

Original Research Article

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Effect of Different Doses of Plant Nutrients on Sheath Blight and Phenolic Content of Rice

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ABSTRACT

Present study was undertaken to evolve an efficient and reliable measure for management of sheath blight of rice through application of judicious doses of plant nutrients. In this investigation, the effect of different doses i.e. control dose (naturally available in soil), deficient dose, recommended dose and excess dose of macronutrients (N, P &K) & micronutrients (S, Zn & Fe) were assessed on incubation period for first appearance of sheath blight, disease severity and on phenolic contents in inoculated rice plants. The experimental results exhibited that enhancement in dose of nitrogen and phosphorus, decreased the incubation period of sheath blight and phenolic contents in inoculated rice plants and increased the sheath blight severity. However, enhanced dose of potassium increased incubation period and quantity of phenols in inoculated rice plants and decreased the sheath blight severity. Application of recommended dose of sulphur increased incubation period and phenolic contents in inoculated rice plants and decreased disease severity, followed by excess or deficient dose. Recommended dose application of zinc or iron resulted in increased incubation period and phenolic contents and decreased sheath blight severity followed by deficient dose. Application of excess dose of zinc or iron reduced incubation period and phenolic contents up to a greater extent and increased sheath blight severity.

Keywords

Plant nutrient,
Phenolic content,
Rhizoctonia solani,
Rice, Sheath blight

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Introduction

Rice (*Oryza sativa* L.), is the world's single most important food crop serving as a primary food source for more than half (60%) of the world population. In India, it is cultivated under varied situations, ranging from below

sea level (Kerala) to about 2000 meters above sea level in Himalayan region and from 8⁰N in Kanya Kumari to 35⁰N in Kashmir. It thrives well in sandy loam to heavy black cotton clay soils ranging from normal to saline alkali conditions. These diverse agro-ecological situations demand location-specific

management technology for realization of full yield potential (Venkateswarly, 1992). To meet the growing food needs of increasing population in the country and more so in the state of Uttar Pradesh, there is a need to raise rice productivity in the region. Efforts for enhancing the productivity are limited by a number of biotic and abiotic stresses.

The crop suffers from a number of devastating diseases and it cause annual crop loss to the tune of 12 to 25 per cent. Fungal diseases alone contribute 12 to 20 per cent in crop losses (Rajan, 1987). Among the diseases, sheath blight, caused by *Rhizoctonia solani* Kuhn [teleomorph *Thanatephorus cucumeris* (Frank) Donk], earlier considered a minor disease, is currently ranked second only to, and often as a rivals of the blast disease. The pathogen is regarded as an unspecialized organism with indefinite pathogenic races (Kotasthane *et al.*, 2004). Under the given circumstances management of sheath blight will require sharply focused approach. Higher doses of Nitrogen and Phosphorus led to higher levels of sheath blight incidence and severity, whereas balancing these with addition of potash and micronutrients reduced the incidence. Enhancement in nitrogen dose interferes with plant metabolism in such a way that quantity of phenols gets reduced, whereas with reduced or no application of nitrogen, quantity of phenols gets increased (Krauss, 2001).

In view of above facts, investigations were carried out to assess the effect of different doses of plant nutrients viz., Nitrogen (N), Phosphorus (P), Potassium (K), Sulphur (S), Zinc (Zn) and Iron (Fe) on sheath blight development and phenolic content of rice, so, that a module can be developed for management of this disease through application of judicious doses of macro and micronutrients.

Materials and Methods

Source and maintenance of culture

The culture of *R. solani* Kuhn used in the present investigation was isolated from the diseased rice plant cv. Pusa Basmati-1 which was collected from the students' instructional farm of Narendra Deva University of Agriculture and Technology (NDUAT), Ayodhya, Uttar Pradesh, India. *R. solani* was isolated, purified and multiplied by following the standard procedure. After isolation, the fungal growth was transferred to the potato dextrose agar slants and after 5-6 days, formation of sclerotia confirmed the *R. solani*. The re-confirmation of *R. solani* was done by Koch's postulates.

Pathogenicity test (Koch's postulate)

Pathogenicity test was done by following sheath inoculation method. For this purpose, at least three rice plants per pot (5 kg capacity) were maintained in three replications. White milky sclerotial stage of *R. solani* was used for inoculation to test Pathogenicity. Five millimetre mycelial bit containing milky sclerotia was placed inside the sheath of rice plant and wrapped with moist absorbent cotton to provide continuous moisture to the culture as well as the plant. The process was done in the month of August and if there was no rain, the cotton wrap was regularly wetted to provide constant moisture. After 3-4 days, typical sheath blight symptoms appeared. Re-isolation of *R. solani* from inoculated rice plant confirms *R. solani*.

Pot experiment for study the effect of nutrients on sheath blight

The experiment was conducted with 4 treatments replicated four times under completely randomized design. In treatments, four doses (control dose- naturally available

in soil, deficient, recommended and excess doses) i.e. 120, 80, 120 & 160 kg/ha of N; 9, 50, 75 & 100 of P; 156.50, 40, 60 & 80 of K; 13.28, 20, 30 & 40 of S; 0.712, 3, 5 & 7 of Zn and 13.72, 1, 1.5 & 2 kg/ha of Fe; were assessed against sheath blight using Pusa Basmati-1 as a test variety under artificially inoculated pot conditions. The weight of one hectare soil (furrow slice) is 2×10^6 kg and a healthy soil contains N, P, K, S, Zn and Fe more than 250, 40, 250, 30, 2.4 and 16 kg/ha, respectively. Prior to pot filling, the soil analysis was done to determine the availability of these nutrients in soil and to decide the dose to be applied as deficient, as per recommendation and excess in the pot containing 2.5 kg soil.

The formulation used for supply of N, N+P, P, K, S, Zn and Fe was Urea (46% N), Diammonium Phosphate-DAP (46% P, 18% N), Single Super Phosphate-SSP (16% P), Muriate of Potash-MOP (60% K), Elemental sulphur (100% S), Zinc oxide (78% Zn) and Ferric oxide (69% Fe), respectively.

Raising nursery for pot experiment

Healthy seeds of susceptible rice variety Pusa Basmati-1 were soaked in water for 12 hrs in night and then sterilized with 2% sodium hypochlorite solution for ten minutes and thereafter washed, thrice with sterilized distilled water, and dried in shade for 6 hrs. These seeds were sown using broadcast method in nursery during *Kharif* season. Fertilizers were applied as per recommended doses and agronomic practices were followed as per recommendation. Twenty five days old seedlings were used for transplanting in pots containing natural soil without any amendment and amended with deficient, recommended and excess doses of N, P, K, S, Zn and Fe. Three seedlings per hill were transplanted and each pot contains 3 hills. The nutrients were supplied in the pot soil

according to the doses as described above. Except nitrogen all the nutrients were applied as basal dose, where as nitrogen was applied in split doses i.e. 50:25:25 ratios as basal dressing and twice top dressing as per recommendation.

Inoculation of rice plants

The transplanted rice plants were inoculated at panicle initiation stage (crop growth stage 5) which comes after 60 days of seed sowing and remain up to 72 days of rice plants. The inoculation was done by placing the ten-days-old mature sclerotium single of *R. solani* inside the sheath. For inoculation leaf sheath was opened carefully and inoculum was placed with the help of sterilized forceps inside the sheath.

Inoculated sheath were wrapped with wet cotton so that moisture is retained after inoculation. A few drops of sterilized water were also added to inoculated sheath. Inoculation was done in the evening and inoculated plants were sprayed with water next morning. These plants were maintained in a net-house.

Appraisal of sheath blight development and severity

After inoculation, crop was regularly watched and monitored for first appearance of disease symptoms. Disease severity was recorded, 7 days after inoculation and further at 5 days interval up to 25 days of inoculation. Observations on sheath blight severity were recorded by measuring lesion length of individual plants inoculated along with plant height of respective plant. Relative lesion height-RLH (disease severity) was calculated using following formula (Meena *et al.*, 2001)

$$\text{RLH} = \frac{\text{Lesion length}}{\text{Plant height}} \times 100$$

Estimation of total phenol

The phenolic contents in rice plants were measured with the help of spectrophotometer following the appropriate methodology (Augustin, *et al.*, 1985). Rice plant samples infected with *R. solani* were collected from the pots containing different level of nutrients and were kept into a hot air oven to dry at $55\pm 5^{\circ}\text{C}$ for 2hrs. Dried samples were cut into small pieces ($<1\text{mm}$) which were mixed and kept into desiccator. Subsequently 1g of the mixed plant sample was ground in a mortar with a pestle along with 20 ml of 80% ethyl alcohol. After grinding of sample, mixture was filtered with the help of Whatman filter paper No. 1. One ml filtrate, 1 ml phenol reagent and 2 ml of Na_2CO_3 solution were taken together and volume was adjusted to 50 ml by adding distilled water. Twenty ml of this preparation was kept in a test tube for 1 hr and its optical density (O.D.) was recorded with the help of a spectrophotometer at 750 nm against blank reagent. Simultaneously a standard curve was prepared by using various concentration of Gallic acid and concentrations of phenols were calculated in the test sample and expressed as phenols weight in mg per 100g dried plant material. Quantity of phenols was calculated with the help of following formula.

$$\text{Total phenol (100g dry wt. basis)} = \frac{\text{Amt. of Gallic acid from std.} \times \text{Sample O.D.} \times \text{Volume made}}{\text{O.D. of known gallic acid} \times \text{Sample wt.} \times \text{Volume or aliquot}}$$

Results and Discussion

The experiment was conducted to assess the efficiency of different doses of macro-nutrients viz. nitrogen, phosphorus and potassium and micro-nutrients viz. sulphur, zinc and iron on incubation period, severity of sheath blight and on phenolic contents in rice plants. The salient findings of this experiment has been presented and discussed in following paragraphs.

Effect of Nitrogen on sheath blight

The excess dose of nitrogen to rice plants decreased incubation period and phenolic contents in rice plants to its lowest extent i.e. 50% and 35.60%, respectively and increased the disease severity up to 60.14% as compared to the plants where no application of nitrogen was done. Application of nitrogen at recommended dose, decreased the incubation period and phenolic contents in rice plants up to 28.13% and 4.24%, respectively, and increased disease severity up to 24.81% severity up to 14.79 per cent. Deficient nitrogen application decreased incubation period and phenolic contents in rice plants up to 15.63% and 2.73%, respectively, and increased disease severity up to 14.79 per cent (Fig. 4.4 and Plate 4.4). According to Krauss (2001) when there was imbalanced ample nitrogen supply, it created high demand for carbon photosynthesis via Krebs cycle for soluble form of organic N compounds and leaving very little carbon for synthesis of secondary metabolite compounds, such as phenols and quinons. Whereas under nitrogen limited conditions however, much more carbon from the Krebs cycle is available for synthesis of phenolic compounds. Phenolic compounds play an important role in the host pathogen relationship and make a basis for defence mechanisms. They act as phytoalexins or as precursors of lignin and suberin which act as mechanical barriers. During present investigation also excess dose of nitrogen enhanced the disease severity. Thus, the findings of present investigation is quite in conformity with the reports of earlier workers like Vijaya Bhaskar *et al.*, (2001) and Krauss (2001). Higher dose of nitrogen promote high vegetative growth and increase succulence in plants, thus, increases water content in the plant tissues which might have increased host proneness and susceptibility in the plants. These alterations might have resulted in

reduced incubation period and increased severity of sheath blight. It also seems that increasing in nitrogen dose, interferes with plant metabolism in such a way that the phenolics get reduced. Whereas with reduced or no application of nitrogen the phenolics get increased. Increased amount of phenolics interferes with pathogenesis and ultimately results in lower severity of rice plants (Hakulinen *et al.*, 1995).

Effect of Phosphorus on sheath blight

Phosphorus application in excess dose to rice plant, decreased incubation period and phenolic contents in rice plants up to 40% and 24.66%, respectively and increased the disease severity up to 31.88% as compared to control dose where no application of phosphorus was done. Application of phosphorus at recommended dose decreased incubation period and phenolic contents in rice plants up to 20% and 12.15%, respectively and increased disease severity up to 13.45 per cent. However, application of phosphorus at deficient dose, decreased incubation period and phenolic contents in rice plants up to 10.00% and 1.06%, respectively and interestingly it decreased disease severity also up to 2.40 per cent (Fig. 4.5 and Plate 4.5). Roy (1986) reported that plants grown with crotonilidene diurea were less susceptible to the disease than those grown with guanylurea phosphate and neem cake and castor cake coated urea. According to a report namely advanced nutrients (2007) plants use phosphorus for photosynthesis, respiration, storing carbohydrates, cell division, energy transport (ATP, ADP), nucleic acids, enzymes and phospholipids. Phosphorus plays a very central role in determining total energy of metabolism of the plant because it forms energy rich phosphate esters (C-P) such as glucose-6-phosphate. Excess phosphorus causes decrease in the uptake of zinc, iron and copper-which starts a

chain reaction of other macro and micro nutrient deficiencies. Hence, deficient or excess phosphorus application affects the defence quality of plants against stresses. In both conditions the defence quality of plants reduced, while defence against stresses in plants determine by phenolic compounds present in the cells and tissues; decreased phenols, decrease resistance in the plants. During present investigation also, excess dose of phosphorus enhanced the disease with reduced phenol contents.

Effect of Potassium on sheath blight

Application of excess dose of potassium to rice plants, increased incubation period and phenolic contents in rice plants up to 68.89% and 67.57%, respectively, and decreased disease severity up to 32.31% as compared to control dose where no application of potassium was done. Application of potassium at recommended dose increased incubation period and phenolic contents in rice plants up to 31.67% and 27.99%, respectively, and decreased disease severity up to 19.73 per cent. Application of potassium in deficient dose, increased the incubation period and phenolic contents in rice plants upto 26.67% and 18.45%, respectively, whereas disease severity was decreased up to 12.58 per cent (Fig. 4.6 and Plate 4.6). Vijaya Bhaskar *et al.*, (2001) reported that incidence of sheath rot of rice caused by *Acrocyldrium oryzae* decreased with increase in potassium level from 0 to 140 kg/ha. The phenol contents in leaf sheath increased with increase in potassium application. According to Krauss, (2001), the ratio between nitrogen and potassium plays obviously a particular role in the host/pathogen relationship. According Perrenoud (1990), it is concluded that the use of potassium decreased the incidence of fungal diseases and increased the crop yields also. An inverse relationship between disease incidence and plant nutrition

with potash in sheath blight, have been reported by Haerdter (1997). Mondal *et al.*, (2001) also found a negative correlation between potassium content in soybean and sesame with disease incidence and a positive correlation between their respective yield. According to Sweeney *et al.*, (2000) the

positive impact on yield could also be attributed to the effect of chloride supplied with KCl Fertilizer. During present investigation also excess dose, of potassium reduced the disease severity and increase the phenol contents which is supported by the findings of all earlier workers.

Fig.1

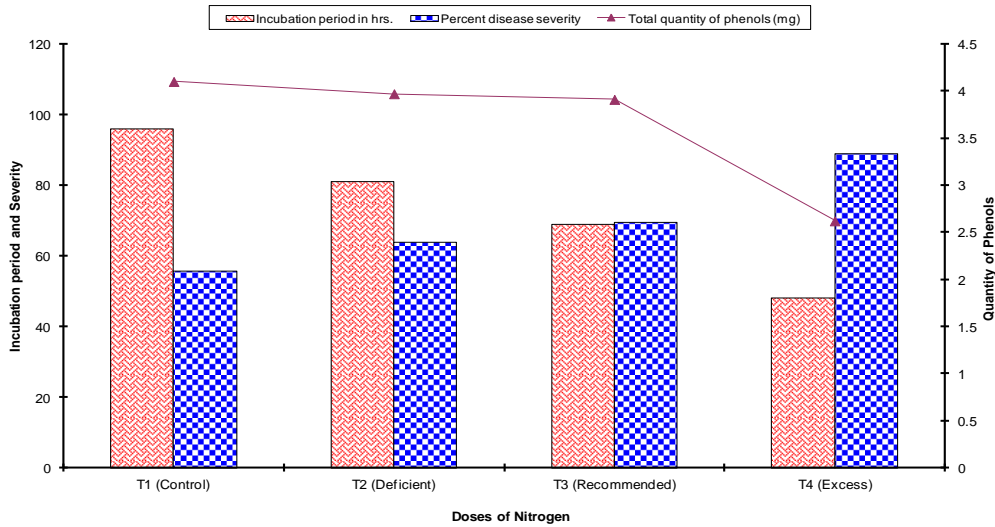


Fig.-4.4: Effect of different doses of Nitrogen on sheath blight.

Fig.2

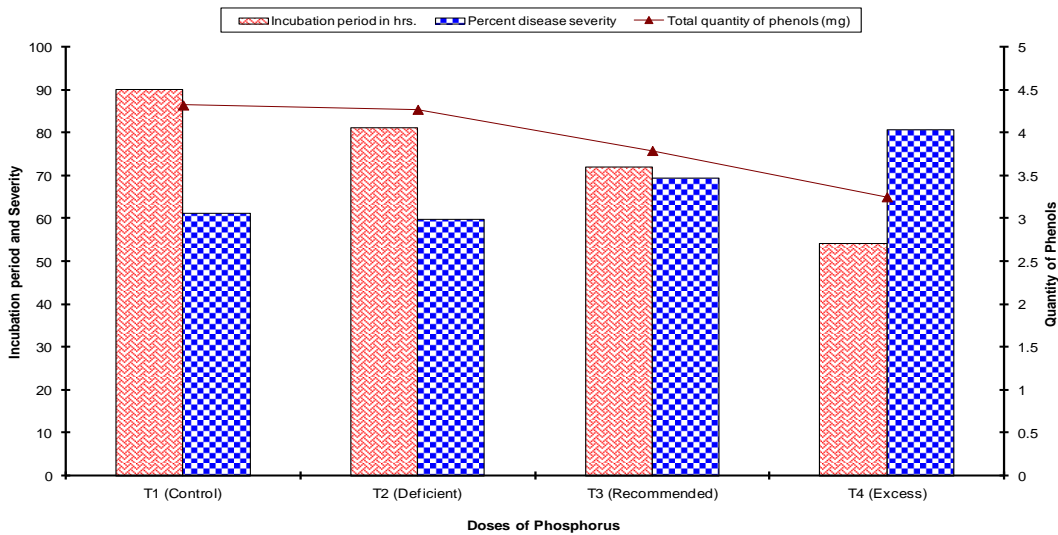


Fig.-4.5: Effect of different doses of Phosphorus on sheath blight.

Fig.3

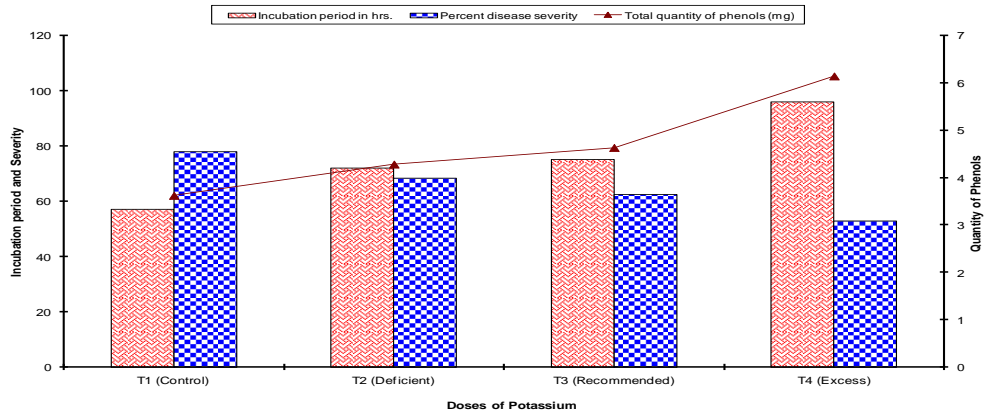


Fig.-4.6: Effect of different doses of Potassium on sheath blight.

Fig.4

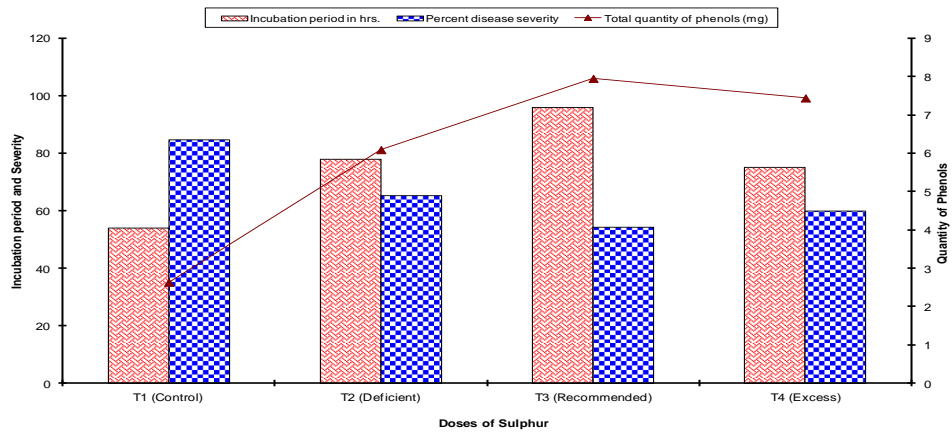


Fig.-4.7: Effect of different doses of Sulphur on sheath blight.

Fig.5

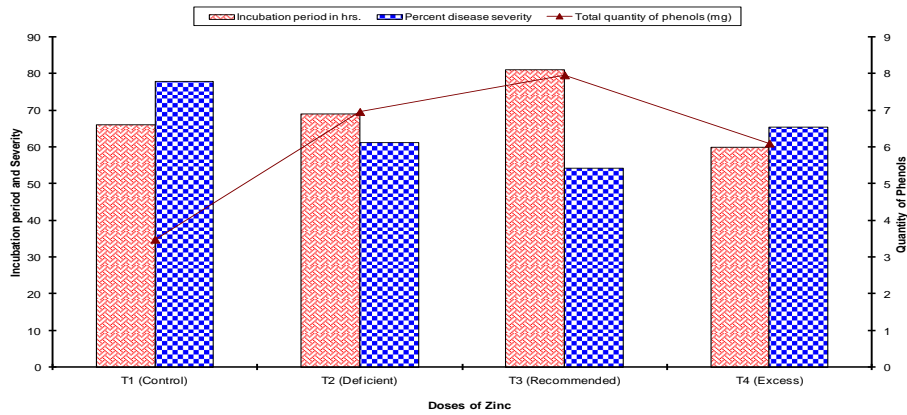


Fig.-4.8: Effect of different doses of Zinc on sheath blight.

Fig.6

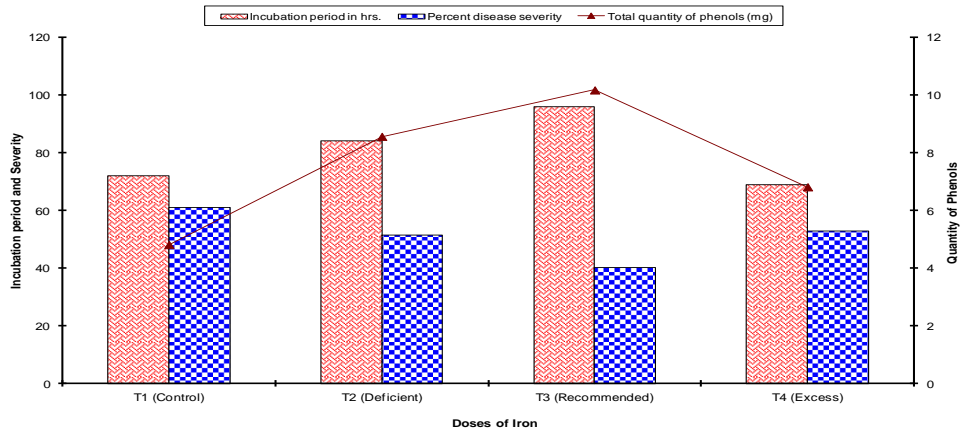


Fig.-4.9: Effect of different doses of Iron on sheath blight.

Fig.7



Fig.8



Effect of Sulphur on sheath blight

Application of sulphur as per recommended dose in sulphur deficient soil, increased incubation period and phenolic contents in rice plants up to 80% and 202.30%, respectively and decreased disease severity up to 36.07% as compared to check where no sulphur application was done. Application of excess dose of sulphur, increased incubation period and phenolic contents in rice plants up to 40% and 183.40%, respectively, and decreased disease severity up to 29.56 per cent. However, application of sulphur in

deficient amount increased incubation period and phenolic contents in rice plants up to 46.25% and 131.41%, respectively, and decreased disease severity up to 22.96 per cent (Fig. 4.7 and Plate 4.7). Datre *et al.*, (2003) reported that zinc sulphate check the growth of *R. solani*. According to Advanced Nutrients (2007), sulphur deficiency causes small and spindly plants with short, slender stalks and reduced growth rate with delayed maturity. An overdose of sulphur can cause premature dropping of leaves. Sulphur is a component of cystine and methionine (amino acids that make up plant proteins). Sulphur is

therefore a component of plant proteins. It also has a major role in root growth and chlorophyll production. It is an enzyme activator and coenzyme compound, it also is a formative part of chloroplasts and nucleic acid proteins. Sulphur deficiency decreases protein synthesis and phenol contents and causes significant reduction in chlorophyll and resistance against stresses. Hence, imbalance sulphur fertilization affects the phenolic contents of plant, its deficiency reduce the phenols and its excessiveness also to be harmful for metabolic activities and therefore affects the defence mechanism with reduction in amount of phenols. During present investigation, different doses of sulphur also affect the phenols and disease severity.

Effect of Zinc on sheath blight

Application of zinc as per recommended dose, increased incubation period and phenolic contents in rice plants up to 24.35% and 130.13%, respectively, and decreased disease severity up to 30.34% as compared to check where no zinc application was done. Application of zinc in excess amount, increased incubation period and phenolic contents in rice plants up to 17.09% and 76.66%, respectively, and decreased disease severity up to 16.04 per cent. Application of zinc in deficient amount, increased incubation period and phenolic contents in rice plants up to 14.96% and 101.89%, respectively, and decreased disease severity up to 21.42 per cent (Fig. 4.8 and Plate 4.8). Datre *et al.*, (2003) reported that zinc sulphate check the growth of *R. solani in vitro*. Kang (2000) reported the incidence of rice diseases due to zinc deficiency (4.4), iron deficiency (2.7), nitrogen deficiency (0.8) and salt injury (0.5). Zinc is essential for growth regulation and also for regulating carbohydrate consumption. Zinc improves chlorophyll function. It is a component in many enzymes and is important

in enzyme systems, particularly for water absorption and usage. It is essential for plant hormone balance, especially auxin (IAA) activity and electron transport. Zinc is essential for protein synthesis and for the activity of RNA polymerase. Zinc also plays a role in the synthesis of tryptophan from indol thus affecting the formation of indol acetic acid by the plant. Deficient plants have mottled leaves with irregular chlorotic areas. Zinc deficiency leads to iron deficiency causing similar symptoms. Excess zinc to be toxic for plant health and it also reduces the resistance by reducing the phenolic. Hence imbalanced zinc application increase the proneness in plants for stresses (Advanced Nutrients, 2007). During present investigation, imbalanced zinc application affects to phenols negatively and enhanced disease severity. However, recommended zinc application enhanced the phenols and reduced the disease severity.

Effect of Iron on sheath blight

Application of iron as per recommendation in iron deficient soil, increased incubation period and phenolic contents in rice plants up to 33.33% and 111.63%, respectively and decreased disease severity up to 34.05% as compared to check where no iron application was done. Application of iron in excess dose increased incubation period and phenolic contents up to 4.15% and 39.64%, respectively, and decreased disease severity up to 13.67 per cent. Deficient iron application seems to be better than excess dose, because it resulted 16.67% and 78.64% enhancement in incubation period and phenolic contents, respectively, and it decreased disease severity up to 16.05 per cent. Sarkar and Sinha (1991) found that ferric chloride gave protection to rice plants against sheath blight (Fig. 4.9 and Plate 4.9). Kang (2000) reported the incidence of rice disease due to zinc deficiency (4.4), iron

deficiency (2.7), N. deficiency (0.8) and salt injury (0.5). Tiwari and Khare (2001) found that healthy seeds and seedlings contained more phenols than the infected seeds and seedlings. The phenolic compounds inhibited the growth of *R. solani*. Iron (Fe) is necessary for enzyme functionality and is important for the synthesis of chlorophyll. It is essential for young, actively growing tissues. Plants use iron for protein and nucleic acid metabolism, chlorophyll formation and electron transport. Enzymes (catalase, peroxidase, cytochromes) and photosynthesis components require iron. Iron deficiencies are indicated by the pale color of young leaves followed by yellowing and large veins. An adequate supply of soluble iron in the plant nutrient also inhibits the formation of phenol compounds (Advanced nutrients, 2007). In present investigation also, excessive iron reduced the phenolic contents in rice plants. Bodegom *et al.*, (2005) reported that ferrous iron (Fe⁺⁺), which is abundant in waterlogged soils, significantly stimulates phenol oxidase activity, both in pure enzyme assays and in waterlogged soil slurries from nutrient-poor dune slacks. In present investigation it was also observed that recommended iron application increased phenols and decreased disease severity, however excess iron decreased phenols and increased disease severity.

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