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EFFECT OF PASSAGES ON THE DEVELOPMENT OF CARBENDAZIM RESISTANCE IN SENSITIVE ISOLATE OF *PHYLLOSTICTA ZINGIBERI* CAUSING LEAF SPOT OF GINGER

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Abstract:
Effect of passage on the development of carbendazim resistance in sensitive isolate, indicated that there is increase in the resistance of pathogen to carbendazim when cultured continuously on the carbendazim for eight successive passages both *in vitro* and *in vivo*. While use of carbendazim alternately and in mixing with kocide, aliette, dhanuka and bordeaux mixture reduced carbendazim resistance significantly.
Keywords: Sensitive, Carbendazim, Ginger, continuous, alternate, Mixed.

Introduction

Ginger is obtained from the rhizomes of *Zingiber officinale* Rosc. It is a herbaceous perennial plant coming under the family zingiberaceae. Ginger is known as sunthi in Ayurveda. It is also indicated in ointment form for local application in pains. It is commonly used to treat various stomach problems, diarrhea and nausea. Such important plant is attacked by *Phyllosticta zingiberi* Ramkr. causing leaf spot disease (Ramakrishnan, 1941). Symptoms are observed on leaves as oval to elongated spots that later turn whitish spots surrounded by dark brown margin with yellowish hallo (Brahma and Nambiar, 1982). Carbendazim was more effective in reducing the severity of the disease (Singh, 2015). Hence this fungicide was undertaken for detailed study. Information on fungicide resistance in plant pathogens of various crop plants is very few. Now-a-days there is increase in fungicide application for the controlling various diseases on plants. Therefore, the aim of the present investigation was to examine the effect of passage on the development of carbendazim resistance in *Phyllosticta zingiberi*.

Material and Method

Thirteen samples exhibiting leaf spot of ginger were collected from different districts of Maharashtra *viz*. Kolhapur (Mudal Titta, Majnal, Kolhapur, Mhalunge and Mangnur), Sangli (Jambhali, Zelam, Islampur, Vadiye Raybag and Tandulwadi) and Satara (Dahiwadi, Koregaon and Jaitapur). Samples are collected in rainy days i. e. from July to October. Collected samples were brought to the laboratory in sterilized bags. The infected portion of leaf was cut in to the size 2 mm and sterilized by using 0.1% $HgCl_2$ and washed with sterilized distilled water (Jadhav *et al.*, 2010). These sterilized leaf portion were kept on Czapek dox agar plates amended with streptomycin sulphate (Patil *et al.*, 2012 and Mali et al., 2015). Inoculated plates were incubated at 28 ± 2^0 C for growth of the fungus and further studies (Mali *et al.*, 2016). After 9-10 days of culture, grayish fungal mass was observed. On the basis of morphological, microscopic characters and following relevant mycological literature the fungal isolate was identified as *Phyllosticta zingiberi* Ramkr. In this manner, 13 isolates were obtained. Carbendazim was used to manage the disease and MIC (Minimum Inhibitory Concentration) of thirteen isolates was carried out both *in vitro* and *in vivo*. The results obtained from the MIC, sensitive and resistant isolate were identified. Sensitive isolate (Pz 1) showed 2 % MIC both *in vitro* and *in vivo* while isolate (Pz 11) was resistant to carbendazim and showed 9 % MIC on agar plates and 8 % on zingiber leaves (Table 1).

After determination of MIC of carbendazim, the effect of continuous and alternate treatments of fungicides and a mixture of both on the development of carbendazim resistance in wild sensitive isolate of *Phyllosticta zingiberi* (Pz-1) was studied *in vitro* and *in vivo*. For this purpose wild sensitive isolate (Pz-1) of *Phyllosticta zingiberi* was cultured for 8 successive passages on the culture medium having carbendazim at its MIC level (2%), continuously (Apte and Kamble, 2013 and Jagtap *et al.*, 2014). During alternate and mixed passage of *in vitro*, same procedure of continuous passage was followed with alternate and in mixture use of fungicides of same concentration respectively. In each passage, linear mycelial growth was measured after 10 days.

During *in vivo* conditions, to study the effect of continuous passage, mycelial suspension of wild sensitive isolate (Pz 1) was prepared. Ten ml mycelial suspension containing 331×10^{-4} spores/ml, was inoculated on healthy ginger leaves (3-4 leaf stage) treated with 2% carbendazim, 24 hours before inoculation

(Jagtap *et al.*, 2014). After 10 days, mycelial suspension from such infected ginger leaves was prepared and applied to healthy ginger leaves (3-4 leaf stage) treated with 2% carbendazim, 24 hours before inoculation. Same procedure was repeated up to 8 passages. During alternate and mixed passage, same procedure of continuous passage was followed with alternate and in mixture use of fungicides of same concentrations (2%) respectively.

In each type of passage mentioned above, the increased mycelial growth from passage to passage was considered as criterion for the development of fungicide resistance.

Results and Discussion

A. Continuous and alternate Passage

It was seen that growing of *Phyllosticta zingiberi* on the medium containing carbendazim for eight successive passages continuously, there was significantly increase the resistance. When carbendazim was altered with, dhanuka, aliette, bourdeaux mixture and kocide fungicides, there was decrease in the development of carbendazim resistance. When carbendazim used alternately with dhanuka and aliette, there was reduction in resistance at 2nd passage, and when carbendazim alternate with kocide and bordeaux mixture there was complete inhibition of pathogen at 3rd passage in *in vitro* condition. (Table: 2).

In *in vivo* studies, in alternate passage, significant reduction in the disease at 2nd passage in case of dhanuka, aliette and bourdeaux mixture. There was complete reduction of the disease at 3 rd passage, when *Phyllosticta zingiberi* inoculated on *Zingiber officinale* alternately with Kocide. (Table: 2).

The results are in agreeing with other workers. Use of ediphenphos alternate with benomyl reduced benomyl resistance in *Septoria nodorum* and *Cercosporella herpotrichoides* (Horsten,1979). A mathematical model to test different fungicides for their alternate use was given by (Kable and Jafferey, 1980). Multisided action of benomyl with mancozeb, benomyl, captafol and thiram might be responsible for the complete inhibition of the development of resistance in the *Macrophomina phaseolina* causing charcoal rot of potato (Kamble, 1991). Alternate treatment with copper oxychloride, ziram and mancozeb significantly reduced aluminium phosphite resistance in *Pythium aphanidermatum* from passage to passage (Gangawane and Shaikh, 1988). Use of ridomil altering with kavach, roko, carbendazium and benomyl checked the resistance of the *Rhizopus artocarpi* causing fruit rot of jackfruit (Dalvi et al., 2016).

Table 1: MIC (Minimum Inhibitory Concentration) of carbendazim against *Phyllosticta zingiberi* isolates causing Leaf spot of *Zingiber officinale.*

Locality	**Isolate**	***in vitro* MIC**(%)	***in vivo* MIC** (%)
Mudal titta	Pz 1	2	2
Majnal	Pz 2	4	4
Kolhapur	Pz 3	5	4
Mangnur	Pz 4	3	2
Mhalunge	Pz 5	6	5
Jambhli	Pz 6	6	4
Zelam	Pz 7	3	3
Islampur	Pz 8	5	3
Dahivadi	Pz 9	7	5
Koregaon	Pz 10	4	3
Jaitapur	Pz 11	9	8
Vadiye Raybag	Pz 12	7	6
Tandulwadi	Pz 13	8	6

Table 2: Effect of exposure of *Phyllosticta zingiberi* (*In vitro / in vivo*) to carbendazim continously and alternating with other fungicides on the development of resistance during eight successive passages.

Fungicides mg/ml		**Passage Number**							
		1	2	3	4	5	6	7	8
Carbendazim continuous	***In vitro***	12.33	13.66	14.33	15.5	15.83	16.66	17.33	18.66
	in vivo	0.1	0.1	1	1	1	5	5	5
Carbendazim alters Dhanuka	***In vitro***	12.33	0	0	0	0	0	0	0
	in vivo	0.1	0	0	0	0	0	0	0
Carbendazim alters Aliette	***In vitro***	12.33	0	0	0	0	0	0	0
	in vivo	0.1	0	0	0	0	0	0	0
Carbendazim alters Bourdeaux mixture	***In vitro***	12.33	19.33	0	0	0	0	0	0
	in vivo	0.1	0	0	0	0	0	0	0
Carbendazim alters Kocide	***In vitro***	12.33	17.66	8	8	8	8	8	8
	in vivo	0.1	0.1	0	0	0	0	0	0

Table 3: Effect of exposure of *Phyllosticta zingiberi* to the mixture of carbendazim with other fungicides (*In vitro / in vivo*) on development of resistance during eight successive passages.

Fungicides mg/ml		**Passage Number**							
		1	2	3	4	5	6	7	8
Carbendazim continuous	***In vitro***	12.33	13.66	14.33	15.5	15.83	16.66	17.33	18.66
	in vivo	0.1	0.1	1	1	1	5	5	5
Carbendazim + Dhanuka	***In vitro***	0	0	0	0	0	0	0	0
	in vivo	0	0	0	0	0	0	0	0
Carbendazim +Aliette	***In vitro***	9	0	0	0	0	0	0	0
	in vivo	0	0	0	0	0	0	0	0
Carbendazim +Bourdeaux mixture	***In vitro***	15.66	0	0	0	0	0	0	0
	in vivo	0	0	0	0	0	0	0	0
Carbendazim +Kocide	***In vitro***	12.83	0	0	0	0	0	0	0
	in vivo	0.1	0	0	0	0	0	0	0

Figure 1: *In vitro* continuous and alternate passage

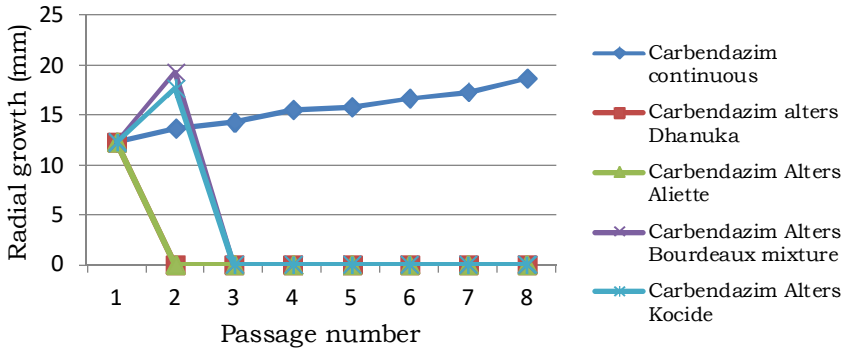


Figure 2: *In vivo* continuous and alternate passage

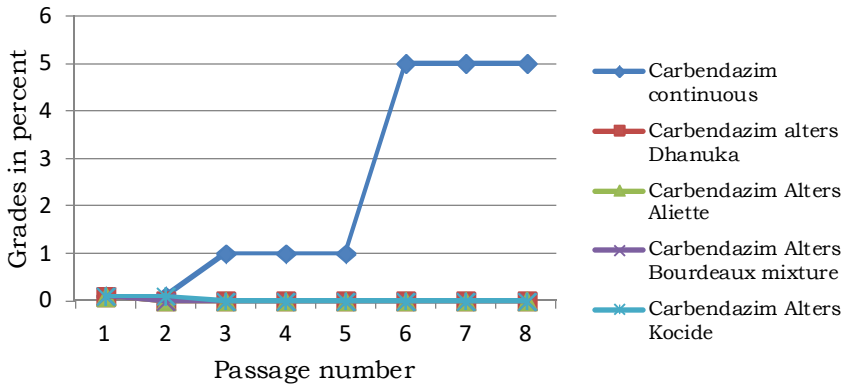


Fig. 3 *In vitro* continuous and mixed passage

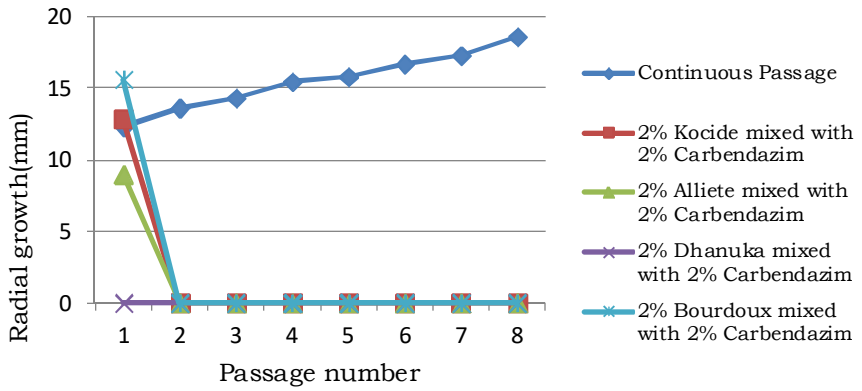
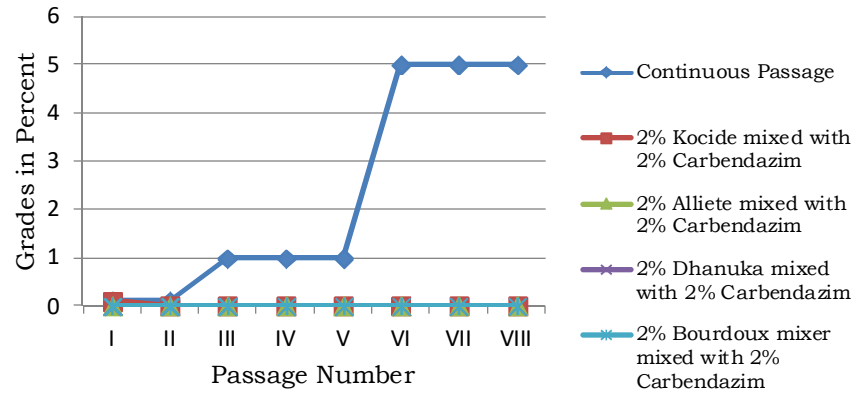


Fig. 4 *In vivo* continuous and mixed passage



A. Continuous and Mixed Passage

Culturing of sensitive isolate of *Phyllosticta zingiberi* on carbendazim continuously for eight successive passages significantly increased the fungicide resistance. It was observed that, carbendazim when used in mixture with dhanuka, aliette, bourdeaux mixture and kocide there was reduction in carbendazim resistance. Carbendazim when used in mixture with dhanuka, completely inhibited the growth of *Phyllosticta zingiberi* at first passage only, while in mixture with aliette, bourdeaux mixture and kocide at second passage *in vitro*. (Table: 3)

In vivo studies shows, carbendazim when used in mixture with dhanuka, aliette and bourdeaux mixture there was complete reduction in the disease at first passage only and when carbendazim used in mixture with kocide there was complete reduction in the disease at second passage. (Table: 3)

The results are agreeing with other workers also. Metalaxyl and difolatan inhibited the growth of *Phytophthora infestans* causing late blight of potato at 2nd and 6th passage (Kamble and Gangawane, 1999). Wadikar, (2002) found that carbendazim with thiram inhibited growth of *M. phaseolina* causing charcoal rot of pigeonpea completely at 7th passage. Sable and Gangawane, (2012) reported that mixing of carbendazim with dithane Z-78 completely inhibited the growth at 3rd passage only while mixing with mancozeb inhibited the growth at 4th passage.

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