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Biochemical changes in *Rumexacetosa* infected with Mancozeb resistant *fusarium proliferatum*

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ABSTRACT

Biochemical changes were observed from healthy and artificially inoculated mancozeb resistant (At_6) and sensitive (At_5) isolates of Chuka (*Rumex acetosa* L.) caused by *Fusarium proliferatum*. There was found significant variation between healthy and infected leafy vegetables showed significant changes with respect to estimation of Moisture content, total sugar reducing sugar, non-reducing sugar, total ash, crude fiber, phosphorus, fat, ascorbic acid, calcium, iron and crude protein. Among them, moisture in the *Rumex acetosa* infected with sensitive and resistant strains was decreased. Among them total ash was increased in healthy leaf, sensitive and resistant followed by Crude protein, Phosphorus and Ascorbic acid, healthy leaf sensitive and resistant. In case of total suger it was in reduced infected leaf. But Calcium and Fat however was decreased due to infection of both isolates.

Key word: *Rumex acetosa*, mancozeb, *Fusarium proliferatum*, biochemical changes.

INTRODUCTION

Vegetables are very important ingredients in human diet. The nutritive values of healthy vegetables are altered because of fungal attack and sometimes fungi produce certain mycotoxins in them and make them unsuitable for human consumption. Leafy vegetables have more nutrition per calorie than any other food. Chuka (*Rumex acetosa* L.) of family Polygonaceae often simply called sorrel is a perennial herb that is cultivated as leafy vegetable and medicinal properties[1]. In small quantities of sorrel is harmless, in large quantities it can be fatal. Several subspecies have been named not all are cultivated i.e. *Rumex acetosa* sp. *acetosa, R.acetosa* sp. *ambiguous, R. acetosa* sp. *arifolius, R. acetosa* sp. *hibernicus, R. acetosa* sp. *hirtulus* and *R.acetosa* sp. *Vinealis* [2,3].

Children and pregnant and nursing women should not used sheep sorrel. The herb is not recommended for people with kidney stones arthritis, endometriosis, gout and hyperacidity, since it may aggravate those conditions, due to its high acidic content and large amounts of sorrel should be avoided [4]. Many examples of fungicide resistance including mancozeb in pathogen of various crops have been reported [5,6,7]. The present investigation was made to evaluate the. Biochemical changes due to *Fusarium proliferatum* inciting leaf spot of *Rumex acetosa*.

MATERIAL AND METHODS

Isolation of *Fusarium proliferatum*: A survey which was commenced from June 2008 to July 2009 in growing season conducted in field and markets of Marathwada region of Maharashtra state. Delicious leafy vegetable crops are attacked by fungal diseases like, leaf spot showing symptom of fungal infection were collected and symptomatology of the disease was studied under natural and artificial conditions. Microscopic slides were prepared from the infected leafy vegetable to observe mycelium, conidiophores and conidia of the pathogens. Isolation of pathogens was done from each distinct type of symptom produced on leafy vegetable. Infected leaf spots were cut into small pieces by sterilized blade then surface sterilized with mercuric chloride (0.1%) for 1 min. The pieces were then washed with sterilized water and dried by sterilized blotting paper. These pieces were placed on potato dextrose agar (PDA) medium and incubated at 28±1°C. The pathogens were isolated and maintained on PDA medium. The fungi were identified *Fusarium proliferatum* (Fries) Wiltshire. The cultures were deposited at Agharkar Research Institue (ARI), Pune and Department of Botany. Arts, Science and Commerce College Naldurg. Morphological characters of the pathogen were studied and pathogens were identified by Ellis [8].

Sensitivity of the pathogens against fungicide (MIC): Sensitivity of ten isolates was tested against mancozeb (75%WP) by food poisoning technique [9]. Czapek Dox agar plates were prepared containing differ ent concentration of (100 to 5000µg/ml) fungicide for treating both isolates. Eight days fresh culture disc (8mm) of isolates were inoculated at the centre of plates in triplicate. The plates were incubated at 28±1°C in the dark and radial growth was measured at different intervals. Plates without fungicides treated as control.

Biochemical analysis: Biochemical analysis of sensitive and resistant isolates by inoculating *Rumex acetosa* leaves were inoculated with spore suspension (105/ml) of pathogen. Uninocoulated plants served as control. After 40 days the infected and healthy leaves were collected and dried in oven at 60°C for 24 hrs. The dried samples were crushed in grinder. 10gms of samples were extracted in 100 ml ethanol and analyses for 12 parameters such as Moisture, Fat, crud protein [10] Crude fiber [11], Total sugar [12], Reducing sugar [13], Non reducing sugar, Total ash [14] and [15], Ascorbic acid [16], Phosphorus, Iron and Calcium [17, 18].

RESULTS AND DISCUSSION

Isolation of *Fusarium proliferatum* (Fries) Wiltshire: After two-three days of inoculation, ash green colonies with whitish peripheral concentric rings are formed. Isolated fungus was identified as *Fusarium proliferatum*. On the basis of cultural and morphological

characteristics the radial growth of the fungus in culture was uniform.

Sensitivity of the pathogens against fungicide MIC:

Sensitivity (MIC) *Fusarium proliferatum* was tested against Mancozeb, The results indicated in (Table 1) that there was large variation in the sensitivity of these isolates. Some isolates were sensitive At_5 (100 µg/ml) while others were resistant Fp_3(1900 µg/ml). Sensitivity ranged from (200 to 700 µg/ml) and sensitive isolates are Fp_1, Fp_4 Fp_2 and Fp_3 resistant isolates Fp_5 (3900 µg/ml). Among them, Fp_3 for resistant isolate were used for biochemical analysis.

Table1: MIC of carbendazim against *Fusarium proliferatum* isolates causing leaf spot of *Rumex acetosa*.

Isolates	MIC µg/ml
Fp_1	1500
Fp_2	200
Fp_3	1900
Fp_4	1000
Fp_5	1600

Biochemical analysis:

Biochemical analysis was determined from leafy vegetable *Rumex acetosa* are shown in (Table.2).). It was noted that the content of all parameters in the pathogen varied in sensitive and resistant strains. Moisture in the *Rumex acetosa* infected with sensitive and resistant strains was decreased. Among them total ash was increased in healthy leaf, sensitive and resistant followed by Crude protein, Phosphorus and Ascorbic acid, healthy leaf sensitive and resistant. In case of total sugar it was in reduced infected leaf. But Calcium and Fat however was decreased due to infection of both isolates.

MIC:Minimum Inhibitory Concentration.*- Sensitive. +- Resistant.

There are reports supporting the characteristic of resistant isolates. According to Sindhan and Parashar [19] the concentration of total sugars, reducing sugars, non reducing sugars, nitrogen and phosphorus was decreased due to infection by early and late leaf spot pathogens of groundnut but the phenol content was increased. The changes of total sugar, reducing sugar, non-reducing sugar, starch, protein, ash and lipid contents decreased remarkably but those of Vitamin-C,

Table 2: Biochemical characteristics of the *Rumex acetosa* infected with mancozeb resistant and sensitive isolates of *Fusarium proliferatum*

Sr. No.	Estimation	Healthy	Sensitive (Fp5)	Resistant (Fp3
1	Moisture (%)	49.34	35.40	30.20
2	Fat (%)	0.92	0.86	1.06
3	Crude protein (%)	20.81	13.20	20.93
4	Crude fiber	0.6	0.4	0.5
5	Total sugar (mg/g)	7.5	3.4	2.7
6	Reducing sugar (mg/g)	2.20	2.0	2.7
7	Non reducing sugar (mg/g)	5.3	1.4	0.55
8	Total Ash (%)	26.11	20.63	27.96
9	Ascorbic acid	12	7.30	8.5
10	Phosphorus	18	12.35	16.4
11	Iron (mg/100gm)	9.80	4.1	4.1
12	Calcium	0.7	0.3	0.4

phenol, pectin and mineral contents increased significantly in leaves of disease affected mulberry as compared to those in healthy mulberry [20]. Biochemocal changes in pomegranate fruit infected with carbendazim resistant of *Fusarium proliferatum* showed more loss of reducing and non-reducing sugar as compared to healthy one [21]. Bhale [22] reported total sugars were reduced in infected leaves of spinach when compared with healthy one. Tofazzal Hossain [23] reported that the content of total sugars, reducing sugars was reduced due to infection by *Colletotrichum gloesporiodes*. Khillare [24] reported total sugars, total amino acids, crude protein DNA and RNA contents increased in their quantity due to infection by both the isolates of fruit rot of grape.

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