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Original Article

Effect of passage on the development of Benomyl resistance in *Fusarium udum* (Butler) causing wilt in Pigeon pea

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Abstract

By culturing the sensitive *Fusarium udum* (Butler) isolate on fungicide Benomyl, continuously for eight consecutive passages significantly showed increase in resistance. Whereas use of Benomyl altering fungicide Blitox and Kocide reduced the resistance while fungicides Kavach and Roko helped in complete inhibition of the pathogen. When fungicides were used in mixture there was complete inhibition of radial mycelial growth, hence effect of all fungicides together will prove to be promising for inducing resistance in Pigeon pea.

Key words: Pigeon pea wilt, *Fusarium udum*, Benomyl, Fungicides.

1. Introduction

Pigeon pea (*Cajanus cajan* L.) Millsp. a member belonging to family Fabaceae is one of the most essential leguminous food crop cultivated in tropical and subtropical countries like, Madagascar, India, Myanmar, Philippines, Australia. India, Myanmar, Malawi, Tanzania and Kenya are the top 5 producers of this crop. Amongst them India holds a major contribution of 90% of total world production. India engages an area of 3.85 million hectare with an annual production of 2.68 million tonnes (Anonymous, 2002). The plant helps in re-establishing soil productivity by atmospheric nitrogen fixation (Reddy et al., 1990). Pigeon pea is a commercially important neutraceutical crop as it contains high level of amino acids like methionine, lysine tryptophan, vitamin B and proteins. The content of protein in seeds is almost similar to Soybean (*Glycine max*) which ranges from 21-28 % (Phatak et al., 1993). Inspite of this, *Cajanus cajan* is affected by various serious diseases and leads to heavy destruction. Pigeon pea is bombarded by numerous bacteria, viruses, fungi but amongst them just a few of them cause a negative impact on the plant. The wilt caused by *Fusarium udum*, is the most destructive disease (Kannaiyan et al., 1984). Genus *Fusarium* account to the most significant group of ascomycetous fungi, whose members are liable for enormous economic loss due to depletion in yield, quality and quantity of pea (Nelson et al., 1983; Leslie and Summerell, 2006). Many members of *Fusarium* produces type A and B trichothecene mycotoxins that cause toxicosis in humans and animals (Mali et al., 2015). Several *Fusarium* species cause catastrophic diseases on cereal grains (White, 1980; Parry et al., 1995; Nyvall et al., 1999; Goswami and Kistler, 2004), some are responsible for vascular wilts or root rots on many important vegetable, ornamental and field crops (Kraft et al., 1981; Linderman, 1981) while cankers are produced by others on soft and hardwood trees (Bloomberg, 1981; Dwinell et al., 1981, 2001; Wingfield et al., 2008).

2. Material and Methods

2.1 Collection of material

Fifteen isolates of infected pigeon pea plants were collected from Kolhapur, Sangli districts of Maharashtra and Dharwad, Vijapura (Bijapur) and, Belgavi (Belgaum) districts of Karnataka. The infected plant materials were brought to the laboratory and were cut into small pieces (0.5-1.0cm length) along the symptomatic region of stem, root, leaves and subsequently surface sterilized by sequential dipping in 70% ethanol for 30 s and in 0.1% $HgCl_2$ for 1 min., rinsed in sterilized distilled water, and then cultured on Czapek Dox agar (CDA)/ Potato dextrose agar (PDA) amended with 25 mg/L of streptomycin sulphate (Patil et al. 2012; Jadhav et al., 2010). Plates were incubated at 25± 2°C for 6 days. A *Fusarium* sp. was consistently isolated from infected tissues, and was purified by single-spore culture (Mali et al., 2015).The plates were observed for fungal outgrowth through the symptomatic parts of plants. After 5-6 days of culture, white cottony fungal mass was observed. On the basis of visual

Table 1. Effect of Exposure of *Fusarium udum* (*in - vitro*) continuous to Benomyl continuously and alternating with other fungicides on the development of resistance during successive passages.

Treatment (1µg/ml 0.5µg/ml)	Passage Number							
	I	II	III	IV	V	VI	VII	VIII
Benomyl Continuous	8.0	9.1	10.9	13.4	16.6	19.8	24.3	25.0
Benomyl alters Kavach	8.0	8.9	10.0	9.0	9.0	9.0	9.0	0.0
Benomyl alters Kocide	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Benomyl alters Blitox	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Benomyl alters Roko	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Benomyl alters Bavistin	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0

Table 2. Effect of Exposure of *Fusarium udum* (*in - vivo*) to Benomyl continuously and alternating with other fungicides on the development of resistance during successive passages.

Treatment (1µg/ml 0.5µg/ml)	Passage Number							
	I	II	III	IV	V	VI	VII	VIII
Benomyl Continuous	60%	60%	60%	80%	100%	100%	100%	100%
Benomyl alters Kavach	60%	60%	80%	100%	100%	100%	100%	100%
Benomyl alters Kocide	40%	60%	100%	100%	100%	100%	100%	100%
Benomyl alters Blitox	40%	80%	100%	100%	100%	100%	100%	100%
Benomyl alters Roko	40%	80%	100%	100%	100%	100%	100%	100%
Benomyl alters Bavistin	60%	80%	100%	100%	100%	100%	100%	100%

Table 3. Effect of Exposure of *Fusarium udum* to the mixture of Benomyl with other fungicides (*in - vitro*) on the development of resistance during successive passages.

Treatment (1µg/ml 0.5µg/ml)	Passage Number							
	I	II	III	IV	V	VI	VII	VIII
Benomyl Continuous	8.0	8.01	8.02	8.06	8.0	8.0	8.0	8.0
Benomyl +Kavach	8.0	8.01	8.04	8.04	8.0	8.0	8.0	8.0
Benomyl +Kocide	8.0	8.01	8.04	8.04	8.0	8.0	8.0	8.0
Benomyl + Blitox	8.0	8.02	8.06	8.02	8.0	8.0	8.0	8.0
Benomyl +Roko	8.0	8.02	8.06	8.02	8.0	8.0	8.0	8.0
Benomyl +Bavistin	8.0	8.02	8.06	8.0	8.0	8.0	8.0	8.0

Table 4. Effect of Exposure of *Fusarium udum* to the mixture of Benomyl with other fungicides (*in - vivo*) on the development of resistance during successive passages.

Treatment (1µg/ml 0.5µg/ml)	Passage Number							
	I	II	III	IV	V	VI	VII	VIII
Benomyl + Kavach	0%	0%	0%	0%	0%	0%	0%	0%
Benomyl + Kocide	0%	0%	0%	0%	0%	0%	0%	0%
Benomyl + Blitox	0%	0%	0%	0%	0%	0%	0%	0%
Benomyl + Roko	0%	0%	0%	0%	0%	0%	0%	0%
Benomyl + Bavistin	0%	0%	0%	0%	0%	0%	0%	0%

morphological characters and microscopic characters the fungal isolate was identified as *Fusarium udum* (Butler). The sensitivity of *Fusarium udum* was carried out by using Food Poisoning Technique (Dekker and Gielink, 1979) by deploying various concentrations of Benomyl, systemic benzimidazole fungicide. The treatment was carried out by preparing Benomyl dilutions from 1000 µg/ml stock solution by dissolving it in sterilized distilled water and then mixed in autoclaved Czapek Dox Agar (CDA). The mixture was prepared in proportion of 1:1 and final volume was made up to 30 ml. The media containing Benomyl solution of various concentrations was poured into Petri plates until solidification of media. Pure actively growing fungal mycelium was transferred on the solidified culture media plates by cutting 8 mm diameter discs. These plates were then incubated at 28-30°C in dark and then continuous growth was measured after various time intervals. A plate without Benomyl was served as control.

For *in-vitro* experiment, the work was carried out in triplicates. After determining Minimum Inhibitory Concentration (MIC) of Benomyl effects of passage on the development of Benomyl resistance was studied in continuous, alternate and mixed pattern along with different fungicide for *in-vivo* and *in-vitro* experiments.

For the study of *in-vitro* experiment, wild sensitive isolate named as Fu-2 was cultured on culture media amended with 1 µg/ml Benomyl. All the experiment was performed in triplicates and studied for resistance of pathogen (*Fusarium*) to fungicide (Benomyl). Discs from the previous passage of the same isolate were then transferred to next passage and successively, they were placed in the centre of the plate in each experiment. During each experiment of passage, linear growth of the fungal mycelium was measured and the development of fungal resistance was observed till 8th passage. All the sets of passage were then repeated for alternate and in mixed form with other fungicides viz., Kavach, Roko, Kocide, Bavistin and Blitox.

Subsequently, *in-vivo* experiment was performed. Mycelial suspension of fungal isolate Fu-2 (sensitive) was prepared and then inoculated on the healthy roots of *Cajanus cajan* treated with 5.0 ml Benomyl and 5.0 ml fungal mycelial suspension. The experiment was carried out with five plant individuals. The spore suspension was measured up to 50ml (10ml for each plant). It was repeated in alternate and in mixture with Roko, Kavach, Kocide Bavistin and Blitox. The severeness of the wilt on the plant thus determines the wilting in the plant.

3. Result and Discussion

In case of *in vitro* experiment, it was noticed that during continues passage there was noteworthy increase in resistance of the pathogen. However, during alternate passage i. e, by using Benomyl altering, Kavach, Roko, Kocide, Blitox and Bavistin there was decrease in the resistance of the pathogen. During *in vivo* study use of Kavach and Kocide along with Benomyl showed complete hindrance of the pathogen at 3rd passage itself.

In case of *in vitro* experiment of mixed passage, it was very interesting to note the mixture of Kavach, Kocide and Benomyl inhibited the growth of *Fusarium udum*. During *in vivo* experiment, Benomyl in combination with Blitox, Roko and Kocide was proved to be very effective against sensitive isolate Fu-2. It showed complete inhibition of the pathogen at 3rd stage of the passage.

Table 1 and 3 depicts that, while performing *in-vitro* experiment, it is concluded that continuous use of Benomyl show an increase in resistance of fungal pathogen. Whereas, use of other fungicide like Kavach and Kocide alternately with Benomyl, showed a significant result of decrease in resistance of the fungal pathogen. It was interesting that there was a prominent depletion of fungal pathogen growth when it was cultured in Benomyl along with Blitox, Roko and Bavistin.

During *in-vivo* experiment, as shown in Table 2 and 4 it was observed that with the use of Benomyl continuously for 8 successive passages, there was increase in the resistance of the fungal pathogen. Nonetheless treatment of Benomyl in alternating with Kavach, Kocide, Blitox, Roko and Bavistin there was a notable reduction of resistance of the pathogen. Use of Benomyl in mixture with Kavach, Kocide, Blitox, Roko and Bavistin proved to be most convenient method in managing wilt of *Cajanus cajan*. The present study corroborates with the previous findings that, resistance of pathogen significantly increases with continuous exposure of similar fungicide (Khilare and Chavan, 2011). Therefore in the current findings, there might have been no sufficient time or period for pathogen to acquire the resistance towards the fungicides. With *Penicillium digitatum* against thiophanate-methyl and in in *Alternaria alternata* against aureofungin work similar results were obtained by the author (Khilare and Chavan, 2011). These results are also in agreement with previous work in case of *Macrophomina phaseolina* against carbendazim (Patil and Kamble, 2011).

4. Conclusion

From current study it can be concluded that, mixture of different fungicides proved to be an effective strategy for the management of wilt of pigeon pea caused by *Fusarium udum.* Mixed and alternate implementation of fungicide must have a different mode of action which is important for induction of resistance.

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