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CONSERVATION OF WILD TAXA: PRESENT SCENARIO

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STUDIES ON GROWTH OF *MACROPHOMINA PHASEOLINA* WITH EFFECT OF DIFFERENT SOLVENT CONCENTRATIONS OF RHIZOME EXTRACT OF *ZINGIBER OFFICINALE*

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ABSTRACT:

Macrophomina phaseolina (Tassi) Goid is a soil borne fungus causes root rot diseases to Sarpagandha (*Rauwolfia serpentina*). The fungus infects the root and lower stem of over 500 plant species and is widely distributed in the United States (Wyllie, 1988). The efficacy of *Zingiber officinale* rhizome extract against *Macrophomina phaseolina* was studied by using aqueous, Methanol and Acetone solvents at different concentrations i.e., 1.00, 2.00, 3.00, 4.00 and 5.00 % for their antifungal efficacy.

KEYWORDS:- *Macrophomina phaseolina*, Sarpagandha, *Zingiber officinale*, Methanol, Acetone, etc.

MATERIALS AND METHODS:

In order to study of antifungal activity of *Zingiber officinale* leaf extract on *Macrophomina phaseolina.* Locally available rhizome of *Zingiber officinale* used i.e., rhizome of *Zingiber officinale* was tested by poisoned food technique in vitro as used by Shiva et.al, (2008) and Francis Borgio, et.al, (2008) to know their inhibitory effect on the growth of *Macrophomina phaseolina.*

1) Preparation of aqueous plant part extracts:

Healthy fresh leaves, rhizomes and bulbs were collected from locally available these different plants, washed thoroughly with fresh water and finally rinsed with sterile distilled water.

Fifty gram of leaves, rhizomes and bulbs of different plants were cut into small pieces and grinded in a grinder by adding 50 ml sterile distilled water. Extracts thus, obtained were

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filtered through double layered muslin cloth in 150 ml flasks and plugged. The concentration of extract was considered as 10 %. The extracts then autoclaved at pressure 15 lbs for 20 minutes. Potato Dextrose Agar (PDA) medium was prepared and sterilized at 15 lbs pressure for 20 minutes. The sterilized extracts were considered as standard plant extracts and used for the testing of antifungal activity.

The different concentrations were prepared i.e. 1.00, 2.00, 3.00, 4.00, 5.00, 6.00, 7.00, 8.00, 9.00 and 10.00 percent. The 10 ml extracts of different concentrations were individually added in 10 ml melted, cooled and sterilized PDA at the time of pouring in the petriplates and incubated at room temperature. After solidification, a 5 mm disc of actively growing 7 days old pure culture of *Macrophomina phaseolina* was incubated aseptically in the centre of plate. Three repetitions were made for each treatment. Medium without phytoextracts served as control. The observations of fungal growth in diameter were observed and recorded and percent growth inhibition was also worked out as per the method given by.

2) Preparation of Methanolic plant part extracts:

Healthy fresh leaves of Ashwagandha (*Withania somnifera L.)* was taken, washed thoroughly with fresh water and finally rinsed with sterile distilled water and dried.

Fifty grams leaves of Ashwagandha (*Withania somnifera L.)* were cut into small pieces and grinded in a grinder to make fine powder and then extracted in 50 ml Methanol. Extracts thus obtained were filtered through double layered muslin cloth in 150 ml flasks and plugged. The extracts then autoclaved at pressure 15 lbs for 20 minutes. Potato Dextrose Agar (PDA) medium was prepared and sterilized at 15 lbs pressure for 20 minutes. The sterilized extract was considered as standard plant extract and used for the testing their antifungal activity.

The different concentrations were prepared i.e. 1.00, 2.00, 3.00, 4.00, 5.00, 6.00, 7.00, 8.00, 9.00 and 10.00 percent. The 10 ml extracts of different concentrations were individually added in 10 ml melted, cooled and sterilized PDA at the time of pouring in the petriplate and incubated at room temperature. After solidification a 5 mm disc of actively growing 7 days old pure culture of *Macrophomina phaseolina* was inoculated aseptically in the centre of plate. Three repetitions were made for each treatment. Medium without phytoextracts served as control. The fungal growth in diameter were observed and recorded and percent growth inhibition was also calculated as per the procedure given by Syeda Fakehha et.al. (2012).

3) Preparation of Acetone plant part extracts:

Healthy fresh leaves, rhizomes and bulbs were taken, washed thoroughly with fresh water and finally rinsed with sterile distilled water and dried.

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Fifty gram of dried leaves, rhizomes and bulbs of different plants were cut into small pieces and grinded in a grinder to make fine powder and then extracted in 50 ml Acetone. Extracts thus, obtained were filtered through double layered muslin cloth in 150 ml flasks and plugged. The extracts then autoclaved at pressure 15 lbs for 20 minutes. Potato Dextrose Agar (PDA) medium was prepared and sterilized at 15 lbs pressure for 20 minutes. The sterilized extracts were considered as standard plant extracts and used for the testing of antifungal activity. The different concentrations were prepared i.e., 1.00, 2.00, 3.00, 4.00, 5.00, 6.00, 7.00, 8.00, 9.00 and 10.00 percent. The 1 ml extracts of different concentrations were individually added in 15 ml melted, cooled and sterilized PDA at the time of pouring in the petriplates and incubated at room temperature. After solidification a 5 mm disc of actively growing 7 days old pure culture of *Macrophomina phaseolina* was incubated aseptically in the centre of plate. Three repetitions were made for each treatment. Medium without phytoextracts served as control. The observations of fungal growth in diameter were observed and recorded and percent growth inhibition was also worked out. Khajista, et.al., (2013).

The sclerotial formation was determined by observing number of sclerotia per microscopic field.

Experimental results AND DISCUSSION:

The efficacy of *Zingiber officinale L.* against *Macrophomina phaseolina* with aqueous extract treatment treated at different concentrations i.e., 1.00, 2.00, 3.00, 4.00 and 5.00 % for their antifungal efficacy using standard Poisoned Food Technique.

The treatment of 1 % concentration of aqueous solution shows 11.34 to 81.75 %, at 2% concentration gives 18.77 to 83.15 %, at 3 % concentration shows 23.25 to 85.38 %, at 4 % concentration gives 28.85 to 90.00 % and at 5 % concentration gives 28.94 to 93.50 % inhibition of the pathogen growth with water solvent viz. recorded at 1 to 7 days of incubation period. The efficacy of *Zingiber officinale,* at 5 % concentration gives maximum inhibition of growth of pathogen. The increase in concentration gives maximum inhibition of pathogen i.e. 93.50 % at 5 % concentration on 7 days of incubation as mentioned in table 1

The efficacy of *Zingiber officinale L.* against *Macrophomina phaseolina* by using methanol extract was evaluated at different concentrations i.e., 1.00, 2.00, 3.00, 4.00 and 5.00 % for growth control by using standard Poisoned Food Technique as shown in table 2.

Zingiber officinale efficacy at 1 % concentration shows 12.75 to 80.77 % in 1 to 7 days of incubation periods, at 2% concentration gives 21.22 to 89.20 %, at 3 % concentration shows 25.35 to 87.95 %, at 4 % concentration gives 27.90 to 90.55 % and at 5 % conc. gives 28.98 to

92.47 % inhibition of the growth of the pathogen with methanol solvent viz. recorded at 1 to 7 days of incubation period. The efficacy of *Zingiber officinale,* at 5 % conc. gives maximum inhibition of growth of pathogen with increase in incubation period.

For the control of growth of *Macrophomina phaseolina, Zingiber officinale L.* rhizome acetone extract was tested at different concentration percentage i.e., 1.00, 2.00, 3.00, 4.00 and 5.00 % for their antifungal efficacy by using standard Poisoned Food Technique as shown in table 3.

Zingiber officinale efficacy at 1 % concentration shows 14.25 to 81.90 %, at 2% concentration gives 19.49 to 86.10 %, at 3 % concentration shows 24.85 to 86.90 % , at 4 % concentration gives 27.76 to 88.35 % and at 5 % concentration gives 28.75 to 90.27 % inhibition of the growth of the pathogen with acetone solvent viz. recorded at 1 to 7 days of incubation period. The efficacy of *Zingiber officinale,* at 5 % concentration gives maximum inhibition of pathogen growth with increase in incubation period.

Table -1: Effect of aqueous rhizome extract of *Zingiber officinale* on growth of *Macrophomina phaseolina.*

Incubation Period (Days)	Percent inhibition Concentration (%)				
	1.00	2.00	3.00	4.00	5.00
1	11.34 (6.51)	18.77 (10.81)	23.25 (13.44)	28..85 (16.76)	28.94 (16.82)
2	20.30 (11.71)	26.35 (15.27)	33.45 (19.54)	35.50 (20.79)	36.89 (21.64)
3	36.72 (21.54)	41.27 (24.37)	48.32 (28.89)	49.75 (29.83)	51.87 (31.24)
4	51.82 (31.20)	50.95 (30.62)	55.25 (33.53)	58.50 (35.80)	64.34 (40.04)
5	62.22 (38.47)	67.39 (42.67)	68.39 (43.14)	72.42 (46.90)	79.90 (53.92)
6	70.45 (45.20)	72.34 (46.33)	76.80 (50.81)	82.26 (56.48)	85.65 (60.12)
7	81.75 (55.90)	83.15 (57.14)	85.38 (60.32)	90.00 (64.17)	93.50 (69.26)

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S.E ±	3.07	2.95	3.51	2.78	3.24
C.D at 5%	9.44	9.08	1.08	8.56	9.96

Figures in parenthesis are ARCSIN transformed value.

Table -2: Effect of methanolic rhizome extract of *Zingiber officinale* on growth of *Macrophomina phaseolina.*

Incubation Period (Days)	Control (Methanol)	Percent inhibition				
		Concentration (%)				
		1.00	2.00	3.00	4.00	5.00
1	7.15 (4.64)	12.75 (7.32)	21.22 (12.25)	25.35 (14.68)	27.90 (16.19)	28.98 (16.84)
2	9.25 (5.30)	24.30 (14.06)	32.35 (18.87)	35.44 (20.75)	36.58 (21.04)	37.85 (22.23)
3	12.10 (6.94)	43.76 (25.94)	47.39 (28.28)	50.67 (30.44)	50.75 (30.49)	52.45 (31.63)
4	15.35 (8.82)	57.85 (35.34)	58.67 (35.92)	65.25 (40.72)	69.80 (44.26)	73.74 (47.50)
5	18.44 (10.62)	72.46 (46.43)	74.35 (48.46)	74.36 (48.57)	78.80 (52.59)	79.90 (53.85)
6	21.56 (12.45)	76.46 (50.37)	79.85 (53.70)	79.88 (53.01)	82.65 (56.68)	86.65 62.16)
7	22.75 (13.14)	80.77 (54.84)	89.20 (63.13)	87.95 (64.42)	90.55 (64.90)	92.47 (67.64)
S.E ±	0.42	2.90	2.97	3.91	3.23	3.76
C.D at 5%	1.30	8.93	9.15	12.02	9.93	11.58

Figures in parenthesis are ARCSIN transformed value.

Table -3: Effect of acetone rhizome extract of *Zingiber officinale* on growth of *Macrophomina phaseolina.*

Incubation Period (Days)	Control (Acetone)	Percent inhibition				
		Concentration (%)				
		1.00	2.00	3.00	4.00	5.00
1	8.15 (5.22)	14.25 (8.19)	19.49 (11.23)	24.85 (14.38)	27.76 (16.11)	28.75 (16.70)

2	10.24 (5.87)	33.60 (19.63)	40.80 (24.07)	44.48 (26.40)	49.50 (29.66)	50.72 (30.47)
3	11.55 (6.63)	48.95 (29.30)	52.00 (31.33)	54.75 (33.19)	55.75 (33.88)	57.15 (34.85)
4	13.00 (7.46)	58.75 (35.97)	62.37 (38.58)	65.35 (40.80)	67.75 (42.64)	68.30 (43.47)
5	16.44 (9.46)	69.35 (43.90)	72.40 (46.38)	74.58 (48.22)	75.45 (49.60)	76.30 (49.72)
6	18.35 (10.57)	75.55 (49.69)	78.75 (52.69)	81.50 (55.63)	85.25 (59.93)	87.35 (62.39)
7	22.10 (12.76)	81.90 (55.77)	86.10 (61.33)	86.90 (61.76)	88.35 (62.07)	90.27 (64.52)
S.E ±	0.42	2.86	3.66	3.41	3.38	3.11
C.D at 5%	1.30	8.82	11.26	10.50	10.42	9.58

Figures in parenthesis are ARCSIN transformed value.

REFERENCES:

1. Choudhari, S. S, and B. M. Kareppa. (2013). Identification of bioactive compounds of *zingiber officinale roscoe* rhizomes through gas chromatography and mass spectrometry. Int. J. Pharmaceutical Research and Development (IJPRD). 5 (8) : 16-20.
2. Choudhari, S. S. and Kareppa B. M., (2013). Studies on Biochemical Changes in Healthy and infected Ginger (*Zingiber Officinale Roscoe*). Int. J. Biological & Pharmaceutical Research. 4 (8): 556-558.
3. Cloud, G. L. and Rupe, J. C. 1991. Comparison of three media for enumeration of sclerotia of Macrophomina phaseolina. Plant Disease 75:771-772.
4. Dhinga, O. D. and Sinclair, J. B. 1977. An annotated bibliography of *Macrophomina phaseolina*. 1905-1975. Universidade Federal de Vicosa, Minas Gerais, Brazil.
5. Dohrro, N. P., O. Sharma., M. Sharma and Sarlaca, R. S. (1994). Effect of organic amendments of soil on rhizome rot, nematodes and rhizosphere mycoflora of ginger (*Zingiber officinale Roscoae*). Annals of Biology. 10 (2): 208-210.

6. Francis, Borgio, J., Jesvin Bency, B. and Neha Sharma. (2008). Compatibility of *Metarhizium anisopliae* (metsch) sorok with *Oscimum sanctum L. (*Tulsi) extracts. J. Ethnobotanical leaflets. 12 : 698-704.

7. Hesamedin, Ramezani. (2008). Biological control of root-rot of Eggplant caused by *Macrophomina phaseolina.* American-Eurasian J. Agric. & Environ. Sci. 4 (2) : 218-220.

8.Hina, Ashraf and Arshad Javaid. (2007). Evaluation of antifungal activity of Meliaceae family against *Macrophomina phaseolina*. Mycopath.5(2) : 81-84.

9. Khajista, Jabeen, Nidra Waheed and Sumera Iqbal. (2013). Antifungal Potential of *Calotropis procera* against *Macrophomina phaseolina,* Life Sci. J. 10 (12s).

10. Mukadam, D.S,. Patil M.S., Chavan A.M and Anjali R. Patil 2006. The illustrations of Fungi.

Saraswati Printing Press, Aurangabad.

11. Mukerji, K. G. and Jayanti Bhasin. (1986). Plant Diseases of India- A Source Book. TataMcGraw-Hill Publishing Company New Delhi. : 212.

12. Nagmani, A., Kulkarni I. K. and Manoharachary, C. (2006). Hand book of soil fungi. I. K. International Pvt. Ltd., New Delhi.

13. Rao, T. G. N., B. Sasikumar and J. K. George. (1995). Field reaction of ginger germplasm *to Phyllostica zingiberi.* Indian Phytopath. 48 (4) : 463-465.

14. White, D. G.1999. Fungal stalk rots. Compendium of Corn Diseases 3rd Edition. D. G. White

ed. APS Press. St. Paul, MN.

15. Wyllie, T. D. 1988. Charcoal rot of soybean-current status. In Soybean diseases of the north Central region. T. D. Wyllie and D. H. Scott, eds. APS Press, St. Paul, M N.

16. Shiva, N., Ganesan S., Banumathy N. and Muthuchelian. (2008). Antifungal effect of leaf extract of some medicinal plant against *Fusarium oxysporium* causing wilt disease of *Solanum melogena L.* Ethnobotanical leaflets. 12 : 156-163

17. Syeda, Fakehha Naqvi, Arshad Javaid and Amna Shoaib. (2012). Evaluation of antifungal activity of methanolic extracts of *Dicanthium annulatum* for the management of *Macrophomina phaseolina.* African J. Microbiology Research. 6 (29): 5882-5886.