ISSN 2250-3153

Antifungal Properties of certain Plant Extracts against *Rhizoctonia Solani* Causing Root Rot of French Bean In Organic Soil of Manipur

H.C.Mangang, G.K.N.chhetry

Department of Life Sciences Manipur University, Canchipur 795003, India

Abstract- *Rhizoctonia solani* is a major pathogen, causing root rot disease of French bean *(Phaseolus vulgaris*).The pathogen is worldwide in distribution and is observed in almost all bean growing countries.[1].This pathogen is also found to cause rot of french bean in the organically managed farms of Manipur. [2]. It is a major menace to the bean cultivators. Although chemical use could check the pathogen yet, it's use in the organic farming system is not permitted. Hence, the use of plants for their anti fungal properties which could be used against the pathogen in the organic farming system becomes an area of interest for the eco friendly mode of disease management. Also the local farmers are using these eco-friendly means like using cows urine, neem leaf extracts etc for controlling plant diseases. So ten commonly available medicinal plants were selected based on their wide application in the state and for their value in folk medicine. Cold water extracts of the ten plants were tested in vitro against *R.solani* and extracts of plants viz. *Artemisia vulgaris, coix lacryma jobi, Lantana camera, Michelia champaka, Passiflora foetida, punica granatum*, and *Strobilanthes flaccidifolius* showed 50% or more mycelial inhibition. . Among the plant extracts *Coix lacryma jobi* showed maximum percent mycelia inhibition with respect to control. The plant extracts were then tested in pot cultures and the plant extracts decrease rot incidence significantly as compared with control pots. In field trials Lantana camera showed maximum decrease in disease incidence. So these plants with anti rhizoctonia properties could be utilized against the pathogens, at least to lessen the impact of the pathogen .Similar eco friendly means of disease control has been appreciated by the present environment conscious generation.Thus exploring new plants for their anti fungal activity would bring about more resource base for use in eco friendly and sustainable mode of agriculture especially in organic farms.

Index Terms- plant extracts, antifungal, organic agriculture, Rhizoctonia

I. INTRODUCTION

French bean (Phaseolus vulgaris) locally known as *coli hawai / tankhul hawai / konsam hawai* (in Manipuri dialect) is a common crop of the state. It is widely cultivated in the state and is a favourite for the preparation of a variety of curries. Most of the marginal farmers of the state grow the crop in the organic mode without the addition of synthetic chemical inputs. In fact their croplands could be considered to be organic by default. Root rot of French bean caused by Rhizoctonia solani is an important fungal disease of French bean causing considerable damage to the crop. Various methods for the management of the disease have been studied by various workers in other states. There are reports that foliar spray of carbendazim (0.1%) and teubucomazole (0.05%) were most effective in reducing the disease.[3] However due to residual effect of synthetic fungicides, there is demand for more eco friendly substances like bio pesticides. Moreover such synthetic chemicals are not permitted in the organic farming system. As such exploration of plant resources for their antifungal potential against the pathogen is quite inevitable for a sustainable and eco friendly management of the pathogen. Further these plant extracts could be readily used by the farmers to lesion the impact of the pathogen on their crop. Using plant resources for its antifungal activity is an attractive avenue for the development of sustainable mode of agriculture in organic farming system. Hence, new plants especially locally available, need to be explored for their antifungal property. Thus ten plants locally used in medicinal purposes were selected based on their abundant availability during the cropping season and for their use in folk medicine.

II. MATERIALS AND METHODS

1. Pathogen isolation

The pathogen is isolated from the infected seedling whose symptom is marked by sharp edge reddish circular or elongated lesions on the hypocotyls of French bean. The pathogen is first isolated in water agar [4] and then maintained on PDA plates at 25±5 OC in BOD incubator and transferred on fresh PDA at regular interval for further study.

2. Plant extracts preparation

Ten plants viz. *Artemisia nilarigica, Artocarpous integefolia, Citrus maxima, Coix lacryma jobi, Hedychium coronarium, Lantana camera, Michelia champaka, Passiflora foetida, Punica granatum* and *Strobilanthes flaccidifolius* were selected for the study. Healthy non infected leaves of the ten plants were collected from the local area ie. Kakwa, Manipur, India. The leaves were washed with running tap water and finally rinsed with distilled water. It is then blotted with filter paper. Preparation of the plant extract is done as given by M.N.Khare.[5].Twenty grams of the plant material were taken and ground well with mortar and pestle. Then water is added in the ratio of 1:2 (weight by volume). It is strained through muslin

cloth. The extract is allowed to settle for a while and the supernatant is passed through filter paper. The filtrate was used for the test. The concentration of the extract thus prepared is taken as 100% . Poisoned food technique is employed to undergo the test for the antifungal activity of the plant extracts.PDA with 2% agar was used as culture medium. Varying amounts of plant extract were added to PDA to get a final concentration of 5%, 10%, 15%and 20% to access their effect on the mycelial growth of the test pathogen.

3. Inoculation

The PDA mixed with the plant extracts were poured in Petri plates and allowed to set. Then, one disc (7mm) of the test fungus taken from the margin of five days old culture were taken and placed in the reversed orientation at the centre of the Petri plates. Three replications were set up for each treatment. The whole set up is placed in BOD incubator with temperature set at 25^{O}C for five days. Pathogen grown on PDA plates with no plant extracts but with only distilled water acts as control plate. Percent inhibition is calculated as,

Percent inhibition = ((a-b)/a) x 100, where 'a' is the radial growth of the pathogen in the control medium and 'b' is the radial growth of the pathogen in the test medium.

4. Pot culture

Inoculation of soil is done as performed by Sachin Upamanyu *et al* [3] . The mass culture of R. *solani* was prepared on sand corn meal medium which was inoculated with mycelia bits and incubated for fifteen days at 25 ± 5^{0}C in BOD incubator. The mass culture of R *solani* was mixed in steam sterilized soil @ 10g/pot. The inoculated pots were sprayed with sterile water and established under polythene cover for two days. The seeds were sown in the inoculated pots. Six plants which show 50%mycelial inhibition over control in *in vitro* plates were used for pot culture experiment. Plant extracts with 20% concentration were used for the pot experiment. Each plant extract were applied in pathogen inoculated and non inoculated pots at an interval of three days up to 15^{th} day after sowing of seed. Distilled water is applied in control pot. The disease incidence is calculated by dividing the total no of plants showing disease symptoms by the total seeds sown and then multiplying by hundred. The data observed on the 20th day after sowing is used for statistical analysis.

5. Field Trials

Field trials were conducted for two consecutive growing seasons (2009 &2010) at Kakwa a small hamlet at the outskirt of the Imphal city of Manipur, India. The field shows rhizoctonia rot incidence during 2008 cropping season. Hence experiment is carried out at the natural inoculums potential of the soil. Field preparation were done during January .FYM at the rate of 20 tonnes /hectare , as recommended by Jasrotia RS and Sharma CM [6], were applied in the experimental plot one week prior to the sowing of the seeds. The experiment was conducted in 2x2 m^2 plots with three replications. In one treatment plant extracts with 20% concentration were applied at three days interval after sowing of seeds. Soil drench treatment as done by Sunita chandel & Manica Tomar [7] was followed. In control plots distilled water was applied. The total disease incidence at 20 days after sowing of seeds is used for statistical analysis .Rhizoctonia rot is more prominient during the earlier growth stages of the plants. Hence disease parameter is studied only at the young stages of the crop.

6. Statistical analysis

The data obtained was analysed using technique of ANOVA as given by Ronald E Walpole [8] to test the effectiveness of the plant extracts and if there is any significant difference in the antifungal properties of the plant extracts.

III. RESULTS

1 In vitro mycelia inhibition

The results as presented in Table 1 shows that the plant extracts were effective in significantly reducing the growth of mycelia as compared with control plates. Again 50% or more mycelia inhibitions as compared with control plates were observed with aqueous extracts of the plants viz. A. *vulgaris*, C. *lacryma jobi*, L. *camera*, M. *champaka*, P. *foetida*, P.*granatum*, and S. *flaccidifolius*, But A.*integefolia* ,C. *maxima* and H. *coronarium* showed less than 50% mycelia inhibition. The effect of concentration gradient from 5%to 20% showed no statistical difference. This might be due to the effectiveness of the plant extracts at further lower concentrations. For this minimum inhibition concentration need to be studied

2 *In vivo* disease incidence

The extract treated pots showed significant reduction in disease incidence with respect to control treatments. As shown in table 2, the application of the plant extracts showed decrease in the number of plants rotted. As per our data L. *Camera* showed maximum decrease in disease incidence. The finding showed the anti fungal effect of the six plants extract against R. *solani*.

3. Field Trials

From the result as sown in table 3, the plant extracts showed significant differences in DI % as compared with control treatment. L. Camera showed minimum percent disease incidence and it shows 61.07% reduction in DI% over control treatment plots.

IV. TABLES

Table 1: Radial mycelia growth and percent mycelia inhibition of R.solani with different plant extracts

concentration	5%		10%		15%		20%		Mean
Name of plants	*Radii in mm	% inhibition	*Radii in mm	% inhibition	*Radii in mm	% inhibition	*Radii in mm	% inhibition	Radii in mm
A.vulgaris	22.33	39.65	15.00	60.87	15.00	57.95	14.00	62.50	16.58
A.integefolia	28.67	22.51	30.33	20.87	25.33	28.99	23.67	36.59	27.00
C.lacrymajobi	11.33	69.38	15.33	60.01	11.33	68.24	11.00	70.53	12.25
C.maxima	27.00	27.03	32.67	14.77	28.67	19.62	31.00	16.96	29.84
H.coronarium	30.33	18.03	32.00	16.51	30.00	15.90	22.67	39.27	28.75
L.camera	18.67	49.54	18.00	53.04	11.33	68.24	11.33	69.65	14.83
M.champaka	18.67	49.54	16.33	57.40	16.67	53.27	14.33	61.61	16.50
P. foetida	20.33	45.05	16.67	56.51	18.67	47.66	15.33	58.93	17.75
P.granatum	23.00	37.84	15.00	60.87	20.33	43.01	17.67	52.67	19.00
S.flacidifolius	20.33	45.05	16.33	56.17	18.60	47.86	15.33	58.93	17.77
control	37.00	0	38.33	0	35.67	0	37.33	0	37.08

*Mean of three replicates
CD (P<0.05) between treatment means =3.57

Table 2: DI % of *R.solani* on French bean with different plant extracts in pot culture.

Name of the plants	DI	% control
A. vulgaris	45.26(42.28)	54.74
C. lacryma jobi	39.45(38.91)	60.55
L. camera	30.34(33.42)	69.66
M. champaka	36.65(37.26)	63.35
P.foetida	47.12(43.35)	52.88
P.granatum	50.23(45.13)	49.77
S. flaccidifolia	49.96(44.98)	50.04
Control	100(90)	

Values in parentheses are arcsine transformed values.
CD(P<0.05) between plant extracts=3.74

Table 3: DI % of *R.solani* on French bean with different plant extracts in field experiment.

Name of the plants	DI % control	% reduction over
A. vulgaris	22.23*(28.13)	31.09
C. lacryma jobi	18.40(25.40)	42.96
L. camera	12.56(20.76)	61.07
M. champaka	16.74(24.15)	48.11
P.foetida	16.48(23.93)	48.92
P.granatum	20.39(26.84)	36.79
S. flaccidifolia	17.64(24.83)	45.32
Control	32.26(34.61)	

*mean of three replicates
Values in parentheses are arcsine transformed values.
CD(P<0.05) between plant extracts=3.31

V. DISCUSSION

Rhizoctonia solani is a necrotropic soil borne pathogen with high competitive saprophytic activity[9]. Hence regular application of the fungicide is needed especially in organic soil where organic matter is usually high. Synthetic chemicals might successfuly control the disease but its application might is against the logic of organic farming. Hence exploration of alternative anti fungal agents, especially the plant extracts has merits. Plant extracts as potential antifungal substance has been explored against several fungal diseases [5] ,[10] In our study six plants showed 50% or above fungal mycelium inhibition activity against the pathogen in in vitro experiment . These plants have been reported to possess antifungal properties against different fungi. Lantana camera is found to to effective against Fusarium solani [11]. Chhetry and Belbahri [12] reported the utility of C lacryma jobi in biological management of pest and disease in jhum cultivated crops. Passiflora species have been shown to possess anti fungal activities to a number of fungi [13]. P granatum shows anti fungal activity against Aspergillus spp.[14]. G.R.Shinde and R.C.Paled [15] have found garlic extract to inhibit R.solani. In our results C. lacrymajobi showed maximum percent mycelia inhibition in vitro. Although C.lacryma jobi, L.camera and M campaka showed above 60% control over non treatments in pot experiments .Only L camera showed 61% reduction of DI% in natural field condition. A.K Srivastava [16] was of the view that thymol present in L. Camera is responsible for the fungicidal activity. Although none of the plants under study showed 100% mycelia inhibition plant yet most of them showed anti fungal activity against R.solani. From the in vitro , pot culture and field results, it can be safely concluded that the aqueous extracts of the six plants could be used in the organic farming environment to lessen the impact of the pathogen on bean crop although complete control could not be attained. Yet based on their wide availability and ease of application it could be used in wide scale in the organic farms for bean cultivation. Even though more useful oils and other components could be extracted through the use of other synthetic solvent and refined techniques yet, their use by the marginal farmers in the organic environment is limited .Hence the use of aqueous extracts has merits and is simple and could be easily followed even by a layman. This study would benefit the farmers who wish to lessen the impact of rhizoctonia rot on bean production. More and more plants, locally available need to be explored for a fruitful sustainable agriculture.

VI. CONCLUSIONS AND FORWARD LOOK

From the results some plants under study showed significant inhibitory effect even though none of the plant extracts shows cent percent mycelia inhibition. They are widely available in the state. Hence these plants could be used in the organic farming environment to lessen the impact of the pathogen in bean production in Manipur. More novel plants need to be explored to increase the resource base for use in organic farming system in a sustainable mode.

REFERENCES

[1] K.A. Mathew and S.K.Gupta ,1996, Studies on web blight of French bean caused by Rhizoctonia solani and its management . Journal of Mycology and plant pathology.26, 171-177.

[2] G.K.N Chhetry. and H. C Mangang 2011 Effect of soil amendments on soil borne pathogens of French bean (Phaseolus vulgaris L) in organic farming system of Manipur. Journal of Agricultural science and Technology.

[3] S.K. Sachin Upamanyu,Gupta and K.R.Shyam,2004, Innovative approaches for the management of root rot and web blight (Rhizoctonia soalni) of French bean.Journal of Mycology and plant Pathology 32(3),317-331.

[4] W.A Guttierrez, H.D.,Shew and T.A. Melton,1997,Source of inoculums and management of Rhizoctonia solani causing damping off on tobacco transplants under greenhouse conditions. Plant Diseases 81,604-608.

[5] M.N Khare. and B.N., Shukla 1998, Utility of plants in crop disease control.Vasundhara. 3, 1-15

[6] R.S Jasrotia and C.M., Sharma 1999, Fertiliser News 44,59-61

[7] Sunita Chandel and Manica Tomar,2008, Effectiveness of bioagents and neem formulations against fusarium wilt of carnation , Indian Phytopathology 61(2),152-154.

[8] Ronald E. Walpole, 1982, Introduction to Statistics, 3rd edn. Macmillan Publishing Company

[9] Garett,1970, Pathogenic root infecting fungi, Cambridge University press.

[10] P.Singh, R Shukla , B Prakash., A. S Kumar Singh., PK Mishra., NK.Dubey,2010, Chemical profile ,antifungal,antiaflatoxigenic and antioxidant activity of citrus maxima Burm. And Citrus sinensis (L.) Osbeck essential oils and their cyclic monoterpene, DL-limonene. Food Chem Toxicol.48(6),1734-40

[11] T. Mamatha and V Ravishankar Rai., 2004, Evaluation of Fungicides and plant extracts against Fusarium solani, leaf blight of Terminalia catapa. Journal of Mycology and Plant Pathology.34(20),306-307.

[12] G.K.N Chhetry. and Lassaad Belbahri, 2009, Indigenous pest and disease management practices in traditional farming systems in north east India. A review. Journal of plant breeding and crop science 1(3),28-38

[13] M. Joan Nicolls ,1970, Antifungal activity in Passiflora Species,Ann.Bot.34,229-37

[14] S Satish., D.C.Mohana, M.P. Ranhavendra and K. A Raveesha.,2007,Antifungal activity of some plant extracts against important seed borne pathogens of Aspergillus sp. Journal of Agricultural Technology. 3(1),109-119

[15] G.R Sindle.and R.L. Patel,2004, Evaluation of plant extracts against Rhizoctonia solani incitant of black scruf disease of potato.Journal of mycologyand plant pathology.32(2),284-286

[16] A.K Srivastava.Lal B .1997, Studies on biofungicidal properties of leaf extract of some plants .Indian Phytopathology 50,408-411

AUTHORS

First Author – H C Mangang, Research student, M.Sc., Department of Life Sciences, Manipur University ,Canchipur. Imphal ,India. email huidromc@gmail.com (corresponding author)

Second Author – G.K.N.Chhetry, Professor, Department of Life Sciences Manipur University, Canchipur, Imphal India.