Dhaka Univ. J. Biol. Sci. **31**(2): 281-288, 2022 (July) DOI: https://doi.org/10.3329/dujbs.v31i2.60886

IN VITRO SCREENING OF FUNGICIDES AND PLANT EXTRACTS AGAINST TWO PATHOGENIC FUNGI OF *CHRYSANTHEMUM MORIFOLIUM* RAMAT

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Key words: Fungicides, Plant extracts, Pathogenic fungi, *Chrysanthemum morifolium*, Leaf blight

Abstract

Chrysanthemum morifolium is one of the most famous cut flowers with a high ornamental value, occupying an irreplaceable position in international flower commerce. But most frequently occurred fungal diseases limit the production of this ornamental plant. A total of five fungicides *viz*., CM-75 WP, Dithane M 45, Ridomil Gold MZ 68 WG, Rovral 50 WP and Score 250 EC were selected to evaluate *in vitro* efficacy at 100, 200, 300, 400 and 500 ppm concentrations against two pathogenic fungi of *Chrysanthemum morifolium* namely, *Curvularia lunata* and *Fusarium moniliforme*. Rovral 50 WP showed complete growth inhibition of *C. lunata* and CM 75 WP showed complete growth inhibition of *F. moniliforme* at all concentrations used. Five plant extracts *viz*., *Azadirachta indica*, *Citrus limon*, *Datura metel*, *Psidium guajava* and *Vitex negundo* were selected to evaluate *in vitro* efficacy at 5, 10, 15 and 20% concentrations against the test pathogens. Out of the five plant extracts, *A. indica* showed complete growth inhibition of *C. lunata* at 15 and 20% concentrations. On the other hand, *P. guajava* showed complete growth inhibition of *F. moniliforme* at 20% concentrations.

Introduction

Chrysanthemum morifolium Ramat is one of the most economically valuable ornamental plants worldwide and commonly known as florist's daisy and Hardy garden mums belongs to the Asteraceae family[1]. In China, the Chrysanthemum is traditionally offered to the elderly as they symbolize long life as well as good luck in the home. In many countries, including the United States and Japan, it is considered as the number one crop[2]. The flowers of *C. morifolium* have been used in Vietnam and other Asian countries for the treatment of eye diseases, headaches, insomnia and hyperglycemia. Extracts of *C. morifolium* have antioxidant, cardiovascular protective and anti-inflammatory functions and potent neuroprotective activity and therefore, might be a potential candidate in neurodegenerative diseases such as Parkinson's disease[3-4]. In Bangladesh, BARI (Bangladesh Agriculture Research Institute, Gazipur) has developed

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two Chrysanthemum cultivars[5]. Fungal diseases are the major constraint for the Chrysanthemum flowers production. In New Zealand at least five fungi namely, *Alternaria alternata*, *Botrytis cinerea*, *Itersonilia perplexans*, *Mycosphaerella ligulicola* and *Stemphylium vesicarium* was found in the flower blight complex[6-7].

Proper management strategy of leaf blight of Chrysanthemum is very essential for the economical and ornamental point of view. Now-a-days, many inorganic and organic fungicides are used frequently to control plant diseases. Use of chemical pesticides provides excellent control of the diseases and result in improved yield. But fungicide's toxicity is not always restricted to the target pest organism. For the ecofriendly management researchers have focused their efforts on developing alternative inputs to synthetic chemicals for controlling diseases. One of them is the use of plant extracts. Plant extracts can be successfully exploited in modern agriculture. Plant extracts are cheap, can be easily prepared and whenever required. Plant parts and their constituents of some higher plants have already been reported to be successful natural fungitoxicants because of their lesser phytotoxicity, easily biodegradability and favorable effects for the growth of the host[8]. In recent years, some research on the fungitoxicity of extracts of various parts of higher plants have indicated the possibility of their exploitation as natural fungitoxicants for controlling plant diseases[9-11]. Lot of research have been done on Chrysanthemum plant and its diseases in abroad, but in Bangladesh research on different diseases of Chrysanthemum is inadequate. The present study was conducted to find out the *in vitro* efficacy of fungicides and plant extracts against two pathogenic fungi of *Chrysanthemum morifolium* Ramat.

Materials and Methods

The diseased samples of *C. morifolium* were collected from Agargoan Nursery, Dhaka and Botanical Garden, Curzon Hall Campus, University of Dhaka during June 2017 to June 2018. Fungi associated with diseases *C. morifolium* were isolated following 'Tissue Planting' method on PDA medium[12]. Identification of the isolated fungi was determined following standard literatures[13-15]. Pathogenecity test of the fungi associated with *C. morifolium* was carried out following 'Detached leaf technique'[16]. Five fungicides *viz*., CM 75 WP, Dithane M-45, Ridomil Gold MZ 68 WG, Rovral 50WP and Score 250 EC were collected from local market, Dhaka. *In vitro* fungitoxicity of these fungicides at 100, 200, 300, 400 and 500 ppm concentrations were evaluated against two pathogenic fungi *viz., Curvularia lunata* and *Fusarium moniliforme* isolated from Chrysanthemum according to Akhtar and Shamsi 2018[16]. A total of five plant parts namely, *Azadirachta indica*, *Citrus limon*, *Datura metel*, *Psidium guajava* and *Vitex negundo* were collected from yhe campus of Dhaka University. *In vitro* efficacy of selected plant parts extract at 5, 10, 15 and 20% concentrations were evaluated against the test pathogens following the method described by Helal and Shamsi[11].

The fungitoxicity of the fungicides and plant parts extracts in terms of percent inhibition of mycelial growth were calculated by 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 inhibition percentage of the radial growth of the pathogen in mm in each replication and evaluated by analysis of variance by using STAR statistical program and means were compared using Duncan's Multiple Range Test.

Results and Discussion

Two types of leaf blight symptoms were found in *C. morifolium.* Type-1 showed sub circular to rectangular brown necrotic lesions on leaves. Type-2 showed light brown irregular large lesions with yellowish surrounding. Total 11 species of fungi were isolated from the diseased leaves of *C. morifolium.* The isolated fungi were *Alternaria alternata* (Fr.) Keissler, *Aspergillus flavus* Link, *A. fumigatus* Fresenius, *A. niger* van Tieghem, *Cladosporium cladosporioides* (Fresen). De Vries, *Colletotrichum gloeosporioides* (Penz.) Sacc., *Curvularia lunata* (Wakker) Boedijin, *Fusarium moniliforme* J. Sheld., *Penicillium* Link, *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill and *Trichoderma viride* Pers. Among the isolated fungi *Curvularia lunata* and *Fusarium moniliforme* were found to be pathogenic to *Chrysan themum* during pathogenicity test and selected as test pathogens (Fig. 1. A-D).

In the present investigation, complete inhibition of growth of *C. lunata* was observed with all the concentrations) 100,200,300, 400 and 500 ppm) of fungicides. Roval 50 WP and and Score 250 EC showed complete inhibition of the aforesaid fungus at 400 and 500 ppm concentrations. Fungicides CM75 WP, Diathane M 45 and Ridomil Gold MZ 68 WG did not inhibit growth of *C. lunata* completely at their highest concentration 500 ppm as well as all other lower concentrations (Table 1).

The growth of *F. moniliforme* was inhibited completely By CM 75 WP at all the concentrations. Score 250 EC inhibited the growth of the fungus completely at 400 and 500 ppm concentrations. All the other fungicides namely Ridomil Gold MZ 68 WG Roval 50Wp and Dithane M 45 did not inhibit the growth of *F. moniliforme* completely even at their highest concentrations 400 and 500 ppm (Table 2). Mamun *et al.* (2016)[17] found that the growth of *Curvularia lunata* was completely checked with Tilt 250 EC, Dithane M-45, Greengel and Capvit at 500 ppm concentration. Chowdhury *et al*. (2015)[18] observed complete inhibition of radial growth of *C. lunata* with Dithane M 45 and Ridomil at 500 ppm concentration. Khatun and Shamsi (2016)[19] showed that Bavistin 50 WP and Greengel 72 WP were responsible for complete growth inhibition of *C. lunata* at 400 and 500 ppm concentrations. It was reported by Chowdhury *et al.* (2015)[18] that Dithane,

Ridomil and Sulphur at 300, 400 and 500 ppm concentrations showed the complete inhibition of radial growth of *F. moniliforme.* Bavistin also showed complete growth inhibition at 400 and 500 ppm concentrations.

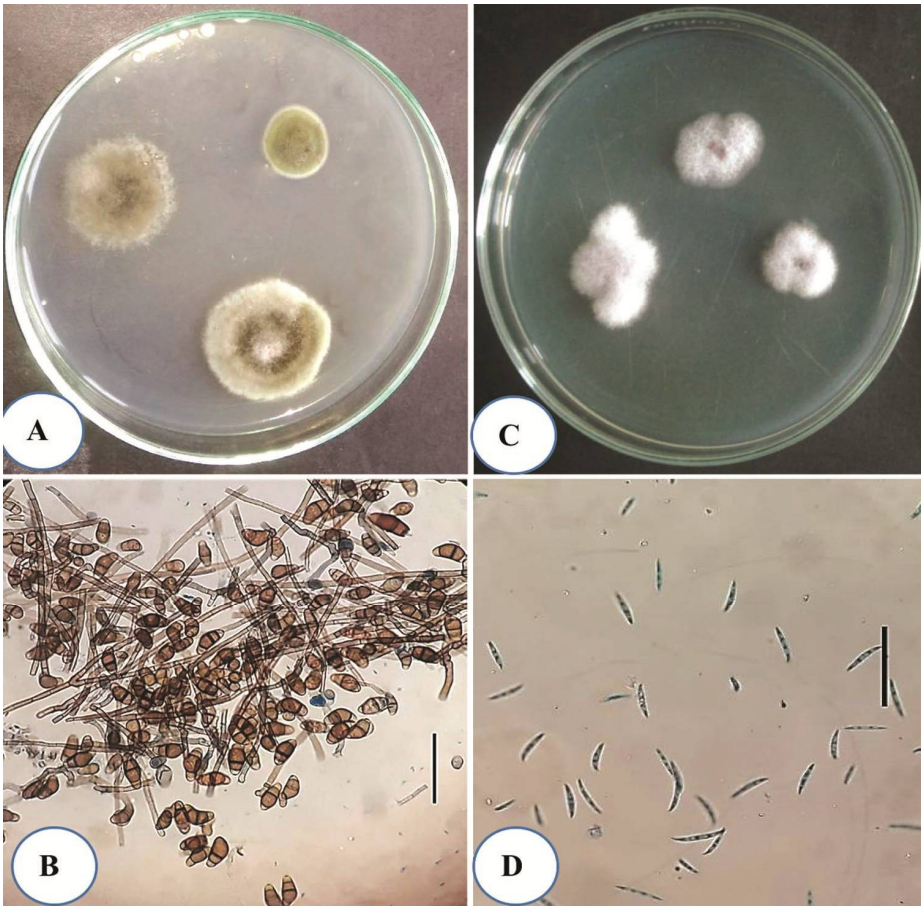

Fig. 1. Colonies and conidia of re-isolated pathogens. A- B. *Curvularia lunata*, C- D. *Fusarium moniliforme* (Bar = 50 μm)

Results of plant extract on the radial growth of *C. lunata* and *F. moniliforme* at 5, 10, 15 and 20% concentration are presented in Tables 3 and 4. Out of the five plant extracts, *Azadirachta indica* showed complete inhibition of radial growth of *C. lunata* at 20% concentration which was followed by *P. guajava* (80.90%), *V. negundo* (77.45%), *D. metel* (60.29%) and *C. limon* (52.08%). Out of the five plant extracts, *P. guajava* (84.55%) showed highest radial growth inhibition of *F. moniliforme* at 20% concentration which was followed by *A. indica* (76.51%), *C. limon* (72.70%), *V. negundo* (69.73%) and *D. metel* (47.40%). The inhibition of the pathogen increases with the increase of the concentration of the plant extracts in culture medium.

Table 1. Growth inhibition of *Curvularia lunata* at different concentrations of fungicides.

Name of fungicides	% inhibition of radial growth at different concentrations (ppm)				
	100	200	300	400	500
CM 75 WP	13.50^{e}	41.75^{c}	44.2^{e}	51.75^{d}	61.22^{d}
Dithane M-45	67.23^{b}	74.63^{b}	79.9^{b}	80.33^{b}	90.85^{b}
Ridomil Gold MZ 68 WG	43.90^{d}	46.34^{d}	54.87^{d}	63.41^{c}	65.85^{c}
Rovral 50 WP	100^{a}	100a	100a	100a	100a
Score 250 EC	56.75^{c}	65.41^{c}	69.18^{c}	100a	100a
CV(%)	2.76	1.53	1.88	1.41	1.58

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

Table 2. Growth inhibition of *Fusarium moniliforme* at different concentrations of fungicides.

Name of fungicides	% inhibition of radial growth at different concentrations (ppm)				
	100	200	300	400	500
CM 75 WP	100a	100a	100a	100a	100a
Dithane M-45	4.45^{e}	14.9^{e}	15.9^{e}	18.85^{d}	30.63^{d}
Ridomil Gold MZ 68 WG	35.48^{c}	42.92^{c}	45.90^{d}	58.80^{b}	69.97^{c}
Rovral 50 WP	25.18^{d}	31.25^{d}	36.07^{c}	42.33^{c}	55.96^{d}
Score 250 EC	56.9^{b}	65.58^{b}	69.15^{b}	100a	100a
CV(%)	6.41	3.15	5.24	4.78	2.67

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

Table 3. Effects of plant extracts on the growth of *Curvularia lunata*.

Name of plants	Plant part	% inhibition of radial growth of the pathogen at different concentrations (%)			
		5	10	15	20
Azadirachta indica	leaf	64.10^{a}	66.84^{b}	100a	100a
Citrus limon	”	38.75^{c}	42.50^{d}	46.53^{e}	52.08^{e}
Datura metel	”	33.82^{d}	35.29^{e}	55.88^{d}	60.29^{d}
Psidium guajava	”	65.55^{a}	78.17^{a}	79.76^{b}	80.95^{b}
Vitex negundo	”	60.48^{b}	61.54^{c}	68.97^{c}	77.45^{c}
CV (%)		2.64	1.87	1.94	2.34

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

Table 4. Effects of plant extracts on the growth of *Fusarium moniliforme*.

Name of plants	Plant part	% inhibition of radial growth of the pathogen at different concentrations (%)			
		5	10	15	20
Azadirachta indica	leaf	63.76[a]	64.88[a]	69.79[a]	76.51[b]
Citrus limon	”	5.49[d]	48.79[c]	51.89[b]	72.70[c]
Datura metel	”	26.43[c]	29.43[e]	33.93[d]	47.40[d]
Psidium guajava	”	29.43[c]	42.42[d]	47.45[c]	100a
Vitex negundo	”	55.95[b]	60.81[b]	67.57[a]	69.73[c]
CV (%)		5.02	3.52	3.73	3.21

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

In contrast to the present study, Khatun and Shamsi (2016)[19] reported that *A. indica*, *D. metel* showed complete growth inhibition of *C. lunata* at 20 % concentration whereas *P. guajava* showed 81.82% growth inhibition at 20% concentrations. Tamuli *et al.* (2014)[20] tested the antifungal activity of ethanolic leaf extract of *V. negundo* against *A. alternata*, *C. lunata* and *S. sclerotiorum*. The results showed that the antifungal activity increases with the increase in concentration of the extract. The maximum zone of inhibition formed by the test extract at 2% concentration was 30.32, 32.24 and 37.22 mm in *A. alternata*, *C. lunata* and *S. sclerotiorum*, respectively. Srivastava *et al.* (2012)[21] reported that the essential oil of *Thuja orientalis* showed antifungal activity against *Alternaria alternata* and *Curvularia lunata* in a direct bioautography assay by lipophilic leaf extract of *T. orientalis*. Mahmud *et al.* (2009)[22] reported that crude extract of fruits (seed) of *Vitex negundo* showed excellent inhibition of growth of *Fusarium solani* (90%). Alkhail (2005)[23] showed that extracts of *Azadirachta indica* presented remarkable biological activity when tested against fungi viz., *F. oxysporum*, *Botrytis cinerea*.

Best performed fungicides, CM 75 WP and Rovral 50 WP as well as plant extracts *A. indica* and *P. guajava* need field trials to extend the efficacy in controlling leaf blight of Chrysanthemum.

Acknowledgement

The first author expresses heartfelt gratitude to the Ministry of Science and Technology, People’s Republic of Bangladesh for providing financial assistance to this research work through NST fellowship program.

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(Manuscript received on 10 November, 2021; accepted on 20 June, 2022)