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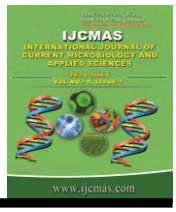

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Assessment of Antifungal Activities of Phytoextracts against *Rhizoctonia solani, Kuhn* Causing Sheath Blight of Rice

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A B S T R A C T

In vitro evaluation of twelve phytoextracts (aqueous and methanolic extract) of locally available plants having antimicrobial properties were done against *Rhizoctonia solani*, Kuhn by sequentially highering the concentration of the extracts i.e. at 1%,2.5%, 5% and 10%. The poisoned food technique employed for the purpose.Among all phytoextracts, clove extracts of garlic gave highest inhibition against the fungal growth in all concentrations of its both solvent extracts *i.e.* 43.23% to 82.73% in aqueous extract and 51.50% to 90.27% in methanolic extract followed by turmeric and datura respectively. The highest radial growth inhibition was achived by methanolic extract of garlic @ 10% (90.27%) followed by its aqueous extract(82.73%) and turmeric aqueous extract(65.23%) respectively in the same concentration. The best performing phytoextracts were tested *in vivo* in pot culture and in the field(2018 kharif & 2019 kharif) to confirm their efficacy against the fungus inciting sheath blight in rice. The garlic methanolic extract was treated along with its nano-emulsion *in vivo* condition and nano-emulsion of methanolic extract was found most effective by reducing the percent disease index in both the years(2018 kharif and 2019 kharif). Carbendazim 50WP(1l/lit) was used as standard fungicide for comparison.

Introduction

Rice (*Oryza sativa*) is the cereal having a world consumption of 450 million tonnes 90% of this rice consumed in Asia, including the region's 560 million people still affected by hunger (IRRI strategic plan 2017-25). India is the second largest country in terms of production and consumption of rice in the world. In India it is cultivated in 42 million ha (Prasad, 2001). There are about 37 fungal diseases that have been reported in rice (Ou,

1985). Sheath blight is a fungal disease of rice which economically comes second after blast disease. The fungus belongs to the basidiomycetes group *i.e.* anastomosis and non-spore forming. It is soil-borne, necrotroph in nature and producing sclerotia in soil for its survival and perpetuation from season to season. This fungus is highly polyphagous in nature and ability to form complexes by intermingling with other anastomosis group that is why no resistant variety sustained against the disease till now. In the present time, the indiscriminate use of synthetic pesticides has created a threat to mankind by polluting the environment and causing other major issues like pest resurgence (minor pest becomes major one), reducing the sustainability of the land in terms of agricultural production. So, moving towards organic farming, use of phytoextracts for plant disease management can be a substitute to the synthetic chemicals. As the botanicals have antimicrobial properties and have the ability to induce resistance in plant against the pathogens, here an attempt has been made to report the botanicals that has been used in these years to manage the particular disease.

Materials and Methods

Twelve locally available plants having antimicrobial properties were collected, washed properly and air dried. For aqueous extract preparation, 50g of plant material along with 50 ml double distilled water (1:1w/v) was taken and grinded. The extract was collected in a muslin cloth and squeezed. The filtrate was collected and centrifuged @ 5000rpm for 10 minutes. The supernatant was collected and passed through coarse filter paper followed by fine filter paper twice. The filtrate was then passed through Tarson's filter apparatus which contains 0.2µm pore size filter paper with the help of a vaccum suction pump to make the final extract bacteria and other microbe free. The concentration of the extract was 100%, the required amount of concentration of the extract was made by diluting the particular extract with required amount of molten potato dextrose agar media. The procedure was practised in sterilized condition. For methanolic extract preparation, the plant materials were subjected to pulverization after shade drying to get coarse powder. The particular plant powder was placed in soxhlet apparatus with methanol (80%) and the extraction was done at 55° C for 6 hours. Then, the extract was placed in rotary evaporator @120 rpm at 227-228 mp vaccum in 45° C for 5 hours. The final crude extract obtained after the extraction was taken as stock solution (100%) and further dilution was made by adding the required amount molten media. For preparation of nano-methanolic emulsion, 1ml of garlic methanolic extract was mixed with 1ml of Tween 20 and 8 ml of distilled water by vortexing (2 minutes).

After vortexing, the tube containing the mixture was placed inside sonicator (37% ampl., room temperature) for 10 minutes for formation of nano-emulsion. For stability checking, phase separation and particle size analyzer was used.

Preliminary screening of the phytoextracts were made by Poisoned food technique (Vincent, 1947). 90mm petriplates containing 20ml of poisoned potato dextrose agar (PDA) media were used. Calculated amount of extracts were mixed with the molten media and plating was done for solidification. For each treatment, three replications were maintained. 5mm disc of hyphal mat was cut from edges of 5 days old culture plate of fungus and inoculated at the centre of the poisoned plate and incubated at 27±1℃. The radial growth measurement of the fungus was done in regular interval till the control plate showed the full growth. The efficacy of the

phyto extracts was expressed by per cent radial growth inhibition over the control which was determined by the formula(Vincent,1947)

$$\% \text{ Radial growth inhibition (I)} = \frac{\text{Radial growth in control (C)} - \text{Radial growth in treatment (T)} \times 100}{\text{Radial growth in control (C)}}$$

The best performing phytoextracts of *in vitro* study were tested in pot culture and in field condition to know their efficacy in semi-natural and natural environment. In pot culture experiment, (45cm X 30 cm) sized pot was used. Each pot contained 4 kg of soil. 21 days old seedling was transplanted in each pot. Three number of hills were maintained in each pot. After 25 days of transplanting, three primary tillers were selected and inoculated with 5mm mycelial disc cut from edges of 7 days old fresh culture plate. In order to avoid the falling of disc from sheath, the disc was wrapped with wet cotton around the sheath. Two sets of treatments were maintained. In one set, there were pre-inoculation spray of treatments before 24 hours of inoculation and in another; there were post-inoculation spray after 24 hours of the inoculation. The spraying was done by atomizer. Since, the pathogen requires high humidity for infection initiation, spraying of water was done every day near the point of inoculation of the plants. In control pots, solvents were sprayed to evaluate whether the solvent has any hazardous impact on plant or not. Absolute control was maintained and spray with 1% carbendazim was done for comparative study. Data was recorded in each 7 days interval after first appearance of the disease symptom i.e. after 3 days of inoculation(DAI) upto 17 DAI. Here, Relative Vertical Spread (RVS) was taken in to account for disease severity study. The vertical spread and total plant height were measured and relative vertical spread (RVS) was calculated using the formula given by Ahn *et al.*, (1986).

$$\text{RVS} = \frac{\text{Vertical spread}}{\text{Plant height}} \times 100$$

Rice sheath blight grade chart (IRRI, 1996)

Disease Vertical spread grade	
0	No infection observed
1	Lesion limited to lower 20 per cent of the height of the plant
3	Lesion limited to 21-30 per cent of the height of the plant
5	Lesion limited to 31-45 per cent of the height of the plant
7	Lesion limited to 46-65 per cent of the height of the plant
9	Lesion more than 65 per cent of the height of the plant

Field experiment was conducted in Randomized Block Design. Each treatment had three replication. (6m X 3m) sized plot were taken for each replication. Judicious use of fertilizer was used and water level was maintained up to optimum level to facilitate disease initiation. In field, inoculation was done in 'straw bead method' *i.e.,* at the active tillering phase of the plants. Like pot culture experiment, two sets of treatments were maintained for pre-inoculation spray and post-inoculation spray. Sprays were done as per pot culture experiment. Data was recorded before the spray and in each 10 days interval after inoculation up to 30 days. For disease severity scoring was recorded by following the scale of SES, IRRI, 1996. Per cent Disease Index (Wheeler, 1969) and Grain Yield (kg/ha) were the parameters which taken in to consideration for data analysis.

$$\text{Per cent Disease Index} = \frac{\text{Sum of rating}}{\text{Number of plants observed}} \times \frac{100}{\text{Maximum Scale}}$$

Results and Discussion

Primary screening of the phytoextracts with two different solvents(aqueous and methanol) was done by poisoned food technique. Initially 12X2 = 22 plant extracts were tested in four concentration i.e.1%, 2.5%, 5% and 10%. Among all extracts methanolic extract of garlic @ 10% was shown the highest inhibition of radial growth *i.e.* 84.67% followed by garlic aqueous extract (82.73%), turmeric aqueous extract (65.23%) and datura (*Datura metel*) aqueous extract (53.10%) respectively at that same concentration. *In vitro* study revealed that both the extracts of garlic in all concentrations showed significant superiority than other phyto extracts after 72hours of inoculation (Table 1). The garlic (*Allium sativum*) extract also restricted the formation of sclerotia in the plates. This finding is in agreement with the work of Kumar *et al*., (2017) who found garlic bulb extract gave highest radial growth inhibition of *Rhizoctinia solani* (80.19%) @ 10% and also with the finding of Yadav (2007). The nano-emulsion of methanlolic extract and methanolic extract of garlic has the significant effect on inhibition of the fungus. This implies methanol is a better solvent for consistant extraction of active ingredients of the plant extracts as compared to other solvent as reported by Lin *et al*., (1999) and Eloff *et al*., (1998). In pot experiment and field experiment, pre-inoculation spray of phytoextracts was given better result than the post-inoculation spray(Table 2 and Table 3). This result corroborates with the finding of Choudhary *et al*., (2017) who also found the pre-inoculation spray of botanicals, specially clerodendrum choloform extract had given highest inhibition of disease in green house as well as in field condition. The nano-emulsion of methanolic extract of garlic @ 1% (10,000ppm) was given significant reduction in percent disease index 31.15% over control was suggested that nano-emusion is more target specific and consumes less active ingredient of the phytoextract for effective result. This finding is in confirmation with the works of Ali *et al*., (2017) who found the nano-emulsion of neem and citronella oils most effective against *Rhizoctonia solani* and *Sclerotium rolfsii in vitro*. After garlic extract, turmeric (*Curcuma longa*) aqueous extract (10%) was the second effective extract against the fungus in pot culture (RVS ranges from 24.04% to 32.79%).

Methanol extracts of several plants of Zingiberaceae family showed great potential as antifungal and antibacterial properties (Yusuf *et al.*, 2001), little deviates from the present findings as the extracts of turmeric also belonged to the same family but the aqueous extract works better. In the field condition the effect of turmeric and datura aqueous extract were at par i.e. 16.49% and 18.50% over control respectively. The effect of datura(*Datura metel*) was not significant on disease control. This finding is in contrast to the reports of Kagale *et al*., (2004), Bhattacharya *et al*., (2013). Kagale *et al*., (2004) found methanolic extract of *Datura metel* inducing systemic resistance against *R.solani* and Bhattacharya *et al.*, also reported that ethanolic extract of *Datura metel* induced systemic resistance in rice against sheath blight fungus.

From the experiment, it was found that extracts of garlic(*Allium sativum*) was the most effective in managing the sheath blight fungus in a greater extent. It may be because of the biologically active compounds present in it which affect a wide range of soil-borne fungal pathogens as reported by Kyung and Lee(2001). Nano emulsion of garlic methanolic extract has the potential to manage the disease in a greates extent as it is target specific and ecofriendly and biodegradable.

Table.1 *In vitro* evaluation of plant extracts against *Rhizoctonia solani*

Sl. No	Plant Extract	Plant Parts used	Per cent Radial growth Inhibition(%) of *Rhizoctonia solani*							
			1%		2.5%		5%		10%	
			A	M	A	M	A	M	A	M
1	Ginger *(Zingiber officinalae)*	Rhizome	**17.27 (24.55)**	10.50 (18.9)	**28.30 (32.13)**	17.63 (24.83)	**35.30 (36.44)**	23.80 (29.18)	**40.57 (39.56)**	31.90 (34.38)
2	Kalmegh *(Andrographis paniculata)*	Leaves	6.80 (15.11)	5.60 (13.67)	9.27 (17.72)	7.57 (15.95)	11.97 (20.21)	9.30 (17.71)	17.53 (24.75)	13.23 (21.33)
3	Clerodendrum *(Clerodendrum infortunatum)*	Leaves	5.30 (13.31)	8.70 (17.15)	11.20 (19.49)	13.70 (21.66)	13.87 (21.86)	17.80 (24.95)	17.27 (24.55)	25.77 (30.50)
4	Carrot grass *(Parthenium hysterophorus)*	Leaves	13.40 (21.44)	2.80 (9.63)	24.60 (29.76)	3.90 (11.29)	27.27 (31.43)	7.27 (15.63)	28.13 (32.01)	13.67 (21.7)
5	Neem *(Azadirachta indica)*	Leaves	2.70 (9.46)	7.87 (16.28)	7.67 (16.42)	11.93 (16.32)	9.13 (17.89)	19.53 (26.22)	11.60 (19.90)	25.83 (24.78)
6	Tulsi *(Ocimum sanctum)*	Leaves	10.27 (18.69)	2.87 (9.72)	17.80 (24.95)	9.57 (17.97)	20.40 (26.83)	14.80 (22.58)	23.20 (28.78)	17.57 (34.96)
7	Begunia *(Vitex negundo)*	Leaves	9.23 (17.67)	11.53 (19.85)	13.80 (21.80)	17.43 (24.62)	22.23 (28.11)	28.73 (32.41)	24.20 (29.46)	32.83 (72.14)
8	Garlic *(Allium sativum)*	Cloves	**43.23 (41.11)**	51.50 (45.86)	**61.60 (51.71)**	72.83 (58.6)	**76.57 (61.12)**	84.67 (72.79)	**82.73 (65.48)**	90.27 (72.14)
9	Turmeric *(Curcuma longa)*	Rhizome	**16.20 (23.73)**	11.17 (19.45)	**34.23 (35.81)**	20.43 (26.87)	**43.50 (41.25)**	37.47 (37.72)	**65.23 (53.88)**	50.10 (44.02)
10	Datura *(Datura metel)*	Leaves	**13.73 (21.74)**	9.37 (17.80)	**22.17 (28.08)**	15.27 (22.92)	**32.60 (34.81)**	19.27 (26.02)	**53.10 (46.78)**	31.47 (34.09)
11	Ber/Common jujube *(Zizyphus jujuba)*	Leaves	5.80 (13.93)	2.97 (9.88)	9.20 (17.66)	5.73 (13.74)	11.63 (19.94)	9.97 (18.37)	14.40 (22.27)	13.57 (21.61)
12	Pink morning glory *(Ipomoea carnea)*	Leaves	7.47 (15.85)	9.13 (17.57)	12.73 (20.89)	14.63 (22.45)	15.43 (23.13)	17.83 (24.97)	17.43 (24.68)	21.87 (27.87)
13	Control	-	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SEm			0.29	0.60	0.43	1.40	0.95	1.50	0.72	0.96
CD at 5%			0.86	1.76	1.25	4.08	2.77	4.38	2.12	2.81

Table.2 Pot culture experiment of plant extracts against *Rhizoctonia solani*

Sl. No.	Treatment details	Relative Vertical Spread(%)			Per cent reduction over control(%)		
	Pre-inoculation Spray	3 DAI	10 DAI	17 DAI	3 DAI	10 DAI	17 DAI
1	Spray with garlic aqueous extract@ 10%	10.11 (18.54)	21.4 (27.56)	26.31 (30.86)	43.26 (41.13)	40.06 (39.26)	37.24 (37.60)
2	Spray with garlic methanolic extract@10%	8.80 (17.26)	19.42 (26.15)	23.90 (29.27)	48.82 (44.38)	44.41 (41.79)	42.96 (40.96)
3	Spray with garlic methanolic nano-emulsion@ 1%	7.10 (15.45)	15.71 (23.35)	19.50 (26.21)	58.24 (49.74)	55.01 (47.88)	53.46 (46.99)
4	Spray with turmeric aqueous extract @ 10%	10.50 (18.91)	23.40 (28.93)	28.16 (32.05)	41.01 (39.83)	34.45 (35.94)	32.79 (34.93)
5	Spray with ginger aqueous extract @ 10%	12.30 (20.53)	28.30 (32.14)	37.80 (37.94)	30.90 (33.77)	20.73 (27.08)	11.48 (19.80)
6	Spray with datura aqueous extract @ 10%	11.30 (19.64)	24.80 (29.87)	31.80 (34.33)	36.52 (37.29)	30.53 (33.70)	25.53 (30.35)
7	Spray with water(C)	17.8 (24.95)	35.70 (36.68)	42.7 (40.80)	0	0	0
8	Spray with solvent(C)	17.2 (24.50)	34.93 (36.22)	41.9 (40.37)	0	0	0
9	Absolute Control(No inoculation, No spray)	0	0	0	0	0	0
10	Spray with Carbendazim 50WP(1g/lit.)	0	0	0	0	0	0
SEm		0.38	0.90	1.17			
CD at 5%		1.13	2.67	3.45			

Table.3 Pot culture experiment of plant extracts against *Rhizoctonia solani*

Sl. No.	Treatment details	Relative Vertical Spread(%)			Per cent inhibition over control(%)		
	Post-inoculation Spray	3 DAI	10 DAI	17 DAI	3 DAI	10 DAI	17 DAI
1	Spray with garlic aqueous extract@ 10%	10.47 (18.86)	22.10 (28.04)	30.27 (33.36)	41.42 (40.06)	38.10 (38.12)	29.12 (32.66)
2	Spray with garlic methanolic extract@10%	9.23 (17.62)	22.40 (28.23)	29.10 (32.64)	46.42 (42.95)	15.15 (22.88)	30.44 (33.47)
3	Spray with garlic methanolic nano-emulsion@ 1%	8.27 (16.70)	14.20 (22.12)	23.80 (29.18)	52.03 (46.16)	46.21 (42.83)	43.11 (41.04)
4	Spray with turmeric aqueous extract @ 10%	14.23 (22.10)	25.30 (30.22)	32.43 (34.69)	20.34 (26.79)	29.13 (32.67)	24.04 (29.36)
5	Spray with ginger aqueous extract @ 10%	13.90 (21.89)	31.20 (33.95)	40.10 (39.29)	22.20 (28.11)	12.61 (20.80)	6.09 (14.19)
6	Spray with datura aqueous extract @ 10%	14.77 (22.59)	29.50 (32.89)	35.83 (36.70)	17.35 (24.62)	17.37 (24.63)	16.08 (23.64)
7	Spray with water(C)	17.87 (24.97)	35.70 (36.71)	42.70 (40.79)	0	0	0
8	Spray with solvent(C)	17.23 (24.53)	26.40 (30.87)	41.83 (40.29)	0	0	0
9	Absolute Control(No inoculation, No spray)	0	0	0	0	0	0
10	Spray with Carbendazim 50WP(1g/lit.)	0	0	0	0	0	0
SEm		0.73	0.90	1.20			
CD at 5%		2.15	2.65	3.55			

Table.4 Field experiment on efficacy of plant extracts against *Rhizoctonia solani*

Sl. No	Plant Extracts	Per cent Disease Index(PDI)			% reduction over Control	Yield (kg/ha)			% Yield increase over control
		2018 *kharif*	2019 *kharif*	Pooled		2018 *kharif*	2019 *kharif*	Pooled	
1	Spray with garlic aqueous extract	35.13 (36.63)	36.33 (37.07)	36.70	18.50	1774.67	1777.67	1776.17	13.18
2	Spray with garlic methanolic extract	32.77 (36.57)	33.27 (35.22)	35.05	21.90	2050.67	1975.33	**2013.00**	24.87
3	Spray with garlic methanolic nano-emulsion	25.43 (31.88)	27.40 (31.55)	30.90	31.15	2246.00	2440.00	2343.00	31.20
4	Spray with turmeric aqueous extract	38.50 (33.83)	38.70 (38.47)	37.48	16.49	1678.33	2175.67	1927.00	24.97
5	Spray with datura aqueous extract	40.90 (40.34)	43.80 (41.44)	40.59	9.86	1577.33	1744.33	1660.83	7.6
6	Spray with Carbendazim	18.60 (26.42)	18.90 (25.77)	25.63		2631.33	2754.33	2692.83	13.18
7	Spray with water(C)	49.73 (47.01)	50.40 (45.23)	45.03		1553.00	1531.67	1542.33	
8	Spray with solvent(C)	49.00 (43.68)	51.20 (45.69)	44.88		1495.00	7320.33	1612.00	
SEm		3.84	1.10	0.85		57.65	55.53	62.98	
CD(5%)		11.63	**3.32**	**2.47**		171.26	164.97	182.59	

There is need of further study of active ingredients present in phyto extracts responsible for inhibiting the growth of the fungus and attempts should to made to know and enhance the shelf life and stability of the extracts so that commercial production of the ecofriendly products can be possible for future use of the farmers for sustainable agriculture.

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