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IN VITRO INHIBITORY EFFECTS OF MEDICINAL PLANTS EXTRACTS ON *SCLEROTIUM ORYZAE*– A FUNGI CAUSING STEM ROT DISEASE IN PADDY

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ABSTRACT

Stem rot of rice is caused by Sclerotium oryzae continues to be a major constraint in rice production. Since, the existing chemical control measures being costly and may favour development of resistance in pathogens, the potential alternative methods have been explored in the present studies. Fifteen medicinal plants extracts viz Andrographis paniculata, Calotropis procera, Pongamia glabra, Azadirachta indica, Terminalia alata, Cassia montana, Cissampelos pareira, Leucas aspera, Vitex leucoxylon, Caesalpinia pulcherrima, Datura stramonium, Aristolochia indica, Rinchosia beddomi, Phyla arvencis and Eukaliptas globules were evaluated for their efficacy against stem rot of rice. The results concluded that the amendment of 2% of extracts of Andrographis paniculata Calotropis procera and Eucalyptus globules were found significantly more effective as an alternative to conventional chemical fungicide.

KEY WORDS

In vitro, antifungal activity, plant extract, Sclerotium oryzae, Rice.

INTRODUCTION

Rice is an important cereal crop affected by fungal diseases, amongst stem rot is the most important devastating rice disease. The fungal pathogen *Sclerotium oryzae* catt. Perfect state *Magnaporthe salvinni catt.,* found to be most destructive under favorable weather conditions in rice growing areas of the world which eventually causes substantial spectrum of disease. The disease causes yield loss up to 75% [1] through reduced tillering unfilled panicles; chalky grain decreased milling yields and increased lodging. Such as Physical, chemical and biological control methods have been employed for effective control the disease. Increasing awareness about the risks involved in chemical pesticides, these chemicals are not readily biodegradable; and develop new physiological races of pathogens [2,3] This led to the immediate need for the development of novel fungicides that are more effective, economically feasible and eco-friendly than the conventional fungicides. Antifungal compounds from plant origin are most suitable being less toxic and more environmentally compatible by nature. From past decades variety of plants have been screened for antifungal activities and valuable results have been achieved [4,5,6]. Keeping this in mind, our present investigation was aimed at phytochemical screening and antifungal assay of fifteen medicinal plant extracts against *Sclerotium oryzae*.

MATERIAL AND METHODS

Preparation of medicinal Plant extracts

Fifteen medicinal plant leaves were collected from the Sri Venkateswara University campus, and were identified by the taxonomist, the voucher specimens were deposited in Department of Botany. The leaves

were thoroughly washed, shade dried at room temperature and grounded using mechanical grinder, leaf powder was extracted in distilled water. Fifty grams of leaf powder was soaked in 300ml of distilled water in a conical flask and loaded on to an orbital shaker at a speed of 120 rpm for 24 hours, the mixture was filtered using whatmann No-1 filter paper and filtrate was concentrated using rotary evaporator and dried using a lyophilizer. The dried extract was collected in an air tight container and stored at 4°C [7].These extract was used for inhibitory studies on *Sclerotium oryzae.*

Preliminary phytochemical analysis; dried and powdered plants leaves material was used in the photochemical studies. The presence or absence of phytoconstituents such as alkaloids, steroids, flavonoids, tannins, saponins, diterpenoids, and amino acid in leaf extracts were assessed by standard phytochemical methods [8, 9].

Isolation of *Sclerotium oryzae*

Stem rot disease infected plant parts were collected from the rice fields of Nellore district. Plant sections of 3-5 mm^2 were cut from the margin of the infected lesions and sterilized for one minute in 1.0% sodium hypochlorite solution and rinsed three times in sterilized distilled water. The sterile pieces were blot dried and placed on potato dextrose agar plates. The plates were incubated at ambient conditions of light and temperature at 25±2°C and observed every day until five days, which were subsequently sub cultured to obtain axenic cultures.

Anti-fungal activity assay

Determination of percent mycelial inhibition by dry mycelial weight technique

The aqueous extracts of 15 plant species were amended to Richard's solution to achieve 10% concentration of the plant extract in the liquid medium. Fifty ml of extract amended media which was taken in a 100 ml Erlenmeyer conical flask and autoclaved. Richard's solution without any aqueous extract of test plants served as control. The flasks were inoculated with 5 mm diameter mycelia disc of *Sclerotium oryzae* taken from 7 days old culture and incubated for 7 days at 22 ± 1°C temperature under alternate cycles 12 h. light and 12 h. darkness. After incubation the content of the each flask were poured into a preweighed Whatman No.1 filter paper. The filter paper with the mycelial mat was dried in an oven at 60°C until a constant weight was reached. Three replicates were maintained for each treatment [10].The percent inhibition of mycelial growth was calculated using the formula:-

Percent inhibition = C – T / C x 100

Where C = Mycelial weight in control and T = Mycelial weight in treatment.

RESULTS AND DISCUSSION

Exploitation of naturally available chemicals from plants, which retards the reproduction of undesirable microorganisms, would be a more realistic and ecologically sound method for plant protection and will have a prominent role in the development of future commercial pesticides for crop protection strategies, with special reference to the management of plant diseases [11, 12]. Considering these as a first step in the present investigation, fifteen plants were screened in vitro for antifungal activities against important phytopathogenic fungi *Sclerotium oryzae*. These plants were selected based on traditional medicine knowledge and random choosing from the local flora.

Investigations on the phytochemical screening of 15 plant extracts (Table 2) revealed the presence of saponins, steroids, tannins, glycosides, alkaloids and flavornoids. These compounds are known to be biologically active and therefore aid the antimicrobial activities. The mycelial growth was inhibited significantly (Table1). The highest percentage inhibition (69.46%) was observed in *Andrographis paniculata* extracts which was statistically significant compared to control and other treatments. The second highest inhibition (66.40%) was observed in *Calotropis procera* extract the lowest percentage inhibition (45.54%) was observed in *Cassia montana* leaf extract (Table1). Inhibitory effects of plant leaf extracts have also been observed on viruses and other soil fungi [13]. Hence exploration of alternative anti fungal agents, especially the plant extracts has merits, to explore against several fungal diseases [14, 15]. In our study six plants exhibited more than 50% of inhibition activity against the pathogen in in-vitro experiment. These plants have been reported to possess antifungal properties against different fungi,

[16] studied antifungal characteristics of *Ocimum sanctum* L. and found that its leaf extract completely inhibited the growth of *Sclerotium rolfsii* and other fungi. Leaf decoction of *Acacia nilotica, Calotropis procera, Datura stramonium, Dodonea vicosa* and *Rhazya stricta* were found to be effective in processing urediospore germination on detached leaves of wheat [17]. Leaf extract of *Datura stramonium* reduced the development of rust pustules on the leaves of wheat [18]. *Andrographis paniculata* has significant inhibition for a gram positive microbes, *Staphylococcus aureus* and *Bacillus subtilis*, [19] Aqueous leaf extract of *Allium sativum, Datura alba* and *Withana somnifera* inhibited the growth of *Alternaria alternata*, *A. brassicola* and *Myrothecium roridum*[20]. Aqueous plants extracts of *Allium cepa, Calotropis procera*, *Chenopodium album, Chenopodium murale, Azadirachta indica* and Cannabis *sativa* were used for antifungal activity against *Acrophomina phaseolina, Alternaria radicina, Helminthosporium tusricum* and *Ascochyta rabiei*[16] .

Table 1: Effect of aqueous leaf extracts of some medicinal plants on *Sclerotium oryzae*.

Sr. No	Plant Name	Mycelial dry weight (mg)at different Concentrations		
		0.5%	1.0%	2.0%
1	*Andrographis Paniculata*	150	100	70
2	*Calotropis procera*	160	130	85
3	*Pongamia glabra*	220	165	110
4	*Azadirachta indica*	155	110	90
5	*Terminalia alata*	240	180	130
6	*Cassia montana*	245	180	150
7	*Cissampelos pareira*	205	145	105
8	*Leucas aspera*	190	150	125
9	*Vitex leucoxylon*	215	160	130
10	*Caesalpinia pulcherrima*	230	175	140
11	*Datura stramonium*	190	120	100
12	*Aristolochia indica*	200	150	105
13	*Rinchosia beddomi*	185	145	100
14	*Phyla arvencis*	180	135	90
15	*Eukaliptas globules*	175	120	100
16	Control	350	350	350

Table 2: Preliminary photochemical studies

Phytoconstituents	ap	cp	pg	az	ta	cm	cap	la	vx	csp	ds	ai	rb	pa	eu
Alkaloid	-	+	+	-	-	-	+	+	+	+	+	+	+	-	-
Steroid	+	+	+	-	+	-	+	+	+		+	-	-	+	+
Flavonoid	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+
Tannin	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+
Saponin	-	+	+	-	+	-	-	+	+	-	-	+	+	-	+
Aminoacid	-	-	-		-	+	+		+	+	-		+	-	-
Diterpenoid	+	-	+	+	+	-	+	+	+	+	+	+	+	+	-

Abbreviations:

ap = *Andrographis Paniculata,* **cp** = *Calotropis procera,* **pg**= *Pongamia glabra,* **az** = *Azadirachta indica,* **ta** = *Terminalia alata,* **cm** = *Cassia Montana,* **cap**=*Cissampelos pareira,* **la** = *Leucas aspera,* **vx** = *Vitex leucoxylon,* **csp** = *Caesalpinia pulcherrima,* **ds** = *Datura stramonium,* **ai** = *Aristolochia indica*, **rb** = *Rinchosia beddomi,* **pa** = *Phyla arvencis*, **eu** = *Eukaliptas globu*

The plant world is a rich storehouse of natural chemicals that could be exploited for use as pesticides. The total number of plant chemicals may exceed 4,000,000 and of these 10,000 are reported to be found secondary metabolites which play a major role in the plants is reportedly defensive[21]. Higher plants are much more important in the production of economically important organic compounds, pharmaceuticals and pesticides [22]. Many species of medicinal plants have not been surveyed for chemical or biologically active constituents and new sources of commercially valuable pesticides [23]. This is mainly due to lack of information on the screening/evaluation of diverse plants for their antibacterial activity. Biologically active plant derived pesticides are expected to play an increasingly significant role in crop protection strategies.

REFERENCE

1. *Kumar, A. Ram Singh and Jalali, B.L. Management of stem rot of rice with resistance inducing chemicals and fungicides. Indian Phytopath. 56: 266-269, (2003)*
2. *Ocamb, C. M., Hamm, P. B. and Johnson, D. A. Benzimidazole resistance of Fusarium species recovered from potatoes with dry rot from storages located in the Columbia basin of oregon and Washington. American Journal of Potato Research, 84,169-177, (2007)*
3. *Siripornvisal, S. Antifungal Activity of Ajowan Oil against Fusarium oxysporum KMITL Sci. Tech. J. Vol. 10 No. 2 (Jul. - Dec. 2010)*
4. *Bansal, R.K., and Gupta, R.K., Evaluation of plant extracts against Fusarium oxysporum, wilt pathogen of fenugreek, Indian Phytopathology, 53, pp 107-108, (2000)*
5. *Zaker, M and H. Mosallanejad, Antifungal activity of some plant extract on Alternaria alternata, the causal agent of alternaria leaf spot of potato. Pak. J.Biol.Sci., 13:1023-1029,(2010)*
6. *Dwivedi SK, Neetu Dwivedi. Antifungal activity of some plant extracts against guava wilt pathogen. International Journal of Environmental Sciences Volume 3 No.1, (2012).*
7. *Telang S.M and M.M.V. Baig,. Effect of different storage periods on seed mycoflora, seed germination and seedling emergence of Brinjal Var, Local Seeds treated with leaf powder of Azadirachta indica, Deccan Current Science, Vol. 3 No. II, cPp 220-226, (2010)*
8. *Siddiqui AA, Ali M Practical Pharmaceutical chemistry. Ist ed., CBS Publishers and Distributors, New Delhi, pp. 126-131,(1997)*
9. *Evans WC, Trease and Evan's Pharmacognosy. 5th ed., Haarcourt Brace And Company, pp. 336,(2002)*
10. *Kumar, S. and Prasad, G. Efficacy of medicinal plant (Andrographis paniculata) extract on aflatoxin production and growth of Aspergillus flavus. Letters in applied Microbiology 15: 131-132.(1992)*
11. *Varma, J. and Dubey, N.K. Prospectives of botanical and microbial products as pesticides of Tomorrow. Current Science 76: 172-179,(1999)*
12. *Gottlieb, O.R., Borin, M.R. and Brito, N.R. (2002). Integration of ethnobotany and phytochemistry: dream or reality. Phytochemistry 60: 145-152,(2002)*
13. *Baker KF, .Evolving concepts of biological control of plant pathogens. Annual Review Phytopathol., 25: 66-87,(1997)*
14. *Khare. M N and Shukla B.N, Utility of plants in crop disease control.Vasundhara. 3, 1-15 (1998)*
15. *P.Singh, R Shukla, B Prakash., A. S Kumar Singh., PK Mishra.,NK.Dubey, 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 ,(2010)*
16. *Jalal AO, Ghaffar A, Antifungal properties of Ocimum sanctum L. National Symposium on the Status of Plant Pathology in Pakistan. Univ. of Karachi., pp. 283-287,(1992)*
17. *Rahber-Bhatti MH, Antifungal properties of plant leaf decoctions against leaf rust of wheat. Pak.J. Bot., 20:259-263,(1998)*
18. *Hussain I, Nasir MA, Haque MR, Effect of different plant extracts on brown rust and yield of wheat. J. Agric. Res., 30:27-131, (1992)*
19. *Hosamani P A, Lakshman H C, Sandeepkumar K and Rashmi C Hosamani Antimicrobial Activity of Leaf extract of Andrographis paniculata Wall. Science Research Reporter 1(2): 92 - 95, Sept. (2011)*
20. *Mughal MA, Nasir MA, Bokhari SAA, Antifungal properties of some plant extracts. Pak. J. Phytopath., 10: 62-6,(1998)*
21. *Grayer, R.J. and Harborne, J.B. Survey of Antifungal compounds from higher plants 1982-1993-review. Phytochemistry 37:19-42.(1994)*
22. *Hostettmann, K. and Wolfender, J.L. The search for biologically active secondary metabolites. Pesticide Science 51: 471-482,(1997)*
23. *Varma, J. and N.K. Dubey, 1999. Prospective of botanical and microbial products as pesticides of tomorrow. Curr. Sci. 76 (2): 172–179.(1997).*

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