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Changes in amino nitrogen, protein and starch content of rice var. ADT 36 as influenced by application of integrated approaches and *Bipolaris oryzae* inoculation

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Abstract
The Pot culture studies were undertaken to investigate the changes of amino nitrogen, protein and starch content 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 the minimum amount of amino nitrogen content whereas protein and starch content increased when compared to comparison fungicide and control treatments. Protein content increased up to the 14th day of sampling and then slightly reduced in all the treatments. The minimum protein content was recorded in control at final sampling

Keywords: Amino nitrogen, protein, starch, rice VAR, ADT 36

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) [17] and rice provides more calories per ha than any other cereal food grains. In India 136.5 million tonnes of rice was produced from an area of 44.0 million ha with the productivity of 2915 kg per ha (Anonymous, 2008). In Tamil Nadu, rice is cultivated in an area of 2.05 million ha with a production of 7.2 million tonnes (Tamil Nadu Statistical Report, 2007) [16].

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 bio protectant knowledge which required the integrated component approach in Indian farmer's level which will be improve growth and disease suppression.

The development of disease and pathogen establishment involves chemical interactions between host and pathogen. The host plant reacts to the stimulus of the intruding pathogen which is manifested by the altered metabolism of the plant. Alterations in the host physiology as a result of pathogenic invasion have been well recognized in a number of host parasite interactions. Biochemical reactions will determine the nature and some extent of resistance or susceptibility. The initial response of a plant to infection was presumably induced by a parasite and subsequent alterations were the results of host's re-adjustments to the pathogens (Sridhar and Nayak, 1990) [14].

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 amino nitrogen, protein and starch 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, 30x45cm 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) [7]

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 what man 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
Amino nitrogen	Moore and Stein, 1958
Starch	Summer and Somers, 1949
Total Protein	Lowry *et al.* 1951

Results and Discussion

Post inflectional biochemical changes: Changes in Amino nitrogen

Application of ZS, SA and PS altered the amino nitrogen content in rice variety ADT 36. Application of SA at 15 DAT or its combination with ZS, and PS decreased the amino nitrogen content. The maximum content of 7.77 mg was recorded in control at the end of the sampling period. The minimum content of 4.19 mg was observed in T_6 (ZSS + SA_1 + PS_2). Amino nitrogen content was gradually increased up to 14^{th} day of sampling and then decreased to the initial level in T_6 treatment on 21^{st} day of sampling. Application of SA at 15 DAT reduced the amino nitrogen content than application of SA at 30 DAT (Table 1).

Changes in Protein

Protein content was significantly altered by ZS, SA and PS application (Table 2). Application of SA either on 15 DAT or on 30 DAT or in combination with PS increased the protein content in ADT 36. The maximum level of protein was

observed in T_6 with 55.82 mg/g at the initial sampling, whereas at the final sampling it was increased to the maximum (66.48 mg/g). Protein content increased up to the 14th day of sampling and then slightly reduced in all the treatments. The minimum protein content was recorded in control at final sampling (40.62 mg/g).

Starch

ZS, SA and PS application significantly influenced the starch content in rice variety ADT 36 when compared to control. Maximum content of starch was observed in T_6 (ZSS + SA_1 + PS_2) which recorded 53.15 mg/g at the 14th day of the sampling. In control, the starch content was the least with 33.35 mg at the end of the sampling period. Starch content gradually increased up to 14th day of sampling and then slightly decreased in all the treatments. The treatment combinations with SA recorded significant increase in starch content (Table 3).

Treatment combinations with $ZnSO_4$, salicylic acid and potassium silicate decreased the amino nitrogen content and increased the protein content in rice variety ADT 36 (Table 1 and 2).

Nitrogenous compounds are important for the growth and multiplication of invading pathogens. Miswa and Miyasakai (1972) [9] observed that bacterial leaf blight susceptible rice variety contained greater amount of amino nitrogen than the resistant variety. Close correlation between blast susceptibility and soluble nitrogen was reported by Chen (1989) [3]. Karpagavalli and Ramabadran (1996) [5] reported reduction in amino nitrogen content due to lignite fly ash application. Ragavan (2003) [12] and Jaiganesh (2005) [4] stated reduction in amino nitrogen content due to chemical inducers like salicylic acid and nicotinic acid application.

Involvement of protein components in plant disease resistance has been documented in several plant patho-systems (Carvalho *et al*., 2006; Aboshosha *et al.*, 2008; Mishra *et al.*, 2011) [2, 1, 8]. Increased protein content due to salicylic acid application in rust infected groundnut leaves (Siddaramaiah *et al.*, 1983) [13] and blast affected rice (Jaiganesh, 2005) [4] has also been reported. These reports lend support to the present findings.

The combination treatment consisting of ZSS, SA_1 and PS_2 (T_6) reduces the amino nitrogen and increases the protein compound when compared to control and fungicide treatments.

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

T. No	Treatments	Amino nitrogen (mg/g)			
		0 (days)	7 (days)	14 (days)	21 (days)
1	ZSS + ZSF_1 + ZSF_2	5.01	5.92	9.07	4.92
2	ZSS + SA_1 + SA_2	4.52	5.19	8.43	4.44
3	ZSS + PS_1 + PS_2	4.93	5.73	8.91	4.76
4	ZSS + ZSF_1 + SA_2	4.46	5.14	8.31	4.40
5	ZSS + SA_1 + ZSF_2	4.40	5.06	8.23	4.34
6	ZSS + SA_1 + PS_2	4.34	4.98	8.12	4.19
7	ZSS + PS_1 + SA_2	4.57	5.32	8.56	4.60
8	ZSS + PS_1 + ZSF_2	4.80	5.67	8.86	4.69
9	ZSS + ZSF_1 + PS_2	4.69	5.42	8.67	4.57
10	Carbendazim	5.82	6.82	9.89	5.48
11	Control	7.92	8.55	11.89	7.77

Table 2: Changes in Protein content of rice var. ADT 36 as influenced by application of ZS, SA, PS and *B .oryzae* inoculation

T. No.	Treatments	Protein content (mg/g)			
		0 (days)	7 (days)	14 (days)	21 (days)
1	ZSS + ZSF_1 + ZSF_2	52.04	56.92	58.61	53.19
2	ZSS + SA_1 + SA_2	53.51	57.86	59.66	54.83
3	ZSS + PS_1 + PS_2	52.35	57.05	58.66	53.27
4	ZSS + ZSF_1 + SA_2	52.70	57.23	58.80	53.64
5	ZSS + SA_1 + ZSF_2	53.63	58.12	59.92	54.09
6	ZSS + SA_1 + PS_2	55.82	61.93	70.56	66.48
7	ZSS + PS_1 + SA_2	54.14	59.06	62.16	57.81
8	ZSS + PS_1 + ZSF_2	53.37	57.61	59.45	55.06
9	ZSS + ZSF_1 + PS_2	53.12	57.49	59.23	55.00
10	Carbendazim	43.90	47.28	48.33	44.86
11	Control	36.29	40.08	43.88	40.62

Table 3: Changes in Starch content of rice var. ADT 36 as influenced by application of ZS, SA, PS and *B. oryzae* inoculation

T. No.	Treatments	Starch content (mg/g)			
		0 (days)	7 (days)	14 (days)	21 (days)
1	ZSS + ZSF_1 + ZSF_2	34.94	42.49	47.13	44.91
2	ZSS + SA_1 + SA_2	36.27	45.43	49.82	48.12
3	ZSS + PS_1 + PS_2	35.05	42.82	47.66	45.63
4	ZSS + ZSF_1 + SA_2	36.69	45.68	52.74	50.86
5	ZSS + SA_1 + ZSF_2	37.05	45.93	52.87	51.04
6	ZSS + SA_1 + PS_2	37.48	46.13	53.15	51.80
7	ZSS + PS_1 + SA_2	37.25	46.04	52.98	51.36
8	ZSS + PS_1 + ZSF_2	35.17	43.31	48.26	46.87
9	ZSS + ZSF_1 + PS_2	35.89	43.68	48.99	47.00
10	Carbendazim	33.60	37.23	42.58	39.61
11	Control	31.64	32.42	35.99	33.35

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