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IN VITRO SCREENING OF FUNGICIDES AND PLANT EXTRACTS AGAINST PATHOGENIC FUNGI ASSOCIATED WITH INFECTED FRUITS OF *CARICA PAPAYA* L.

REZUANA BINTE HELAL[1] AND SHAMIM SHAMSI[*]
Department of Botany, University of Dhaka, Dhaka – 1000, Bangladesh

ABSTRACT

Five fungicides *viz*., Autostin 50 WDG, Dithane M 45, Greengel 72 WP, Ridomil Gold MZ 68 WG and Tilt 250 EC exclusively were tested against three pathogenic fungi *viz.,* C*olletotrichum gloeosporioides* (Penz.) Sacc., *Fusarium nivale* (Fr.) Ces. and *Fusarium* sp. associated with *Carica papaya* L. following poisoned food technique. At 100 ppm Autostin showed complete growth inhibition of *C. gloeosporioides, F. nivale* and *Fusarium* sp., while Tilt 250 EC showed complete growth inhibition of *C. gloeosporioides* at the same concentration. These fungicides at 400 and 500 ppm showed complete growth inhibition of *F. nivale.* Tilt 250 EC showed complete growth inhibition of *Fusarium* sp. at 300, 400 and 500 ppm. Eight plants extract, *viz., Adhatoda vasica, Azadirachta indica, Curcuma longa, Ocimum sanctum, Lantana camara, Tagetes erecta, Thuja orientalis* and *Vitex negundo* were also tested for their efficacy at 5, 10, 15 and 20% concentrations against these pathogenic fungi. Out of the eight plant extracts, *Ocimum sanctum* showed highest (88.57%) radial growth inhibition of *C. gloeosporioides* at 20% concentration. *Curcuma longa* showed highest (88.55%) radial growth inhibition of *F. nivale* and *Adhatoda vesica* showed highest (80.02%) radial growth inhibition of *Fusarium* sp. at the same concentrations. The present investigation suggests Autostin 50 WDG and Tilt 250 EC as best inhibiting chemical fungicides for *C. gloeosporioides*, *F. nivale* and *Fusarium* sp. Extracts of *O. sanctum, C. longa* and *Adhatoda vesica* were found to be superior to other plant extracts tested.

Keywords: *In vitro* screening, Fungicides, Plant extracts, Pathogenic fungi, *Carica papaya* L.

INTRODUCTION

Papaya (*Carica papaya* L.) plant is known to have uterotonic, nephroprotective, anti-inflamatory and anti-tumor properties (Milind and Gurditta 2011). Papaya is a good source of nutrient and vitamin A and C (Duke 1984). Glucose is the main sugar in papaya but sucrose content increases during ripening and can reach up to 80% of the total sugars (Parni and Verma 2014). The availability of the fruit is reduced due to high level of post-harvest loss (Mondal *et al*. 1995). Sawant and Gawri (2011) reported that fungal infection reduced the nutritional value of the fruits. Approximately 41 % post-harvest loss of papaya was recorded in Bangladesh (Hassan 2010). Various fungi cause rots in fruits of papaya.

Due to environmental concerns of controlling fungal disease by toxic chemicals, researchers have focused their efforts on developing alternative methods of controlling fungal diseases (Baker 1987, Cook 1993 and Heydari and Pessarakli 2010). One is use of biological antagonists and plant extracts. Plant extracts can be successfully exploited in modern agriculture which has recently attracted attention of several workers. Plant constituents have been reported to be successful fungitoxicants because of low phytotoxicity, systemicity, easy biodegradability and favorable effects for the growth of the host (Fawcett and Spencer 1970 and Panday *et al*. 1983). Extracts obtained from many plants have recently been studied for their antifungal

* Corresponding author: <prof.shamsi@gmail.com>.
[1] The experiment was conducted in the laboratory of Mycology and Plant Pathology, Department of Botany, University of Dhaka, Dhaka – 1000, Bangladesh

activities (Monoharachary and Reddy 1978, Ahmed and Sultan 1984, Miah *et al*. 1990, Hosen *et al*. 2016).

The present investigation was undertaken to evaluate the toxicity of some chemical and plant extracts against test pathogens.

MATERIALS AND METHODS

Infected ripe fruits of *Carica papaya* L. having characteristic symptoms were collected from five different markets of Dhaka City, during April and May, 2016 in separate sterile polyethylene bags, labeled properly and brought to the laboratory. The associated fungi were isolated following Tissue planting method on PDA medium (Anonymous 1968). Identification of the fungi were done following standard literature. Pathogenecity of the isolated fungi were done following fruit inoculation technique. Among the isolated fungi *Colletotrichum gloeosporioides*, *Fusarium nivale* and *Fusarium* sp. were found to be pathogenic to papaya fruit (Helal 2017). Five fungicides with different active ingredients, *viz*., Autostin WDG, Dithane M 45, Greengel 72 WP, Ridomil Gold MZ 68 WG and Tilt 250 EC were collected from the Krishi Upokoron Biponi Kendro at Khamarbari, Farmgate, Dhaka (Table 1). Of the five fungicides used in the present investigation, Autostin 50 WDG and Dithane M 45 are systemic while Tilt 250 EC and Greengel 72 WP are protectant fungicides and Ridomil gold MZ 68 WG is both systemic and protectant fungicide. For each fungicide, a stock solution having the concentrations of 10,000 ppm was prepared. The calculated amount of the stock solution of a fungicide was supplemented with sterilized PDA medium to get the conc. of 100, 200, 300, 400 and 500 ppm. In the control set required amount of sterile water instead of fungicide solution was added to the PDA medium.

A total of eight plants were selected for evaluating their effect on the radial growth of *C. gloeosporioides*, *F. nivale* and *Fusarium* sp. follwing Rai and Bashar (1991). The desired parts of each plant were thoroughly washed in tap water, air dried and then used for fresh extract preparation. 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 minutes to remove particulate matter. The supernatants were filtered through Whatman filter paper and the filtrate was collected in 250 mL Erlenmeyer flasks. In this method, the requisite amount of the filtrate of each plant extract was mixed with PDA medium to get 5, 10, 15 and 20% concentratios.

Both the sets i.e. medium with fungicides or plant extracts thus prepared were poured into sterilized Petri plates and was allowed to solidify. Each Petri plate was inoculated centrally with a 5 mm agar disc cut from the margin of actively growing 7 day culture of the test pathogens. In the control set, a Petri plate containing PDA medium with the requisite amount of distilled water instead of a fungicide or a plant extract was added then agar disc with mycelia of each test pathogen was inoculated in the centre of Petri plate. Three replications were maintained for both the experiments and control sets. The inoculated Petri plates were incubated at 25±2˚C. The radial growth of the colonies of the test pathogens was measured after 5 days of incubation.

The fungitoxicity of the fungicides and plant parts extracts in terms of percentage inhibition of mycelial growth was 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 results were statistically analyzed by “t” test following Steel and Torrie (1960).

RESULTS AND DISCUSSION

Three pathogenic fungi C*olletotrichum gloeosporioides* (Penz.) Sacc., *Fusarium nivale* (Fr.) Ces., and *Fusarium* sp. were isolated from infected papaya fruits. Five fungicides *viz*., Autostin 50 WDG, Dithane M 45, Greengel 72 WP, Ridomil Gold MZ 68 WG and Tilt 250 EC were selected to evaluate *in vitro* efficacy at 100, 200, 300, 400 and 500 ppm concentrations against three pathogenic fungi.

In the present investigation, complete inhibition of the radial growth of *C. gloeosporioides* was observed with Autostin 50 WDG and Tilt 250 EC at all the concentrations used. Among the fungicides, Dithane M 45 showed complete growth inhibition of *C. gloeosporioides* at 400 ppm and 500 ppm concentrations. Greengel 72 WP and Ridomil 68 WG showed complete growth inhibition of the fungus at 500 ppm respectively. The toxicity of these fungicides against *C. gloeosporioides* at 100 ppm in descending order was Autostin 50 WDG=Tilt 250 EC> Greengel 72 WP> Ridomil Gold MZ 68 WG> Dithane M 45 (Table 1).

On the other hand, complete inhibition of the growth of *Fusarium nivale* was observed with Autostin 50 WDG at all the concentrations used. Tilt 250 EC showed 100% growth inhibition of the fungus at 400 ppm and 500 ppm concentrations. Ridomil gold MZ 68 WG, Dithane M 45 and Greengel 72 WP showed highest 78.02%, 71.42% and 70.32% growth

Table 1. Per cent inhibition of radial growth of *Colletotrichum gloeosporioides* at different concentrations of fungicides

Name of fungicides	% inhibition of radial growth at different concentrations (ppm)				
	100	200	300	400	500
Autostin 50 WDG	100[a]	100[a]	100[a]	100[a]	100[a]
Dithane M-45	51.21[b]	56.91[b]	67.47[a]	100[a]	100[a]
Greengel 72 WP	60.97[b]	66.67[a]	68.29[a]	78.04[a]	100[a]
Ridomil Gold MZ 68 WG	55.28[b]	60.97[b]	70.73[a]	81.30[a]	100[a]
Tilt 250 EC	100[a]	100[a]	100[a]	100[a]	100[a]

a, b and c indicate significance of ‘t’ value at p = 0.001 and 0.01 and 0.05, respectively.
In a row, figures with same letter do not differ significantly, whereas figures with dissimilar letter differ significantly (as per DMRT), NS = Not significant. Efficiency gradient of fungicides against *Colletotrichum gloeosporioides* at 100 ppm concentration: Autostin 50 WDG=Tilt 250 EC> Greengel 72 WP> Ridomil Gold MZ 68 WG> Dithane M 45.

Table 2. Per cent inhibition of radial growth of *Fusarium nivale* at different concentrations of fungicides

Name of fungicides	% inhibition of radial growth at different concentrations (ppm)				
	100	200	300	400	500
Autostin 50 WDG	100[a]	100[a]	100[a]	100[a]	100[a]
Dithane M-45	58.24[b]	60.43[a]	64.83[a]	69.23[a]	71.42[a]
Greengel 72 WP	51.64[b]	56.04[b]	58.24[a]	64.83[a]	70.32[a]
Ridomil Gold MZ 68 WG	59.34[b]	62.63[a]	63.29[a]	67.03[a]	78.02[a]
Tilt 250 EC	86.90[a]	93.78[a]	98.20[a]	100[a]	100[a]

a, b and c indicate significance of ‘t’ value at p = 0.001, 0.01 and 0.05, respectively.
In a row, figures with same letter do not differ significantly, whereas figures with dissimilar letter differ significantly (as per DMRT), NS = Not significant. Efficiency gradient of fungicides against *Fusarium nivale* at 100 ppm concentration: Autostin 50 WDG =Tilt 250 EC>Ridomil Gold MZ 68 WG> Dithane M-45>Greengel 72 WP.

inhibition of *Fusarium nivale*, respectively at 500 ppm. The toxicity of these fungicides against *Fusarium nivale* at 100 ppm in descending order was Autostin 50 WDG> Tilt 250 EC> Ridomil Gold MZ 68 WG> Dithane M 45> Greengel 72 WP (Table 2).

Complete inhibition of the growth of *Fusarium* sp. was observed with Autostin 50 WDG, at all concentrations used. Tilt 250 EC showed 100% growth inhibition of the test fungi at 300 ppm, 400 ppm and 500 ppm concentrations. Dithane M 45 showed highest 99.50% growth inhibition of *Fusarium* sp. at the 500 ppm concentration. At 500 ppm concentration Ridomil 68 WG and Greengel 72 WP showed complete inhibition of *Fusarium* sp. The toxicity of these fungicides against *Fusarium* sp. at 100 ppm in descending order was Autostin 50 WDG> Tilt 250 EC> Dithane M-45> Greengel 72 WP> Ridomil Gold MZ 68 WG (Table 3).

due to selection of different test pathogens. Efficacy gradients observed in the present study expressed that Autostin 50 WDG, Tilt 250 EC, Dithane M-45, Greengel 72 WP and Ridomil 68 WG were the best inhibiting agent against the *in vitro* growth of the test pathogens. In contrast to the present study, Chakraborty *et al.* (2009) reported that, at 0.5% dose, Bavistin happened to be the most efficient with the highest inhibition (83.7%) of growth of *Fusarium solani* causing wilt of brinjal *in vitro*. Bashar (1992) while reporting the results of laboratory evaluation of some pesticides on *F. oxysporum* f. sp. *Ciceri* causing wilt of chick pea found that Bavistin checked the growth of the pathogen completely at 100 ppm. Bashar also noted that Dithane M 45

Table 3. Per cent inhibition of radial growth of *Fusarium* sp. at different concentrations of fungicides

Name of fungicides	% inhibition of radial growth at different concentrations (ppm)				
	100	200	300	400	500
Autostin 50 WDG	100[a]	100[a]	100[a]	100[a]	100[a]
Dithane M-45	78.30[a]	84.90[a]	86.79[a]	90.00[a]	99.5[a]
Greengel 72 WP	75.47[a]	81.13[a]	82.07[a]	82.07[a]	100[a]
Ridomil Gold MZ 68 WG	46.22[b]	74.52[a]	81.13[a]	98.70[a]	100[a]
Tilt 250 EC	95.80[a]	99.90[a]	100[a]	100[a]	100[a]

a, b and c indicate significance of ‘t’ value at p = 0.001, 0.01 and 0.05, respectively. In a row, figures with same letter do not differ significantly, whereas figures with dissimilar letter differ significantly (as per DMRT), NS = Not significant. Efficiency gradient of fungicides against *Fusarium* sp. at 100 ppm concentration: Autostin 50 WDG> Tilt 250 EC> Dithane M-45>Greengel 72 WP>Ridomil Gold MZ 68 WG

Efficacy of various fungicides against the three test fungi pathogens indicates that Autostin 50 WDG, Tilt 250 EC, Dithane M-45, Greengel 72 WP and Ridomil 68 WG showed promising results. The same fungicides also showed different effects on three test pathogens in the present investigation. This variation might be failed to check the growth of the pathogen completely even at 3,000 ppm.

All the fungicides tested caused partial or complete inhibition of *C. gloeosporioides*, *F. nivale* and *Fusarium* sp. at 500 ppm concentration (Tables 1-3). Similar observations have also been reported for other fungicides by

Vishwakarma and Chaudhury (1992) and Bashar (1992). Singh and Singh (1970) observed that reaction of *Fusarium* spp. to fungicides varies from species to species and sometimes even from isolate to isolate of the same species. It was reported by Chowdhury *et al.* (2015) that Dithane, Ridomil and Bavistin at 400 and 500 ppm showed complete inhibition of radial growth of *F. moniliforme*.

Results of plant extracts on the radial growth of *Colletotrichum gloeosporioides, Fusarium nivale* and *Fusarium* sp. are presented in Tables 4-6. All the plant extracts showed varied degree of growth inhibition of the pathogens at different concentrations. Out of the eight plant extracts, *Ocimum sanctum* (88.57%) showed best results for inhibition of radial growth of *Colletotrichum gloeosporioides* at 20% concentration which was followed by *Curcuma longa* (84.00%), *Azadirachta indica* (82.79%), *Adhatoda vasica* (80.99%), *Thuja orientalis* (71.00%), *Lantana camara* (67.32%), *Tagetes erecta* (65.59%) and *Vitex nigundo* (42.77%). The inhibition of the pathogen increases with increase in concentration of plant extracts in culture medium. The order of effectiveness against *C. gloeosporioides* at 20% concentration was *Ocimum sanctum*> *Curcuma longa*> *Azadirachta indica*> *Adhatoda vasica*> *Thuja orientalis*> *Lantana camara*> *Tagetes erecta*> *Vitex negundo* (Table 4).

Table 4. Effects of plant extracts on the radial growth of *Colletotrichum gloeosporioides* at different concentrations

Name of plants	% inhibition of radial growth of the pathogen at different concentrations (%)			
	5	10	15	20
Adhatoda vasica	51.78[b]	62.77[b]	78.23[a]	80.99[a]
Azadirachta indica	54.78[b]	70.45[a]	77.84[a]	82.79[a]
Curcuma longa	50.00[b]	52.50[b]	67.50[a]	84.00[a]
Lantana camara	40.34[c]	48.99[b]	56.20[b]	67.32[b]
Ocimum sanctum	62.23[b]	50.14[b]	71.28[a]	88.57[a]
Tagetes erecta	31.18[c]	49.46[b]	48.38[b]	65.59[b]
Thuja orientalis	27.66[c]	32.65[b]	65.22[a]	71.00[a]
Vitex nigundo	18.98[NS]	36.70[c]	39.24[b]	42.77[b]

a, b and c indicate significance of 't' value at p = 0.001, 0.01 and 0.05, respectively. In a row, figures with same letter do not differ significantly, whereas figures with dissimilar letter differ significantly (as per DMRT), NS = Not significant. Efficiency gradient of plant extracts against *Colletotrichum gloeosporioides* at 20% concentration: *cimum sanctum*> *Curcuma longa*> *Azadirachta indica*> *Adhatoda vasica* > *Thuja orientalis*> *Lantana camara*> *Tagetes erecta*> *Vitex negundo.*

Chowdhury *et al.* (2015) reported that ethanol extract of *A. indica* showed complete radial growth inhibition of at 10 and 20% concentration whereas *T. erecta* showed complete growth inhibition at 20% concentrations. Prasad and Anamika (2015) reported that extracts of *L. camara* were found most effective for the control of the *C. gloeosporioides*. Imtiaj *et al.* (2005) found that plant extracts such as *Curcuma longa, T. erecta* and *Zingeber officinale* were also highly effective against *C. gloeosporioides*.

Out of the eight plant extracts, *Curcuma longa* showed highest (88.55%) radial growth inhibition of *Fusarium nivale* at 20% concentration which was followed by *Ocimum sanctum* (87.65%), *Azadirachta indica* (75.64%), *Tagetes erecta* (69.09%), *Lantana camara* (57.78%), *Adhatoda vasica* (56.79%) , *Vitex negundo* (49.03%) and *Thuja orientalis* (47.00%). The inhibition of the pathogen increases with the increase of the concentration of the plant extracts in culture medium (Table-5).

The order of effectiveness against *Fusarium nivale* at 20% concentration was *Curcuma longa*> *Ocimum sanctum*> *Azadirachta indica*> *Tagetes erecta*> *Lantana camara*> *Adhatoda vasica*> *Vitex negundo*> *Thuja orientalis* (Table 5). Out of the eight plant extracts, *Azadirachta indica* showed highest (90.19%) radial growth inhibition of *Fusarium* sp. at 20% concentration which was followed by *Adhatoda vasica* (80.02%), *Ocimum sanctum* (79.89%), *Thuja orientalis* (69.34%), *Lantana camara* (67.99%), *Cucurma longa* (67.64), *Tagetes erecta* (65.93%) and *Vitex negundo* (58.75%). The inhibition of the pathogen increases with the increase of the concentration of the plant extracts in culture medium. The order of effectiveness against *Fusarium* sp. at 20% concentration was *Azadirachta indica*> *Adhatoda vasica*> *Ocimum sanctum*> *Thuja orientalis*> *Lantana camara*> *Cucurma longa*>*Tagetes erecta*> *Vitex negundo* (Table 6). In contrast to the present study, Bashar and Chakma (2014) reported that the plant extract of *A. indica* showed 62.03% growth inhibition of *F. oxysporum* at 20% concentration. The same plant extracts also showed different effects on different pathogens in the present investigation. This variation might be due to selection of different test pathogens. Singh *et al.* (1993) reported the effectiveness of aqueous extracts of *A. indica* in the control of disease development in banana. Plant

Table 5. Effects of plant extracts on the radial growth of *Fusarium nivale* at different concentrations

Name of plants	% inhibition of radial growth of the pathogen at different concentrations (%)			
	5	10	15	20
Adhatoda vasica	32.78^{c}	45.66^{b}	45.66^{b}	56.79^{b}
Azadirachta indica	40.45^{c}	55.67^{b}	65.54^{a}	75.64^{a}
Curcuma longa	69.44^{b}	72.22^{a}	79.00^{a}	88.55^{a}
Lantana camara	26.90^{c}	30.33^{c}	56.44^{b}	57.78^{b}
Ocimum sanctum	38.27^{c}	67.90^{b}	70.37^{a}	87.65^{a}
Tagetes erecta	36.36^{c}	63.63^{b}	56.81^{b}	69.09^{b}
Thuja orientalis	30.12^{NS}	35.22^{c}	45.50^{c}	47.00^{c}
Vitex negundo	18.75^{NS}	31.25^{c}	47.50^{c}	49.03^{b}

a, b and c indicate significance of 't' value at p = 0.001, 0.01 and 0.05, respectively. In a row, figures with same letter do not differ significantly, whereas figures with dissimilar letter differ significantly (as per DMRT), NS = Not significant. Efficiency gradient of plant extracts against *Fusarium nivale* at 20% concentration: *Curcuma longa*> *Ocimum sanctum*> *Azadirachta indica*> *Tagetes erecta*> *Lantana camara*> *Adhatoda vasica*> *Vitex negundo*> *Thuja orientalis.*

Table 6. Effects of plant extracts on the radial growth of *Fusarium* sp. at different concentrations

Name of plants	% inhibition of radial growth of the pathogen at different concentrations (%)			
	5	10	15	20
Adhatoda vasica	66.50^b	69.45^b	75.78^a	80.02^a
Azadirachta indica	58.92^b	75.27^b	82.25^a	90.19^a
Cucurma longa	58.82^b	61.76^a	64.70^a	67.64^a
Lantana camara	29.77^c	34.46^b	58.92^a	67.99^a
Ocimum sanctum	43.15^c	53.68^b	64.21^b	79.89^a
Tagetes erecta	24.57^b	34.74^b	54.91^a	65.93^a
Thuja orientalis	32.14^c	45.67^b	56.88^b	69.34^a
Vitex negundo	27.02^b	37.50^a	46.25^a	58.75^a

a, b and c indicate significance of 't' value at p = 0.001, 0.01 and 0.05, respectively. In a row, figures with same letter do not differ significantly, whereas figures with dissimilar letter differ significantly (as per DMRT), NS = Not significant. Efficiency gradient of plant extracts against *Fusarium* sp. at 20% concentration: *Azadirachta indica*> *Adhatoda vasica*> *Ocimum sanctum*> *Thuja orientalis*> *Lantana camara*> *Cucurma longa*> *Tagetes erecta*> *Vitex negundo*

parts and their constituents of some higher plants have already been reported to be of successful nature of fungitoxicants, lesser phytotoxicity, systemicity, easy biodegradability and favourable effects of the growth of the host (Fawcett and Spencer 1970, Panday *et al.* 1983). Chakraborty *et al.* (2009) reported the efficacy of various cell free extracts of the plants against the growth inhibition of the pathogen. The effectiveness of extracts varied significantly with dosage, where 100% inhibition of the pathogen was achieved both with neem and garlic extracts.

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