J. Agrofor. Environ. 5 (2): 17-20, 2011 ISSN 1995-6983

In vitro effect of plant extracts, fungicides and antibiotics on the fungal isolates associated with damping-off disease of crucifers

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Abstract: Damping off of seedlings of Cabbage, Cauliflower and Kohlrabi is very common in the nursery beds of the Dhaka city. *Fusarium oxysporum*, *F. pallidoroseum* and *Rhizoctonia solani* were found to be consistently associated with higher frequency with the diseased seedlings. The effect of extracts of 10 angiospermic plants, 7 fungicides and 3 antibiotics on the vegetative growth of these 3 isolates were studied *in vitro*. The plant extract of *Datura innoxia* was found to be most inhibitory on the growth of *F. oxysporum* and *F. pallidoroseum* followed by that of *Polygonum orientale*. The plant extract of Allium sativum and D. innoxia caused complete growth inhibition of R. solani followed by those of Asparagus racemosus and P. orientale. The growths of 3 test isolates were inhibited completely with Bavistin and Knowin at all the concentrations applied. The inhibitory effect of Calixin, Cupravit and Dithane M-45 was also promising. Of the 3 antibiotics, Grisovin and Ketoral effectively inhibited the growth of *F. oxysporum* and *F. pallidoroseum* but Streptomycin stimulated the growth of the 3 fungal isolates. The result of this study will be helpful in suggesting some effective chemical control measures of damping-off disease of these 3 crucifers.
Key words: Crucifer, Damping-off, Crucifers, Antibiotics, Fungicide, Plant extract, *Rhizoctonia*, *Fusarium*

Introduction

The seedlings of Cabbage (*Brassica oleracea* var. *capitata*), Cauliflower (*B. oleracea* var. *botrytis*) and Kohlrabi (*B. oleracea* var. *gangyloides*) were found to be infected lesser to moderately with damping-off disease in some plant nurseries of the Dhaka city. Damping-off disease of seedlings occurs worldwide in tropical and temperate climates and in greenhouse. Several species of *Fusarium*, *Phytophthora*, *Pythium* and *Rhizoctonia* cause this disease (Agrios, 1997). A survey of the relevant literatures indicated that very few attempt has been made in Bangladesh, to study the damping-off disease of winter vegetable crops like Cabbage, Cauliflower and Kohlrabi (Ishaque and Talukder, 1967; Talukder, 1974 and Hossain, 1993).

Thus, an attempt was made to find out the fungi associated with damping-off infected seedlings of Cabbage, Cauliflower and Kohlrabi and to assess the effect of some angiospermic plant extracts, fungicides and antibiotics on the vegetative growth of some isolates *in vitro*. It is expected that the results of this study will be helpful in suggesting some effective chemical control measures of damping-off disease of Cabbage, Cauliflower and Kohlrabi.

Materials and Methods

Fifteen to twenty five days old damping-off infected seedlings of Cabbage, Cauliflower and Kohlrabi were collected from 3 selected plant nurseries at Kamlapur, Mohammadpur and Uttara in Dhaka city during November, 2006. The seedlings were raised from seeds those were mainly imported from Netherlands and were treated with a seed dressing fungicide (Thirum). However, about 10 to 35% seedlings of the crucifers were found to be affected by damping-off disease in the selected nursery beds. The diseased seedlings were carefully dug out with the help of sterilized trowel and were gently tapped to remove the loosely adhering soil particles. The shriveled and blackened portions with some adjacent healthy parts of the infected seedlings were cut off, kept in a sterilized polyethylene bag and brought at the laboratory for isolation work.

Isolation of the pathogen: Fungi associated with diseased parts of seedlings of each crucifer were isolated separately following tissue planting method (Ashraffuzzaman, 1976). In each case, a total of 200 inocula were placed in 50 Petri plates containing PDA medium. Percentage frequency of the occurrence of each fungal isolates was calculated by adopting the following formula.

$$\% \text{ Frequency} = \frac{\text{Number of inocula from which fungal isolate was obtained}}{\text{Number of inocula cultured}} \text{ X } 100$$

Identities of the isolates were determined following standard literatures (Booth, 1971 and Barnett and Hunter, 1972).

Effect of plant extracts, fungicides and antibiotics on three isolates: Three fungal species, viz., *Fusarium oxysporum* Sah., *F. pallidoroseum* (Cooke) Sacc. and *Rhizoctonia solani* Kühn which were found to be consistently associated in higher frequency with damping-off infected seedlings were selected as test isolates. Ten angiospermic plant parts, 7 fungicides and 3 antibiotics were selected to evaluate their effect on the test isolates. All these were collected from the Dhaka city and their particulars are shown in Table 2 and 3. Extract of each desired plant parts and medium preparation was done by following same methods described in Hossain (1993). The effect of each plant extract was tested following poison food technique at 20% concentration whereas it was 25, 50, 100, 200, 400 and 800 ppm in case of fungicides and antibiotics. The radial growths of the colonies were measured after 3 days in case of *R. solani* while 7 days in case of *Fusarium* spp.

The effect was expressed as percentage of inhibition/ stimulation of growth of the test pathogen and was calculated by using the formula given below:

$$\text{Percent growth inhibition/stimulation} = \frac{C - T}{T} \text{ X } 100$$

Where, C = growth in control, T = growth in treatment, Inhibition = C > T, Stimulation = C < T (+ sign was put before the calculated value).

The results were statistically analyzed by t-test following Steel and Torrie (1960).

Results and Discussion

Fungi found to be associated with damping-off infected seedlings of Cabbage, Cauliflower and Kohlrabi were distributed over 4 genera (Table 1). The average percentage frequency of occurrence of *Rhizoctonia solani* was highest (43.67%) followed by *Fusarium oxysporum* and *F. pallidoroseum* (27.33%). Amongst the isolates, *R. solani* is known as the causal agent for damping-off of seedling of Cabbage and Cauliflower (Rai *et al*., 1974; Roy, 1975 and Singh & Pavgi, 1980). A number of strains of *F. oxysporum* are recorded as serious wilt pathogens of Cabbage, Cauliflower and Kohlrabi. *F. pallidoroseum* is known as a secondary invader of plant tissues and often associated with disease complexes. Its contribution to the damping-off of tomato seedlings in cultural studies was demonstrated by some workers (Booth, 1971). The role of these *Fusarium* species in the damping-off of seedlings of Cabbage, Cauliflower and Kohlrabi is yet to be determined.

Table 1. The fungi associated with damping-off infected seedlings of Cabbage, Cauliflower and Kohlrabi of nursery beds of the Dhaka city.

Fungi isolated	% frequency in different host			Average % frequency
	Cabbage	Cauliflower	Kohlrabi	
Alternaria spp.	6	4	7	5.67
Curvularia spp.	8	11	6	8.33
Fusarium spp. (*F. oxysporum* Sch. *F. pallidoroseum*(Cook) Sacc.)	23	32	27	27.33
Rhizoctonia solani Kühn	38	44	49	43.67

Effect of plant extracts, fungicides and antibiotics on three isolates: Effect of plant extracts of 10 selected angiospermic plants on the growth of 3 test isolates, viz., *Fusarium oxysporum*, *F. pallidoroseum* and *Rhizoctonia solani* is presented in Table 2. The plant extracts as used in the present work, differed with respect to their effect on growth of the test isolates. Out of the 10 plant extracts, only 6 obtained from *Allium cepa*, *Azadirachta indica*, *Cassia alata*, *Curcuma longa*, *Datura innoxia* and *Polygonum orientale* were found to inhibit the growth of all the 3 isolates. However, the leaf extract of *Mikania scandens* stimulated the growth of all the test isolates. Several studies also demonstrated the inhibitory and stimulatory effects of plant extracts on various pathogens (Saha, 1997; Eksteen *et al*.2001 and Hasan *et al*. 2005).

Table 2. Effect of plant extracts on the growth of *Fusarium oxysporum* [FO], *F. pallidoroseum* [FP] and *Rhizoctonia solani* [RS] at 20% concentration

Plant species [Native name]	Plant parts used	% growth inhibition/stimulation of 3 isolates at 20% concentration		
		FO	FP	RS
Allium cepa L. [Piaz]	Bulb*	3.05	6.81^{b}	26.39^{a}
Allium sativum L. [Rosoon]	Bulb*	$+42.38^{a}$	$+38.35^{a}$	100.00^{a}
Ampelygonum salarkhanii Hasan [Girish sovon shak]	Leaf**	4.54	6.98^{c}	$+23.4^{b}$
Asparagus racemosus L.[Satamuli]	Root*	$+13.37^{b}$	$+ 8.12^{c}$	59.29^{a}
Azadirachta indica A.Juss.[Neem]	Leaf**	9.64^{c}	10.80^{c}	8.12^{c}
Cassia alata L. [Dadmordan]	Leaf**	3.98	3.10	1.23
Curcuma longa L. [Holud]	Rhizome*	12.49^{a}	19.47^{b}	26.76^{b}
Datura innoxia Mill. [Dhutra]	Leaf**	61.37^{a}	52.61^{a}	100.00^{a}
Mikania scandens Cl. [Assamlata]	Leaf**	$+76.92^{a}$	$+89.44^{a}$	$+61.87^{a}$
Polygonum orientale L.[Bishkatali]	Leaf**	25.26^{b}	30.11^{b}	41.81^{a}

* Extract prepared without adding water, ** Distilled water was added in the ratio of 1:1(w/v) during extract preparation, a, b and c significant at p=0.001, 0.01 and 0.05 respectively, + indicates stimulation of growth.

The plant extract of *D. innoxia* was found to be most inhibitory to *F. oxysporum* and *F. pallidoroseum* (61.37% and 52.61% respectively) followed by that of *P. orientale*. The inhibitory effects of plant extracts of *A. cepa*, *A. indica*, *C. alata* and *C. longa* against these 2 isolates were not so encouraging. The maximum inhibition (100.0%) of

R. solani was found with plant extracts of *A. sativum* and *D. innoxia* followed by *A. racemosus* (59.29%). It was also noticed that the sclerotia formation of *R. solani* was directly related with the degree of inhibition of its mycelial growth.

The bulb extract of *A. sativum* showed 100% inhibition of *R. solani* but stimulated the growth of *F. oxysporum* and *F. pallidoroseum*. Similar trends of results were also noticed in case of *A. racemosus*. These results are in good agreement with the findings of Hossain (Hossain, 1993). It is evident from the foregoing findings that the toxicity of a plant extract may differ with the fungal pathogenic species. A plant extract which is inhibitory to a number of pathogens may have also a stimulatory effect on other fungal pathogen. The inhibitory effect of a plant extract is owing to the presence of antifungal agents of various groups like antibiotics, phenolic compounds etc. (Agrios, 1997). These antifungal agents may be of narrow spectrum and effective against a particular fungus or a group of fungal pathogens but can not inhibit others. Likewise, the presence of one or more growth promoting or nutritional factors in a plant extract may attribute for its stimulatory effect on some fungal pathogen. In fact, many fungal pathogens are grown in culture medium supplemented with extracts of plant parts, particularly the extracts of plant parts of the host plant for growth and sporulation (Anonymous, 1968).

Table 3. Effect of fungicides and antibiotics on the growth of *Fusarium oxysporum* [FO], *F. pallidoroseum* [FP] and *Rhizoctonia solani* [RS] at different concentrations

Name [Active ingredient/s]	Isolates	Per cent growth inhibition / stimulation of three isolates at different concentrations (ppm)					
		25	50	100	200	400	800
Fungicides:							
Bavistin [50% Carbendazim]	FO	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}
	FP	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}
	RS	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}
Calixin [75% Tridemorph]	FO	53.55^{a}	66.71^{a}	71.56^{a}	82.04^{a}	87.01^{a}	95.67^{a}
	FP	75.78^{a}	77.28^{a}	79.06^{a}	83.07^{a}	86.38^{a}	93.89^{a}
	RS	46.19^{a}	56.97a	66.67^{a}	78.59^{a}	80.61^{a}	91.13^{a}
Cupravit [50% Copper oxychloride]	FO	9.06^{b}	18.67^{b}	35.54^{b}	67.63^{a}	95.68^{b}	100.00^{a}
	FP	5.46^{b}	9.28^{b}	21.33^{c}	45.02^{b}	86.99^{b}	100.00^{a}
	RS	0.00	4.23	7.84^{b}	15.17^{b}	23.51^{c}	30.49^{a}
Dithane M-45 [80% Mancozeb]	FO	68.19^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}
	FP	15.98^{a}	24.30^{a}	31.41^{a}	59.59^{a}	80.94^{a}	84.39^{a}
	RS	21.76^{b}	34.13^{b}	44.35^{a}	54.73^{a}	69.83^{a}	74.29^{a}
Knowin [50% Carbendazim]	FO	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}
	FP	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}
	RS	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}
Macuprax [58% Neutral Bordeaux Mixture + 7% Cufraneb	FO	0.00	1.72	3.89^{c}	11.37^{b}	28.60^{a}	38.93^{a}
	FP	1.80	3.19^{b}	7.29^{b}	16.13^{b}	23.61^{b}	29.76^{a}
	RS	2.15	6.94^{c}	16.17^{c}	21.19^{c}	26.87^{c}	29.37^{b}
Thiovit [80% Sulpher]	FO	3.86	7.01^{c}	9.66^{b}	14.80^{b}	20.96^{c}	29.43^{b}
	FP	5.01	7.44^{c}	9.64^{b}	12.14^{c}	14.59^{c}	18.75^{b}
	RS	11.16^{b}	17.35^{b}	25.84^{b}	29.88^{b}	35.66^{b}	42.17^{a}
Antiboitics:							
Grisovin-FP [Griseofulvin, 500 mg/tablet]	FO	40.36^{a}	42.02^{a}	44.81^{a}	45.33^{a}	48.23^{a}	50.88^{a}
	FP	48.16^{a}	52.39^{a}	54.86^{a}	55.91^{a}	55.27^{a}	57.81^{a}
	RS	30.49^{a}	32.05^{a}	33.12^{a}	33.43^{a}	34.11^{a}	35.98^{a}
Ketoral [Ketoconazole, 200 mg/tablet]	FO	64.23^{a}	68.99^{a}	74.77^{a}	83.34^{a}	88.28^{a}	91.35^{a}
	FP	67.71^{a}	69.67^{a}	71.26^{a}	71.55^{a}	72.64^{a}	73.59^{a}
	RS	17.34^{b}	18.23^{c}	18.74^{c}	19.88^{b}	20.91^{b}	21.39^{b}
Streptomycin [Streptomycin sulphate, 1g/ampule]	FO	+7.89^{c}	+10.11^{c}	+12.40^{b}	+14.79^{b}	+18.84^{c}	+20.31b
	FP	+12.14^{b}	+13.20^{b}	+13.76^{b}	+14.39^{b}	+14.89^{b}	+15.61c
	RS	+93.64^{a}	+94.88^{a}	+95.87^{a}	+97.64^{a}	+99.37^{a}	+102.89^{a}

a, b and c significant at p=0.001, 0.01 and 0.05 respectively, + indicates stimulation of growth.

Amongst the 7 fungicides used in the present investigation, Bavistin and Known and Calixin are systemic while Cupravit, Dithane M-45, Macuprax and Thiovit are non-systemic, contact fungicides. The 3 antibiotics– Grisovin, Ketoral and Streptomycin also used in the present investigation are systemic in their action.

All the fungicides inhibited the radial growth of the 3 isolates (Table 3). The effect of inhibition varied amongst the 3 isolates, however, inhibition of fungicides increased with their increasing concentrations. The complete inhibition of the growth of 3 isolates was observed with Bavistin and Knowin at all concentration used. The complete inhibition was also observed in *F. oxysporum* and *F. pallidoroseum* with Dithane M-45 and Cupravit at 50 and 800 ppm concentrations respectively. Calixin inhibit the growth of 3 isolates significantly however inhibitory effects of Macuprax and Thiovit are insignificant.

Bashar (1992A) reported that Bavistin check the growth of *F. oxysporum* Sah. f.sp. *cicei* (Padwick) Snyd. and Hans. at 100 ppm while Dithane M-45 fail to check the growth of the pathogen even at 3,000 ppm concentration. Sen and Kapoor observed that Bavistin and Dithane M-45 were most effective in inhibiting the growth of *R solani* causing collar rot of cauliflower at 100 ppm concentration (Sen and Kapoor, 1975).

It is evident from the foregoing discussions that considerable differences exist in the findings of various workers as regards the effect of some fungicides like Bavistin and Dithane M-45 on pathogenic fungi like *F. oxysporum* and *R. solani*. It is not unlikely that different isolates of *R. solani* or different strains of *F. oxysporum* will react differently against one or more fungicides. This is because of the differences in biology of different isolates or strains of a pathogenic fungal species (Singh and Singh, 1972).

The effect of these 3 antibiotics on the growth of 3 test isolates is shown in Table 3. The antifungal ones– Grisovin-FP and Ketoral caused inhibition and the antibacterial antibiotic– Streptomycin caused stimulation of growth of the 3 isolates. Ketoral caused maximum growth inhibition of *F. oxysporum* (91.35%) and *F. pallidoroseum* (73.59%) at 800 ppm concentrations. The effects of Ketoral and Grisovin on the growth of *R. solani* are found insignificant. For obvious reasons the antifungal antibiotics are always more effective against fungal pathogens. This observation is in accord with the finding of Basher (1992B).

It is anticipated from the present study that the *A. sativum* and *D. innoxia* may serve as candidate plant species for their exploitation as potent fungitoxicants for controlling damping-off disease of Cabbage, Cauliflower and Kohlrabi. More over, Bavistin, Knowin and Calixin can be used in suitable chemical control measures of damping-off disease of these 3 crucifers.

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