ISSN 2250-3153

Evaluation of antifungal activities of certain plant against *Fusarium udum* Butler causing wilt in pigeonpea (*Cajanus cajan* (L.) Millsp.)

Tongbram Ranjana Devi and GKN Chhetry

Department of Life sciences, Manipur University, Canchipur, Imphal – 795003

Abstract- The antifungal effect of aqueous extracts of locally available plant species have been investigated *in vitro* conditions against *Fusarum udum* , the causal agent of *Fusarium* wilt of pigeonpea. The plant extracts were screened against mycelial growth and spore germination of *F. udum* at different concentrations of 5%, 10%, 15%, and 20% using poisoned food technique and cavity slide method. Among the tested plants higher inhibition was noticed in *Allium sativum, Azadirachta indica,Spilanthes acmella and Adhatoda vasica.*The remaining plants produced less inhibitory effect. Among them *A. sativum* at 20% alone recorded 100% inhibition of mycelial growth and spore germination. In field experiment, aqueous extract of *A. sativum* showed highest percentage of disease control.

Index Terms- Antifungal properties, *Fusarium udum*, Pigeonpea, Plant extract, Wilt

I. INTRODUCTION

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is an important grain legume crop of the family Fabaceae. It is the most important kharif pulse crop with much higher productivity. In Manipur the crop is cultivated on marginal lands by resource-poor farmers, who commonly grow traditional medium- and long- duration landraces. It is cultivated either as sole crop or mixed with pearlmillet (*Pennisetum glaucum* L) and maize (*Zea mays* L) [3]. Diseases are major biological constraints to production. Of these *Fusarium* wilt are widespread and causes heavy damage. As the management of this soil borne disease through conventional technology such as growing resistant varieties, fungicidal seed treatment, single treatment of fungicide or bio-agent cannot provide a remedy for disease control [6], a need was felt to develop botanical based biofungicides for control of plant diseases. Because non judicious use of synthetic fungicides since last four decades led to several problems to human and animal health besides environmental problems. This scenario, therefore, calls for alternative approaches which are economically feasible and ecofriendly to increase yield of pigeonpea. In view of the hazardous effect of synthetic fungicides, that too for the soil borne ones the present investigation has been carried out for evaluating the phytotoxic activity of locally available plants against wilt disease of pigeonpea.

II. MATERIALS AND METHODS

Ten plants viz., *Allium cepa, Allium sativum, Carica papaya, Citrus limon, Eryngium foetidum, Gynura angulosa, Aloe vera, Azadirachta indica, Spilanthes acmella, Adhatoda vasica*, locally known for their medicinal values for the treatment of common human diseases were selected to determine the antifungal activity against *F. udum*. Extracts of plant parts such as leaf, flower, bulb, clove, etc. were prepared by the standard method used by Gerard Ezhilan *et al.* [2]. Fresh plant parts were washed with tap water followed by sterile distilled water, processed with sterile distilled water @1mlg^{-1} of plant tissue (1:1v/w) with pestle and mortar and filtered through a double layered cheese cloth. The filtrate so obtained formed the standard plant extract solution. The plant extract so prepared were screened *in vitro* against *F. udum* using poisoned food technique. Stock solution 5, 10, 15, and 20 ml were mixed respectively with 95, 90, 85 and 80 ml of sterilized molten Potato Dextrose Agar (PDA) media to obtained 5, 10, 15 and 20 percent concentration of plant extract. The mixed medium was thoroughly shaken to ensure uniform mixing of extract. 20 ml of poisoned PDA was poured into sterile petriplates. Five replications were maintained for each concentration. After solidification of poisoned media, the plates were inoculated with mycelium disc (4mm diameter) of vigorously growing pure culture colony of *F. udum*. The control petriplates in five replications were maintained using only sterile water without any plant extract but with mycelium disc (4mm) for comparison . The inoculated petriplates were incubated at 25 ± 1^{0}C. The radial growth of *F. udum* was measured in treated and control petriplates after seven days of incubation . The percent growth inhibition was calculated following the formula given by Vincent [8].

$$I = \frac{C - T}{C} x100$$ where, I=percent inhibition

C = growth in control
T = growth in treatment

Effect of plant extracts on the spore germination of *Fusarium udum.*

Antifungal activity of aqueous extract of the test plants on the

spore germination of *F. udum* was assayed by cavity slide method. One drop(about 0.1ml) of spore suspension containing 40-50 spores per microscopic field (10×40x) was put over a concentrations viz. 5,10,15 and 20 percent .The slides were kept in moist chamber prepared by putting two folds of filter paper in both sides of petriplates. These plates were incubated at 25± 1^0 C for 24 hours. Cavity slides with sterilized distilled water serve as control. Three replications were kept for each treatment. Spore germination was calculated by observing the number of spores germinated / microscopic field. Percent inhibition of germination over control was calculated.

FIELD EXPERIMENT:

The experiments were carried out in Chirang a village located at the outskirt of Imphal city where pigeonpea was grown regularly in a randomized block design for two consecutive years (2009 and 2010). A wilt susceptible local variety was shown in third week of April in each plot (2×2m^2).

Plant extracts that produced high percentage of inhibition at 20% concentration viz. *A.sativum, A. indica, S.acmella, A. vasica* were tested further to see their effect *in vivo* conditions. Plant extracts of test plant were prepared afresh on the day of foliar application and used for spray immediately after preparation. The spray treatments were started after 45 days of planting followed by two subsequent sprays at 15 days interval. A standard check with untreated control (water spray) was also maintained. Data on disease incidence were recorded and calculated by using the formula:

Percent Disease incidence

$$(DI\%) = \frac{Number\ of\ plants\ infected\ by\ the\ disease}{Total\ number\ of\ plants\ observed} \times 100$$

III. RESULTS

Result showed significant effect of plant extracts against the mycelial growth of *F. udum* causing wilt of pigeonpea (table 1). Among them, extract of *Allium* sativum showed complete inhibition of radial growth of *F. udum* followed by *Azadirachta indica* (79.4%), *Spilanthes acmella* (68.8%) and *Adhatoda vasica* (58.8%), *Gynura angulosa* (55.9%), Aloe vera (55.9%), *Eryngium foetidum* (50%).The other test extracts showed less than 50% inhibition of mycelial growth. Result presented in table 2 showed that highest inhibition of spore germination was observed in *A. sativum* extract followed by *A.indica*(60.7%), *S.acmella* and *A. vasica* showed more or less similar inhibitory effect. The other tested plants showed less inhibitory effect. . Under field conditions, among the four plants, maximum control of disease was observed with *A. sativum*(35.3%), followed by *A. indica*(25.9%), *S acmella*(14.2%) and *A. vasica*(10.2%). The results proved that application of bulb extract of A. sativum could be biopesticidal and ecofriendly substitute for chemical fungicide. However, further studies are needed to standardized the dose of bulb extract for foliar spray in the field to achieve better results.

IV. TABLES

Table1: Effect of plant extracts on the radial growth of mycelium of *F. udum*

Botanicals	**Parts used**	**Local name**	***Mycelial growth (cm)**				**mean**	**Percent inhibition of mycelium growth over control**			
			5%	10%	15%	20%		5%	10%	15%	20%
Eryngium foetidum	leaf	awaphadigom	2.9	2.9	1.9	1.7	2.35	14.7	14.7	44.1	50.0
Carica papaya	leaf	awathabi	2.7	2.6	2.2	1.9	2.35	20.6	23.5	35.3	44.1
Adhatoda vasica	leaf	Nongmangkha angouba	2.5	2.4	1.8	1.4	2.03	26.5	29.4	47.1	58.8
Allium cepa	bulb	tilhou	3.0	2.9	2.9	2.7	2.86	11.8	14.7	14.7	20.6
Citrus limon	leaf	champra	2.9	2.7	2.4	2.4	2.60	14.7	20.6	29.4	29.4

Spilanthes acmella	flowe r	chinlengbi	2.5	2.1	1.3	0.9	1.70	26.5	37.7	61.2	68.8
Gynura angulosa	leaf	terapaibi	2.5	2.5	1.8	1.5	2.08	26.4	29.4	47.1	55.9
Allium sativum	clove	chanam	1.5	0.3	00	00	0.45	55.9	92.1	100	100
Aloe vera	leaf	ghritakumari	2.5	2.3	1.5	1.5	1.95	26.5	32.4	55.9	55.9
Azadirachta indica	leaf	neem	1.9	1.5	0.9	0.7	1.25	44.1	55.9	73.5	79.4
Control			3.5	3.5	3.2	3.4	3.40				
Mean			2.6	2.3	1.8	1.7					
CD@5%	Between plant extracts : 2.7 Between concentrations:4.4										

*mean of five replications

Table2: Effect of plant extrac ts on the spore germination of *F. udum*

Botanicals	***Spore germination (%)**				**Mean**	***Percent inhibition**			
	5%	10%	15%	20%		5%	10%	15%	20%
E.foetidum	82.6	78.2	72.3	65.6	74.68	10.3	15.1	21.5	28.8
C.papaya	82.8	78.3	72.8	70.2	76.03	10.1	15.0	20.9	23.8
A. vasica	81.1	74.9	71.0	61.3	72.73	11.9	18.7	22.9	33.4
A.cepa	86.5	80.4	75.4	73.5	78.95	6.1	12.7	18.1	20.2
C.limon	85.1	78.6	73.4	72.6	77.43	7.6	14.7	20.3	21.2
S. acmella	75.8	74.1	68.1	60.6	69.65	17.7	19.5	26.1	34.2
G.angulosa	81.7	75.4	71.6	65.5	73.55	11.3	18.1	22.3	28.9
A. sativum	9.2	7.0	4.5	00	5.12	9.0	92.4	95.1	100
A.vera	79.0	74.3	69.1	63.9	70.93	14.2	19.3	25.0	30.6
A. indica	60.5	40.6	45.5	36.2	45.70	34.3	55.9	50.6	60.7
Control	93.0	92.1	92.5	90.8	92.1				
Mean	74.3	68.5	65.1	60.0					
CD@5%	Between plant extracts : 2.6 Between concentrations : 4.4								

*mean of three replications

Table 3: Effect of plant extracts on wilt disease of pigeonpea under field condition

Name of plants	***DI%**		**Pooled mean**	**% Disease control**
	2009	**2010**		
A. sativum	61.1 (51.41)	62.8 (52.42)	62.0 (51.94)	35.3
A. indica	71.1 (57.48)	71.1 (57.48)	71.1 (57.48)	25.9
S. acmella	76.7 (61.14)	87.8 (69.56)	82.3 (65.12)	14.2
A. vasica	82.2 (65.05)	90.0 (71.57)	86.1 (68.11)	10.2
Control	94.5 (76.44)	97.2 (80.37)	95.9 (78.32)	

*average value of the occurrence of disease
*figure in parentheses are arcsin transformed values

V. DISCUSSION

The major setback in the control of *Fusarium udum* has been low capita per income, with household poverty which has hindered farmers acquiring synthetic chemicals to control the disease. Locally available plants have been tested as an alternative to synthetic chemicals. A. sativum extract completely inhibited mycelial growth and spore germination.A.indica also inhibited the growth of the pathogen above 50%.The effectiveness of garlic and neem extract in micelial growth and spore germination might be due to the presence of antifungal compounds like diallyl disulphide and diallyl trisulphide and

azadirachtin. Effectivenees of garlic and neem as bio-fungicides has already been reported by many against different fungi. Garlic extract at 15% inhibited the growth of the pathogen (88.26%) [1]. Pradeep Kumar Singh *et al* [5] also reported highest inhibition of radial growth of F. udum by A. indica(67.8%) at 5%. Effectiveness of garlic and neem extract in different fungi is also reported by many workers [4,7].

VI. CONCLUSION

Present investigation suggests that locally available plant resources such as A. sativum and A. indica may be of use for possible control of F.udum. However, further work is needed to explore potential of selected plants at the field level.

REFERENCES

[1] Ankita Shukala and S. K. Dwivedi.(2012). Bioefficacy of plant extracts against Fusarium species causing wilt in pulses.IOSR Journal of Engineering. 2(1): 136-144.

[2] Gerard E .Chandrasekar and V. Kurucheve. (1994).Effect of six selected plant products and oil cakes on the sclerotial production and germination of Rhizoctonia solani. Indian Phytopath. 47 (2): 183-185.

[3] G.K.N. Chhetry.(2004). Dispersion statistics and sequential sampling plan for timing fungicides application against Cercospora cajani leaf spot in pigeonpea. J. Mycol. Pl. Pathol. 34 (2):554-557.

[4] N. Siva, S.Ganesan, N. Banumathy and Muthuchelian.(2008). Antifungal effect of leaf extract of some medicinal plants against Fusarium oxysporum causing wilt disease of Solanum melogena L. Ethnobotanical Leaflets 12: 156-163.

[5] Pradeepkumar Singh, Anish Khan, Robin Gogoi and Ritesh Kumar Jaiswal (2010). Plant leaf extracts and bioagents for eco-friendly management of wilt of pigeonpea caused byFusarium udum. Indian Phytopath. 63 (3) : 343 – 344.

[6] R. M. Gade , K.K. Zote and C. D. Mayee. (2007). Integrated management of pigeonpea wilt using fungicide and bio-agent. Indian Phytopath. (60) 1: 24-30.

[7] R. R. Sharma, H.N. Gour and Pankaj Sharma.(2005). Effect of plant extracts on growth of Rhizoctonia solani and disease development in maize. J. Mycol. Pl. Pathol. 35(2): 377-379.

[8] Vincent JM. (1947). Distortion of fungal hyphae in the presence of certain inhibitors. Nature 150:850.

AUTHORS

First Author – Tongbram Ranjana Devi. Ph,D Student, Department of Life Sciences, Manipur University, Imphal, Canchipur,795003
Email address: ranjanatongbram@yahoo.in

Second Author – GKN Chhetry, Professor, Department of Life Sciences, Manipur University, Imphal, Canchipur,79500

.