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Effect of Passage on the Development of Carbendazim Resistance in *Gloeosporium ampelophagum* Causing Anthracnose of Grapes

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Fungicide carbendazim is recommended to manage anthracnose of grapes in orchards which is important disease in India. The wild sensitive isolate GA-1 was studied both *in vitro* and *in vivo* on grapes. Culturing wild type isolate continuously for five successive passages on carbendazim individually increased resistance significantly. However, reduced resistance was observed when pathogen was cultured alternately or in mixture with different fungicides of amide and conazole groups. Similar type of results was obtained on the grape berries. Use of difenoconazole and myclobutanil alternately and difenoconazole, myclobutanil and propiconazole in mixture appeared to be most useful to break the development of carbendazim resistance in pathogen.

Key words: Fungicide resistance, Carbendazim, Anthracnose of grapes, *Gloeosporium ampelophagum*

The grape (*Vitis vinifera*) is one of the most economically important fruit crops in the world[1]. Anthracnose is one of the most damaging diseases of grape and is caused by *Gloeosporium ampelophagum* (Pass.) Sacc. and responsible for yield losses in commercial grape production. In wet humid regions the disease incidence and severity on various cultivars of grape can be very serious[2]. Infection may occur on all succulent plant material but is most common on fruit and shoots. Lesions on berries are initially small, circular and reddish in color. Acervuli are also produced in these lesions. Leaf spots are often numerous and resemble those on fruit. The center often drops out leaving a shot hole appearance. Young leaves are more susceptible than older leaves and are malformed when veins become infected. Fungicides have been extensively used to control anthracnose of grape, but cause environmental pollution and leave residues in the agricultural soil and on products. Chemical usage has been effective, although resistance to these fungicides is developing. The development of carbendazim resistance against *G. ampelophagum* in Maharashtra[3] and other States of India was studied by many workers[4-10].

MATERIAL AND METHODS

Sensitivity of pathogen

The infected samples of grapes were collected from different districts known as 'grape belt' of Maharashtra like Ahmednagar, Nashik, Pune

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and Solapur during 2009 & 2010 crop seasons. Isolation of pathogen was done by inoculating the samples on Czapek-Dox agar medium. The cultures were further purified and maintained on same medium at 27± 1° C. A total of 37 isolates were purified and tested against carbendazim fungicide by 'Poisoned Food Technique'[11] to check their sensitivity. Czapek-Dox agar medium (2X) was prepared and it was then sterilized and 10 ml of this was properly mixed with 10 ml of fungicide (2X a.i. concentrations) selected for study in sterile Perti plates. A series of concentrations was prepared; the fungicide was thoroughly mixed with medium and allowed to solidify. A 4 mm disc of the fresh grown *G. ampelophagum* isolates was transferred aseptically at the centre of Petri plate. On the basis of minimum inhibitory concentration (MIC) the resistant and highly resistant population was calculated by multiplying four times to the sensitive baseline dose as per guidelines given by fungicide resistance action committee (FRAC). The data of radial growth was analyzed for MIC and essential dose (ED_{50}) by using following equation[12].

$$Y = \frac{H}{1 + Exp\ (a+bx)}$$

(Where, Y = radial growth as percentage of control, H= upper limit of curve, Exp= logarithmic exponent, a= regression constant, b= regression coefficient and x= measured points).

Study of passage

In order to study the effect of passage *in vitro* wild sensitive isolate GA-9 in each passage was cultured on agar plates containing sub-lethal dose of carbendazim (0.3 µg/ml). The plates without fungicide served as control. A 4 mm diameter disc of freshly grown culture taken from the culture of previous passage of the same isolate was placed at the centre of each plate. In each passage linear growth was measured after eight days. Percentage increase of growth of the isolate from passage to passage was considered as increase in carbendazim resistance or *vice-versa*.

The development of resistance thus was studied up to 5th passage. Alternate passage carbendazim with triadimefon, metalaxyl, difenoconazole, myclobutanil, propiconazole and mixed passage with the same fungicide were also carried out.

Passage studies were also carried out on the grape berries (*Vitis vinifera* L. *var. Thomson seedless*). The grape berried were inoculated with spore suspension taken from the culture of previous passage of the same isolate was inoculated on grape berries. The concentration of carbendazim and wild sensitive isolate GA-9 was kept same as used *in vitro* studies. At each passage percent disease index (PDI) was calculated. PDI increased from passage to passage considered as increase in the carbendazim resistance. The development of resistance was studied up to 5th passage both in alternate and in mixture of different fungicides.

RESULTS AND DISCUSSION

Results in Table 1 indicate that in vitro individually culturing of the pathogen in carbendazim increased growth significantly up to 5th passage. However, alternate culturing of pathogen on difenoconazole and myclobutanil

Table 1. Effect of continuous exposure to carbendazim and to carbendazim alternately with other different fungicide on growth of *Gloeosporium ampelophagum* on agar medium during five successive passages

S. No.	Fungicides (0.3µg/ml)	Passage number				
		I	II	III	IV	V
1	Carbendazim continuous	18.57	24.35	48.57	70.00	73.61
2	Carbendazim alters triadimefon	18.57	94.40	35.71	87.14	70.83
3	Carbendazim alters metalaxyl	18.57	95.83	32.85	88.57	34.72
4	Carbendazim alters difenoconazole	18.57	51.38	34.28	57.14	09.16
5	Carbendazim alters myclobutanil	18.57	97.20	35.71	81.42	11.08
6	Carbendazim alters propiconazole	18.57	48.61	72.85	80.00	61.11

reduced growth significantly. This reduction was more prominent with myclobutanil than other fungicides. Interestingly there was also significant reduction in the growth of pathogen when cultured on the carbendazim in combination with difenoconazole and myclobutanil on grape berries (Table 2). *In vivo* results are given in Table 3 and 4. It was seen that again treatment of carbendazim to grape berries for five successive passages increased PDI on grapes. However, treatment of carbendazim alternately with triadimefon, metalaxyl, difenoconazole, myclobutanil and propiconazole reduced PDI significantly. Use of carbendazim with difenoconazole, myclobutanil and propiconazole were most useful in controlling grape anthracnose. There are some evidences for increase of resistance due to continuous exposure of pathogen to the fungicides[12, 13]. Alternate or mix application of fungicide must have different mode of action[14] and in the present investigation there might have less

Table 2. Effect of continuous exposure to carbendazim and to carbendazim alternately with other fungicides on growth of *Gloeosporium ampelophagum* on grape berries during five successive passages

S. No.	Fungicides (0.3µg/ml)	Passage number				
		I	II	III	IV	V
1.	Carbendazim continuous	52.22	59.78	65.12	70.14	75.43
2.	Carbendazim alters triadimefon	52.22	88.28	44.30	75.18	66.40
3.	Carbendazim alters metalaxyl	52.22	60.12	47.14	76.44	32.18
4.	Carbendazim alters difenoconazole	52.22	82.20	56.16	78.15	21.85
5.	Carbendazim alters myclobutanil	52.22	94.12	62.18	81.00	15.22
6.	Carbendazim alters propiconazole	52.22	62.46	78.15	84.67	58.10

Table 3. Effect of continuous exposure to carbendazim and to carbendazim mixed with other different fungicide on growth of *Gloeosporium ampelophagum* on agar medium during five successive passage

S. No.	Fungicides (0.3µg/ml)	Passage number				
		I	II	III	IV	V
1	Carbendazim continuous	21.25	33.33	38.57	39.18	40.00
2	Carbendazim mixed triadimefon	34.61	48.33	39.74	32.50	32.89
3	Carbendazim mixed metalaxyl	37.17	50.00	44.44	35.00	36.52
4	Carbendazim mixed difenoconazole	34.28	21.93	16.11	07.17	05.52
5	Carbendazim mixed myclobutanil	40.00	38.70	37.50	35.89	35.52
6	Carbendazim mixed propiconazole	22.85	27.41	22.36	24.35	20.68

Table 4. Effect of continuous exposure to carbendazim and to carbendazim mixed with other different fungicide on growth of *Gloeosporium ampelophagum* on grape berries during five successive passages

S. No.	Fungicides (0.3µg/ml)	Passage number				
		I	II	III	IV	V
1.	Carbendazim continuous	23.78	43.69	57.42	66.90	72.14
2.	Carbendazim mixed triadimefon	38.15	49.77	43.24	40.78	40.00
3.	Carbendazim mixed metalaxyl	42.66	56.90	46.11	41.89	42.00
4.	Carbendazim mixed difenoconazole	36.08	14.15	13.80	09.68	09.32
5.	Carbendazim mixed myclobutanil	41.90	39.00	37.08	34.88	32.18
6.	Carbendazim mixed propiconazole	27.10	34.11	27.00	30.83	21.66

chances to mutate or to adopt resistance in *G. ampelophagum* due to use of other group of fungicides. These results also agree with the earlier work in case of *Septoria nodorum* against carbendazim[12]. Similar results were obtained by author in *Penicillium digitatum* against thiophanate-methyl and in *Alternaria alternata* against aureofungin[15,16] causing fruit rot of grapes.

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