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Use of phytoextracts and non-conventional chemicals for management of brown leaf spot disease of rice

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Abstract
Nine phytoextracts and five non-conventional chemicals were evaluated against *Bipolaris oryzae* (causal agent of brown leaf spot disease of rice) under *in vitro* and field conditions. Out of nine phytoextracts, evaluated under *in vitro* conditions, garlic cloves extract inhibited the mycelial growth of fungus by 95.04% followed by neem (84.85%) and calotropis (78.79%) leaf extracts at 30% concentration. Five better phytoextracts were also evaluated as foliar spray under field conditions and the maximum per cent disease control was found in garlic cloves extract treated plots (15.04%). Among five non-conventional chemicals ferric chloride was found superior in which per cent disease control was observed as 31.28% followed by magnesium sulphate.

Keywords: Rice, brown leaf spot, management, phytoextracts, non-conventional chemicals

1. Introduction
Rice (*Oryza sativa* L.) is important staple food for many countries including India that suffers from a number of fungal diseases and many of them occur at times in fairly severe form and cause substantial loss to the crop yield. Among the major fungal diseases of rice, brown leaf spot occupies important position because of its historical importance as the disease was the main reason for occurrence of Bengal Famine in India in 1942 due to which more than 4 million people died due to starvation. Rice brown spot is also called as orphan disease of rice still the disease adversely affects million of hectares worldwide every year [21]. In Haryana, it occurred as emerging disease in direct seeded rice. Earlier studies showed loss in grain weight to the extent of 4.3 - 29.0 per cent [5] and also losses ranging between 26.2 to 51.8% due to heavy grain infection [7]. In severely infected crop the number of tillers and grains are reduced including reduction in quality and weight of individual grains resulting in a loss of 30-43 per cent while it was only 12 per cent under moderate and non-significant (negligible) at lower infection site or soil with moderate conditions [14]. Earlier studies also showed that reduction in mycelial growth of pathogen by 64 per cent by [6] after application of leaf extracts of *Juglans regia* (walnut) while aquous extracts of *Acorus calamus* (sweet flag) reduced hyphal growth by 80 per cent along with 45.3 per cent reduction in brown spot incidence [11]. Among different bio-pesticides, Biotos 2.5 ml/l, Tricure 5 ml/l, Achook 5 ml/l [12], Neemazal 3 ml/l and Wanis 5 ml/l [17] effectively reduced disease severity and also increased the grain yield significantly. Methanolic extracts from some medicinal plants like *Bergia capensis*, *Lippi anodiflora*, *Marseli aquadrifolia*, *Eclipta prostrate* and *Commleina clavata* [13] whereas essential plant oils from basil (*Ocimum basilicum*) and sweet fennel (*O. gratissimum*) gave good result by their inhibitory activity against *B. oryzae* [15]. Ferric chloride [19], calcium chloride and amino-n-butyric acid [4] when applied as foliar spray induced resistance in rice plants against *B. oryzae* while chitosan at 1000 ppm [16] caused 100% inhibition of the pathogen. Amongst nine non-conventional chemicals, sodium salenate, ferric chloride and nickel nitrate successfully reduced both the phases of the disease considerably but the latter two chemicals were found to have some phytotoxic effect on leaves [17].

2. Material and Methods
2.1 Plant materials and pathogen
The studies were conducted at the CSSHAU, RRS, Kaul, Kaithal (Haryana) on aromatic rice cultivar CSR 30. Sowing of crop was done in June, 2019. Plot size was kept 5×2 m^2 and spacing was maintained 20×15 cm^2, following randomized block design with four replications of each treatment.

Crop was raised by following package of practice of *kharif* crops of the Haryana (Anonymous, 2013) [3]. *Bipolaris oryzae* was isolated from the diseased leaves of rice taken from RRS, Kaul. The fungus was purified by hyphal tip method. Pure culture was obtained in 8-10 days and sub cultured after every 15 days.

2.2 Preparation of plant extracts

Healthy fresh leaves and cloves of the given plants samples were taken and washed thoroughly in running water. The leaves and the cloves were then surface disinfected with ethanol and again washed in water for 2-3 times. Then equal amount of surface washed plant parts with equal amount of water were macerated in the grinder. The content was allowed to grind until they were uniformly crushed to give fine meshed product. Then the meshed product was further subjected to centrifuge at 8000 rpm, 48˚C for 15 min to remove the plant parts and extracts (as supernatant). The supernatant that was obtained after centrifugation was designated as concentrated leaf extract. Supernatant was then passed through bacterial proof filter paper lined over Buchner's funnel. Serial dilutions (1:10) were made from this concentrated extract by mixing equal amount of mixture of plant extract and water on weight basis was considered to be 100% (stock solution) from which further dilutions was made (Harish *et al.*, 2008) [9].

To estimate the inhibitory effect of different plant extracts against *Bipolaris oryzae*, poisoned food technique was used. Extracts were added to double strength PDA medium to make the concentrations *viz.*, 10, 20 and 30% (v/v) and were mixed thoroughly before solidification at 50 to 60˚C and poured in Petri plates under aseptic condition in laminar air flow chamber. Petri plates were inoculated with equal discs (10mm) of pathogen taken from the mother culture. To compare the growth inhibition, a control plate containing PDA medium was also kept along and inoculated with pathogen in the similar way. Mycelial growth was recorded when there is 90 mm growth in control plates at 28±2˚C and per cent growth inhibition was calculated by using the formula given by Vincent (1927) [20].

Growth Inhibition (%) = [(Growth in control – growth in treatment) / Growth in control] × 100

Only five superior phytoextracts were taken and were mixed with water to prepare the solution and sprayed in the field at different concentration with the help of Knapsack sprayer during disease initiation and 50% panicle emergence stage in four replications by following Random Block Design. The plot sprayed with plain water was taken as control. After appropriate appearance of brown leaf spot disease symptoms on the leaves, 100 randomly selected leaves were collected from each plot and disease severity at 15 days before harvest was observed as per Standard Evaluation System for Rice. Grain yield (q/ha), per cent disease control and per cent increase in yield over control was also calculated. Disease severity was done as per 1-9 scale (Anonymous, 2013) [2] as given in table 1 and disease severity was calculated by following formula:

$$\text{Per cent disease severity} = \frac{\text{Sum of all numerical rating}}{\text{Total no. of leaves observed x maximum disease grade}} \times 100$$

Five non-conventional chemicals were mixed in water to prepare the solution and were applied in the field with the help of sprayer during disease initiation and 50% panicle emergence stage. The plot sprayed with plain water was taken as control. Non-conventional chemicals were applied at following concentration, ferric chloride @ 1g/l, magnesium sulphate @ 1g/l, nickel nitrate @ 2g/l, potassium chloride @ 1g/l and sodium salenate @ 1g/l of water. Disease severity (%), yield (q/ha) and per cent disease control was observed from 100 randomly selected leaves from four replications 15 days before the crop harvest.

3. Results and Discussion

Nine phytoextracts were evaluated for their efficacy against *Bipolaris oryzae* under *in vitro* conditions. Table 2 data shows that all phytoextracts significantly reduced the radial growth of fungus but garlic cloves extract was found to be superior followed by neem, calotropis, bittergourd and banyan leaves. Garlic (*Allium sativum*) at 30% concentration showed maximum per cent growth inhibition upto 95.04% followed by neem (*Azadirachta indica*), calotropis (*Calotropis gigantia*), bittergourd (*Momordica charantia*) and banyan (*Ficus bengalensis*) upto 78.79, 78.46 and 64.25% respectively. Tulsi was the least effective among all phytoextracts in which per cent growth inhibition value at 30% concentration was recorded as 40.45%. Ahmed *et al.*, (2002) [1] also observed that garlic followed by neem extract inhibited the mycelial growth of *B. oryzae in vitro* up to 91.7 and 83.3%, respectively. Similarly, Harish *et al.*, (2007) [10] observed the growth inhibition per cent of fungal mycelium by neem under *in vitro* conditions was 80.18% and growth inhibition per cent by *Calotropis gigantia*, *Ficus bengalensis*, *Ficus religiosa*, *Momordica charantia* and *Ocimum basilicum* to be 68.9, 54.1, 45.8, 68.1 and 29.6%, respectively. Five better phytoextracts *viz.* garlic, neem, calotropis, bittergourd and banyan were evaluated against brown leaf spot disease under field conditions. All phytoextracts significantly controlled the brown spot of rice in field conditions as given in table 3.Garlic extract was found superior that reduced the disease severity from 60.67 to 51.54% and increased the yield from 17.52 q/ha to 18.45 q/ha. Per cent disease control and per cent increase in yield over control was reported as 15.04 and 5.31 per cent.

It was followed by neem, which reduced the disease severity from 60.67 to 52.54% and increased the yield from 17.52 q/ha to 18.13 q/ha. The per cent disease control in above case was reported as 13.4% and increase in yield over control was found as 3.48 per cent. The value of disease severity in calotropis, bittergourd and banyan was reported as 53.5, 53.62 and 54.55% respectively, over control (60.67%). The per cent increase in yield over control in case of calotropis, bittergourd and banyan was reported up to 1.99, 1.19 and 0.79 per cent respectively. Harish *et al.*, (2007) [10] reported that out of different plant extracts evaluated, neem cake extract was found superior in reducing 70% disease and per cent increase in yield (23%) was also found maximum followed by *Nerium oleander* extract, *Pithecolobium dulce* and mahua cake extract. Judicious use of combination of phytoextracts and bio-control agents can significant reduce use and subsequently cost of chemical pesticides and thereby contribute to sustainable development of agriculture.

Five non-conventional chemicals were evaluated for their effect on yield and defence against brown leaf spot of rice under field conditions. Table 4 data reveals that all non-conventional chemicals significantly reduced brown leaf spot

severity. Ferric chloride when applied @ 1g/l as foliar spray was found superior among all non-conventional chemicals and reduced disease severity from 61.92 to 42.55% and increased the yield from 17.11 q/ha to 19.80 q/ha. Per cent disease control and per cent increase in yield over control was found to be 31.28 and 15.72% in this case. It was followed by magnesium sulphate @ 1g/l in which disease severity was reduced from 61.92 to 48.31% and yield was increased from 17.11 to 18.33 q/ha. Per cent disease control and per cent increase in yield was reported as 21.98 and 7.13% over control. Nickel nitrate @ 2g/l, potassium chloride @ 1g/l and sodium salenate @ 1g/l reduced the disease severity from 61.92 to 54.17, 54.41 and 51.20% and increased the yield from 17.11 q/ha to 18.24 q/ha, 17.98 q/ha and 17.88 q/ha respectively. Whereas per cent disease control and increase in yield over control was reported as 12.51, 12.12 and 17.31% and 6.6, 5.08 and 4.5% respectively. Per cent disease control in case of sodium salenate was higher than nickel nitrate and potassium chloride but per cent increase in yield over control was lower than mentioned treatments. Sunder *et al.,* (2010) [17] also reported that out of nine non-conventional chemicals, ferric chloride, sodium salenate and nickel nitrate provided more than 26% reduction in severity and 14.7-24.2% reduction in stalk rot phase of the brown spot. They also observed some phytotoxic effect of sodium salenate and nickel nitrate on leaves. Spray application of non-conventional chemicals lead to induction of host resistance by production of phytolexins following SAR pathway and provide defense against the disease thereby reduce disease severity (Sunder *et al.,* 2010; Giri and Sinha, 1983; Trivedi and Sinha, 1980) [17, 8, 18].

Table 1: Brown Leaf Spot Rating Scale (1-9)

Scale	Reaction	Affected leaf area (%)
1	Resistant (R)	<1%
2		
3	Moderately resistant (MR)	1-10%
4		
5	Moderately susceptible (MS)	11-25%
6		
7	Susceptible (S)	26-50%
8	Highly susceptible (HS)	>51%
9		

Table 2: Effect of different phytoextracts under *in vitro* conditions against *Bipolaris oryzae*

Phytoextracts	Concentration (per cent) *			Mean *	EC_{50}	EC_{90}
	10	20	30			
	Per cent growth inhibition					
Calotropis	58.35 (49.79)	68.56 (55.87)	78.79 (62.55)	68.57 (56.07)	0.82	1.77
Banyan	44.65 (41.91)	54.54 (47.58)	64.25 (53.26)	54.48(47.58)	1.15	2.14
Bittergourd	58.30 (49.76)	68.12 (55.60)	78.46 (62.32)	68.29 (55.89)	0.83	1.80
Jamun	42.59 (40.73)	52.67 (46.51)	62.59 (52.27)	52.62 (46.50)	1.19	2.17
Tulsi	20.48 (26.89)	30.48 (33.50)	40.45 (39.48)	30.47 (33.29)	1.74	2.71
Peepal	35.29 (36.43)	45.30 (42.29)	55.18 (47.96)	45.26 (42.23)	1.38	2.35
Garlic	70.68 (57.19)	80.86 (64.03)	95.04 (77.12)	82.19 (66.11)	0.6	1.42
Mehndi	37.48 (37.73)	47.49 (43.55)	57.63 (49.37)	47.53 (43.55)	1.32	2.29
Neem	64.60 (53.47)	74.63 (59.73)	84.85 (67.07)	74.69 (60.09)	0.66	1.62
CD (p = 0.05)	(0.15)	(0.08)	(0.31)	-	-	-

*Mean of four replications
Figures in parenthesis indicate angular transformed values

Table 3: Effect of different phytoextracts on brown leaf spot severity (%) and grain yield of rice cv. CSR 30 under field conditions

Superior Phytoextracts	Disease severity (%)*	Disease control (%)	Grain yield (kg/plot)*	Grain yield (q/ha)*	Percent increase in yield over control
Garlic @ 0.05%	51.54 (45.86)	15.04	1.84	18.45	5.31
Neem @ 0.05%	52.54 (46.44)	13.40	1.81	18.13	3.48
Calotropis @ 0.05%	53.50 (46.99)	11.81	1.78	17.87	1.99
Bittergourd @ 0.05%	53.62 (47.05)	11.62	1.77	17.73	1.19
Banyan @ 0.05%	54.55 (47.59)	10.03	1.76	17.66	0.79
Control	60.67 (51.17)	-	1.75	17.52	-
CD(p= 0.05)	(1.03)	-	0.01	0.12	-

*Mean of four replications
Figures in parenthesis indicate angular transformed values

Table 4: Effect of different non-conventional chemicals on brown leaf spot severity (%) and grain yield of rice cv. CSR 30

Non-conventional Chemicals	Disease severity (%)*	Disease control (%)	Grain yield (kg/plot) *	Grain yield (q/ha) *	Percent increase in yield over control
Ferric chloride @ 1g/l	42.55 (40.69)	31.28	1.98	19.80	15.72
Magnesium sulphate @ 1g/l	48.31 (44.01)	21.98	1.83	18.33	7.13
Nickel nitrate @ 2g/l	54.17 (47.37)	12.51	1.82	18.24	6.60
Potassium chloride @ 1g/l	54.41 (47.51)	12.12	1.79	17.98	5.08
Sodium salenate @ 1g/l	51.20 (45.67)	17.31	1.78	17.88	4.50
Control	61.92 (51.88)	-	1.71	17.11	-
CD (p= 0.05)	(0.27)	-	0.03	0.34	-

*Mean of four replications
Figures in parenthesis indicate angular transformed values

4. Conclusions

Rice (*Oryza sativa* L.) suffers from a large number of fungal, bacterial and viral diseases among which, brown spot occupies an important position, as it occur in severe form in direct seeded rice especially in aromaticrice varieties. Yield losses in relative terms may vary widely from 4 to 52 per cent. In the present studies, attempts were made for management of brown leaf spot using biorational approaches *viz.* use of botanicals extracts and development of resistance using non-conventional chemicals.

In case of plant extracts that were evaluated under *in vitro* conditions, garlic clove extract was found to be superior followed by neem, calotropis, bittergourd and banyan leaves extracts. The brown leaf spot severity was found minimum in plot treated with garlic followed by neem, calotropis, bittergourd and banyan. There was considerable difference in the severity when compared with control. Similarly, per cent increase in yield was also found maximum in garlic treated plot followed by neem treated plot. Five non-conventional chemicals were evaluated against brown leaf spot of rice. Ferric chloride was found maximum effective among all other non-conventional chemicals and the disease severity was reduced to maximum extent.

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