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EVALUATION STUDIES OF SOME MEDICINAL PLANT EXTRACTS AND FUNGICIDES AGAINST *ALTERNARIA SOLANI.*

Phalisteen S*, Ishaq S, Amardeep K, Arif J and Sami S

Department of Microbiology, IIIM, (CSIR)-Sanatnagar, Srinagar-190005. (Formerly, RRL)

*Division of Microbiology, Indian Institute of Integrative Medicine (CSIR)– Sanatnagar Srinagar – 190005
Email: *shakir21@rediffmail.com* Tel.: + 91-94-2431253

ABSTRACT

Alternaria is a polyphagus fungus that occurs frequently on dead and decaying organic material and is responsible for causing leaf spot disease. In Indian subcontinent, there are different varieties of plants showing antimicrobial and other medicinal properties which can be employed in plant disease management to reduce the chemical load from the environment. The present investigation has been taken to evaluate the effect of medicinal plant (leaf) extracts and their combination with fungicides (Carbendazim) against radial growth of *Alternaria solani.*. The fungus was collected from infected leaf spots of potato plant and grown in PDA (potato dextrose agar) media. The water extracts of medicinal plants viz. *Azadirachata indica* (3%), *Calotropis procera* (3%), *Nerium oleander* (3%), *Ocimum sanctum* (3%) fungicide, carbendazim (1%) and their combinations were prepared. On the basis of results, the medicinal plant extracts Neem (3%), Nerium (3%) added with carbendazim (1%) showed maximum inhibition of fungus (*Alternaria solani*).

Key words: Polyphagus, radial growth, *Alternaria solani*, medicinal plant extracts and potato dextrose agar.

INTRODUCTION

Agriculture is the inseparable and integral part of Indian economy. As largest private enterprise, Indian agriculture contributes nearly quarter of the national GDP (Bureau of Statistics), sustain livelihood of more than 60% of population and is the backbone of agro-based industry (1). Since 1950, the productivity gained by nearly 3.3 times in vegetables(2). Vegetables constitute an important item of human diet, vegetables are richest source of protein and vitamins. A large number of vegetables are grown in India. A strong vegetable sector will lead to economic growth throughout the country. India is the largest producer of vegetables in the world (3), Surpassed only by China. In 2002, India has produced 78.2 million tons of vegetables from 5.73 million hactares of land (4). Indian farmers grow about 175 different types of vegetables. Among these, potato, tomato, onion, cabbage and cauliflower account for 60% of total production. It is projected that the domestic vegetable requirement rises from current 83-91 million tons to 151-193 million tons by 2030 (5).

India is the major exporter of vegetables; exporting worth approx. $245 million of vegetables annually (10th in the world) (6). Indian vegetables were restricted to potatoes and fresh onions. But now the government of India sees great opportunities for extending export of several other vegetables including dettuce, fresh peppers, tomatoes, squash and gherkins. Agriculture and its

allied sciences aim to maximize the production of plants for food fibers, building material, fuel and some essential drugs etc. Various groups of pathogens (bacteria, fungi, viruses and insects) are known to cause losses to agricultural yield allover the world including India. In India total loss due to pest, diseases and weed is approximately 18% of our total production or Rs 5000 crores annulay (7).There are many methods being presently used for pest control as cultural, biological, physical and chemical etc.(8). Among these the chemical control has been most useful and widely adopted strategy and has been yielding phenomenal results in reducing the disease severity. Excess use of these chemicals resulted in degradation of environment and cause ecological imbalances viz. accumulation of high amount of residues in food material and natural resources, depeletion of nutrients in the soil etc. Hence, there is a need of judicious use of such chemicals with suitable alternatives which are eco-friendly and leave no residue and are thus safe for mankind. Among the several methods available to control pests, plant based alternative strategy has been found to offer promising results. Hence, botanical pest control is gaining importance and recognition as a possible alternative and practical method to control diseases of many crop plants.

Potato, a vegetable crop is widely adopted for cultivation in India. The potato has been cultivated in India since its introduction in early part of the seventeenth century by English or portguese. It is grown from October to February. At the inception of CPRI in 1999, India used to produce 1.54 million tons of potato out of 0.234 million hectares with an average yield of 6.58 tons/hac from 1999-2001(9). India has produced 23.63 million tones of potatoes from 1.29 million hectares of land with an average of 18.23/ hac. In north Indian plains, potatoes can be grown successfully almost around the year. According to the projections of international food policy Research Institute (IFPRI) and International Potato Centre (IPC), world demand for potatoes shall increase by 40% upto 2020 (8). In potato cultivation several diseases infects the crop, black wart disease of potato, leaf spot of potato, early blight of potato caused by *Alternaria solani*. *A. solani* is an important constraint in cultivation of vegetables (10). Use of plant products or extracts was explored to control various diseases and mycoplasma etc, antifungal effects of Neem and other plant products or extracts are used in management of plant diseases caused by *Alternaria sp*. Earlier it has been reported that volatile component of crude aqueous extracts of garlic bulbs inhibits the germination of micro conidia and hyphal extension of *Fusarium oxysporum* and *Alternaria zinniae* in culture(11).

The present study was conducted to evaluate the antifungal effect of extracts of common weed *Calotropis procera* (Madar), *Azadirachta indica* (Neem) *Nerium oleander* (Kandar) and *Ocimum tenuiplorum* (Tulsi).

Azadirachta indica is found throughout India. The leaves, bark, seed and flower are bitter, astringent, acrid, refrigerant, depurative and toxic. It is also found effective against soil borne pathogens such as *Fusarium oxysporum* and some other fungi(12). *Calatropis procera* Linn belongs to the family Asclepiadaceae and found throughout dry waste places in India. *Nerium oleander* belongs to the family Apocynaceae, cultivated throughout India. Having leaves in a whorl, shortly. stalked linear, down green and shiny above. Leaves are powerful repellants and are used for seabies and hemorrhoids. *Ocimum tenuiflorium* ,(*Ocimum sanctum*) also known as

holy tulsi. It is distributed all over India. An erect branched softly pubescent under shrub, 30-60cm in height with red or purple sub-quadrangular branches. Leaves are simple, opposite elliptic oblong or acute entire, serrated or dentate, pubescent on both sides minutely gland dotted, petiole slender & hairy. The plant is bitter, acrid, aromatic, stomachic, demulcent, diaphoretic, digestive, vermifuge and alexetoxic. Bavistin is a product name of carbendazim. Carbandazim is the common name of Methyl-2-bendzimidazole carbamate. Bavistin have been applied for seed treatment, soil drench, soil mix and foliar spray. It is also used to control powdry mildew of apple-scab, brown rot of stone fruit by foliar spray. The present study was undertaken with the objectives, To study the effect of medicinal plant (leaf) extracts against growth of *Alternaria solani and* to evaluate effect of combined ability of medicinal plant extracts with carbendazim on *Alternaria solani*.

Materials & Methods

The glasswares, conical flasks, Petri dishes, pipettes and test tubes were thoroughly washed and dried. Petri dishes and pipettes were sterilized in hot air oven at 160°c for 4 hours. For isolation of fungus, potato dextrose agar (PDA) media was used. The procedure adopted for the preparation of media is based on the method of Rickian, 1936. Peel and boil the potato with 500ml water in a pan. After boiling, filter through cloth in another vessel and add agar at the rate of 1.5 – 2%. After boiling for sometime, add dextrose, boil again for some more time and make volume upto 1000ml and adjust pH to 6.5. The media was sterilized at 121^0 C for 20 mins in the autoclave.

Isolation of fungus

Infected plant material (leaves) having typical leaf spot symptoms of *A. salani* were collected from the nearby field. Leaves were examined under the microscope to confirm the presence of pathogen *Alternaria*. The infected parts of the leaves (Fig.1) were cut into small pieces, surface sterilized with 0.1% mercuric chloride solution for 30 seconds, washed three times by distilled H_2o and transferred onto Petri plates containing solid PDA (Potato dextrose agar) media. The inoculated plates were incubated at 25 ^{0}c for 4-6 days. The characteristic features of *Alternaria* are production of beaked, pigmented, conidia with relatively thin transverse and longitudinal septa. The pathogen *Alternaria* has septate, dark colored mycelia and produces short erect conidiophores that bear single or branched chains of conidia (Fig.2). The *Alternaria spp.* was purified from isolated dishes and maintained by periodic sub-culturing on PDA slants after every 15 days. The fungus was treated with T1, (Neem leaf extracts 3%), T2 (Madar leaf extracts 3%), T3 (Kaner leaf extract 3%), T4 (Tulsi leaf extract 3%), T5 (Carbendazim 1%), T6 (Neem 3% + Carbendazim 1%), T7 (Madar 3% + Carbendazim 1%), T8 (Kaner 3%+Carbendazim 1%), T9 (Tulsi 3%+Carbendazim 1%) and T_0 (control).

Preparation of plant extracts

Fresh leaves of medicinal plants were collected from nearby research field. 200 grams of leaves of each plant species were taken in 100ml beaker crushed with the help of mixer and filtered the extract in test tubes. 1% of fungicide (carbendazim) was prepared by weighing the required amount of fungicide and dissolve in 100 ml distilled water. The stock solution prepared

was used for making the required concentration by serial dilution method.

Inoculation of test organism

Arrange the sterilized petri plates such that each treatment was replicated 3 times. The measured quantities of stock solution were added to the petri plates to make resultant dilution and the treatment without any addition was considered as control. When the media was solidified, then with the help of sterilized cork borer, dishes of 0.3cm diameter from actively growing fungal cultures were cut and placed in centre of each dish containing the poisoned food (extracts). Inoculated petri plates were incubated at 25°c and the radial growth of colonies (in cms) were measured after 4 days of inoculation. The F-test as suggested by Fischer and Yartes was used to determine differences.

Results

Effect of treatments

The radial growth of fungus was measured against control and Neem extract in combination was found to have significant inhibitory effect on the growth of fungus. The order of their effectiveness was, T1>T8>T3>T9> T5>T6>T2>T7>T4>T0. (Fig.3,4,5,6) However after the 96 hours of the treatment T1 (Neem leaf extract 3%) and T8 (Kanaer (3%) + carbendazim 1%) were recorded equally effective in inhibiting the radial colony growth of test fungus. The effectiveness was recorded in this order, T1>T8>T9>T5>T6>T3>T2>T7.T4>To (Table 1).

Effect of period

The maximum inhibition of mycelial growth was found after 96 hours in comparison to 48 hours. The mean radial growth after 96 hours of treatment was 1.843. But time periods did not significantly effect the radial growth of fungus *Alternaria solani*.

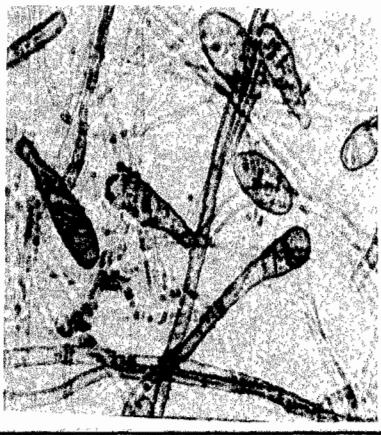
Azadirachta indica extracts inhibits the radial growth of *A. solani* after 48 hours of treatment in comparison to carbendazim (1%) in combination of extracts. However, after 96 hours the most effective combination was T1 and T8 in comparison to control.

Table 1: Mean radial growth of *A.solani* after 48 hours and 96 hours of treatment.

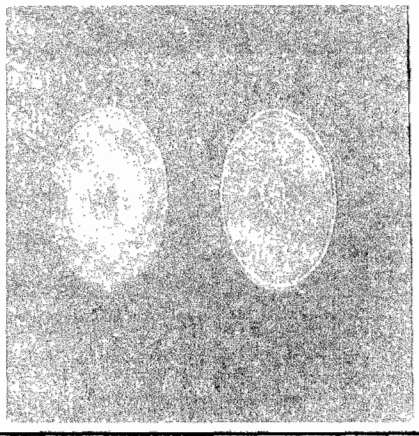
Treatment	Time		
	48 hours	96 hours	Mean
To	3.30	5.70	4.50±1.2
T1	1.20	1.25	1.28±0.02
T2	1.90	2.23	2.07±0.16
T3	1.28	2.02	1.68±0.37
T4	2.50	2.80	2.65±0.15
T5	1.50	1.60	1.55±0.05
T6	1.80	1.97	1.89±0.08
T7	2.40	2.50	2.45±0.05
T8	1.25	1.25	1.25±0.01
T9	1.30	1.37	1.33±0.03

Figures

in leaves.



Alternaria solani.



Calotropis 3% (T2).

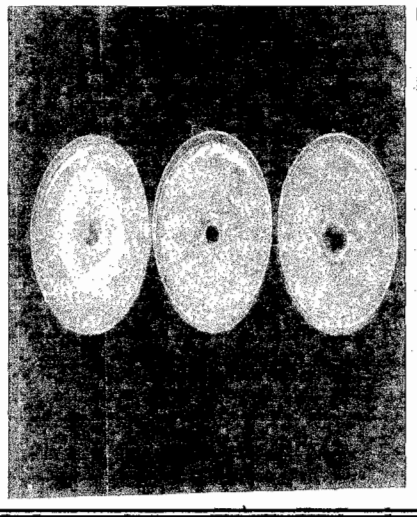


Fig.4. Control (To) compared with Nerium 3% (T3) and Ocimum 3% (T4).

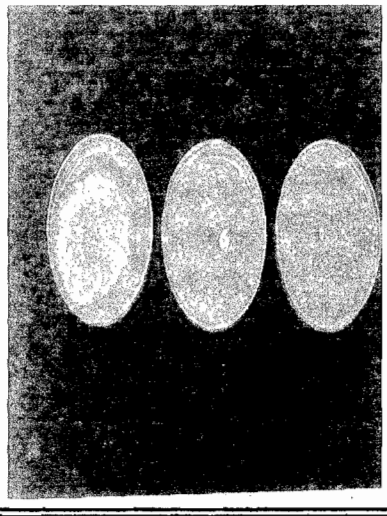


Fig.5. Control (To) compared with Neem 1% (T1) and Bavistin 1% (T5).

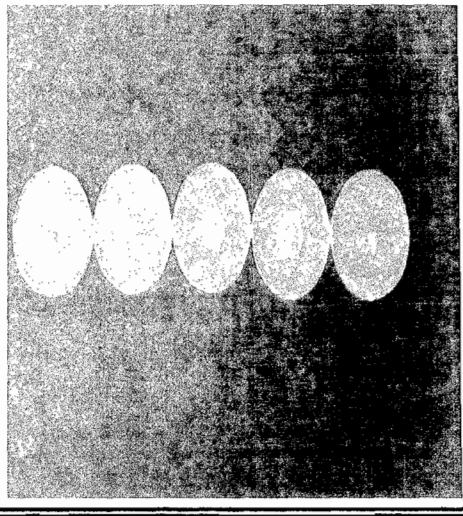


Fig.6. Control To compared with Neem 3% + Bavistin 1%, T6, Calotropis 3%+ Bavistin 1% and T7,Ocimum 3% .

DISCUSSION

The effect of leaf extracts of *Azadirachta indica, Calotropis procera, Nerium oleander, Ocimum sanctum,* fungicide, carbendazim and their combination on *Alternaria solani* were studied. The results of the present study highlighted that Neem extract in combination with carbendazim (1%) and Nerium (3%) were effective in reducing the growth of *Alternaria solani* as compared to control (13). Carbendazim alone was also effective in achieving the better results over *Alternaria solani*. The results of the experiment were in agreement with the results obtained (14), by using the fresh plant species of *Allium cepa, A. sativum, A. indica, Calotropsis procera, Datura stremonium* and *Ocimum*

sanctum, Polyalthia longifolia, Tagetus erecta, Vinca rosea and *Withania somnifera* showed antifungal properties against five pathogens *A. solani, Colletotrichum capsici, Fusarium oxysporum, Rhizoctonia solani and Sclerotina sclerotoirum* However, *A. indica, D. stramonium, Ocimum sanctum, Polyoelthia longifolia* were found to be more effective, It was also reported the seed mycoflora of chick pea was effectively controlled by plant extracts of *Azadirachta indica, Calotropis procera. Nerium oleander* compared with fungicide (Benlate, Thiram etc) against *Rhizopus nigricans, Colletotrichum dermatum* and *Alternaria solani* (15). In present study Neem extract has been found to be effective and promising botanical pesticide for further exploration and control of *A. solani*. In the present scenario there is an urgent need to develop eco-friendly, cost effective botanical pesticides as an alternative of chemical fungicides. From this study it is concluded that botanicals can be used as an alternative of fungicides in future and can be employed for sustainable agriculture. It is also evident that botanicals like Neem, Kaner and Madar leaf extracts alone and together with the fungicide can be used for the management of fungal diseases caused by *A. solani* and will reduce the chemical load, but before applied to field it needs further investigations.

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