

Biochemical changes in spinach infected with carbendazim resistant *Alternaria spinaciae*

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Spinach (*Spinacea oleracea* L.) is one of the important vegetables in Maharashtra. It is infected with *Alternaria spinaceae*. Many examples of fungicide resistance including carbendazim in pathogens of various crops have been reported (9, 8, 5, 2, 4). It appears that nearly 18 pathogens showed resistance against 24 fungicides from different parts of our country. In the present paper effect of *A. spinaceae* resistant to carbendazim on the biochemical changes in spinach leaves have been described.

A total of 21 isolates of *A. spinaceae* were isolated from infected leaves of spinach throughout Maharashtra using Czapek Dox agar medium. Carbendazim resistance of all the isolates were studied by food poisoning technique on Czapek Dox agar medium (6, 8) at different concentrations of containing carbendazim (a.i) for evaluation of MIC. The isolate indicating R/S factor more than three grown in the earthen pots using garden soil. Leaves were inoculated with spore suspension (10^5 /ml) of resistant and sensitive isolates of pathogen. Uninoculated 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 analysed for 11 parameters such as Nitrogen (3), Phosphorus, Calcium (1), Phenols, Crude Protein (1), Sugar and total sugars (11) and DNA and RNA (10).

It was seen that total sugars were reduced in infected leaves of spinach when compared with healthy ones. Total sugars in the leaves infected with sensitive and resistant strains were variable. MIC of isolates against carbendazim was more variable among the isolate. It ranged from 350 µg/ml to 700 µg/ml. Highest (700 µg/ml) was seen in isolate AS-6 and AS-15. Thus according to FRAC isolate AS-21 was resistant and isolates AS-6 and AS-15 were sensitive. Other isolates were moderately resistant (MIC 375 to 675 µg/ml) (Table 1). In India and abroad, many examples of fungicide resistance have been recorded (11, 4, 8).

There are many reports indicating the biochemical characteristics in different host (12, 13) infected with pathogens. There was slight increase in total sugars due to

resistant isolate. But crude protein and nitrogen DNA and RNA however were decreased due to infection of both the isolates. There was slight increase in phenols in the leaves inoculated with resistant sensitive isolates in the healthy leaves. In case of phosphorus and calcium were reduced in infected leaves (Table 2). There are many reports supporting these. Therefore it is necessary to study the different isolates even in the absence of fungicides.

Table 1. MIC of Carbendazim against *Alternaria spinaciae* isolates causing leaf spot of spinach

Isolates	MIC* µg/ml
AS-1	450
AS-2	375
AS-3	600
AS-4	600
AS-5	400
AS-6	350
AS-7	550
AS-8	500
AS-9	650
AS-10	475
AS-11	550
AS-12	450
AS-13	450
AS-14	400
AS-15	350
AS-16	525
AS-17	650
AS-18	500
AS-19	425
AS-20	675
AS-21	700
CD 5%	60.7%

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MIC*: Minimum Inhibitory Concentration

Table 2. Biochemical characteristics of the leaves infected with carbendazim resistant and sensitive isolates of *Alternaria spinaciae* after 10 days

S. No.	Biochemical composition (mg/g & %)	Spinach leaves		
		Healthy	Sensitive AS-15	Resistant NMU AS-1
1	Nitrogen %	4.17	2.84	1.67
2	Phosphorus %	0.09	0.07	0.08
3	Calcium %	0.44	0.35	0.31
4	Crude protein %	26.04	17.7	6.25
5	Total ash %	19.00	14.00	14.00
6	Non reducing sugar mg/g	0.75	0.48	0.39
7	Reducing sugar mg/g	2.25	1.14	1.27
8	Total sugar mg/g	3.00	1.25	2.00
9	DNA mg/g	1.00	0.11	0.22
10	RNA mg/g	5.60	1.30	2.33
11	Phenols mg/g	1.08	1.44	1.48
	CD 5%	6.63	4.74	3.14

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