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EVALUATION OF FUNGICIDES AND PLANT EXTRACTS AGAINST PATHOGENIC FUNGI ASSOCIATED WITH *BASELLA* SPP.

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Abstract

Five fungicides *viz*., CM 75, Dithane M 45, Knowin 50 WP, Nativo 75 WG and Rovral 50 WP were evaluated against *Colletotrichum lindemuthianum, Drechslera sacchari* and *Fusarium semitectum* following poisoned food technique. Out of five fungicides complete inhibition of radial growth of *C. lindemuthianum* was observed in Nativo 75 WG at 100 ppm. On the other hand, the complete inhibition of the growth of *D. sacchari* was observed with Rovral 50 WP at 400 ppm, whereas Nativo 50 WP showed complete growth inhibition at 500 ppm. CM 75 WP, Knowin 50 WP and Nativo 75 WG showed complete growth inhibition of *F. semitectum* at 100 ppm. Five different plant leaf extracts *viz*., *Azadirachta indica* A. Juss., *Heliotropium indicum* L., *Lippia alba* L., *Michelia champaca* L. and *Thuja occidentalis* L. were tested against the test pathogens. Of the five plant leaf extracts, *Lippia alba* showed the highest growth inhibition in *C. lindemuthianum, D. sacchari* and *F. semitectum* at 20% concentration.

Key words: Fungicides, Plant extracts, Pathogenic fungi, *Basella* spp.

Introduction

Basella is a popular tropical leafy-green vegetable, belongs to *Basellaceae* (Sushila *et al.* 2010) and has two chief cultivars namely, *Basella alba* and *B. rubra* (Cook 2010). A vast amount of yield is lost in terms of quantity and quality due to various constraints (Hasan *et al.* 2016). Proper management strategy of leaf spot of *Basella* spp. is very essential for the economical point of view. Various workers in different countries of the world evaluated the efficacy of various fungicides against *Colletotrichum* spp., *Macrophomina phaseolina*, *Fusarium* spp., *C. gloeosporioides* and *Alternaria* spp. under laboratory and field conditions (Hossain and Bashar 2011, Ahmed *et al.* 2014, Chowdhury *et al.* 2015, Mamun *et al.* 2016, Hosen *et al*. 2016, Islam *et al.* 2017). Some researchers have used different chemical fungicides to control leaf spot disease of *Basella* and have achieved various degree of success (Khan and Smith 2005). The residue of chemical fungicides poses potential health hazard (Alemu *et al*. 2014). The alternate approaches like using plant extracts were found to be effective against the pathogen (Uddin *et al.* 2013, Maketon *et al.* 2008). Plant extracts are considered as new rays of hope because they are

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eco-friendly and can be used as an alternative measure to control plant diseases. Recently, some researchers have indicated the possibility of their exploitation as natural fungitoxicants for controlling plant diseases (Sharmin and Shamsi 2013, Mamun *et al.* 2016, Hosen *et al.* 2016, Islam *et al.* 2017). The aim of this study was to evaluate the common and easily available fungicides as well as selected plants to determine minimum inhibitory concentration (MIC) values of various levels to find out the most suitable one to reduce yield and post-harvest losses caused by leaf spot of *Basella* spp.

Materials and Methods

The samples were collected from three markets of Dhaka city *viz.*, Karwan Bazar, Anando Bazar and Polashi Bazar during April to November, 2017. Fungi associated with the leaf spot of *Basella* spp. were isolated following Tissue planting method (CAB 1968). Pathogenicity test has been done according 'detached leaf assay' followed by Azad and Shamsi (2011) with slight modification. Five fungicides with different active ingredients *viz*., CM 75 WP (Mancozeb 63% + carbendazim 12%), dithane M 45(80% Mancozeb), Knowin 50 WP [50% carbendazim (methyl benimidazol-2-ylcarbamate)], Nativo 75 WG (500 g tebuconazol, 250 g trifloxy-strobin) and Rovral 50 WP (Iprodione) were collected from the Siddique Bazar, Fulbariya, Gulistan, Dhaka. These fungicides were evaluated for their *in vitro* efficacy at different concentrations (100, 200, 300, 400 and 500 ppm) against pathogenic fungi associated with *Basella* spp.

A total of five different plant leaf extracts *viz.*, *Azadirachta indica* A. Juss., *Heliotropium indicum* L., *Lippia alba* L., *Michelia champaca* L. and *Thuja occidentalis* L. were screened against the selected test pathogens. Leaves of *Lippia alba* L. were collected from Bhairab, Narsingdi and the rest of the plant's leaves were collected from the Botanical garden of Curzon Hall campus, University of Dhaka. *In vitro* efficacy of selected plant leaf extracts at 5, 10, 15 and 20% concentrations were evaluated against the test pathogens following the method described by Helal and Shamsi (2018). The desired leaves of each plant were thoroughly washed in tap water, air dried and were prepared by crushing to known weight of fresh materials with distilled water in ratio of 1:1 (w/v). The pulverized mass of a plant part was squeezed through four-folds of fine cloth and the extracts were centrifuged at 3000 rpm for 20 min to remove particles. The supernatants were filtered through Whatman filter paper No. 1 and the filtrate was collected in 250 ml Erlenmeyer flask. The requisite amount of the filtrate of each plant extract was mixed with PDA medium to get 5, 10, 15 and 20% concentrations. In control, required amount of water was used instead of plant extract. All the Petri plates were incubated at $25\pm2^{o}C$.

The radial growth of the test pathogen colonies was measured after 7 days. The per cent growth inhibition of each test pathogen was calculated using the following formula:

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

where, I = Per cent growth inhibition, C = Growth in control, T = Growth in treatment.

The data were collected as per cent inhibition of the radial growth of the test pathogens in mm and evaluated by ANOVA by using STAR statistical program and means were compared using DMRT.

Results and Discussion

Ten fungi *viz.*, *Alternaria alternata*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Colletotrichum dematium*, *C. lindemuthianum*, *Curvularia lunata*, *Drechslera sacchari*, *Fusarium semitectum* and *Penicillium* sp. were isolated from the leaf spot of *Basella* spp. Among the isolated fungi, *C. lindemuthianum, D. sacchari* and *F. semitectum* were selected as test pathogens owing to their pathogenic potentiality.

Amongst the five fungicides, complete inhibition of the radial growth of *Colletotrichum lindemuthianum* was observed with Nativo 75 WG at 100 ppm concentration. Out of rest four fungicides, the highest growth inhibition of *C. lindemuthianum* was observed with CM 75 WP (68.84%) which was followed by Knowin 50 WP (63.08%), Rovral 50 WP (60.25%) and Dithane M 45 (59.10%) at 500 ppm concentration (Table 1).

Ann *et al.* (2017) reported the application of Nativo which significantly suppressed the development of leaf anthracnose and black berries disease caused by *Colletotrichum gloeosporioides*. Rajesha *et al.* (2010) reported that mancozeb completely inhibited the radial mycelial growth of *C. lindemuthianum* at 400 ppm concentration. Suresh and Ekbote (2005) also observed carbendazim as most effective in inhibiting the growth of *C. lindemuthianum.*

On the other hand, the complete inhibition of growth of *D. sacchari* was observed with Rovral 50 WP at 400 ppm concentration, whereas Nativo 75 WG showed complete growth inhibition at 500 ppm concentration and the highest growth inhibition (84.41%) at 400 ppm concentration (Table 2). Jadon and Shah (2012) observed that CM 75 WP at 500 ppm concentration completely inhibited the mycelial growth of *Drechslera bicolor*. Wahid *et al.* (1992) found thiophanate methyl as the best fungicide followed by captan against *Drechslera sacchari*. CM 75 WP, Knowin 50 WP and Nativo 75 WP showed complete growth inhibition of *F. semitectum* at all the treated concentrations whereas Rovral 50 WP

Table 1. Inhibition of radial growth (%) of *Colletotrichum lindemuthianum* at different concentrations of fungicides.

Name of fungicides	% inhibition of radial growth at different concentrations (ppm)				
	100	200	300	400	500
CM 75 WP	50.14[b]	56.58[b]	59.05[b]	61.42[b]	68.84[b]
Dithane M 45	14.18[c]	46.73[c]	49.17[c]	52.01[cd]	59.10[d]
Knowin 50 WP	57.05[b]	58.05[b]	59.06[b]	60.73[bc]	63.08[c]
Nativo 75 WG	100[a]	100[a]	100[a]	100[a]	100[a]
Rovral 50 WP	15.40[c]	21.38[d]	36.78[d]	50.57[d]	60.23[d]
CV (%)	8.62	3.70	5.82	5.50	2.60

Means followed by the same letter within a column did not differ significantly at 5% level by DMRT.

Table 2. Inhibition of radial growth (%) of *Drechslera sacchari* at different concentrations of fungicides.

Name of fungicides	% inhibition of radial growth at different concentrations (ppm)				
	100	200	300	400	500
CM 75 WP	36.59[b]	52.51[b]	61.45[b]	64.24[b]	65.64[c]
Dithane M 45	22.11[c]	57.19[b]	60.35[b]	60.70[b]	73.68[b]
Knowin 50 WP	11.08[d]	18.18[c]	22.72[c]	26.14[c]	38.35[d]
Nativo 75 WG	74.03[a]	79.74[a]	80.00[a]	84.41[a]	100[a]
Rovral 50 WP	71.80[a]	72.58[a]	84.33[a]	100[a]	100[a]
CV (%)	6.56	7.03	4.41	3.11	3.57

Means followed by the same letter within a column did not differ significantly at 5% level by DMRT.

showed complete growth inhibition at 500 ppm concentration. Rovral 50 WP showed 62.46 and 75.44% growth inhibition at 300 and 400 ppm concentrations, respectively. Dithane M 45 showed 16.91, 20.69, 53.93, 57.72 and 69.39% growth inhibition at 100, 200, 300, 400 and 500 ppm concentrations, respectively (Table 3). It is also noticed from the results that the per cent growth inhibition of the test pathogens gradually increased with the increase of concentration of the fungicides in culture media.

Hoque *et al.* (2016) reported that radial colony diameter of *F. semitectum* was significantly reduced over control due to amendment of PDA with Bavistin (carbendazin). The aforesaid fungicide showed 100% radial growth inhibition of *F. semitectum*. Pramesh *et al.* (2016) also found effective against blast and sheath blight diseases of rice by using Nativo 75 WG.

In vitro efficacy of various fungicides against the test pathogens indicated that CM 75 WP, Nativo 75 WG and Rovral 50 WP showed promising results as compared to others (Tables 1-3). The same fungicides also showed different effects on tested fungi in the present investigation. This variation might be due to selection of different strains of test pathogens.

Table 3. Inhibition of radial growth (%) of *Fusarium semitectum* at different concentrations of fungicides.

Name of fungicides	% inhibition of radial growth at different concentrations (ppm)				
	100	200	300	400	500
CM 75 WP	100[a]	100[a]	100[a]	100[a]	100[a]
Dithane M 45	16.91[b]	20.69[c]	53.93[b]	57.72[c]	69.39[b]
Knowin 50 WP	100[a]	100[a]	100[a]	100[a]	100[a]
Nativo 75 WG	100[a]	100[a]	100[a]	100[a]	100[a]
Rovral 50 WP	21.05[b]	28.77[b]	62.46[b]	75.44[b]	100[a]
CV (%)	7.00	6.71	3.03	2.80	0.69

Means followed by the same letter within a column did not differ significantly at 5% level by DMRT.

Results of leaf extracts on the radial growth of *Colletotrichum lindemuthianum, Drechslera sacchari* and *Fusarium semitectum* are presented in Tables 4-6. All the plant extracts showed distinct degree of growth inhibition of the test pathogens at 5, 10, 15 and 20% concentrations. Among the five plant extracts, *Lippia alba* showed complete growth inhibition of *C. lindemuthianum* at 20% concentration which was followed by *Azadirachta indica* (47.59%), *Thuja occidentalis* (46.05%), *Heliotropium indicum* (24.67%) and *Michelia champaca* (23.89%) (Table 4). The inhibition of the pathogen increases with the increase of the concentration of the plant leaf extracts in culture medium. Choudhary *et al.* (2017) reported that leaf extract of *A. indica* significantly reduced (78.83%) the mycelial growth of *C. lindemuthianum* at 10 ppm.

Out of five plant extracts, *Lippia alba* showed complete radial growth inhibition of *D. sacchari* at 20% concentration which was followed by *A. indica* (50.09%), *Thuja occidentalis* (50%), *Heliotropium indicum* (40.49%) and *Michelia champaca* (28.19%) (Table 5). Miah *et al.* (2017) reported that BARI Gom-26 variety showed the lowest fungal infection (6%) owing to *A. indica* and *Thuja occidentalis* plant extract followed by *Citrus limon* (8%), *Allium sativum* (10%) and *Datura metel* (10%). Jadon and Shah (2012) found *A. indica* as the best mycelial growth inhibitor among the perennials against the *Drechslera bicolor*.

Table 4. Effect of plant leaf extracts on the radial growth of *Colletotrichum lindemuthianum* at different concentrations.

Name of plants	Per cent inhibition of radial growth at different concentrations (%)			
	5	10	15	20
Azadirachta indica	26.62[a]	31.87[b]	37.12[b]	47.59[b]
Heliotropium indicum	14.80[b]	17.92[c]	19.48[c]	24.67[c]
Lippia alba	39.68[b]	41.90[ab]	45.39[a]	100[a]
Michelia champaca	12.46[b]	14.80[c]	19.48[c]	23.89[c]
Thuja occidentalis	27.91[a]	40.69[a]	44.65[a]	46.05[b]
CV (%)	15.99	12.89	9.85	5.81

Means followed by the same letter within a column did not differ significantly at 5% level by DMRT.

Table 5. Effect of plant leaf extracts on the radial growth of *Drechslera sacchari* at different concentrations.

Name of plants	Per cent inhibition of radial growth at different concentrations (%)			
	5	10	15	20
Azadirachta indica	30.34[b]	36.80[b]	38.34[b]	50.09[b]
Heliotropium indicum	28.05[b]	34.84[b]	38.25[c]	40.49[d]
Lippia alba	47.07[a]	54.39[a]	62.23[a]	100[a]
Michelia champaca	16.86[c]	19.27[c]	23.61[d]	28.19[e]
Thuja occidentalis	27.78[b]	39.33[b]	45.11[b]	50.00[c]
CV (%)	9.13	9.48	5.28	3.32

Means followed by the same letter within a column did not differ significantly at 5% level by DMRT.

The highest inhibition of radial growth of *F. semitectum* was observed with *Lippia alba* (100%) at 20% concentration which was followed by *A. indica* (46.47%), *T. occidentalis* (41.46%), *M. champaca* (20.73%) and *H. indicum* (17.97%) (Table 6). Sinha and Varma

(2017) reported that the ethanolic, methanolic and aqueous extracts of *M. champaca* L. exhibited antioxidant and free radical activity.

Out of the five plant extracts *Lippia alba*, *Azadirachta indica* and *Thuja occidentalis* showed maximum radial growth inhibition of *C. lindemuthianum*, *D. sacchari* and *F. semitectum* at 20% concentration. But *Heliotropium indicum* and *Michelia champaca* showed minimum growth inhibition of the test pathogens.

Table 6. Effect of plant leaf extracts on the radial growth of *Fusarium semitectum* at different concentrations.

Name of plants	Per cent inhibition of radial growth at different concentrations (%)			
	5	10	15	20
Azadirachta indica.	29.05[a]	38.59[a]	40.87[a]	46.47[b]
Heliotropium indicum	7.83[d]	11.59[d]	14.49[d]	17.97[d]
Lippia alba	15.20[c]	39.18[a]	44.44[a]	100[a]
Michelia champaca	10.36[d]	14.14[c]	18.29[c]	20.73[d]
Thuja occidentalis	26.83[b]	31.71[b]	37.80[b]	41.46[c]
CV (%)	14.12	6.93	6.41	5.16

Means followed by the same letter within a column did not differ significantly at 5% level by DMRT.

Basella spp. is an important plant for its nutritional, medicinal point of view. So, the production of the vegetables by controlling various diseases it is necessary to identify the most prevalent pathogen causing leaf spot and to reduce the yield as well as post-harvest loss of the vegetables. The results of this investigation identified Nativo and Rovral as the best inhibiting fungicides against leaf spot of *Basellla* spp. Leaf extracts of *Lippia alba* and *Azadirachta indica* identified as an effective botanical against for further testing against pre-harvest diseases of *Basella* spp.

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