# Changes in Phenolic compound of rice var. ADT 36 as influenced by application of Integration of components and *Bipolaris oryzae* inoculation

**Jaiganesh, V.**
Assistant Professor, Department of Plant Pathology,
Faculty of Agriculture, Annamalai University, Annamalai nagar
Cuddalore DT, Tamil Nadu.

**Abstract**

The Pot culture studies were undertaken to investigate the changes of Total phenol and O.D. phenol compound in rice as influenced by application of Zinc sulphate and foliar application of salicylic acid and Potassium silicate and Brown spot pathogen *Bipolaris oryzae* inoculation. The results revealed that Soil application of Zinc sulphate @ 25 kg/ ha along with foliar application of plant activator Salicylic acid @ 50 ppm on 15 days after transplanting and Foliar spray of silicon based nutrient potassium silicate @ 3 % recorded the minimum disease incidence and maximum biometrics of rice. The same treatment recorded highest O.D. phenol and total phenol content when compared to comparison fungicide and control treatments. The phenol content gradually increased with the sampling period.

## Introduction

Rice (*Oryza sativa* L.) is the second most cultivated crop worldwide and it has been estimated that half the world's population survives wholly or partially on this crop (Van Nguyen and Ferrero, 2006) and rice provides more calories per ha than any other cereal food grains. Also, rice crop has been under cultivation from time immemorial, being grown under varying climatic conditions in different parts of the world. The majority of the rice (90%) is being produced in Asian countries (IRRI, 2008) and global rice production was approximately 680 million tonnes in 2009.

Rice crop is widely affected by a number of diseases caused by fungi, bacteria, viruses and mycoplasma which results in considerable yield losses (Ou, 1985). Among the various fungal diseases of rice, brown spot or sesame leaf spot incited by *Helminthosporium oryzae* (Breda de Haan) Subram. and Jain (Current name: *Bipolaris oryzae* (Breda de Haan) Shoemaker) is found to occur in most rice growing areas.

Currently the disease is being managed by application of fungicides. Due to pesticides hazards, pollution effect, fungicide resistant, bio control agent resistant strains, lack of bioprotectant knowledge which required the integrated component approach in Indian farmer's level which will be improve growth and disease suppression.

Toyoda and Suzuki (1960) showed that the rapid browning at the fungal infection locus of the rice leaf tissue is closely related to the defense reaction of host tissue and browning of the tissue occurred through the following metabolic changes in the host tissue.

1. Increase in the content of poly phenols
2. Increase in the peroxidase activity
3. Decrease in the catalase activity and
4. Rise in $H_2O_2$ level, as the result of alteration of terminal oxidase from metal protein and flavoproteins.

Therefore, with an aim to develop an integrated strategy involving the use of certain macro-micro nutrients, silicon based nutrients and resistance inducing chemicals for the successful sustainable management of rice brown spot. Hence, the present studies were undertaken to investigate the changes of Phenol content by application of Macro-micro nutrient, Salicylic acid, potassium silicate along with pathogen inoculation.

## Materials and Methods

### Crop, Variety and Source

Crop : Rice (*Oryza sativa* L.)

Variety : ADT 36

Source : Tamil Nadu Rice Research Institute (TRRI),
Aduthurai, Tamil Nadu.

### Pot culture studies

The pot culture studies was conducted to test the efficacy of certain macro-micro nutrients, silicon based nutrients and certain resistance inducing chemicals for assessing their influence on the incidence of brown spot of rice with various treatment and combinations. The brown spot susceptible variety ADT 36 grown in rectangular pots of size, 30x45 cm was used for the study. The plants were given artificial inoculation by spraying the spore suspensions with adequate spore load (50,000 spores/ml) at 15 DAT in the evening hours. The crop was maintained in a poly house with frequent spraying of water to provide adequate moisture and relative humidity to enable successful infection by the pathogen. The experiments were conducted in a randomized block design with three replications for each treatment and a suitable control. The fungicide carbendazim 50 WP @ 0.1 per cent was used for comparison and the standard agronomic practices as recommended by the State Agricultural Department were followed.

The effective treatments observed in different experiments conducted under pot and field conditions were pooled together and a new schedule of treatments in combination was evolved for the effective management of brown spot disease of rice. Also, zinc sulphate @ 25 Kg/ha was applied as basal application to the entire treatments (ZSS) except control and comparison. The treatment details are given below;

Treatment schedule

$T_1$ – ZSS + $ZSF_1$ + $ZSF_2$

$T_2$ – ZSS + $SA_1$ + $SA_2$

$T_3$ – ZSS + $PS_1$ + $PS_2$

$T_4$ – ZSS + $ZSF_1$ + $SA_2$

$T_5$ – ZSS + $SA_1$ + $ZSF_2$

$T_6$ – ZSS + $SA_1$ + $PS_2$

$T_7$ – ZSS + $PS_1$ + $SA_2$

$T_8$ – ZSS + $PS_1$ + $ZSF_2$

$T_9$ – ZSS + $ZSF_1$ + $PS_2$

$T_{10}$ – Carbendazim 50 WP @ 0.1 per cent as foliar spray (comparison)

$T_{11}$ – Control

$ZnSO_4$ @ 25 Kg/ha was applied as basal application to the entire treatments (ZSS) except control and comparison. The treatment details are given below;

**$T_1$** – ZSS + Two sprays of zinc sulphate @ 3 % on 15 and 30 DAT

**$T_2$** - ZSS + Two sprays with salicylic acid @ 50 ppm on 15 and 30 DAT.

**$T_3$** - ZSS + Two sprays with potassium silicate @ 3 % on 15 and 30 DAT.

**$T_4$** - ZSS + First spray with zinc sulphate @ 3 % on 15 DAT + second spray with salicylic acid @ 50 ppm on 30 DAT.

**$T_5$** - ZSS + Second spray with zinc sulphate @ 3 % on 30 DAT

**$T_6$** - ZSS + First spray with salicylic acid @ 50 ppm on 15 DAT + second spray with potassium silicate @ 3 % on 30 DAT

**$T_7$** - ZSS + First spray with potassium silicate @ 3 % on 15 DAT + second spray with salicylic acid @ 50 ppm on 30 DAT

**$T_8$** - ZSS +First spray with potassium silicate @ 3 % on 15 DAT + second spray with zinc sulphate @ 3 % on 30 DAT

**$T_9$** - ZSS + First spray with zinc sulphate @ 3 % on 15 DAT + second spray with potassium silicate @ 3 % on 30 DAT

**$T_{10}$** – Carbendazim (0.1 %) – Comparison

**$T_{11}$** - Un treated control.

**Phenolic changes - Method of sampling**

Samples of plant materials from each treatment were taken at 0, 7, 14 and 21 days after inoculation both in healthy and inoculated plants for estimating the changes in the biochemical constituents *viz.*, reducing sugars, non-reducing sugars, total sugars, starch, ortho dihydroxy phenols, total phenols, amino nitrogen, protein and enzymes like peroxidase, polyphenol oxidase, phenylalanine ammonia lyase and ascorbic acid oxidase.

**Preparation of ethanol extracts (Mahadevan and Sridhar, 1986)**

Plant materials of both healthy and infected were collected and 4 g samples were taken. They were chopped and then extracted in 16 ml of boiling 80 per cent ethanol for 5 min. and cooled in running tap water. The material was homogenized by grinding in a porcelain pestle and mortar and squeezed through two layers of cheese cloth. The residue was transferred back to 5 ml of boiling 80 per cent ethanol and reextracted for 5 min. cooled and filtered. Both the extracts were pooled and filtered through Whatman No.41 filter paper. A jet of ethyl alcohol was used to wash the filter paper and the final volume was adjusted to 20 ml with 80 per cent ethyl alcohol, so as to get 5 ml of the extract representing every g of plant tissue. The ethanol extract was used for the estimation of sugars, phenols, amino nitrogen and protein. The biochemical constituents were assessed based on standard procedures.

| Biochemical constituents | References |
|---|---|
| Total phenol | Bray and Thorpe, 1954 |
| Ortho-dihydroxy phenol | Johnson and Schaul, 1957 |

**Results and Discussion**

**Post infectional biochemical changes - Total Phenols**

Generally, there was a gradual increase in total phenol content throughout the sampling period in all the treatments with increase in the duration of sampling period. The total phenol content was found to be profoundly increased (5.54 mg/g) by the combined application of ZSS, $SA_1$ and $PS_2$ ($T_6$) in pathogen inoculated plants on $21^{st}$ day observation after inoculation (Table 1). The plots which received soil application and foliar spray with $ZnSO_4$ twice recorded 4.68 mg/g of total phenol content. The least phenol content was observed in control. The total phenol content increased with the age of the plant, i.e., the content gradually increased with sampling intervals and attained a maximum on $21^{st}$ day of sampling.

**Ortho-dihydroxy Phenols**

Ortho-dihydroxy phenol content was very much altered by the application of combination of ZSS, $SA_1$ and $PS_2$ ($T_6$). The maximum conc. of O.D. phenol was observed in $T_6$ (ZSS + $SA_1$ + $PS_2$) which recorded 5.55 mg/g in ADT 36 (Table 2) at the $21^{st}$ day of sampling after inoculation. Also, it was found that the treatments with SA in combination with potassium silicate or zinc sulphate significantly influenced the O.D. phenol content ($T_6$, $T_7$, $T_5$ and $T_4$). The O.D. phenol content gradually increased with sampling period.

Generally, there was a gradual increase in total phenol and O.D. phenol contents throughout the sampling period in all the treatments with increase in the duration of sampling period. Phenolic compounds are the natural constituents in all the plants investigated until now. Phenolics seem to inhibit disease development through different mechanisms involving the inhibition of extracellular fungal enzymes (cellulases, pectinases, laccase and xylanase) (Ashry and Mohamed, 2011). Antimicrobial activity of phenolics has been well documented in various crops (Adesanya *et al*., 1986). Application of SA could have increased the phenolic content of the plants (Klessig and Malamy, 1994). Foliar application of SA, increased the total phenolic content of groundnut plants inoculated with *Cercospora personatum* (Meena *et al*., 2001). An increase in phenolic content was observed in SA treated groundnut leaves and inoculation with *Alternaria alternata* (Chitra *et al.*, 2008).

One of the probable reasons attributed for decrease in disease intensity in macro-micro nutrient spray treatment was increased phenols and reduced sugars leading to the development of resistance in the rice plants as observed by Alagarsamy (1985). Several workers have implicated phenolic conc. as a resistance factor (Khallal, 2007; Khatun *et al.*, 2009; Umamaheshwari *et al.*, 2009; Mishra *et al.*, 2011), because they become highly reactive upon oxidation and may form substances toxic to pathogens or inactivate enzymes including hydrolytic enzymes produced by plant pathogenic fungi (Patil and Dimond, 1967). Also, Sridhar and Nayak (1990) stated that a variety of phenols and related derivatives conferred protection and resistance to rice against blast.

The combination treatment consisting of ZSS, $SA_1$ and $PS_2$ ($T_6$) increased the phenolics (total and O.D. phenol) compound when compared to control and fungicide treatments.

## References

**Adesanya, S.A., O'Neill, M.J.** and **Roberts, M.F.** (1986). Structure related Fungi toxicity of isoflavonoids. *Physiol. Molec. Plant Pathol*., **29**: 95-103.

**Alagarsamy, G.** (1985). Effect of nutrition on sheath rot of rice caused by *Sarocladium oryzae* Gams and Hawksworth. *M.Sc. (Agri.)* Thesis, Tamil Nadu Agricultural University, Coimbatore, pp. 186.

**Ashry, N.A.** and **Mohamed, H.I.** (2011). Impact of secondary metabolites and related enzymes in flax resistance and or susceptibility to powdery mildew. *World Journal of Agri. Sci.,* **7**(**1**): 78-85.

**Bray, H.G.** and **Thorpe, W.V.** (1954). Analysis of phenolic compounds of interest in metabolism. *Meth, Biochem, Anal.,* **1** : 27-52.

**Chitra, K., Ragupathi, N., Dhanalakshmi, K., MareeshWari, P., Indira, N., Kamalakannan, A., Sankaralingam, A.** and **Rabindran, R.** (2008). Salicylic acid induced resistance on peanut against *Alternaria alternate. Archives of Phytopathology and Plant Protection,* **41**(**1**): 50-56.

**IRRI,** (2008). International Rice Research Institute Annual report, Phillipines, 285p.

**Johnson,G.** and **Schaul,L.A.** (1957). Chlorogenic acid and other *ortho* – dihydroxy phenols in scab-resistant Russet Burbank and scab susceptible triumph potato tubers of different maturities. *Phytopathol*.**, 47**: 253-255.

**Khallal, S.M.E.**, (2007). Induction and modulation of resistance in tomato plants against *Fusarium* wilt disease by bioagent fungi (*arbuscular mycorrhiza*) and/or hormonal elicitors (jasmonic acid and salicylic acid): 2-changes in the antioxidant enzymes, phenolic compounds and pathogen related- proteins. *Australian J. Basic and Applied Sci.,* **1**(**4**): 717-732.

**Khatun, S., Bandyopadhyay, P.K.** and **Chatterjee, N.C.** (2009). Phenols with their Oxidizing Enzymes in Defence against Black Spot of Rose (*Rosa centifolia*). *Asian J. Exp. Sci.,* **23(1**): 249-252.

**Klessig, D.F. and Malamy, J.** (1994) The salicylic acid signal in plants. *Plant Mol. Biol.,* **26:** 1439-1458.

**Mahadevan,A.** and **Sridhar,R.** (1986). Methods in Physiological Plant Pathology. III. Edn. Sivakami Pub. Madras. 82p.

**Meena, B., Marimuthu, T.** and **Velazhahan, R**. (2001).Salicylic acid induces systemic resistance in groundnut against late leaf spot caused by *Cercosporidium personatum. Journal of Mycology and Plant Pathology*, **31 :** 139-145.

**Mishra, B.K., MIshra, R.K., Mishra, R.C., Tiwari, A.K., Yadav, R.S.** and **Dikshit, A.** (2011).Biocontrol efficacy of *Trichoderma viride* isolates against fungal pathogens causing disease in *Vigna radiate* L. *Archives of applied science research*, **3**(**2**): 361-369.

**Ou,S.H.** 1985, Rice Diseases, 2nd Edition, Common Wealth Mycological Institute, U.K. 380p.

**Patil, S.S.** and **Dimond, A.E.** (1967). Inhibition of *Verticillium* polygalacturonase by oxidation products of polyphenol. *Phytopathology*, **57**: 492-496.

**Sridhar, R.** and **Nayak, N.** (1990). Physiology of disease resistance in rice. In : *Basic Research for Crop Disease Management* (Edn. By P. Vidhyasekaran), Daya Publishing House, New Delhi, India. 110-120.

**Toyoda, S.** and **Suzuki, N.** (1960). Histochemical studies on rice blast lesions caused by *Pyricularia oryzae* Cav. *Ann. Phytopath, Soc. Japan.***25;** 172-177.

**Umamaheshwari, C., Sankaralingam, A**. and **Nallathambi, P.** (2009). Induced systemic resistance in watermelon by bio control agents against *Alternaria alternate*. *Archives of Phytopathology and Plant Protection,* **44**(**12**): 1187-1195.

**Van Nguyen, N.** and **Ferrero, A.** (2006). Meeting the challenges of global rice production. *Paddy water Environ.*, **4**: 1-9.

**Table 1. Changes in Total phenols of rice var. ADT 36 as influenced by application of ZS, SA, PS and *B.oryzae* inoculation**

| T.No. | Treatments | Total phenols (mg/g) | | | |
|---|---|---|---|---|---|
| | | 0 (days) | 7 (days) | 14 (days) | 21 (days) |
| 1 | ZSS + $ZSF_1$ + $ZSF_2$ | 2.84 | 3.93 | 4.19 | 4.68 |
| 2 | ZSS + $SA_1$ + $SA_2$ | 3.33 | 4.31 | 4.88 | 5.17 |
| 3 | ZSS + $PS_1$ + $PS_2$ | 2.88 | 3.99 | 4.23 | 4.76 |
| 4 | ZSS + $ZSF_1$ + $SA_2$ | 3.41 | 4.37 | 4.95 | 5.23 |
| 5 | ZSS + $SA_1$ + $ZSF_2$ | 3.37 | 4.53 | 5.01 | 5.39 |
| 6 | ZSS + $SA_1$ + $PS_2$ | 3.46 | 4.76 | 5.11 | 5.54 |
| 7 | ZSS + $PS_1$ + $SA_2$ | 3.43 | 4.65 | 5.06 | 5.47 |
| 8 | ZSS + $PS_1$ + $ZSF_2$ | 3.02 | 4.08 | 4.39 | 4.98 |
| 9 | ZSS + $ZSF_1$ + $PS_2$ | 2.99 | 4.17 | 4.59 | 5.08 |
| 10 | Carbendazim | 2.65 | 3.81 | 4.00 | 4.42 |
| 11 | Control | 2.41 | 2.85 | 3.24 | 3.49 |

**Table 2. Changes in Ortho-Dihydroxy phenol of rice var. ADT 36 as influenced by application of ZS, SA, PS and *B.oryzae* inoculation**

| T.No. | Treatments | OD phenols (mg/g) | | | |
|---|---|---|---|---|---|
| | | 0 (days) | 7 (days) | 14 (days) | 21 (days) |
| 1 | ZSS + $ZSF_1$ + $ZSF_2$ | 1.63 | 3.67 | 3.98 | 4.06 |
| 2 | ZSS + $SA_1$ + $SA_2$ | 1.73 | 4.20 | 4.45 | 4.85 |
| 3 | ZSS + $PS_1$ + $PS_2$ | 1.65 | 3.70 | 3.99 | 4.12 |
| 4 | ZSS + $ZSF_1$ + $SA_2$ | 1.75 | 4.29 | 4.67 | 5.17 |
| 5 | ZSS + $SA_1$ + $ZSF_2$ | 1.80 | 4.32 | 4.88 | 5.21 |
| 6 | ZSS + $SA_1$ + $PS_2$ | 1.85 | 4.38 | 5.04 | 5.55 |
| 7 | ZSS + $PS_1$ + $SA_2$ | 1.83 | 4.35 | 4.90 | 5.28 |
| 8 | ZSS + $PS_1$ + $ZSF_2$ | 1.67 | 3.98 | 4.03 | 4.34 |
| 9 | ZSS + $ZSF_1$ + $PS_2$ | 1.70 | 4.11 | 4.22 | 4.60 |
| 10 | Carbendazim | 1.51 | 3.45 | 3.55 | 3.92 |
| 11 | Control | 1.36 | 1.49 | 2.00 | 2.52 |