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RESEARCH ARTICLE

Biochemical changes in rice plants due to application of bioinoculant, organic product, plant activator and moculation of *Pyricularia oryzae*

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ABSTRACT

The present study were undertaken to investigate the changes of biochemical and enzymatic constituents in rice plants due to application of bioinoculant, *Serratia marcescens,* plant activator, Nicotinic Acid (NA), organic product *Panchakavya* (PK) and *Pyricularia oryzae* inoculation under pot culture conditions. Among the various treatments, combined application of SMS (seed treatment with *S.marcescens* @ 10g/kg of IR 50 rice seed), NA_1 (foliar application of NA @ 0.1 per cent for 15 days after transplanting (DAT)) and PK_2 (foliar application of PK @ 5 per cent on 30 DAT) was significant changes of biochemical and enzymatic constituents in rice plants. The phenolic content (total and O.D. phenol) increased with application of SMS, NA and PK. Reducing sugars were found generally decreased after the initial sampling. Application of SMS, NA_1 and PK_2 combinations reduced the accumulation of non-reducing and total sugars. The starch content was found increased due to combined application of SMS with NA_1 and PK_2 and *P.oryzae* inoculation. Blast infection increased the protein content of rice leaves. Application of SMS, NA_1 and PK_2 reduced the protein accumulation. The increased activity of PO and PPO was observed due to SMS, NA_1 and PK_2 application at all the sampling periods. Sampling after *P.oryzae* inoculation influenced the ascorbic acid oxidase and peroxidase activity. The level increased up to 14^{th} day of sampling and then reduced.

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INTRODUCTION

Rice continues to be the major staple food crop for human population. With the twin forces of population growth and economic expansion particularly in Asia, world rice requirements are expected to increase by 1.7 per cent annually between 1990 and 2025. Although seemingly small, this growth rate translates into an additional requirement of 13 million tonnes of rice per year. With less land available to expand rice-growing areas with competing demands from urbanization and industrialization on existing rice lands and irrigation water, production increase should come from intensive agriculture in existing lands of favourable and less favourable areas. This can yield some negative non-target effects such as serious increases in pest and disease pressure, for instance the catastrophe caused by rice blast (Zeigler *et al.,* 1994).

Blast of rice caused by *Pyricularia oryzae* Cavara (*Magnaporthe grisea*) is found to occur in almost all the rice growing countries and is the most destructive fungal disease of rice causing loss up to 90 per cent (Mehrotra, 1980) despite, decades of research towards its control. The possible control measures of blast disease are the use of fungicides, growing resistant varieties, application of organic amendments, balanced nutrition, biological agents and resistance inducing chemicals. The indiscriminate use of chemical fungicides

resulted in environmental pollution and ill health to biotic community as a whole. Also, a biological method of disease management has so far not proved as efficient in bringing down the disease incidence below the Economic Threshold Level (ETL). Hence, it was thought that the development of integrated approach with various ingredients would enhance disease suppressing mechanisms.

Serratia marcescens produces chitinolytic enzymes which causes degradation of the fungal cell walls, induction of plant defence reaction and certain antifungal low molecular weight molecules (Someya *et al.,* 2000). Many reports are available indicating induction of resistance of rice plants to *Rhizoctonia solani* by using non-conventional chemicals, which are known as inducers of phytolaxenins and/or elicitors of resistance in different plants (Dantre *et al.,* 2003). The application of chemical fertilizers has undoubtedly increased the production at the same time led to the accumulation of hazardous pollutants and undesirable effect on soil sustainability in the long term, however, it increases the incidence the disease. In this context there is an imperative need to improve the production of food grains without affecting the environment and the quality of food grains with improved agricultural technology. Based on these concepts the use of *Panchakavya* is gaining momentum among the farmers (Natarajan, 1999). Therefore, the present study was undertaken to investigate the changes of biochemical and enzymatic constituents in rice plants due to application of bioinoculant, *Serratia marcescens,* plant activator, nicotinic acid (NA), organic product, *Panchakavya* or *Panchagavya* (PK) and *Pyricularia oryzae* inoculation under pot culture conditions.

MATERIALS AND METHODS

***Integrated approach (Pot culture conditions)* :**

The treatment with bioinoculant *Serratia marcescens,* plant activator nicotinic acid (NA) and organic product, *Panchakavya* (PK) are individually and combined formed in integrated disease management programme in IR 50 rice crop during Navarai season (December – March).

Treatment combinations :

T_1 – SMS SMF_1 SMF_2
T_2 – SMS NA_1 NA_2
T_3 – SMS PK_1 PK_2
T_4 – SMS SMF_1 NA_2
T_5 – SMS NA_1 SMF_2
T_6 – SMS NA_1 PK_2
T_7 – SMS PK_1 NA_2
T_8 – SMS PK_1 SMF_2
T_9 – SMS SMF_1 PK_2
T_{10} – Control

(Where, SMS -Seed treatment with *S.marcescens* @ 10 g/kg; SMF_1 – foliar application with *S.marcescens* @ 2.5 kg/ha on 15 DAT; SMF_2 - foliar application with *S.marcescens* @ 2.5 kg/ha on 30 DAT ; NA_1- foliar application of Nicotinic acid@ 0.1 % on 15 DAT; NA_2 - foliar application of Nicotinic acid @ 0.1 % on 30 DAT ; PK_1- foliar application of *Panchakavya* @ 5 % on 15 DAT ; PK_2 - foliar application of *Panchakavya* @ 5 % on 30 DAT).Recommended cultural practices for rice cultivars were followed.

Plants were inoculated on 14th day after transplanting by spraying *P. oryzae* spore suspension with atomizer and the control plants were sprayed with dist. water only. High humidity was created by sprinkling the water frequently in the poly house.

Biochemical constituents and enzymes :

***Method of sampling* :**

Sample 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, protein and enzymes like peroxidase (PO), poly phenol oxidase (PPO) and ascorbic acid oxidase.

Preparation of ethanol extract (Mahadevan and Sridhar, 1986):

Plant materials of both healthy and infected were collected and 4 g quantities were taken. They were chopped and then extracted in boiling 80 per cent ethanol and used for the estimation of sugars, phenols and protein.

Quantitative estimation of sugars :

***Reducing sugars* :**

Reducing sugars were estimated by Nelson's (1944) method.

Non-reducing sugars :

Non-reducing sugars with ethanol extract were hydrolyzed and the total sugars were estimated by employing Nelson's method. The total reducing sugars were calculated from glucose equivalents. The final concentration was calculated by deducting the reducing sugar present in the unhydrolysed original sample from the reducing sugar present in the hydrolysed sample. Hydrolysis for non-reducing sugars was done by following the method of Inman (1965).

Estimation of starch :

Starch in the sample was estimated by the method of Sumner and Somers (1949).

Quantitative estimation of phenols :

***Total phenols* :**

Total phenols were estimated by employing folin ciocalteu reagent (Bray and Thorpe, 1954). Ready made

folin ciocalteu reagent obtained from Central Research Laboratories, a division of Central Agro Industries Pvt. Ltd., Mumbai was used for the estimation after making required dilution at the time of estimation.

Ortho-dihydroxy phenols :

Ortho-dihydroxy phenols were estimated by the method described by Johnson and Schaul (1957) employing Arnow's reagent specific to ortho groups.

Estimation of total protein :

Protein was estimated by method of Lowry *et al.* (1951).

Enzyme assays :

***Enzyme extraction* :**

One g of the leaf material was cut in to small bits, crushed in chilled 0.1 M sodium phosphate buffer at pH 7.1, and the volume was made up to 5 ml with the buffer, centrifuged at 2,100 rpm. for 30 min. and the supernatant was used as the enzyme source and all the assays *viz*., poly phenol oxidase, peroxidase and ascorbic acid oxidase were performed in a UV Spectrophotometer at 28 ± 1^0 C (Sridhar *et al.,* 1969). The activity of poly phenol oxidase was estimated by the method of Matta and Dimond (1963), Peroxidase by Hampton (1963) and ascorbic acid oxidase by Oberbachner and Vines (1963). Enzyme activity of PO and PPO was expressed in terms changes in absorbance /minute/mg of protein (Anonymous, 1965). The activity of PAL was expressed as nmol transcinnamic acid min^{-1} mg $protein^{-1}$.

Statistical analysis :

The statistical analysis of the experimental results was performed employing the computer software package 'IRRISTAT', version 90-1, developed by Department of Statistics, International Rice Research Institute, Philippines and as per the procedure of Gomez and Gomez (1976).

RESULTS AND DISCUSSION

The experimental findings of the present study have been presented in the following sub heads:

Post infectional bio chemical changes :

The study was undertaken to find out the effect of different levels of SM, NA and PK application and *P.oryzae* inoculation on the bio chemical changes in rice var. IR 50.

Changes in phenolic compounds :

***Total phenol* :**

The data on the changes in total phenols due to SM, NA, PK application and *P.oryzae* inoculation are recorded in Table 1. 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 profoundly increased by combined application of SMS, NA_1, and PK_2 (T_6) in pathogen inoculated plants. The least phenol content was observed in control.

Ortho-dihydroxy phenols :

The results on the effect of SM, NA, PK application and *P.oryzae* inoculation are presented in Fig. 1. In general, ortho-dihydroxy phenol content was very much altered by the application of SMS, NA_1, and PK2. Maximum concentration of O.D. phenol was observed in T_6 (SMS + NA_1 at 15 DAT + PK_2 at 30 DAT) which recorded 5.92 mg/g in IR 50 at the first stage of sampling. Application of nicotinic acid in combination with SMS and *Panchakavya* 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.

Table 1 : Changes in total phenols of rice var. IR 50 as influenced by application of SM, NA, PK and *P.oryzae* inoculation

Tt.No.	Treatments	Total phenols (mg/g)			
		0 (days)	7 (days)	14 (days)	21 (days)
1	SMS SMF_1 SMF_2	2.65	3.38	3.62	3.78
2	SMS NA_1 NA_2	2.93	3.74	4.15	4.23
3	SMS PK_1 PK_2	2.64	3.28	3.69	3.76
4	SMS SMF_1 NA_2	3.04	3.64	4.07	4.36
5	SMS NA_1 SMF_2	2.96	3.69	4.29	4.41
6	SMS NA_1 PK_2	3.02	4.46	5.04	5.13
7	SMS PK_1 NA_2	2.96	4.17	4.68	4.83
8	SMS PK_1 SMF_2	2.94	3.71	3.87	3.98
9	SMS SMF_1 PK_2	2.87	3.79	3.91	4.01
10	Control	2.47	2.79	3.07	3.21

C.D. (P=0.5), MT – 0.56, ST × MT – 0.16 , ST – 0.30 , MT× ST - 0.14

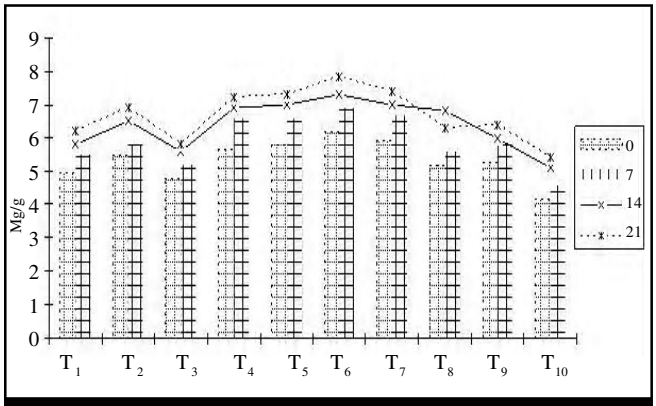

Fig. 1 : Changes in ortho-dihydroxy phenol of rice var. IR 50 as influenced by application of SM, NA, PK and *P. oryzae* inoculation

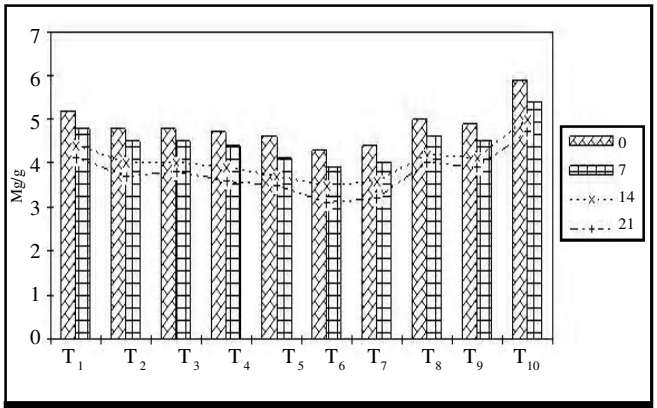

Fig. 2 : Changes in ortho-dihydroxy phenol of rice var. IR 50 as influenced by application of SM, NA, PK and *P. oryzae* inoculation

Reducing sugars :

From Table 2, a general reduction in the quality of reducing sugars due to inoculation and SM, NA, PK application was clearly observed. In inoculated plants the level of reducing sugar was found lesser than in control. Application of NA along with SMS and /or PK (T_6, T_7, T_8 and T_4) reduced the reducing sugar content with increase in time. The reduction in reducing sugar content was more in plants sprayed with nicotinic acid at 15 DAT and *Panchakavya* at 30 DAT.

Non-reducing sugars :

Changes in non-reducing sugars, as influenced by application of SM, NA, PK and *P.oryzae* inoculation is presented in Fig.2. Combinations of spray application of SM, NA and PK significantly influenced the non-reducing sugars. In inoculated plants, the non-reducing sugar content was more in control which recorded 5.9 mg on initial day of sampling. It decreased as the sampling periods increased. Minimum non-reducing sugar content was observed in T_6 at all the sampling periods.

Total sugars :

Changes in total sugars, as influenced by application of SM, NA, PK and *P.oryzae* inoculation is presented in Fig.3.

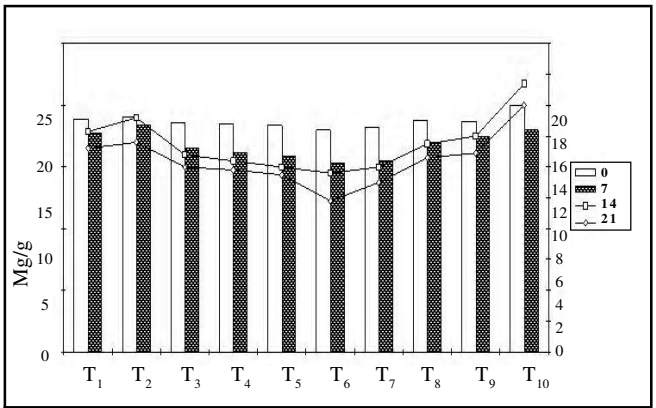

Fig. 3 : Changes in total sugars of rice var. IR 50 as influenced by application of SM, NA, PK and *P. oryzae* inoculation

Table 2 : Changes in reducing sugars of rice var. IR 50 as influenced by application of SM, NA, PK and *P.oryzae* inoculation

Tt.No.	Treatments	Reducing sugars (mg/g)			
		0 (days)	7 (days)	14 (days)	21 (days)
1	SMS SMF_1 SMF_2	36.22	33.92	31.69	30.74
2	SMS NA_1 NA_2	35.15	29.94	27.83	25.14
3	SMS PK_1 PK_2	35.48	33.44	31.29	29.95
4	SMS SMF_1 NA_2	34.62	27.97	23.94	22.64
5	SMS NA_1 SMF_2	34.43	25.46	23.64	20.51
6	SMS NA_1 PK_2	33.77	24.69	18.43	18.06
7	SMS PK_1 NA_2	33.89	24.91	20.36	19.98
8	SMS PK_1 SMF_2	34.87	32.84	30.27	28.00
9	SMS SMF_1 PK_2	34.89	31.96	29.78	27.12
10	Control	36.57	34.63	33.22	32.54

C.D. (P=0.5), MT – 0.62, ST × MT – 2.8, ST – 1.41 , MT× ST - 3.43

SM, NA and PK application reduced the total sugar content. Similar to the trend observed in reducing and non-reducing sugar content, total sugar also showed decreasing trend in the treatments containing nicotinic acid and in combination with PK and SM. Maximum total sugar was recorded in control. Minimum content was recorded in T_6 (SMS + NA_1 + PK_2). An increase in sampling period gradually decreased the total sugar content in all the treatments.

Starch :

Results on the changes of starch due to application of SM, NA, PK and *P.oryzae* inoculation is presented in Table 3. SM, NA and PK application directly influenced the starch content in rice var. IR 50. In control, the starch content was the least with 32.90 mg at the end of the sampling period. Starch content gradually increased up to 14th day of sampling and then slightly decreased at 21st day in all the treatments. The treatment combinations with nicotinic acid recorded significant increase in starch content.

Changes in protein :

Protein content was significantly altered by SMS, NA and PK application (Fig. 4). Application of NA either on 15 DAT or on 30 DAT or in combination with PK increased the protein content in IR 50. The maximum level of protein was observed in T_6 with 54.56 mg/g at the initial sampling, whereas at the final sampling it was increased to the maximum (68.74 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 (37.50 mg/g).

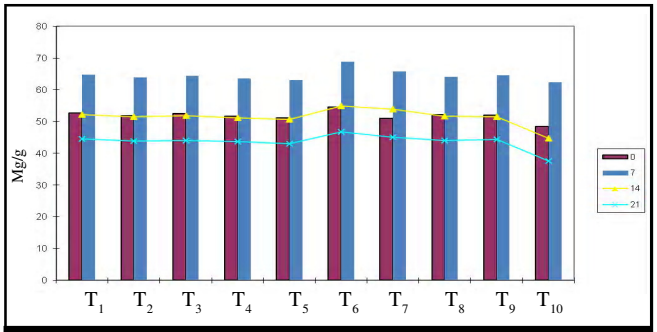

Fig. 4 : Changes in protein content of paddy var. IR 50 as influenced by application of SM, NA, PK and *P. oryzae* inoculation

Effect of SM, NA and PK application and *P.oryzae* inoculation on the enzymatic activity of rice var. IR 50 :

***Poly phenol oxidase (PPO)* :**

Application of SM, NA and PK in combination with each other significantly influenced the poly phenol oxidase activity (Table 4). The maximum content of 2.60 mg was recorded in control at the initial sampling immediately after inoculation. The activity increased from the 7th day of sampling and maximum was observed on 14th day of sampling and there after showed decrease in all the treatments. Inoculation of *P.oryzae* significantly increased the poly phenol oxidase activity in all the treatments. At the end of sampling period, the maximum poly phenol oxidase activity was observed in T_6 (SMS + NA_1 + PK_2).

Peroxidase (PO) :

The results of the studies on the influence of SM, NA, PK application and *P.oryzae* inoculation on peroxidase levels are tabulated in Table 5. The activity of peroxidase was found very less in control (0.55 mg). Application of Nicotinic acid increased the peroxidase activity. Among the treatments, T_6 (2.19 mg) showed the excelled activity of peroxidase throughout the sampling periods. The activity of peroxidase increased up to 14th day of sampling and then decreased in all the treatments.

Ascorbic acid oxidase :

Ascorbic acid oxidase activity was significantly

Table 3 : Changes in starch content of rice var. IR 50 as influenced by application of SM, NA, PK and *P.oryzae* inoculation

Tt.No.	Treatments	Starch content (mg/g)			
		0 (days)	7 (days)	14 (days)	21 (days)
1	SMS SMF_1 SMF_2	34.4	41.18	45.42	42.10
2	SMS NA_1 NA_2	36.26	45.18	50.09	47.67
3	SMS PK_1 PK_2	34.56	41.44	46.42	43.18
4	SMS SMF_1 NA_2	36.47	44.93	48.93	46.61
5	SMS NA_1 SMF_2	35.81	44.38	48.46	46.04
6	SMS NA_1 PK_2	36.81	45.96	53.12	50.79
7	SMS PK_1 NA_2	36.13	45.68	52.89	50.32
8	SMS PK_1 SMF_2	34.91	42.10	46.97	43.24
9	SMS SMF_1 PK_2	35.23	42.63	47.74	44.09
10	Control	33.76	34.40	36.10	32.90

C.D. (P=0.5), MT – 0.93, ST × MT – 1.74, ST – 0.66 , MT× ST - 1.82

influenced by SM, NA, PK application (Table 6). Nicotinic acid application generally decreased the ascorbic acid oxidase activity. Among the different treatments T_6 recorded the least activity with 75.42 mg on 21st day of sampling.

The total phenol content was profoundly increased by the combined application of SMS, NA_1 and PK_2 (T_6) in pathogen

Table 4 : Changes in poly phenol oxidase of rice var. IR 50 as influenced by application of SM, NA, PK and *P.oryzae* inoculation

Tt.No.	Treatments	Poly phenol oxidase (units/min/mg of protein)			
		0 (days)	7 (days)	14 (days)	21 (days)
1	SMS SMF_1 SMF_2	0.54	2.36	22.45	16.26
2	SMS NA_1 NA_2	0.48	2.24	20.99	17.54
3	SMS PK_1 PK_2	0.59	2.32	22.38	16.44
4	SMS SMF_1 NA_2	0.40	2.30	20.99	17.94
5	SMS NA_1 SMF_2	0.36	1.58	20.66	18.24
6	SMS NA_1 PK_2	0.26	2.64	19.99	18.58
7	SMS PK_1 NA_2	0.41	2.08	20.41	18.15
8	SMS PK_1 SMF_2	0.52	2.43	21.47	16.61
9	SMS SMF_1 PK_2	0.480	2.19	21.38	16.94
10	Control	2.60	4.00	19.59	14.70

C.D. (P=0.5), MT – 0.66, ST × MT – 1.09, ST – 0.26 , MT× ST - 1.02

Table 5 : Changes in peroxidase of rice var. IR 50 as influenced by application of SM, NA, PK and *P.oryzae* inoculation

Tt.No.	Treatments	Peroxidase (units/min/mg of protein)			
		0 (days)	7 (days)	14 (days)	21 (days)
1	SMS SMF_1 SMF_2	0.87	12.17	108.85	72.80
2	SMS NA_1 NA_2	0.99	10.24	116.64	76.86
3	SMS PK_1 PK_2	1.19	18.84	119.20	81.69
4	SMS SMF_1 NA_2	0.96	9.32	114.51	74.42
5	SMS NA_1 SMF_2	1.05	19.89	115.82	80.64
6	SMS NA_1 PK_2	2.19	40.90	125.69	82.63
7	SMS PK_1 NA_2	1.19	18.99	119.99	82.08
8	SMS PK_1 SMF_2	1.09	21.64	119.39	81.09
9	SMS SMF_1 PK_2	0.91	14.18	117.34	79.88
10	Control	0.55	7.32	62.80	33.78

C.D. (P=0.5), MT – 0.61, ST × MT – 1.81, ST – 0.50 , MT× ST - 1.69

Table 6 : Changes in ascorbic acid oxidase of paddy var. IR 50 as influenced by application of SM, NA, PK and *P.oryzae* inoculation

Tt.No.	Treatments	Ascorbic acid oxidase (units/min/mg of protein)			
		0 (days)	7 (days)	14 (days)	21 (days)
1	SMS SMF_1 SMF_2	24.68	114.51	111.80	84.25
2	SMS NA_1 NA_2	23.99	111.38	107.10	82.10
3	SMS PK_1 PK_2	24.65	114.68	111.38	84.30
4	SMS SMF_1 NA_2	23.70	110.84	106.35	81.40
5	SMS NA_1 SMF_2	22.65	109.45	104.82	80.98
6	SMS NA_1 PK_2	21.26	103.48	97.12	75.42
7	SMS PK_1 NA_2	22.19	109.12	100.46	77.92
8	SMS PK_1 SMF_2	24.18	118.47	109.49	82.99
9	SMS SMF_1 PK_2	24.12	112.41	108.31	83.10
10	Control	14.91	85.96	79.28	58.56

C.D. (P=0.5), MT – 1.16, ST × MT – 5.81, ST – 3.02 , MT× ST - 6.03

inoculated plants. The amount of total phenol increased in all treatment combinations at all the sampling periods. The least phenol content was observed in control. Resistance to plant pathogens is very often described to the changes in the metabolism of a host (Cruickshank and Perrin, 1964 and Chandramohan *et al.,* 1967). Eswaran (1990) reported that the inoculation of rice with *Aspergillus oryzae* led to the increase in total phenolic content. Regarding the mechanism by which the phenols confer resistance, it is believed that the phenols may be :

- Exerting a toxic effect to the pathogen or,
- Exerting an inhibitory effect through the oxidation products of phenolics which being more toxic towards the pathogen or,
- Interfering with the electron transport system leading to a blockage in the energy release process or,
- Binding with enzymes containing sulphide groups and inactivate them or,
- Oxidizing the pectinolytic enzymes produced by the pathogens or,
- Suppressing the IAA oxidase in plants there by increasing the levels of IAA.

The O.D. phenols content was very much altered by the application of combination of SMS, NA_1 and PK_2. The O.D. phenol content of the inoculated plants showed an increasing trend with the development of disease. Application of resistance inducing chemicals could have increased the phenolic content of the plants (Klessig and Malamy, 1994).

Deranged carbohydrate metabolism of the host in response to infection was investigated by number of workers (Kalyanasundaram, 1986). The level of sugars correlated with disease resistance (Jayapal and Mahadeven, 1968 and Mohan and Subramanian, 1977). Reduction of sugars and accumulation of starch due to the application of lignite fly ash (LFA) and combination of lignite fly ash with potash in blast infected leaves was reported by Mallika and Ramabadran (1995) and Karpagavalli and Ramabadran (1996). These reports are lending support to the present findings.

Nitrogenous compounds were important for the growth and multiplication of invading pathogens. The increased accumulation of amino nitrogen in the inoculated plants might be partly attributed to the syntheses by the pathogen (Rohringer, 1957). Close correlation between blast susceptibility and soluble nitrogen was reported by Chen (1989). Karpagavalli and Ramabadran (1996) reported the reduction in amino nitrogen content due to lignite fly ash application. Alteration of nitrogen metabolism of the host plants in response to pathogenic invasion has been studied in detail by many workers (Sridhar and Mahadevan, 1979; Hwang *et al.,* 1983). All these reports are in line with the present findings.

Miswa and Miyasakai (1972) reported that the bacterial leaf blight susceptible rice variety contained greater amount of amino protein than resistant variety. Siddaramaiah *et al.* (1979) observed increased protein content in rust infected groundnut leaves as observed in the present study.

Sridhar and Mahadevan (1968) reported that blast infected tissue exhibited an increase in ascorbic acid oxidase and peroxidase. Increased activity of peroxidase upon infection might be required for an additional deposition of lignin around the lesions induced by pathogens. According to Robb *et al.* (1964) the increased activity of poly phenol oxidase (PPO) might be due to either solubilization of polyphenols from cellular compartments or activation of latent poly phenol oxidase. Rice leaves infected with *P.oryzae* exhibited increased peroxidase activity (Wang *et al.,* 1991; Aver- Yanov and Lapikova, 1995). PPO is a copper containing enzyme, oxidizes phenolics to highly toxic quinines and involved in the terminal oxidation of diseased plant tissues which was attributed for its role in disease resistance (Kosuge, 1969). All these reports confirm the present findings.

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