the treatment

Sporulation

To determine the sporulation whole petri plate containing fungal growth was washed with total 40 ml of sterile distilled water (wash twice with 20 ml each by rubbing)and thoroughly mixed with the sterile distilled water and spore concentration has been counted using Haemocytometer.

Statistical analysis

All experiments were conducted in a completely randomized design with three repetitions, for each treatment. The statistical analysis of the results was conducted by analysis of variance (ANOVA) in MS excel sheet.

Result and discussion

Inhibition of radial growth

Fungal mycelial growths of both the fungi were restricted by the treatments, although the degree of restriction varies significantly among the treatments. The growth inhibition percent has been presented in table no1 & 2.

Table no.1 indicated that maximum mycelial growth inhibition recorded at T1 and T8 which were treated with Eucalyptus and *Myrraya* leaf extract respectively. Mycelial inhibition decreases along with the increase of incubation period irrespective of the treatments. Tsonchumi *et al*, 2018[32] reported the best inhibitory effect with 40.6% reduction as compared to the control by *Chromolaena odorata* against *Curvularia* sp. Maximum antifungal potential was recorded with the 20%extracts of *Cannabis sativa*, which showed excellent inhibitory activity against *C. lunata* (100%), *A. zinniae* (59.68%), followed by leaf extract of *Parthenium hysterophorus* (50%) against *A. solani*. (Ashwani *et al*, 2011) [6]. Brunda Devi *et al*, 2017 [11] screened three botanicals aqueous extract of which, *Duranta erecta* showed maximum antifungal activity against Rhizopus *arrhizus*, *Sclerotium rolfsii*, *Fusarium solani*, followed by *Lasonia inermis*, Neem oil, and *Cocculus hirsutus.* The degree of inhibition increased correspondingly with increasing concentrations of the plant extracts. 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.(Akhtari Khatoon *et al*, 2017) [2].

Table1: Growth inhibition percent of *Curvularia lunata* with plant extracts at different time intervals

Treatments	24hrs	48hrs	72hrs	96hrs	120hrs	Mean
T1	100.00 (90.00)	78.45 (62.35)	62.78 (52.42)	61.15(51.46)	59.61 (50.55)	72.04(61.35)
T2	75.86 (61.04)	41.39 (40.00)	30.00 (33.12)	28.29 (32.11)	26.58 (31.00)	40.42 (39.45)
T3	57.97 (49.61)	22.40 (28.24)	16.11 (23.66)	13.08 (21.20)	12.11 (20.34)	24.33 (28.61)
T4	34.07 (35.30)	12.06 (20.03)	9.44 (17.59)	8.06 (15.80)	8.05 (15.16)	14.34 (20.78)
T5	21.94 (27.78)	14.64 (22.03)	11.11 (19.16)	11.77 (19.71)	17.19 (24.10)	15.33 (22.56)
T6	21.94 (27.78)	11.20 (19.38)	9.44 (17.79)	8.87 (17.30)	11.17 (19.93)	12.65 (20.43)
T7	35.91 (36.78)	22.40 (28.24)	15.00 (22.77)	10.54 (18.90)	7.04 (12.64)	18.18 (23.87)
T8	94.00 (75.82)	67.30 (55.31)	51.93 (45.62)	50.93 (45.47)	40.04 (39.16)	60.67 (52.28)
SEm(±)	2.96	2.46	2.27	2.76	3.45	
CD(p=0.05)	11.11	9.25	8.54	10.36	12.97	

*Figure in parenthesis is the arcsin transformed value

T1= *Eucalyptus globulus*; T2= *Piper betel*; T3= *Ocimum sanctum*; T4= *Annona raticulata*; T5= *Mikania micrantha*; T6= *Asteracantha longifolia*; T7= *Aegle marmelos*; T8= *Murraya paniculata*

It is clear in table no 2 that all the treatment inhibit the radial mycelial growth of *Thielaviopsis paradoxa* at varied degree, although not very effectively. The inhibition of radial mycelial growth gradually decreases along with the increase of incubation period this may be due to the decrease in the antifungal property in the botanical extracts. The table indicated that only T8, T1 and T2 effectively inhibit the mycelial growth.

Both the fungal mycelial growth were inhibited by the media treated with *Eucalyptus globulus* and *Murraya paniculata* effectively (Fig.1). Maria Diana *et al*, 2016 [23] observed under *in vitro* condition that the selected species, of *Allium sativum*, *Aloe vera*, *Glycyrrihiza glabra*, *Myroxylon balsamum*, *Rhizophora mangle* and *Protium heptaphyllum*, showed consistent antifungal activity against *F. guttiforme* and *T. paradoxa*.

Several earlier works recorded that various plant species possess antifungal and antibacterial properties (Maji *et al*., 2005; Nduagu *et al*. 2008; Yasmin *et al.,* 2008; Harlapur *et al.* 2007 and Akinbode and Ikotun, 2008) [22, 26, 33, 16, 3]. *Murraya paniculata* showed the antifungal activity against *Candida albicans* at different concentration (Sri Agung Fitri Kusuma, *et al*, 2017) [31].

Mehta and Sharma, 2013 [24] observed that *Eucalyptus globules* leaves crude extract prepared in water and alcohol 100% showed maximum inhibition against test pathogens. Our study also agrees with the result of earlier workers.

Table 2: Growth inhibition percent of *Thielaviopsis paradoxa* with plant extracts at different time intervals

0	24hrs	48hrs	72hrs	96hrs	120hrs	Mean
T1	88.66 (71.08)	85.54 (67.70)	77.89 (62.03)	65.96 (54.34)	51.23 (45.70)	73.86 (60.17)
T2	84.29 (66.74)	84.00 (67.06)	70.53 (57.20)	51.93 (46.09)	35.44 (36.42)	65.24 (54.70)
T3	48.31 (44.03)	65.42 (54.05)	58.95 (50.19)	41.05 (39.79)	25.61 (30.03)	47.87 (43.62)
T4	70.88 (57.54)	66.75 (54.88)	30.18 (33.23)	10.88 (18.81)	5.26 (13.26)	36.79 (35.54)
T5	76.51 (61.35)	68.53 (56.32)	50.88 (45.68)	33.33 (34.45)	18.95 (23.14)	49.64 (44.19)
T6	63.87 (53.29)	72.77 (58.60)	48.77 (44.29)	24.56 (29.68)	7.02 (15.15)	43.40 (40.20)
T7	85.48 (68.04)	74.75 (59.85)	52.28 (46.31)	23.16 (28.75)	5.26 (13.26)	48.19 (43.24)
T8	98.30 (83.96)	96.11 (78.70)	93.33 (75.08)	90.18 (71.77)	85.61 (67.81)	92.70 (75.46)
SEm(±)	3.46	2.91	3.14	4.06	4.08	

CD(p=0.05)	12.99	10.92	11.78	15.26	15.32	

*Figure in parenthesis is the arcsin transformed value

T1= *Eucalyptus globulus*; T2= *Piper betel*; T3= *Ocimum sanctum*; T4= *Annona raticulata*; T5= *Mikania micrantha*; T6= *Asteracantha longifolia*; T7= *Aegle marmelos*; T8= *Murraya paniculata*

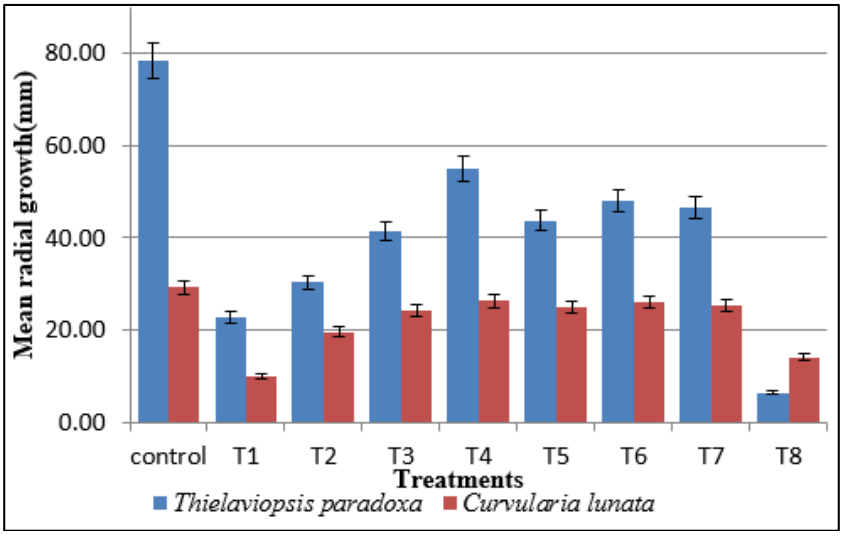

Fig 1: Mean radial growth of *T. paradoxa* and *C. lunata* in different treatments

Effect on sporulation

Regarding sporulation character both the fungi significantly varied in spore production along with the treatments. *Thielaviopsis parradoxa* produced enormous spores in all the treatment except in T8 (0.40 x 10^5) *i.e.* media treated with *Murraya paniculata.* Maximum sporulation recorded in T2 (156 x 10^5) followed by T3 (96 x 10^5) which indicated that these two treatments enhance the sporulation as compare to untreated control (89.60 x 10^5). Whereas in *Curvularia lunata* all the treatments reduce the sporulation as compared to control with no sporulation in T6 and T8 which were treated with *Asteracantha longifolia* and *Murraya paniculata* respectively (table no.3).It is interesting to note that even the T1 treatment reduces the mycelial growth but it did not restrict the spore production in *Curvularia lunata*. Amadi *et al*, 2014[4] found that both extracts of guava leaf and ginger rhizome hindered sporulation and spore germination of *Aspergillus flavus*, *A. Niger*, *Rhizopus stolonifer* and *Fusarium* species. Medicinal plants represent a rich source of antimicrobial agents (Mahesh and Satish, 2008; Adnan *et al.*, 2010) [21, 1].

Table 3: Sporulation count of fungi at different plant extract (X10^5)

Treatments	Sporulation per ml (X 10^5)	
	Thielaviopsis paradoxa	***Curvularia lunata***
T1	15.84	0.40
T2	156.00	0.08
T3	96.00	0.08
T4	76.64	0.16
T5	20.80	0.16
T6	41.12	0.00
T7	31.36	0.08
T8	0.40	0.00
Control	89.60	0.72
SEm(±)	2.14	0.02
CD(p=0.05)	7.58	0.06

T1= *Eucalyptus globulus*; T2= *Piper betel*; T3= *Ocimum sanctum*; T4= *Annona raticulata*; T5= *Mikania micrantha*; T6= *Asteracantha longifolia*; T7= *Aegle marmelos*; T8= *Murraya paniculata*

Conclusion

The screening of these botanicals namely *Eucalyptus globulus* and *Murraya paniculata* were effectively inhibit the radial mycelial growth of both the postharvest pathogens *Thielaviopsis paradoxa* and *Curvularia lunata. Murraya paniculata* also restrict the sporulation of the *Curvularia lunata* and *Thielaviopsis paradoxa* but *Eucalyptus globulus* failed to do so. However for confirmation different concentrations and its field trial need to be conducted before recommendation for environmentally safe and eco-friendly management of pathogens

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