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INHIBITION OF SPORE GERMINATION AND MYCELIAL GROWTH OF THREE FRUIT ROT PATHOGENS USING SOME CHEMICAL FUNGICIDES AND BOTANICAL EXTRACTS

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Abstract: Six chemical fungicides and extracts of 15 locally available plants were tested against three fruit rot pathogens *viz*. *Fusarium oxysporium* f. sp. *capsici*, *Rhizopus artocurpi* and *Alternaria tenuis* for the evaluation of inhibition of spore germination and mycelial growth. Among the fungicides tested, all the concentrations of Ridomil showed 100% inhibition of spore germination and the least inhibition was recorded in case of treatment with Bavistin for *F. oxysporium* f. sp. *capsici*. In case of *R. artocarpi* and *A. tenuis*, Dithene–M 45 showed good inhibitory effects but Thiovit had no inhibitory effect against *A. tenuis*. Both leaf and seed extracts of *Azadirachta indica* showed good inhibitory effect than tested with other plant extracts. *Moringa oleifera* extract showed least inhibitory effect against all the fungi tested. *Datura metel, Plygonum oriantale, Tagestes patula* and *Micania scandens* also showed promising inhibition on spore germination and mycelial growth of all the pathogens tested.

Key words: Fruit rot pathogens, plant extracts, chemical fungicides, spore germination, mycelial growth, PIGR.

mvivsk: ‡¯úvi A¼‡iv`Mg I gvB‡mvjqvg Gi ev×‡Z evav`vb gj¨vq‡bi Rb¨ QqvU QÎvKbvkK Ges GjvKvq mnR cvc¨ c‡bivU Dv™` vZbvU dj cPb Rxevb h_vµ‡g- *F. oxysporium* f. sp. *capsici*, *R. artocarpi* Ges *A. tenuis* Gi vei“‡× civ¶v Kiv nq| civv¶Z QÎKbvkK ¸‡jvi g‡a¨ Ridomill mKj Nb‡Z ‡¯úv‡ii A¼‡iv`M‡g 100% evav`vb K‡i Ges me‡P‡q Kg evav`vb †iKW Kiv nq hLb Bavistin v`‡q *oxysporium* f. sp. *capsici* ‡K vbqš‡bi civ¶v Kiv n‡qvQj| *R. artocarpi* Ges *A. tenuis* Gi †¶‡Î Dithene-M 45 ‡ekx evav`vq‡b cfve c`kY K‡i vKš Thiovit Gi *A. tenuis* vei“‡× evav`v‡b †Kvb cfve bvB| civv¶Z Ab¨vb¨ Dv™` vbhv‡mi †P‡q q *Azadirechta indica* Gi cvZv Ges ev‡Ri vbhvm DfqB ‡ekx evav`v‡b cfve c`kb K‡i | mKj civv¶Z QÎv‡Ki vei“‡×*Moringa olieifera* vbhv‡mi me‡P‡q Kg evav`vb civjv¶Z nq| mKj civv¶Z RxevYi †¯úvi A¼‡iv`Mg I gvB‡mvjqvg Gi ev×i Dci *Datura metel, Plygonum oriantale, Tagestes patula* Ges *Micannia scandens* Gi Avkve¨vÄK evav`vb djvdj †`Lv hvq|

Introduction

Fruit rot is a very common and destructive disease that causes serious economic loss in production mainly of fruits and vegetables. *Alternaria* mediated fruit rot is widespread and it causes highly destructive disease of chilli, and yield loss caused by the disease is up to 100% under congenial environmental conditions (Mahal 2005). Rhizopus rot is also the most common detrimental disease of jackfruit which is caused by *Rhizopus artocarpi* (Alam *et al.* 2002a). Use of chemical fungicides is common in fruit rot disease management but they often result problems of toxic residue. Plant extracts are least expensive and cause less health hazards. Several higher plants and their constituents have shown success in plant disease control and are proved to be harmless and non-phytotoxic unlike chemical fungicides (Singh *et. al.* 1983; Alam *et al*. 2002b). Evidence suggests that plant extracts can be used against microbes causing diseases in plants. Thus remarkable antifungal effects of plant extracts on the germination of fungal spores (Dubey 1991), and extracts of plant parts for controlling the disease (Pandey *et al*. 1983; Chary *et al*. 1984; Singh and Dwivedi 1990) are reported earlier. Here we report the effects of 6 chemical fungicides and extracts of 15 different plants on three fruit rot pathogens, such as *F. oxysporium* f. sp. *capsici*, *R. artocarpi* and *A. tenuis*.

Materials and Methods

Pathogens used: A. tenuis, and *F. oxysporium* f. sp. *capsici* were isolated from fruit rot disease affected chillies; and *R. artocarpi* was isolated from infected jackfruit. The fungal pathogens were cultured on PDA (Potato Dextrose Agar) medium and stored at 4ºC for experimentation.

Effect of chemical fungicides: Spores of three pathogens were taken from 7 day-old cultures on PDA. Spore suspension (10^3 conidia/ml) were made separately against five different concentrations of the chemical fungicides (500, 1000, 1500, 2000 and 2500 ppm) when the threads were thoroughly covered with mycelium and spores. The spores were removed and put in triplicate in fungicidal solution or suspensions in sterile water where different concentrations of each fungicide were used. Five ml suspensions of each were taken in small sterilized Petri dishes (65 mm) and kept at 28 ºC for 30 min. Then a drop of lacto phenol cotton blue was added

to conidial suspension on the slides. The slides were finally examined under microscope (×400) for recording the percentage of conidial germination.

Preparation of plant extracts: Extractions of root, seed, bark and leaf tissues were made in 80% ethyl alcohol following the method described by Mahadevan and Sridhar (1982). Plant tissues (5 g each) were cut into pieces and plunged immediately in boiling alcohol (5-10 ml/g tissues) in a beaker and allowed to boil for 5-10 min. The extracts were made on top of a steam bath and then cooled in a pan of cold water. The tissues were crushed thoroughly in a mortar with a pestle and passed through two layers of cheese-cloth. The ground tissues were re-extracted for 3 min in hot alcohol (2-3 ml/g). The extracts were cooled and passed again through cheese-cloth and filtered through Whatman's No.1 filter paper. Ten ml of the extracts was evaporated on a steam bath to dryness, 1.25 ml of sterilized distilled water was added per 5 g tissues and the extracts were used as test materials.

Inhibition of spore germination by plant extracts: Spores from the culture on PDA plates were taken and suspensions of spores were made separately with different plant extracts. These suspensions (1.25 ml) were taken in small sterilized Petri dishes and were kept at 30 ºC for 30 min. A drop of treated spore suspension (with different plant extract) was taken on separate slides in a moisture chamber for 24 hrs of incubation. Then a drop of lacto phenol cotton blue was placed on the spore suspension on each of the slides. The slides were examined under microscope (×40) for recording the percentage of spore germination.

Mycelial growth inhibition by plant extracts: For growth inhibition tests stock solutions were prepared by crushing known weight of fresh leaves with distilled water (1:1 by W/V). For barks and fruits, the ratio was 1:2 whereas bulbs were crushed without adding water. The pulverized mass of a plant part was passed through a three-fold fine cloth and was centrifuged at 3000 rpm for 15 min. The supernatant was filtered through Whatman's No. 1 filter paper and the filtrate was collected in 250 ml Erlenmeyer flask. The filtrate of each plant extract was mixed with PDA medium to make 5, 10 and 20% concentrations. After autoclaving, a plant extract supplemented medium was poured in sterilized Petri plates and allowed to solidify. Each Petri plate received 20 ml of plant extract supplemented medium. This Petri plates were inoculated at the centre with a 5 mm agar disc, cut from the margin of actively growing culture of the pathogens. In the control, a Petri plate containing PDA medium with requisite amount of sterilized water instead of a plant extract was also inoculated with a plant pathogen. Three replicates were maintained in each case and inoculated Petri plates were kept at 28°C. The radial growth of the colonies was measured after 7 days of incubation. The two readings in the control and treatment lines were transformed into percent inhibition of radial growth (PIRG) using the following formula (Skidmore and Dickenson 1976): % Growth inhibition= (C-T)/C × 100, where C= growth in the control and T= growth in the treatment.

Statistical analyses: For analyzing the experimental data, arcsine angular transformations were made. Least significant differences (LSD) were determined wherever the calculated F values (analysis of variance, ANOVA) were significant at 5% level (Snedecor and Cochran 1980).

Result and Discussion

Effects of chemical fungicides on the inhibition of spore germination: Data presented in Table 1 reveal that Ridomil was the most effective fungicide against *F. oxysporium* while Bavistin had the least effect, Dithene-M 45 showed the highest and Thiovit the lowest against *R. artocarpi*, and Dithene-M 45 had the highest and Bavistin the lowest effects against *A. tenuis* but Thiovit showed no inhibitory effect on this species. All 6 chemical fungicides exhibited significant inhibition of spore germination against *F. oxysporium* ($F_{5,20}$=12.52; P<0.01), *R. artocarpi* ($F_{5,20}$=9.61; P<0.01) and *A. tenuis* ($F_{5,20}$=10.77; P<0.01).

Sekhar *et al.* (1989) reported field trials on *Z. mauritiana* cv. *kajhali* infected by *Prarthgada zizyphi* with fungicides applied as spray and observed that Bavistin at 0.1% gave the lowest disease incidence followed by 0.2% Dithene-M 45. An economically viable measure was suggested by Singh *et al.* (1990) to farmers where *Alternaria brassicae* induced leaf spot under field conditions was controlled by seven fungicides including Bavistin and Dithene-M 45. Ridomil, Dithehe-M 45, Cupravit, Bavistin and Rovral were found effective fungicides against *A. tenuis* in field experiments ((Alam *et al.* 1999). The present results lend support to the above three findings.

Effects of plant extracts on the inhibition of spore germination: Leaf extracts of *A. indica* showed highest inhibitory effect and *M. oleifera* leaf the lowest against *F. oxysporum* f. sp. *capsici*, leaf and seed extracts of *A. indica* inhibited the highest spore germination whereas *Terminalia arjuna* fruit had no effect on *R. artocarpi*, and *A. indica* leaf inhibited the most but *M. oleifera* leaf had none inhibitory effects on *A. tenuis* (Table 2). A significant inhibitory effect of all plant extracts on spore germination in the fruit rot pathogens was evident ($F_{16,32}$= 3.92; P<0.01).

A. indica inhibiting growth of *A. alternate, Bipolaries sorokiniana* and several other fungi have been reported (Singh and Dwivedi, 1990; Alam *et al*. 2002a). As regards the leaf extract, the most promising fungitoxic effect was recorded in case of *M. scendens* (63.10% and 61.80%), *P. orientale* (60% and 58.30%), *D. metel* (55.21% and 57%) and *T. patula* (58.30% and 56.20%) against *F. oxysporum* f. sp. *capsici* and *A. tenuis. R. artocarpi* plant extracts were less effective and there was no effect of fruit extract of *T. arjuna* against *R. artocarpi* and leaf extract of *M. oleifera* against *A. tenuis*. Alam *et al.* (1999) reported the antifungal effects of *V. rosea* and *A. indica* against *A. tenuis* of chilli fruit rot pathogen. Akhter *et al*. (2006) reported that *Vinca rosea*, *Piper betle* and *A. indica* extracts have inhibitory (100%) effect against spore germination of *Bipolaris sorokiniana*. The fungitoxicity of the leaf extract of *Datura* sp. has been reported earlier by several investigators against *F. oxysporum* f. sp. *ciceri* (Podwick) Snyd and Hans (Bashar and Rai, 1991) and *R. solani* (Hossain *et al*., 1993). Hossain *et al*. (1993) and Anwar *et al*. (1994) reported antifungal activity of the leaf extracts of *M. scendens* and *P. orientale* on a number of pathogens including *Alternaria* sp. and *Rhizopus* sp. The present study indicated that the inhibitory effect of the plant parts on spore germination of *F. oxysporum, R. artocarpi, A. tenuis* might be attributed to the presence of some partially effective antifungal ingredients.

Effects of plant extracts on the inhibition of mycelia growth: A. indica leaf extracts at 20% concentration inhibited the highest mycelial growth in all the fruit rot pathogens, whereas the lowest inhibitory effects were shown by *M. oleifera* leaf extracts in *F. oxysporum* f. sp. *capsici* and *R. artocarpi*, and *T. arjuna* bark extracts in *A. tenuis* (Table 3). ANOVA on the mycelial growth inhibition data showed highly significant effects of the plant extracts on *F. oxysporum* ($F_{16,32}$= 31.57; P<0.001), *R. artocarpi* ($F_{16,32}$= 57.23; P<0.001) and *A. tenuis* ($F_{16,32}$=35.14; P<0.001).

In previous work Ogbebor *et al*. (2007) reported that extracts of *Ocimum basilicum* L. and *Allium sativum* L. exhibited total inhibitory effects on the mycelial growth of *Colletotrichum gloeosporioides*. It was also observed that although not promising but still the fungitoxic effect of these plant extracts persisted even at 5% concentration. This observation suggests that fungitoxicity of the plant extracts have been found to be promising against plant pathogens like *Fusarium* sp. and *Alternaria* sp. and can be increased further by using these plant extracts at higher concentrations.

Conclusion

Chemical fungicides and extracts of locally available plants tested against three fruit rot pathogens inhibited spore germination and mycelial growth significantly. Effects of two fungicides (Ridomil and Dithene-M45) and a plant extract (*Azadirachta indica*) were promising compared to others.

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Table 1. Effects of six fungicides on the inhibition of spore germination of three fruit rot pathogens 30 min after immersion.

Pathogens	Fungicides	% inhibition of spore germination at concentrations (ppm)[a]					Correlation (r) values
		500	1000	1500	2000	2500	
F. oxysporum f. sp. *capsici*	Cupravit	23(77)	24(76)	31(69)	33(67)	39(61)	0.977
	Ridomil	100(00)	100(00)	100(00)	100(00)	100(00)	-
	Rovral	10(90)	23(77)	27(73)	33(67)	35(65)	0.954
	Thiovit	25(75)	27(73)	29(71)	32(68)	33(67)	0.992
	Dithene-M 45	11(89)	17(83)	21(79)	33(67)	35(65)	0.979
	Bavistin	7(93)	15(85)	19(81)	22(78)	25(75)	0.973
F value ($LSD_{0.05}$)	12.52** (12.398)						
R. artocarpi	Cupravit	18(82)	29(71)	33(67)	45(55)	70(30)	0.956
	Ridomil	33(67)	37(63)	40(60)	41(59)	45(55)	0.985
	Rovral	18(82)	21(79)	28(72)	57(43)	75(25)	0.948
	Thiovit	25(75)	29(71)	32(68)	38(62)	40(60)	0.991
	Dithene-M 45	33(67)	(69)37	75(25)	77(33)	81(19)	0.914
	Bavistin	31(69)	37(63)	40(60)	43(57)	47(53)	0.990
F value ($LSD_{0.05}$)	9.61* (31.517)						
A. tenuis	Cupravit	87(13)	89(11)	95(05)	98(02)	100(00)	0.983
	Ridomil	81(19)	83(17)	91(09)	95(05)	100(00)	0.988
	Rovral	83(17)	88(12)	93()07	97(03)	100(00)	0.995
	Thiovit	00(100)	00(100)	00(100)	00(100)	00(100)	-
	Dithene-M 45	96(04)	99(01)	100(00)	100(00)	100(00)	0.821
	Bavistin	37(63)	39(61)	41(59)	43(57)	48(52)	0.974
F value ($LSD_{0.05}$)	10.77* (9.489)						

a= mean of three replicates; values in parentheses indicate % spore germination; **= P<0.01.

Table 3. Effects of plant extracts on the inhibition of mycelial growth of three fruit rot pathogens in three different concentrations after 7 days' incubation.

Plant species	Plant parts	% inhibition of mycelia growth in different concentrations (%)[a]								
		F. o. f. sp. *capsici*			*R. artocarpi*			*A. tenuis*		
		5	10	20	5	10	20	5	10	20
Azadirachta indica	Leaf	51.10	56.20	65.30	72.00	87.00	89.00	55.10	57.00	77.00
Azadirachta indica	Seed	50.20	53.10	61.80	76.00	83.00	88.00	51.00	52.00	69.00
Blumea lacera	Leaf	11.30	19.10	27.33	09.50	11.20	17.30	05.20	13.30	16.50
Datura metel	Leaf	42.30	52.10	57.20	11.10	17.20	27.80	28.58	42.60	55.90
Euphorbia plucherrima	Leaf	09.50	11.30	19.80	03.50	05.20	06.23	11.10	19.20	20.10
Mentha spicata	Leaf	07.50	11.50	16.07	02.20	04.20	05.90	08.30	11.20	15.31
Mikania scandens	Leaf	11.15	31.43	61.00	09.70	11.30	19.45	22.70	37.10	63.10
Moringa oleifera	Leaf	05.90	07.50	12.50	00.00	00.00	00.00	05.30	09.10	11.20
Polygonum orientale	Leaf	35.50	47.20	50.30	10.20	13.10	19.50	23.00	30.00	53.00
Psidium guajava	Leaf	12.00	19.50	24.15	03.50	05.20	09.78	11.20	13.30	18.50
Tagestes patula	Leaf	25.10	33.30	47.50	01.20	02.50	05.10	45.10	52.20	58.30
Zingiber officinale	Rhizome	13.00	17.50	22.13	01.00	01.90	02.20	07.50	11.30	19.20
Curcuma longa	Rhizome	21.50	32.50	39.90	32.00	04.10	04.50	31.50	32.50	40.25
Terminalia arjuna	Bark	21.00	27.50	35.50	08.10	09.50	13.50	01.10	02.30	03.31
Terminalia arjuna	Fruit	07.50	11.50	22.83	00.00	00.00	00.00	11.50	19.50	25.50
Allium cepa	Bulb	37.10	39.20	43.20	01.20	02.30	03.10	21.50	27.80	37.50
Allium sativum	Bulb	09.20	11.20	24.51	05.90	06.10	07.06	20.10	25.20	28.21
F value ($LSD_{0.05}$)	Plant extracts	31.57**(5.337)			57.23**(7.127)			38.14**(5.635)		

a=Mean of three replications; **=P<0.01

Table 2. Effects of plant extracts on the inhibition of spore germination of three fruit rot pathogens 30 min after immersion.

Plant species	Plant parts	% inhibition of spore germination[a]		
		F. oxysporum f. sp. *capsici*	*R. artocarpi*	*A. tenuis*
Azadirachta indica	Leaf	87.00(13.00)	100.00(00)	82.00(18.00)
Azadirachta indica	Seed	75.00(25.00)	100.00(00)	66.00(44.00)
Blumea lacera	Leaf	17.33(82.67)	3.55(96.45)	16.15(83.85)
Datura metel	Leaf	55.21(44.79)	27.00(73.00)	57.00(43.00)
Euphorbia plucherrima	Leaf	22.50(77.50)	9.80(90.20)	10.50(89.50)
Mentha spicata	Leaf	25.31(74.69)	21.07(78.93)	5.90(94.10)
Mikania scandens	Leaf	63.10(36.90)	13.00(87.00)	61.80(38.20)
Moringa oleifera	Leaf	2.35(97.65)	2.85(97.15)	0.00(100)
Polygonum orientale	Leaf	60.00(40.00)	21.45(78.55)	58.30(41.70)
Psidium guajava	Leaf	18.82(81.18)	24.10(75.90)	8.20(91.80)
Tagestes patula	Leaf	58.30(41.70)	15.00(85.00)	56.20(43.80)
Zingiber officinale	Rhizome	21.00(79.00)	3.10(96.90)	37.00(63.00)
Curcuma longa	Rhizome	31.00(69.00)	7.00(93.00)	41.00(59.00)
Terminalia arjuna	Bark	32.50(67.50)	19.00(81.00)	31.00(69.00)
Terminalia arjuna	Fruit	22.00(78.00)	0.00(100)	27.00(73.00)
Allium cepa	Bulb	41.20(58.80)	12.00(88.00)	32.20(77.80)
Allium sativum	Bulb	34.10(65.90)	17.00(83.00)	38.20(61.80)
F value ($LSD_{0.05}$)	3.92** (18.443)			

a= mean of three replications; values in parentheses indicate % spore germination; *= P<0.01.

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