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Effect of Carbon, Nitrogen and Vitamins on Epidemiological Components of Sheath Blight of Rice caused by *Rhizoctonia solani* Kühn

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A B S T R A C T

The rice crop is affected by various biotic and abiotic stresses. The fungal diseases come under major biotic stress, among fungal diseases sheath blight of rice is one of the major threat for cultivation of rice crops. The sheath blight pathogen (*Rhizoctonia solani* Kühn) is being affected by different sources of nutrients. In this regards, six carbon, eight nitrogen and eight vitamin source were screened against *R. solani* causing sheath blight of rice. Among the carbon sources maximum number of sheath blight lesions, largest size of sheath blight lesion were recorded when rice plants were inoculated with the inoculums grown on the medium supplemented with sucrose, fructose & lactose together as carbon source. Among the nitrogen sources, significantly, highest level of disease severity (52.09%) was recorded when the *R. solani* inoculums were provided with urea as a source of nitrogen, followed by calcium nitrate (42.48). In case of Vitamins, highest level of disease severity (43.21%) was recorded due to Thiamine, followed by the combination of three vitamins (Biotin+ Calcium pentothenate + Thiamine hydrochloride (30.62%). The results of present finding indicate that sources of carbon, nitrogen and vitamins are affecting the disease severity. Therefore, precautions should be taken when these are applying in the fields.

Introduction

Globally, Rice cultivation is often influenced by various biotic and abiotic stresses and the most common and severe diseases are blast, sheath blight, stem rot, and bacterial blight (Chaudhary *et al.*, 2019). Sheath blight caused by the soil borne necrotrophic fungal pathogen *Rhizoctonia solani* Kühn (teleomorph: *Thanatephorus cucumeris*) is one of the most destructive disease of rice (*Oryza sativa* L.). This disease was first reported from Japan by Miyake in 1910. Since then, sheath blight has been observed in almost all rice growing areas of the world. In India, this disease was first reported from Gurdaspur in Punjab by Paracer and Chahal (1963) and in Uttar Pradesh by Kohli (1966). A modest estimation of losses due to sheath blight of rice in India has been reported to be up to 54.3 % under favorable conditions (Zheng *et al.,* 2013; Shu *et al.,* 2015). In present days, attempts to control rice sheath blight have been directed to the cultural practices, use of resistant cultivars and fungicides, to some extent biological methods. No resistant cultivar is available for practical field use condition (Lal *et al.,* 2012) and the present intensive rice cultivation practices offer a favorable condition for disease development.

Now days, intensive cultivation along with application of fertilizers and other sources of nutrients make luxuriant growth of rice crop. Thereby, incidence of sheath blight disease is increasing. The selection of nutrient sources is paramount for managing sheath blight to some extent without expending much input cost. Disease severity in the crop fields is influence by the aggressiveness of the pathogen, sclerotial size and inoculum density which ultimately influence the inoculums potential. The highly aggressive isolates of *R. solani* produced severe symptoms and crop losses than the less aggressive isolates. It has been reported that pathogens showed different response towards different nutritional sources like, Carbon, Nitrogen and Vitamins (Shrama and Tripathi, 2002; Chauhan, 2006). Determining the nutritional requirements of the pathogen plays the crucial role in better understanding its epidemiology, pathogenicity and aggressiveness. Therefore, the present investigation was taken to evaluate effect on sheath blight disease, when different type nutrients *i.e.* carbon, nitrogen and vitamin were given to *Rhizoctonia solani*.

Materials and Methods

Effects of carbon source

Czapek's broth medium was used as the basal medium for the study of five carbon sources *viz.*, Sucrose (S), Glucose (G), Lactose (L), and Fructose (F) were tested for their effect on pathogenicity of *Rhizoctonia solani*. Combinations of carbon sources used during the present investigation were: T_1: Glucose, T_2: Lactose, T_3:Fructose, T_4:S+G+L+F, T_5:S+F+L, T_6:S+G+F and T_7: Check.

The sucrose was omitted from the basal medium and other carbon sources were added in such a manner so that each combination should provide 12.61 gram of carbon per liter of medium. Amount of each carbon sources were determined according to their molecular weight.

Effects of nitrogen source

Czapek's broth medium was used as the basal medium for the study of four different nitrogen sources *viz.*, Calcium nitrate, Urea, Sodium nitrate and Alanine were tested to determine their effect on pathogenicity of *R. solani*. Quantity of nitrogen sources were determined on the basis of nitrogen content in the sodium nitrate, which was a constituent of basal medium and substituted with other sources mentioned above to provide same amount of nitrogen as provide by the Sodium nitrate *i.e.* 330 mg in one liter medium . Inoculation and observations were recorded in the similar manner as in preceding experiment on carbon sources.

Effects of vitamins source

Czapek's broth was used as a basal medium for the study of four vitamin sources *viz.*, Calcium pentothenate (C) @ 100 µg per liter, Thiamin hydrochloride (T) @ 100 µg per liter, Inositol (I) @500 µg per liter and Biotin (B) @ 5 µg per liter were tested in different combination for their effect on pathogenicity of *Rhizoctonia solani.* Vitamins were provided in following combinations: T_1:Calcium pentothenate (C), T_2:Inosital (I), T_3:Thiamine hydrochloride (T), T_4:Biotin (B), T_5:C+I+T+B, T_6:B+C+T, T_7:C+I+B, T_8:B+I+T and T_9: Check (without vitamin)

The vitamins in appropriate amount were dissolved in de-ionized distilled water to get the required concentration and added to the basal medium before autoclaving. After autoclaving and proper cooling, the flasks containing medium supplemented with different combinations of vitamins or without vitamins were taken out of autoclave after proper cooling. Now these flasks were placed in inoculation chamber under U.V. light for one hrs. After one hrs of UV exposure, flasks were inoculated with 5mm mycelium discs in aseptic condition and placed in incubator adjusted at 28± 1^0C under the darkness for 14 days. Each treatment was replicated thrice. Rest of the procedure of inoculums harvesting and inoculation were same as in case of carbon sources.

Preparation of basal medium and inoculation of *R. solani*

Czapek's broth medium, as basal medium supports good growth of mycelium and sclerotia formation of the *Rhizoctonia solani* (Singh, 2006). The pH (6.5) of the medium was adjusted by adding 0.1N NaOH and HCl. The pH of medium was determined before and after sterilization by Backman pH meter. The flasks containing medium were sterilized in an autoclave at 15 psi. (121.6^0 C) for 20 minutes. A 5 mm diameter mycelial disc from actively growing *Rhizoctonia solani* culture was inoculated in each flask and incubated for 14 days at 28±1°C under dark condition. Each treatment was replicated thrice.

Fourteen days old mycelium and sclerotia were harvested from the flasks supplied with different combinations of carbon sources, nitrogen sources and vitamins and inoculated on the healthy rice plants. Inoculated plants were wrapped with moist absorbent cotton and watered frequently to maintain proper moisture at the point of inoculation and regularly monitored for observations on incubation period, lesion length (cm), No. of lesions and disease severity in all such experiments.

Results and Discussion

Effect of carbon source on incubation period, no. of lesion, lesion length and disease severity of Sheath blight caused by *Rhizoctonia solani* Kuhn

The effect of different carbon sources on incubation period, number and length lesions and disease severity sheath blight disease have been presented in Table 1. Maximum incubation period at 77 hrs was exhibited when inoculums was taken from the medium where sucrose, glucose and fructose were provide together as a source of carbon followed by the inoculums grown on the medium supplemented with fructose at 59 hrs. Maximum number of sheath blight lesions (6.85) were recorded when rice plants were inoculated with the inoculums grown on the medium supplemented with sucrose, fructose and lactose together as carbon source, followed by the inoculums grown on lactose supplemented medium and also on the medium supplemented with all the four carbon sources together *i.e.* sucrose, glucose, fructose and lactose. However, the values obtained due to these three treatments did not differed significantly. Largest lesion lengths of sheath blight (4.66 cm) and (4.17 cm) were recorded from the rice plants inoculated with inoculums obtained from the check (Sucrose) and lactose respectively was provided as source of carbon. Rest of the treatments where either glucose or fructose were provided individually or mixture of 3 or 4 sugars were provided as carbon source could produced comparatively smaller lesion lengths ranged between the length of 1.76 cm to 2.93 cm. The highest sheath blight disease severity (31-83%) was observed when rice plants were inoculated with the inoculums harvested from the medium supplemented with all the four sugars *i.e.* S, G, F and L together as source of carbon. Prasad, (2007) concluded that glucose and sucrose as carbon source were equally effective for the mycelia growth and sclerotia formation in *R. solani*, whereas maltose and lactose were least effective for growth and sclerotia formation. Singh (2007) reported that fructose, glucose and lactose together stimulated the mycelial growth and sclerotia formation. However, Chaudhary *et al.,* (2018) reported that among the different carbon sources, glucose supported fast mycelia growth and highest number of sclerotia formation. Carbon considered as the main source of energy used by the fungus during infection and penetration (Solomon *et al.,* 2003). Prior to penetration, the fungus must rely on stored carbon sources like glycogen, trehalose, sugar alcohol and lipids (Jennings and Lysek, 1996; Thines *et al.,* 2000; Weber *et al.,* 2001).

Effect of nitrogen source on incubation period, no. of lesion, lesion length and disease severity of Sheath blight caused by *Rhizoctonia solani*

The effect of different sources of nitrogen in the basal medium of inoculants fungus and its effect on various component of pathogencity *i.e.* incubation period, number and length of lesions and disease severity have been presented in Table 2. Shortest length of incubation period 44.66 hr was recorded when rice plants were inoculated with the inoculums obtained from the medium provided with alanine as source of nitrogen, followed by the inoculums from the medium provided with calcium nitrate, urea and alanine together. Highest number of sheath blight lesions *i.e.* 7.75 and 7.42 were recorded when the rice plants were inoculated with the inoculums provided with Ca+U+A together and urea alone, respectively. However inoculums provided with sodium nitrate could produce lowest number of lesions *i.e.* 2.24 only and the numbers of lesions recorded due to other treatments were statistically at par with the highest number of lesions recorded. Longest lesion length (4.96 cm) was recorded when rice plants were inoculated with the *R. solani* inoculums provided with calcium nitrate + sodium nitrate + alanine together as source of nitrogen, whereas the smallest lesion length (2.40 cm) was recorded when rice plants were inoculated with the *R. solani* inoculums which was provide with urea. Highest level of disease severity (52.09 %) was recorded when the *R. solani* inoculums was provided with urea as a source of nitrogen, followed by the severity recorded due to inoculums provided with calcium nitrate (42.48 %).

Tandan (1967) concluded that potassium nitrate, calcium nitrate and sodium nitrate were generally utilized as nitrogen source by imperfect fungi. According to Chauhan (2006) the calcium nitrate exhibited good mycelial growth of *Bipolaris sorokiniana*. This also indicates that *B. sorokiniana* and *R. solani* (both are imperfect fungi) have similar preference for sources of nitrogen. Prasad (2007), found that calcium nitrate was effective for mycelia growth. Urea was inhibitory to growth of *R. solani* and for sclerotial formation calcium nitrate and sodium nitrate were equally effective. The interaction of fungal disease and nitrogenous fertilizers is of great practical interest and numerous studies have been conducted so far (Long *et al.,* 2000; Hoffland *et al.,* 2000;

Jensen and Munk, 1997). Application of nitrogen above the recommended rate has often been shown to significantly increase disease incidence and lesion area (Solomom *et al.*, 2003).

Table.1 Response of different sources of carbon against sheath blight of rice

S No.	Treatments	Incubation period	No. of lesion	Lesion length (cm)	Severity (%)
1	T_1:Glucose (G)	52.00	5.61	2.95	24.19
2	T_2:Lactose (L)	47.33	5.87	4.17	24.76
3	T_3: Fructose (F)	59.00	3.12	2.17	29.02
4	T_4:S+G+L+F	46.00	5.37	2.64	31.83
5	T_5:S+F+L	53.00	6.85	2.50	27.35
6	T_6:S+G+F	77.00	2.09	1.76	28.75
7	T_7:Check (Sucrose)	43.00	2.00	4.66	18.88
SE(m)		4.34	1.587	0.625	3.244
CD 5%		13.13	3.394	1.889	9.485

Table.2 Response of different sources of nitrogen against sheath blight of rice

S No.	Treatments	Incubation period	No of lesion	Lesion length (cm)	Severity (%)
1	T_1: Calcium nitrate (Ca)	50.33	4.38	2.84	42.48
2	T_2: Urea (U)	59.33	7.42	2.40	52.09
3	T_3: Sodium nitrate (Na)	97.66	2.24	0.49	27.87
4	T_4: Alanine (A)	44.66	5.56	3.37	31.63
5	T_5: Ca+U+Na+A	47.00	4.12	2.74	31.41
6	T_6: Ca+U+A	45.66	7.75	3.13	30.57
7	T_7: Ca+ Na+A	61.66	6.71	4.96	34.84
8	T_8: Check	56.00	5.85	3.02	18.81
SE(m)		7.706	1.018	0.637	5.563
CD 5%		23.300	3.077	1.926	16.822

Table.3 Response of different source of vitamins against sheath blight of rice

S No.	Treatments	Incubation period	No of lesion	Lesion length (cm)	Severity (%)
1	T_1: Calcium pentothenate	53.66	5.19	3.73	26.27
2	T_2: Inosital	53.33	5.37	4.47	25.80
3	T_3: Thiamine hydrochloride	79.33	2.88	2.30	43.21
4	T_4; Biotin	49.00	4.93	4.01	24.99
5	T_5: C+I+T+B	47.33	6.98	3.22	28.99
6	$T_{6:}$ B+C+T	40.00	6.37	1.64	30.62
7	T_7: C+I+B	47.66	1.45	3.84	26.58
8	$T_{8:}$ Check	52.33	4.03	3.31	21.15
SE(m)		4.282	0.997	0.463	3.757
CD 5%		12.947	3.014	1.401	11.360

Effect of vitamins and their different combination on pathogencity of *R. solani*

Effect of different vitamins added to basal medium on various components of pathogenncity *viz.* incubation period, number& length of lesion and disease severity of sheath blight have been presented in Table 3. Combination of three vitamins *i.e.* biotin+ calcium pentothinate + thiamin resulted in shortest length of incubation period (40 hr) followed by combination of four vitamins (47.33 hr) *i.e.* C+I+B+T. Maximum number of lesions *i.e.* 6.98 were recorded due to combination of 4 vitamins *i.e.* C+I+T+B, followed by combination of three vitamins (6.37) *i.e.* B+C+T. Maximum size of sheath blight lesions *i.e.* 4.47cm were recorded due to application of inositol followed by biotin (4.01cm), which were at par to each other. Level of disease severity *i.e.* 43.21% was recorded due to Thiamine, followed by the combination of three vitamins *i.e.* B+C+T (30.62%) which were significantly different to each other. According to Bilgrami (1963) and Tandan (1967), thiamine is essential for many imperfect fungi for their optimum growth. Biotin requirement among the fungi appears to be next to thiamine. Chauhan (2006) tested four vitamins against *B. sorokiniana* isolates and reported that all isolates exhibited maximum growth on thiamine. Singh, (2007) reported that biotin, calcium pentothenate and thiamin together resulted in highest mycelial growth and sclerotia formation, whereas presence of inositol in any combinations seems to be inhibitory to the fungal growth and sclerotia formation of *R. solani*.

It can be concluded that different carbon, nitrogen and vitamin sources are affecting the sheath blight disease scenario. Therefore, application of these sources must be considered before selection of carbon, nitrogen and vitamin as a nutrients source.

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