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Synergistic bio-efficacy of three plant extracts on sporulation of fruit rotting fungi

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In the present communication the synergistic effects of medicinal plant extracts were carried out and their fungitoxicity against fruit rotting fungi was determined. The different concentrations of leaf extracts of medicinal plants were tested upon the spore germination of five fruit rotting fungi which caused serious diseases in some important fruits in India. When extracts were applied individually it was found that they were not so effective as compared to synergistic application of plant extracts. In this study it was found that synergistic application of *Allium sativum* and *Azadirachta indica* extracts in 1:1 ratio at 700 ppm was sufficient to check spore germination in all the fruit rotting fungi tested. It is considered quite likely that this composition may by useful in controlling fruit rotting diseases under field conditions.

Key Words : Medicinal plant extracts, fruit rotting fungi, fungitoxicity, individual and synergiotic application

INTRODUCTION

In recent years it has become evident, as a result of public opinion and environmental laws, that new and safer alternatives to traditional synthetic pesticides are both desirable and mandated. Several pressures have accelerated the search for more environmentally and toxicologically safe and more selective and efficacious pesticides. The increasing incidence of pesticide resistance is also fueling the need for new pesticides. Thus, natural compounds have increasingly become the focus of those interested in formulation of pesticides.

Plants contain a virtually untapped reservoir of pesticides that can be used directly or as templates for synthetic pesticides. Numerous factors have increased the interest of the pesticide industry and the pesticide market in this sources of natural products as pesticides. These include diminishing returns with traditional pesticide formulation methods, increased environmental and toxicological concerns with syntheitc pesticides, and the high level of reliance of modern agriculture on pesticides.

Tens of thousands of secondary products of plants have been identified. There is growing evidences that most of these compounds are involved in the interaction of plants with other species-primarily the defense of the plant from plant pests. Thus, these secondary compounds represent a large reservior of chemical structures with biological activities. This resource is largely untapped for use as pesticides. These available natural resources, combined with increasing need and environmental pressure, are greatly increasing the interest in plant products as pesticides.

The post-harvest diseases caused by the fungi are the major cause of production loss in many crops. Fruits are very susceptible to the attack by fungi due to their low pH, higher moisture content and nutrient composition, turning them unfit for the

human consumption. To control post-harvest disease, fungicide application is the usual practice. However, using synthetic chemicals to control post-harvest diseases can cause carcinogenicity, terato-genicity, high and acute residual toxicity and other side effects on humans (Unnikrishnan & Nath, 2002).

Allium sativum L. (Liliaceae) and *Azadirachta indica* Juss. (Meliaceae) have well documented reports on their antifungal activity. However, little is known about the effectiveness of these plant extracts on fruit rotting fungi and there are very few reports on antifungal activity of *Adenocalymna alliaceum* Miers (Bignoniaceae). Rana *et al.* (1999) have reported the antifungal activity of aqueous extract of leaves of *Adenocalymna alliaceum.* Among natural fungicidal substances garlic (*Allium sativum*) extract has been found active in various trials. The fungicidal activity of garlic is documented by various workers (Singh *et al.*,1990, 2001; Singh and Singh 2000). *Azadirachta indica* (Neem) leaves have been shown to possess antifungal activity against a number of phyto-pathogens (Mishra and Tewari, 1990; Locke, 1995; Sharma, 1995). These have reduced radial growth and spore germination of *Curvularia lunata* (Bhowmick and Vardhan, 1981), successfully controlled fruit rots of cucurbitaceous plants caused by *Fusarium equiseti* and *F. semitectum* (Krishna *et al.,* 1986), and significantly resuced fruit rot of tomatoes caused by *Aspergillus flavus* and *A. niger* (Sinha and Saxena, 1987). Sharma and Verma (1991) and Sharma (1992) have also reported about various antifungal plant extracts which are active against fruit rotting fungi.

In the present study, the antifungal activity and *in vitro* toxicity of three plant extracts against five post-harvest pathogens of four fruits have been determined. It is anticipated that a synergistic application of these plant extracts can increase efficacy and ultimately serve as a management alternative to control fruit rotting.

MATERIALS AND METHODS

Plant used

Three plants used in the present study were *Adenocalymna alliaceum* Miers (Bignoniaceae), Garlic creeper, *Allium sativum* L. (Liliaceae)-Garlic and *Azadirachta indica* Juss. (Meliaceae)-Neem. They were tested individually and also in combination against five fruit rotting fungi. For preparing plant extracts cloves of *Allium sativum* and leaves of *Adenocalymna alliaceum* and *Azadirachta indica* were used.

Fruit rotting fungi

Strains of *Aspergillus niger* MPPLU 05 (An) and *Botryodiploidia theobromae* MPPLU 07 (Bt) isolated from mango; *Alternaria alternata* MPPLU 01 (Aa) from tomato; *Penicillium expansum* MPPLU 24 (Pe) from apple, and *Cladosporium cladosporioides* MPPLU 14 (Cc) from grapes (from the collection of Mycology and Plant Pathology Division, Botany Department, University of Lucknow) were used. The cultures of these organisms were maintained on Potato Dextrose Agar (PDA) at 4°C.

Preparation of Plant extracts

Fresh plant parts were washed with tap water and sterilized water. Then thoroughly washed with 2% aqueous sodium hypochloride solution and finally with sterile distilled water. It was then grounded with sterilized distilled water at the rate of 1 ml g^{-1} of tissues (1:1 w/v) with the pestle and mortar and filtered through muslin cloth and the liquid was centrifuged at 5000 rpm for 25 minutes. The clear supernatant was decanted and filtered through sterilized bacteria free Millipore filter (0.22 µm pore size) using Swinnex filter adapter attached to a syringe. This solution served as standard plant extract solution (100%), which was stored in the refrigerator at 5 ± 1°C for further use. The solution was used within 24 hrs. Besides during testing of individual extracts, two plant extracts were mixed together in ratos of 1:1, 1:2, 1:3, 2:1 and 3:1 and tested against the organisms.

Spore germination assay

In the spore germination assay, six concentrations (50, 150, 300, 500, 700 and 1000 ppm) of plant extracts of each plant alone and in various combinations were tested against each test fungi.

The fungal spores obtained from 10-day-old cultures of the fungi were taken and placed on the glass slides in triplicate. The slides containing the spores were incubated in a moist chamber at 25 ± 2°C for 24 h. Each slide was then fixed in lactophenol-cotton blue and examined under high power (× 40) microscope for recording number of spore/conidia germinated using haemocytometer. Ahout 200 spores were counted and the number of spores germinated was recorded to calculate the percentage of the spore germination (Surender *et al.*, 1987).

RESULTS

In the present study all the three plant extracts were found effective in checking the spore germination of all the five fruit rotting fungi tested. The bio-efficacy of plant extracts was promising when they were used in various synergistic combinations. The data in Table-1 indicated that when three plant extracts of *Adenocalymna alliaceum* (AA), *Allium sativum* (AS) and *Azadirachta indica* (AI) when tested individually they checked spore germination in increasing order along with increase in concentration of extract. AA plant extract almost completely checked the spore germination in *Botryodiploidia theobromae, Cladosporium cladosporioides* and *Penicillium expansum* at 1000 ppm concentration. Plant extract of AS was found effective in almost checking spore germination in *Cladosporium cladosporioides* and *Penicillium expansum* at 700 ppm while in other three test fungi there was complete inhibition of spore germination at 1000 ppm. The extract of AI was effective only against the *Alternaria alternata, Cladosporium cladosporioides* and *Penicillium expansum* at 1000 ppm. When these three extracts were used in various combinations then promising increase in their activity was observed. In case of *Alternaria alternata* none extract was found effective to check spore germination when used individually, but composition AA + AS (1:2) at 700 ppm, AS + AI (1:1) at 1000 ppm were enough to check germination. When three extracts were used individually only AS at 1000 ppm was effective to check spore germination in *Aspergillus niger* but when three extracts were used in different composition the best compositions to check spore germination of *Aspergillus niger* were AA + AS (1:2 and 1:3) and AA + AI (1:2) at 1000 ppm and AS + AI (1:1) at 700 pm.

Botryodiploidia theobromae spores were completely inhibited to germinate when alone AS was used at 1000 ppm, but synergistic efficacy was observed in composition AA + AS (1:2) at 700 ppm, besides this all compositions of AS + AI at 1000 ppm was also effective to check sporulation. Spores of *Cladosporium cladosporioides* were not able to germinate when AS was used alone at 700 ppm, but composition of AS + AI (2:1 and 3:1) was able to completely check sporulation even at 500 ppm. Besides this AA + AS (1:1, 1:2 and 1:3) at 700 ppm and AA + AI (1:1 and 3:1) at 1000 ppm were the good compositions to check sporulation in *Cladosporium cladosporioides.* To check spore germination in *Penicillium expansum* AA and AS at 1000 ppm individually were sufficient. But the best synergistic composition to check spore germination in *Penicillium expansum* was AS + AI (1:1 and 2:1) at 500 ppm, besides this AA + AS (1:1, 1:2 and 1:3) at 700 ppm and AA + AI (1:3 and 3:1) at 1000 ppm also completely check sporulation.

After clearly examining the data in Table 1 it can be concluded that the best composition which checks the spore germination in almost all the tested fruit rotting fungi is AS + AI i.e. *Allium sativum* and *Azadirachta indica* extracts in 1:1 ratio at 700 ppm.

DISCUSSION

In order to control post-harvest diseases in a more sustainable and environment friendly way, seeking for alternative materials instead of commonly used fungicides is getting much concerned. Plant extracts and plant products provided major sources to realize this aim. All the plant extracts and their compositions used in this experiment were significantly superior in controlling the spore germination of various fruit rotting fungi. It is evident from the experiment that it is better to use plant extracts synergistically rather than using individually, because compositions of plant extracts in various ratio increase the bio-efficacy of plant extracts. The significant inhibitory effect of *Allium sativum* and *Azadirachta indica* extracts in 1:1 ratio at 700 ppm on spore germination of all the fruit rot-

Table 1 : Per cent spore germination inhibition of five fruit rotting fungi by three plant extracts in alone and in various combinations at different concentrations.

			Per cent spore germination inhibition				
Plant extracts	**Composition ratio**	**Concentration (ppm)**	***Alternaria alternata***	***Aspergillus niger***	***Botryodiploidia theobromae***	***Cladosporium cladosporioides***	***Penicillium expansum***
AA	—	50	0.0	0.0	0.0	0.0	0.0
		150	6.0	2.0	18.6	12.3	19.8
		300	16.0	12.0	20.3	21.6	36.9
		500	50.3	40.3	49.4	53.6	61.5
		700	70.2	66.3	78.9	75.3	86.9
		1000	89.6	74.3	96.0	98.7	100.0
AS	—	50	0.0	0.0	2.6	5.6	5.1
		150	8.0	12.6	29.4	31.9	33.6
		300	29.6	39.2	51.3	62.3	60.6
		500	56.5	59.4	74.6	87.6	86.9
		700	88.0	91.0	89.0	100.0	98.6
		1000	96.2	100.0	100.0	100.0	100.0
AI	—	50	0.0	0.0	0.0	0.0	0.0
		150	16.3	9.3	13.6	12.3	15.3
		300	32.6	25.3	21.6	25.6	29.6
		500	66.6	56.4	56.3	51.6	49.8
		700	89.6	74.9	76.3	79.5	79.6
		1000	97.6	89.6	88.0	96.5	98.6
AA+AS	1:1	50	0.0	0.0	2.1	6.3	2.0
		150	26.3	8.0	21.6	35.9	31.9
		300	41.2	21.4	45.6	69.8	63.5
		500	76.0	49.6	73.4	93.6	88.7
		700	98.6	79.0	89.6	100.0	100.0
		1000	100.0	88.0	100.0	100.0	100.0
	1:2	50	0.0	0.0	6.2	7.1	6.1
		150	34.3	15.4	31.3	36.5	33.8
		300	58.3	41.4	61.4	71.6	65.8
		500	89.2	63.6	88.7	95.4	87.9
		700	100.0	96.6	100.00	100.0	100.0
		1000	100.0	88.0	100.00	100.0	100.0
	1:3	50	0.0	0.0	5.2	5.6	5.9
		150	24.3	16.7	29.3	31.6	32.6
		300	46.3	44.5	60.1	68.9	65.4
		500	75.2	67.9	85.3	91.3	87.9
		700	96.3	98.0	98.6	100.0	100.0
		1000	100.0	88.0	100.0	100.0	100.0
	2:1	50	0.0	0.0	0.0	0.0	0.0
		150	12.0	5.0	21.3	26.8	29.6
		300	29.6	19.0	31.5	51.6	31.6
		500	60.3	39.6	53.6	79.8	65.9
		700	79.3	65.3	89.6	95.6	89.9
		1000	94.3	79.6	100.0	100.0	100.0
	3:1	50	0.0	0.0	0.0	0.0	0.0
		150	11.6	4.3	19.3	25.6	25.6
		300	26.3	17.9	29.6	48.9	30.6
		500	57.3	37.6	51.3	76.8	66.5
		700	78.3	69.3	86.3	89.6	88.9
		1000	91.4	76.4	98.0	98.9	100.0
AA+AI	1:1	50	0.0	0.0	0.0	0.0	0.0
		150	18.8	10.3	19.6	21.3	22.6
		300	36.3	24.3	28.3	39.8	41.6
		500	71.2	51.3	51.3	59.8	58.9
		700	93.6	73.4	78.9	79.8	75.1
		1000	100	96.0	91.3	98.9	97.9

	1:2	50	0.0	0.0	0.0	0.0	0.0
		150	20.6	15.3	15.3	25.3	18.9
		300	38.6	26.9	26.3	41.3	39.5
		500	73.4	55.4	49.4	61.5	69.8
		700	96.3	78.6	75.4	79.5	84.6
		1000	100.0	99.0	90.3	97.8	98.9
	1:3	50	0.0	0.0	0.0	0.0	0.0
		150	25.6	14.3	19.3	26.5	23.6
		300	41.4	26.6	28.6	38.7	39.5
		500	68.0	53.4	59.3	58.7	58.9
		700	88.0	76.3	79.3	78.9	82.3
		1000	97.3	96.0	93.6	96.5	99.9
	2:1	50	0.0	0.0	0.0	0.0	0.0
		150	7.3	10.3	21.3	19.8	20.6
		300	19.0	21.6	31.9	35.8	33.2
		500	53.6	44.9	59.6	61.3	58.7
		700	73.6	73.8	79.6	86.9	84.6
		1000	91.3	88.4	98.9	100.0	95.6
	3:1	50	0.0	0.0	0.0	0.0	0.0
		150	7.4	9.3	19.3	16.8	19.9
		300	18.0	20.8	29.3	39.5	35.6
		500	56.3	41.5	55.4	69.5	66.3
		700	75.3	76.6	71.6	88.9	87.9
		1000	90.4	90.9	89.6	99.9	100.0
AS+AI	1:1	50	4.3	6.8	4.6	9.6	12.3
		150	20.3	20.4	38.6	35.9	39.8
		300	51.2	61.4	56.3	68.9	75.6
		500	73.2	88.0	79.6	91.6	100.0
		700	100.0	100.0	94.6	100.0	100.0
		1000	100.0	100.0	100.0	100.0	100.0
	1:2	50	0.0	0.0	2.3	4.6	10.3
		150	18.3	19.4	29.6	29.8	31.6
		300	43.6	53.6	51.3	59.8	69.8
		500	68.0	78.0	74.9	87.9	88.9
		700	91.0	93.0	92.3	100.0	100.0
		1000	100.0	100.0	100.0	100.0	100.0
	1:3	50	0.0	0.0	0.0	5.9	9.6
		150	17.4	17.3	21.3	33.6	33.2
		300	41.8	54.6	50.3	68.9	67.8
		500	65.9	76.3	74.5	79.9	81.3
		700	88.6	90.1	89.5	96.9	96.5
		1000	96.0	99.0	100.0	100.0	100.0
	2:1	50	2.3	5.8	5.2	13.6	16.5
		150	19.3	19.3	36.3	39.8	38.7
		300	45.0	59.3	54.2	73.6	79.8
		500	71.3	81.6	69.6	100.0	100.0
		700	96.0	99.0	91.6	100.0	100.0
		1000	100.0	100.0	100.0	100.0	100.0
	3:1	50	0.0	0.0	3.2	13.5	15.9
		150	15.3	18.3	35.6	37.6	33.6
		300	39.8	53.6	44.6	77.6	72.5
		500	69.4	79.4	65.4	100.0	88.9
		700	95.0	96.3	89.6	100.0	100.0
		1000	100.0	100.0	100.0	100.0	100.0

AA = *Adenocalymna alliaceum*, AS = *Allium sativum* and AI = *Azadirachta indica*

ting fungi tested *in vitro* is an indication of the fact that this composition may be effective in controlling fruit rotting diseases under field conditions.

Various workers have documented the antifungal properties of plant extracts but there is no precise presentation of synergistic bio-efficacy of plant extracts against fruit rotting fungi. Alam *et al.* (1999) have reported that antifungal effects of leaf and root extracts of *Vinca rosea* and leaf, root and seed extracts of *Azadirachta indica* against chilli fruit rot pathogen *Alternaria tenuis.* Singh *et al.* (1993) have reported the antifungal activities of leaf extracts against *Botryodiplodia theobromae, Fusarium oxysporum, Helminthosporium spiciferum, Curvularia lunata, Aspergillus flavus* and *Trichothecium roseum.* Shekawat and Prasada (1971) have reported inhibition of spore germination by various plant extracts. Rai *et al.* (2000) have reported also effect on spore germination by various plant extracts.

Research emphasis in this study has, therefore, been on the development of alternative approaches to control pathogens of fruits utilizing natural plant products. The current focus is on utilizing these well known plant extracts with synergistic approach to control fruit rotting pathogens.

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