Journal Homepage: -www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI:10.21474/IJAR01/7420
DOI URL: http://dx.doi.org/10.21474/IJAR01/7420
ISSN NO. 2320-5407

RESEARCH ARTICLE

IN VITRO EVALUATION OF ANTI FUNGAL ACTIVITY OF SOME MEDICINAL PLANTS AGAINST *HELMINTHOSPORIUM ORYZAE*, THE INCITANT OF BROWN SPOT DISEASE OF PADDY.

Resmy Jayaraj[1], A. S. Rubin Jose[2] and V. Kuruchev[1].
1. Faculty of Agriculture, Annamalai University, Annamalainagar, Tamil Nadu, India.
2. Department of Botany, Fatima Mata National College, Kollam-2, Kerala, India.

Manuscript Info

Manuscript History

Received: 15 May 2018
Final Accepted: 17 June 2018
Published: July 2018

Keywords:-
Medicinal plants, Antifungal activity, *Helminthosporium oryzae*.

Abstract

Brown spot of rice incited by *Helminthosporium oryzae* is a serious disease and cause considerable yield loss. Several fungicides have been reported to control the disease effectively. With the increase in awareness on the toxic hazards of chemicals to crops, consumers and environment, the importance of indigenous natural products in plant disease control has been recognized. In the present investigation, four medicinally important plant species were screened for fungitoxicity against *H. oryzae*. Among the plant species assayed, crude extracts of garlic bulb (20%), *Lawsonia inermis* (80%) and *Vinca rosea* (100%) leaves recorded complete inhibition of mycelial growth and are on par with Hinosan (0.1%) and neem leaf extract recorded 85% reductin in mycelial growth over control at 80 % concentration. Since garlic was found to be more effective than other plant extracts, a detailed *in vitro* screening study was conducted to find out the effective concentration.

Introduction:-

Brown spot of rice incited by *H. oryzae* is a serious disease, occurs in most of the rice growing countries of the world. Now-a -days, farmers use a number of plant protection chemicals for pest control. The extensive and indiscriminate uses of synthetic chemicals to control pathogens, besides having long residual effects, are hazardous for environment (Khoshoo, 1986). With the increase in awareness on the toxic hazards of chemicals to crops, consumers and environment, the importance of indigenous natural products in plant disease control has been recognized. In addition, due to the development of new physiological races of pathogens, many of the synthetic fungicides are gradually becoming ineffective (Wellman, 1977).

Among various bio control agents, green plants appear to be a good reservoir of effective chemotherapeutant and would constitute an exhaustible source of harmless pesticides (Sbragia, 1975 and Swaminathan, 1978). The presence of anti fungal compounds in higher plants has long been recognized as an important factor to disease resistance. So different types of plant extracts were screened *in vitro* against *H. oryzae*.

Corresponding Author:-Dr. A. S. Rubin Jose.
Address:-Deprtment of Botany, Fatima Mata National College, Kollam-2, Kerala, India.

Materials and Methods:-

Preparation of plant extracts:-

Fresh plant materials were used for extraction. The plant materials were first washed with fresh water and finally with sterile water. They were ground with sterile water at the rate of 1g of tissue/ml of water (1:1 w/v) with pistle and mortar and filttered through cheese cloth. This formed standard plant extract solution (100%).

Evaluation of water extract of plant products on the mycelial growth of *H.oryzae*:-

Potato Dextrose Agar (PDA) medium (Ainsworth, 1961) was prepared and autoclaved. Plant extracts were mixed with PDA so as to get a final concentration of 5, 10, 20, 40, 60, 80 and 100 %. 0.1%Hinson was added to the medium for comparison. The medium without any natural product served as control.

Evaluation of water extract of plant products on germination of conidia of *H. oryzae*:-

Test extract of 0.05 ml and spore suspension of the fungus 0.05ml (2000-3000 spores/ml) of the test fungus estimated using haemocytometer were mixed in a cavity slide and incubated for 24 hr in Petri dish glass bridge moist chamber. Evaluation of natural products on seed germination, growth and vigour of rice seedlings (IR 50) was done by Roll Towel Method (Anonymous, 1976).

Results:-

Effect of water extract of plant products on the mycelial growth of *H. oryzae*:-

The effect of water extracts of natural products at various concentrations *viz*., 5, 10, 20, 40, 60, 80 and 100% on the mycelial growth of *H.oryzae* was noticed (Table1). Complete inhibition of *H.oryzae* was obtained with fungicide 0.1 per cent, which was 100 per cent reduction over control. In untreated check, the mycelial growth was 90 mm. Among the four plant extracts tested, bulb extract of *Allium sativum* (20%) showed complete inhibition of *H.oryzae* and it was on par with fungicide Hinosan. Other plant extracts showed complete inhibition in mycelial growth at higher concentrations only i.e. *Lawsonia inermis* (leaf) at 80 per cent and *Vinca rosea* (leaf) at 100 per cent.

Effect of natural products on the conidial germination and sporulation of *H. oryzae*:-

Hinosan at 0.1% concentration recorded complete inhibition of conidial germination and sporulation of test fungus (Table 2). Among the natural products, garlic bulb extract at 10 per cent and *Lawsonia* at 80 per cent and *Vinca rosea* at 100 per cent concentrations recorded complete inhibition of germination of conidia and sporulation of *H.oryzae* and they were on par with Hinosan.

Effect of natural products on seed germination, growth and vigour of rice seedlings:-

Among the natural products tested, garlic (20%) exhibited 95 per cent and Hinosan showed 98 per cent seed germination Table 3). *Lawsonia* and *vinca rosea* at 80 and 100 per cent concentrations recorded 86 and 80 per cent seed germination respectively when compared to control. All the treatments recorded significant results when compared to control. In control, root and shoot length was 3.8 and 5.7 cm and the vigour index was 779.0 only. Hinosan (0.1%) treated seeds recorded more root length (7.5), shoot length (11.0 cm) and vigour index (1813.0) and it was found to be superior, over all the treatments (Table 3). It was followed by garlic treatment.

Discussion:-

Effect of plant extracts on the mycelial growth of *H. oryzae*:-

Hinosan 0.1% recorded total inhibition of the mycelial growth. Among the various natural products screened, bulb extract of *Allium sativam* at 20 per cent concentration and leaf extract of *Lawsonia inermis* at 80 per cent and *V. rosea* at 100 per cent concentration completely inhibited the mycelial growth of *H. oryzae* and were on par with fungicide Hinosan. Among the various plant extracts tested, leaf extract of neem alone had not totally inhibited even in higher concentrations. There are several reports on the antifungal activity of garlic, *Lawsonia* and *V. rosea* controlling various pathogens. Kurucheve and Padmavathi (1997) reported that the extracts from *A. sativum* (bulbs) and *L. inermis* (leaf) inhibited the growth of *Pythium aphanidermetum*. Natarajan and Lalithakumari (1987) has reported the antifungal activity of *L. inermis* against *Drechslera oryzae*. Ganguli (1994) has reported that the leaves of V. rosea effectively inhibited the mycelial growth and spore germination of *H. oryzae* and *P. oryzae*. The inhibitory effect of plant products may be due to the presence of some antifungal compounds in the extracts. The difference in the inhibitory effect of various plant products may be due to the qualitative and quantitative difference

in the antifungal principle present in them. Dubey (1991) reported that effectiveness of plant extracts depended upon the nature and amount of active principle contained. Stoll and Seeback (1951) reported that garlic bulb contained allicin (dialkylthiosulphate) which was responsible for the fungitoxic nature. Natrajan and Lalithakumari (1987) reported that phenol-2- hydroxy- 1, 4-napthoquinone (Lawsone) in the leaf extract of *L. inermis* was responsible for its antifungal activity.

Effect of Plant extracts on the conidial germination and sporulation of H. oryzae:-

Hinosan 0.1% concentration recorded complete inhibition of conidial germination and sporulation of the test fungus. Among the natural products tested garlic bulb at 10 per cent, Lawsoniainermis and *Vinca rosea* at 80 and 100 per cent recorded complete inhibition of germination of conidia and sporulation of *H.oryzae* and they were on par with Hinosan. Bulb extract of garlic, leaf extract of *Lawsonia* and buffalo urine completely inhibited the conidial germination of and sporulation of *Colletotricum falcatum* (Kumar, 2000). Reduction in spore germination and growth rate may be due to the presence of some inhibitory substances in aqueous extracts, which affected the physiological metabolism of the fungus (Bera and Saha, 1983).

Effect of Plant extracts on seed germination, growth and vigour of rice seedlings:-

All the natural products were found to be significantly superior and stimulatory in seed germination and growth of rice seedling over control. Seed soaking with *A. sativum* and bulb extract increased the seed germination, shoot growth, root growth and vigour of various seedlings (Padmavathi, 1994; Sundarraj, 1994 and Raja, 1995) and these are in agreement with our findings.

The enhancement of seed germination and growth of seedlings by natural products might be attributed to several factors such as the fungitoxic action leading to killing of the pathogens present both internally and externally in the seeds. It may be due to the presence of hormone synthesising organisms as phyllosphere population and the presence of auxins in the natural products.

Conclusion:-

Eventhough, the synthetic pesticides contributed greatly in the increase of good production indirectly by controlling insects and pathogens, their side effects spoiled the environment. Economically unimportant organisms assumed pathogenic status as a result of indiscriminant use of pesticides. The very recent typical example of outbreak of eriophyd mite infestation on coconut trees in South India is mainly due to the large scale impoundment of pesticides and fertilizers on other agricultural crops. Thus, garlic *Lawsonia inermis* and *Vinca rosea* could possibly be exploited for effective management of brown spot disease. However, further studies are needed regarding the active principles involved in the fungitoxicity of natural products. Such a control measure would be of more practical value, economical and safer to the consumers and environment due to readily biodegradable nature of natural products.

Table 1:-Screening of some plant extracts against mycelial growth of *H. oryzae*

Sl. No.	Sources	*Diameter of mycelial growth (mm)						
		5	10	20	40	60	80	100
	Plant Extracts							
	A. sativum	24.00	18.00	0.00	0.00	0.00	0.00	0.00
	L. inermis	78.00	69.00	47.00	12.00	10.00	0.00	0.00
	A. indica	44.00	42.00	40.00	25.00	20.00	14.00	10.00
	V. rosea	81.00	74.00	54.00	42.00	26.00	17.00	0.00
	Hinosan (0.1%)	0.00	-	-	-	-	-	-
	Control	90.00	-	-	-	-	-	-

Table 2:-Effect of Plant extracts on the conidial germination and sporulation of *H. oryzae*

Sl. No.	Sources	*Conidial germination (%) after 24 hrs.							sporulation
		5	10	20	40	60	80	100	-
	A. sativum	21.79	0.00	0.00	0.00	0.00	0.00	0.00	-
	L. inermis	-	-	-	42.75	35.14	0.00	0.00	-
	V. rosea	-	-	-	67.71	55.26	36.19	0.00	-
	Hinosan (0.1%)	0.00	-	-	-	-	-	0.00	-

	Control	100	-	-	-	-	-	-	+++

Table 3:-Effect of Plant extracts on seed germination and vigour of rice seeds

Sl. No.	Sources	Concentration	Seed germination (%)	Root length (cm)	Shoot length (cm)
	A. sativum	20	95	6.0	10.2
	L. inermis	20	89	5.2	8.5
	V. rosea	20	85	4.5	7.8
	Hinosan (0.1%)	40	98	7.5	11
	Control	20	82	3.8	5.7

Reference:-

1. Anonymous. Seed SciTechnol 1976; 4: 3-49.
2. Ainsworth GC. Dictionary of Fungi. Commonwealth Mycological Institute, Kew Survey, England, 1961. p.547.
3. Bera AK, Saha, A. J Pl Dis and Prot 1983; 90: 363-365.
4. Dubey RC. Indian Phytopath 1991; 44: 241- 244.
5. Ganguli LK. Environ. and Eco. 1994; 12:731- 733.
6. Kumar KS. Mycotoxic activity of natural products against Colletotrichumfalcatum Went, the incitant of sugarcane red rot. M.Sc.(Ag.) Thesis, Annamalai Univ., Annamalainagar, India. 2000. p. 67
7. Kurucheve V, Padmavathi R. IndianPhytopath 1997; 50: 529- 535.
8. Natarajan MR, Lalithakumari D. Indian Phytopath 1987; 40: 390- 395.
9. Padmavathi R. Studies on antifungal activity of plant products against *Pythium aphanidermatum* (Edons) Fitz. the causal agent of damping-off of chilli. M.Sc.(Ag.) Thesis, Annamalai Univ., India. 1994. p. 76
10. Sundarraj T. Mycotoxic properties of some plant products and animal faeces against *Rhizoctonia solani* Kuhn (*Thanatephorus cucumeris*(Frank. Donk), the causal agent of sheath blight of paddy. M.Sc.(Ag.) Thesis, Annamalai Univ., Annamalainagar India.1994. p. 69.
11. Swaminathan,M. S. (1978). Inaugural Address. First Botanical Conference, Meerut (India), 1-31.
12. Raja J. Fungicidal activity of crude plant extracts and animal products against *Macrophomina phaseolina* (Tassi.) Goid. and *Fusarium oxysporum* f.sp. lycopersici (Sacc.) Snyder and Hansen, M.Sc. (Ag.) Thesis, Annamalai Univ., India. 1995. p. 36.
13. Sbragia RJ. Annu. Rev. Phytopath 1975; 13: 257- 267.