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EFFECT OF ALTERNATE AND MIXED PASSAGES OF FUNGICIDES ON BENOMYL RESISTANCE IN VITRO IN *FUSARIUM SOLANI*

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ABSTRACT: Rhizome rot disease of ginger is commonly caused by *Fusarium solani*. Disease greatly reduced the rhizome yields at field, storage and market levels. Controlling this disease, farmers are using the synthetic fungicides. Continuous use of fungicides causes resistance in the pathogens. Hence, alternate and mixed passages of fungicides were evaluated to examine the potentiality of benomyl resistance in *F. solani* by poisoned food technique. The results revealed that sensitive isolate (5µg/ml) of *F. solani* grown on benomyl continuously for eight consecutive passages increased the resistance. However, pathogen grown alternately and mixed with carbendazim, roko, bordoyash and ridomil fungicides benomyl resistance in *F. solani* was reduced.

Keywords: Ginger rot, *Fusarium solani*, benomyl resistance, passages.

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1. INTRODUCTION

Ginger (*Zingiber officinale* Rosc.) is a crucial tropical plant belongs to Zingiberaceae family. It is cultivated throughout the world for aromatic rhizomes which used as both spice and medicine [1-3]. In India, rhizome rot of ginger has been reported from all the states [4-8]. The disease reduced potential yields to a greater extent in field, storage and market, and economic loss more than fifty to eighty per cent [9]. Ginger is damaged by several fungal pathogens [10] however; rhizome rot is commonly caused by *Fusarium solani* (Mart.) Sacc. [11]. Controlling this pathogen, farmers are using many synthetic fungicides. However, continuous use of fungicides led to accumulation of residues in ginger that adversely affects the health of the consumers and cause serious risk to environmental pollution. It’s imperative to work out the baseline sensitivity for the fungicidal agents against a sensitive strain [12]. Resistance to fungicides has become a tough issue in the management of crop diseases and has threatened the activity of some extremely potent fungicides [13]. The practical issues of fungicide resistance were appeared following the initiation and extensive use of site-specific systemic fungicides in the management of plant diseases. In the early seventies, cases of development of fungicide resistance in the pathogens were observed after large-scale use of systemic fungicides like benomyl and dimethirimol [14-17]. The resistance is occurred owing to hereditary adaptation of a fungal pathogen that causes in decreased sensitivity to a fungicide. The site specific systemic fungicides including benomyl affect the nucleus of fungal pathogens and suppress or interrupt mitosis thereby low binding activity of fungicide to the tubulin protein [18-19]. Many researchers have reported benomyl resistance in the other pathogens [20-27]. However, very scarce information is available on benomyl resistance in *F. solani* causing rhizome rot of ginger. Hence, the present work was undertaken to evaluate the effect of alternate and mixed passages of fungicides on benomyl resistance in *F. solani.*

2. MATERIALS AND METHODS

Minimum inhibitory concentration (MIC) of benomyl against 14 isolates of *Fuarium solani* causing rhizome rot of ginger was determined on Czapek Dox Agar (CDA) plates by poisoned food technique [28]. The benomyl sensitive isolate (5µg/ml) of *F. solani* was used to study the effect of continuous, alternate and mixed passages of fungicides on benomyl resistance development. Benomyl, carbendazim, ridomil, roko and bordoyash fungicides were evaluated *in vitro* in this study.

2.1. Continuous passage

The effect of continuous passages of benomyl in order to develop benomyl resistance, wild sensitive isolate of *F. solani* was cultured on CDA plates containing benomyl (5µg/ml). The concentration of benomyl was kept constant for all the passages. Agar disc (8 mm) of freshly grown culture was taken from the culture of previous passage of same isolate and placed upside down on agar surface.

2.2. Alternate passage

Sensitive isolate was cultured on CDA plates containing 5µg/ml benomyl. After eight days, 8 mm disc was taken from the previous passage and transferred to the plates containing another fungicide having same concentration. Such process of alternate passage of benomyl to another fungicide was continued up to 8^{th} passage.

2.3. Mixed passage

Sensitive isolate was cultured on CDA plates containing benomyl with another fungicide both having equal concentration (5µg/ml) to investigate the effect of mixed passage of fungicides. 8 mm discs of sensitive isolate of *F. solani* culture were taken from the earlier passage and then transferred to the plates containing the same mixture of fungicides after eight days. In each type of passages as mentioned above, passage to passage increased in the radial growth of *F. solani* was considered as increased of benomyl resistance.

All mixed passages were conducted in triplicates and incubated at room temperature (26±3°C). The effect of mixed passages was continued up to 8th passage. The radial growth was measured after 8 days of inoculation for each passage.

3. RESULTS AND DISCUSSION

The sensitive isolate cultured continuously on plates which contained benomyl at its MIC level (5µg/ml) for eight consecutive passages showed that sensitive isolate had significantly increased its resistance towards benomyl with increasing passage number. However, alternate passage of benomyl fungicide with bordoyash, carbendazim, ridomil and roko fungicides showed decrease in benomyl resistance of sensitive isolate of *F. solani.* When benomyl was altered with carbendazim and roko, pathogen growth was completely suppressed at 3rd and 8th passages, respectively. Moreover, benomyl altered with ridomil and bordoyash, growth of *F. solani* was reduced after 8th passage (Table 1, Fig.1). The effect of mixture of benomyl with carbendazim, ridomil, roko and bordoyash on development of fungicide resistance in *F. solani* was also studied. It was interesting to note that benomyl when used in mixture with carbendazim completely inhibited the pathogen at 2nd passage only. This was followed by roko, ridomil and bordoyash at 3rd, 4th and 5th passages respectively (Table 2, Fig. 2).

Table 1: Effect of *F. solani* (*in vitro*) exposure to benomyl continuously and alternating with other fungicides on development of resistance during eight consecutive passages

Fungicides	Passage number							
	1	2	3	4	5	6	7	8
Benomyl continuous (05 µg/ml)	11.00	12.66	13.66	15.33	17.00	17.33	17.33	17.33
Benomyl (05 µg/ml) alters Carbendazim (05 µg/ml)	11.33	10.33	00.00	00.00	00.00	00.00	00.00	00.00
Benomyl (05 µg/ml) alters Ridomil (05 µg/ml)	11.00	69.33	15.33	39.66	12.66	23.66	10.33	12.66
Benomyl (05 µg/ml) alters Roko (05 µg/ml)	11.33	60.66	13.66	35.00	11.66	15.33	09.66	00.00
Benomyl (05 µg/ml) alters Bordoyash (05 µg/ml)	11.33	72.66	14.00	43.00	12.66	21.00	11. 00	14.00

Significance by two way analysis of variance (ANOVA)

Between passages F	6.10
Between fungicides F	5.09
C.D. at P=0.05	10.76
C.D. at P=0.01	19.87

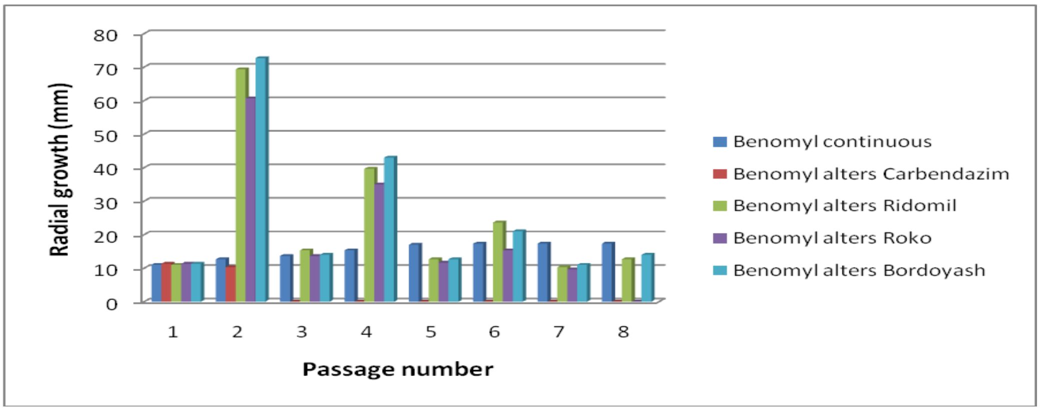

Fig. 1. Effect of benomyl continuously and alternate fungicides on the radial growth (mm) of benomyl sensitive isolate of *F. solani* during eight consecutive passages

Table 2: Effect of *F. solani* (*in vitro*) exposure to the mixture of benomyl with other fungicides on development of resistance during eight consecutive passages

Fungicides	**Passage number**							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Benomyl (05 µg/ml) + Carbendazim (05 µg/ml)	10.33	00.00	00.00	00.00	00.00	00.00	00.00	00.00
Benomyl (05 µg/ml) + Ridomil (05 µg/ml)	11.66	10.33	09.66	00.00	00.00	00.00	00.00	00.00
Benomyl (05 µg/ml) + Roko (05 µg/ml)	10.33	10.00	00.00	00.00	00.00	00.00	00.00	00.00
Benomyl (05 µg/ml) + Bordoyash (05 µg/ml)	10.33	10.33	10.00	09.33	00.00	00.00	00.00	00.00

Significance by two way analysis of variance (ANOVA)

Between passages F	2.63
Between fungicides F	16.27
C.D. at P=0.05	5.02
C.D. at P=0.01	6.76

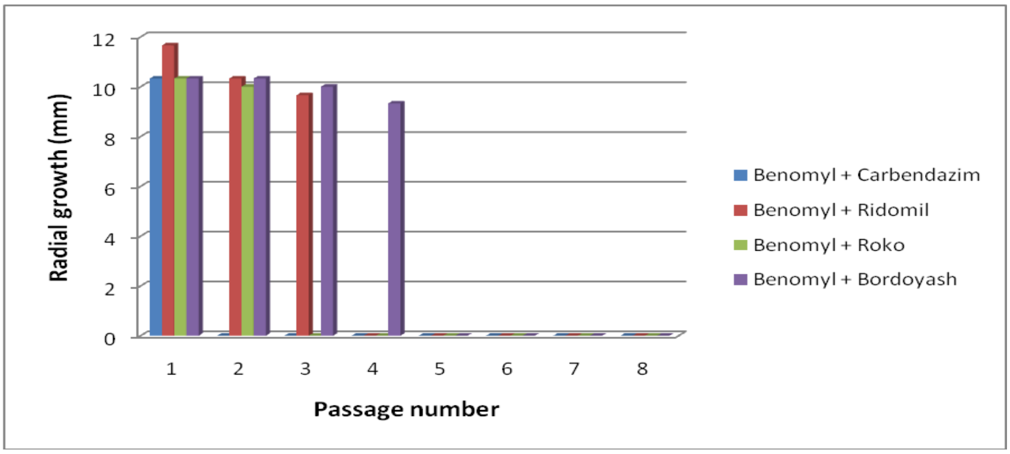

Fig. 2. Effect of mixed fungicides on the radial growth (mm) of benomyl sensitive isolate of *F. solani* during eight consecutive passages

The experiments were conducted to investigate whether benomyl resistance is delayed or prevented in *F. solani* by continuous, alternate or mixed use of two different fungicides. Present study showed that continuous growing of sensitive isolate on benomyl amended plates had increased resistance. There was reduction in benomyl resistance when benomyl used alternately with bordoyash, carbendazim, ridomil and roko. Moreover, using benomyl in mixture with these fungicides also reduced the resistance in *F. solani*. The similar results have been recorded by many researchers. It was shown that *Septoria nodorum* and *Cercosporella herpotrichoides* in cereals have developed carbendazim and edifenphos resistance *in vitro* after continuous exposure to these fungicides [29]. However, resistance was reduced by alternate or mixed use of carbendazim and edifenphos. It was also shown that culturing of *Zizyphus* fruit rot pathogen *Aspergillus niger* alternately or mixed with dithane Z-78 and mancozeb reduced the carbendazim resistance [30]. The effect of benomyl resistance was examined in *F. udum* and shown that continuous use of single fungicide for successive passages increased the resistance in pathogen [25, 31]. However, pathogen grown continuously or mixed with other fungicides reduced the resistance. It was reported that benomyl resistance in *F. oxsporum* f. *conglutinans* was found to reduce by alternate and mixed passages of fungicides [26].

4. CONCLUSION

Culturing of sensitive isolate of *F. solani* on benomyl continuously for eight consecutive passages increased resistance. Use of benomyl alternately with carbendazim, roko, bordoyash and ridomil reduced benomyl resistance. Benomyl when used in mixture with carbendazim roko, ridomil and bordoyash also reduced *in vitro* growth of the pathogen. Eventually, use of alternate and mixed fungicides with different modes of action could reduce increased benomyl resistance in *F. solani*.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The author confirms that the data supporting the findings of this research are available within the article.

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CONFLICT OF INTEREST

No conflict of interest.

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