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Effects of different fungicides on the growth of Fusarium Udum a comparative study

Dr. Udaysingh A. Desai

Research student, Department of Botany, Shivaji University, Kolhapur

Prof. Shivaji S. Kamble

Professor department of Botany, Shivaji University, Kolhapur, Maharashtra

Abstract: Cultures of Fusarium udum obtained from different localities of Maharashtra and Karnataka states of India was cultured in pure Czapekdox agar medium amended with different types of fungicides viz. kavach, roko, kocide, bavistin and blitox along with benomyl. Fungicides showed variation in their resistive nature when used along with benomyl. Mixture of different fungicides proved to be a very effective strategy for the management of wilt of Pigeonpea caused by Fusarium udum.

Key words – Benomyl, Fungicides , Fusarium udum, Pigeonpea.

Introduction

Pigeonpea (*Cajanus cajan* L.) Millsp. belongs to family Fabaceae, it is an essential food crop cultivated in India and neighboring countries like Nepal, Bhutan, Afghanistan, Pakistan etc. It is also cultivated in various other tropical and subtropical countries like, Madagascar, Myanmar, Philippines and Australia. The countries viz. India, Myanmar, Malawi, Tanzania and Kenya are the top 5 producers. India contributes to 90% of total world production engaging an area of 3.85 million hectare with an annual production of 2.68 million tonnes (Anonymous, 2002). The plant establishes soil productivity and increase fertility by fixing atmospheric nitrogen (Reddy et al., 1990). Pigeon pea is a popular and commercially important nutraceutical crop as it contains high level of amino acids like methionine, lysine tryptophan, vitamin B and proteins. The content of protein found in seeds is almost similar to Soybean (*Glycine max*) which ranges from 21-28 % (Phatak et al., 1993). Such nutraceutical and commercially important crop is affected by various serious diseases and leads to heavy destruction. Pigeon pea is bombarded by numerous bacteria, viruses and fungi but amongst them just a few create a negative impact on the plant. The wilt caused by *Fusarium udum*, is one of the most destructive disease affecting *Cajanus cajan* (Kannaiyan et al., 1984). *Fusarium udum* is one of the main constraints in attaining the desired yield of the crop. Genus *Fusarium* account to the most significant group of ascomycetous fungi, whose members are liable for enormous economic loss causing depletion in yield, quality and quantity of pea (Nelson et al., 1983; Leslie and Summerell, 2006). Several *Fusarium* species also cause catastrophic diseases on various 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).

Wilt of *Cajanus cajan* is very much obvious to the eyes by observing the symptoms such as, aerial parts of the plant turning brittle along with discoloration of leaves and stem, plant show loss of turgidity along with chlorosis of internal conducting tissues. Hence, chemical or biological management of *Fusarium udum* is very much essential in order to obtain a proper stable crop yield across the globe especially in Asian countries and India in particular, the reason being

many farmers possess marginal tilling land and lack of sufficient knowledge regarding the management of the infection.

Present work deals in effective management of *Fusarium udum* by chemical methods so as to arrest the growth as well as to combat the spread with the help of different fungicides (systemic as well as contact) in order to attain proper yield. Work was carried out using fungicides in continuous, alternate and mixed method so as to check the effect on growth of *Fusarium udum*.

Material and methods

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 in clean polythene bags, they were cut into small pieces (0.5-1.0cm length) along the symptomatic region of stem, root and leaves, they were subsequently surface sterilized by sequential dipping in 70% ethanol for 30 sec and in 0.1% $HgCl_2$ for 1 min and were later rinsed in sterilized distilled water, and then cultured on Czapek Dox agar (CDA) amended with 25 mg/l of streptomycin. Plates were incubated at 25± 2°C for 6 days. The plates were observed for fungal outgrowth through the symptomatic parts of plants. After a period of 5-6 days white cottony fungal mass was observed. On the basis of visual morphological and microscopic characters the fungal isolate was identified as *Fusarium udum* (Butler). *Fusarium udum* was consistently isolated from infected tissues which were purified by single-spore culture method. The sensitivity of *Fusarium udum* was carried out by using Food Poisoning Technique (Dekker and Gielink, 1979) by deploying various concentrations of benomyl a 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-1 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) i.e., Methyl – methyl 1-[(butylamino) carbonyl]-H- benzimidazol-2-yl carbamate]. 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 (Clorothilonil), Roko (thiophanate methyl), Kocide (copper hydroxide), Bavistin (methyl benzimidazole-2-carbamate) and Blitox (Copper oxychloride). Subsequently, *in vivo* experiment was performed. Mycelial suspension of fungal isolate Fu-1 (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 determined the wilting in the plant.

Continuous use of fungicide for in vitro study

To evaluate the outcome of continuous passage on the development of benomyl resistance for *in vitro* study, sensitive isolate Fu-1 was cultured on petri plates with 10 µg/ml Benomyl in triplicates. The agar discs of 8 mm in diameter which were used in the previous passage was reused and placed in new petri plates at the centre. Henceforth, in each successive continuous passage linear radial growth of mycelium was measured.

Alternate use of fungicide in vitro study

To perform the experiment of alternate passage the sensitive isolate Fu-1 was cultured on czapek dox agar containing 10 µg/ml of benomyl, it was kept for 7 days under observation and radial mycelial growth was noted. After 7 days the same 8 mm disc was transferred to other plate containing another fungicide of the same concentration. This process of repeated alternation of transfer was continued till 8^{th} passage and the readings were recorded.

Mixed use of fungicide in vitro study

During the effect of mixed passage, sensitive isolate of fungal pathogen Fu-1 was cultured on CDA plates comprising benomyl along with other fungicide in uniform proportion i.e. 10 µg/ml. After 7 days the 8 mm disc was transferred to the succeeding plates having the same combination of fungicides.

In every passage referred above, increase in radial mycelial growth from one passage to the other was examined as standard for benomyl resistance development. All these experiment involving passage were conducted at room temperature for 7 days and effect was studied upto 8 passage. The radial growth was measured after 7^{th} day of inoculation. Experiments were carried out in triplicates.

Continuous use of fungicide for in vivo study

To perform this experiment, the fungal mycelial suspension of sensitive isolate Fu-1 was prepared by utilizing a full luxuriantly grown culture plate. 10 ml fungal spore suspension was inoculated on the healthy plants of Pigeon pea treated with 10 µg/ml benomyl before 2 day. After 7 days of incubation the fungus was re-isolated from artificially infected plants and spore suspension was prepared and applied to the healthy plants, treated with same concentration of benomyl. Same process was repeated upto 8^{th} passage.

Alternate use of fungicide in vivo study

For the present study 10 µg/ml benomyl suspension was prepared and was inoculated on healthy Pigeon pea (*Cajanus cajan*) plant treated with 10µg/ml Benomyl 2 days before. After 7 days of incubation fungal pathogen was isolated and then inoculated on new healthy Pigeon pea (*Cajanus cajan*) plants with other fungicides of equal concentrations. Same process was repeated upto 8^{th} passage.

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Mixed use of fungicide in vivo study

During mixed passage fungal spore suspension was inoculated on healthy plants of Pigeon pea (*Cajanus cajan*), treated 2 days prior with Benomyl and other fungicide in equal concentration. After 7 days of incubation, fungal pathogen was isolated from the artificially infected plants, spore suspension was prepared and incubated on healthy Pigeon pea (*Cajanus cajan*) plants treated with combination of other fungicides in equal proportions. Same process was repeated upto 8th passage.

Results

Fig: 1. Effect of exposure of *Fusarium udum* (*in vitro*) to benomyl continuously and alternating with other fungicides on the development of resistance during eight successive passages.

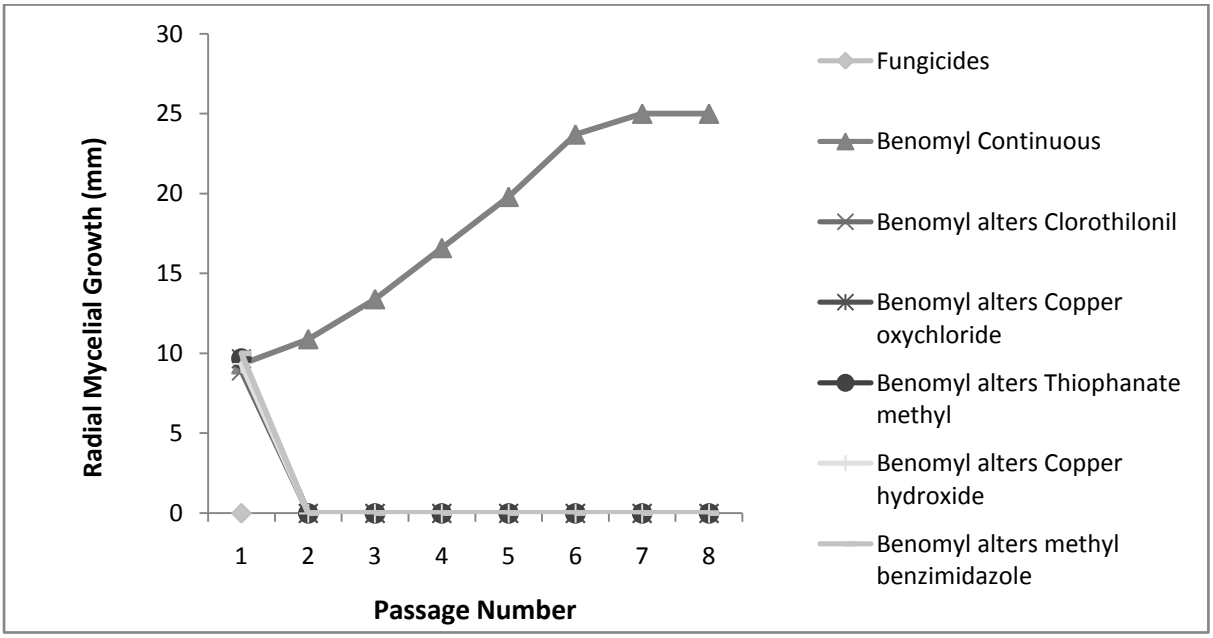


Fig: 2. Effect of exposure of Fusarium udum (*in vitro*) to the mixture of the benomyl with other fungicides on the development of resistance during eight successive passages.

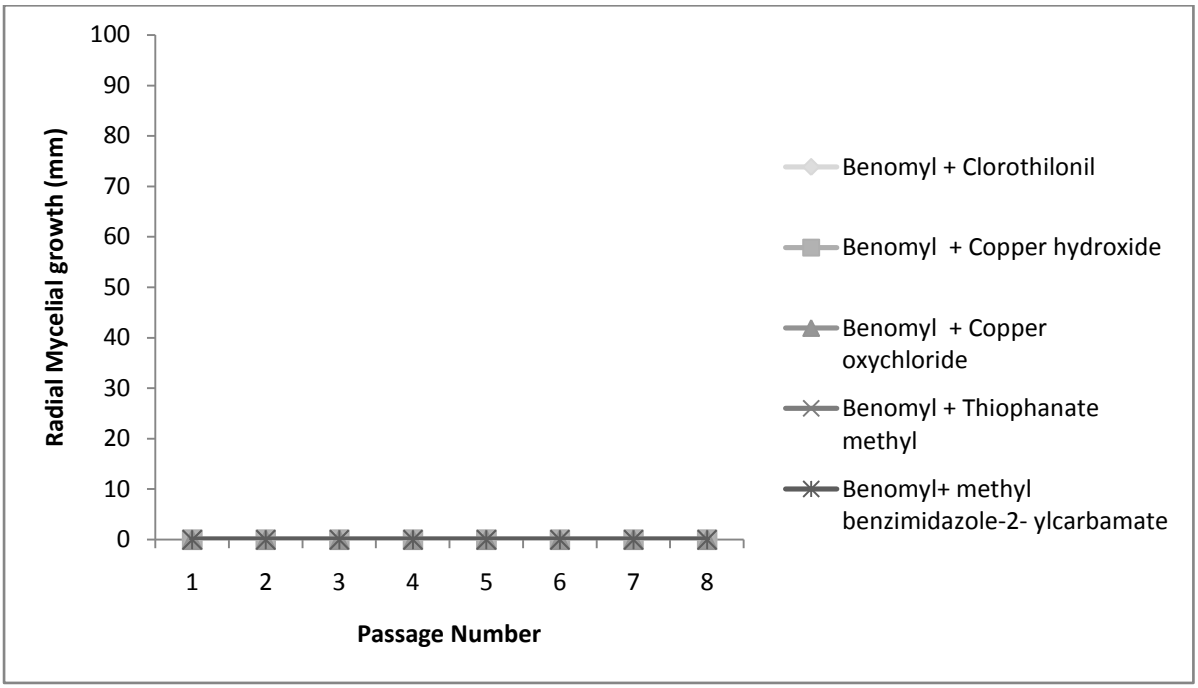


Fig: 3. Effect of exposure of Fusarium udum (*in vivo*) to benomyl continuous and alternating with other fungicides on the development of resistance during eight successive passages.

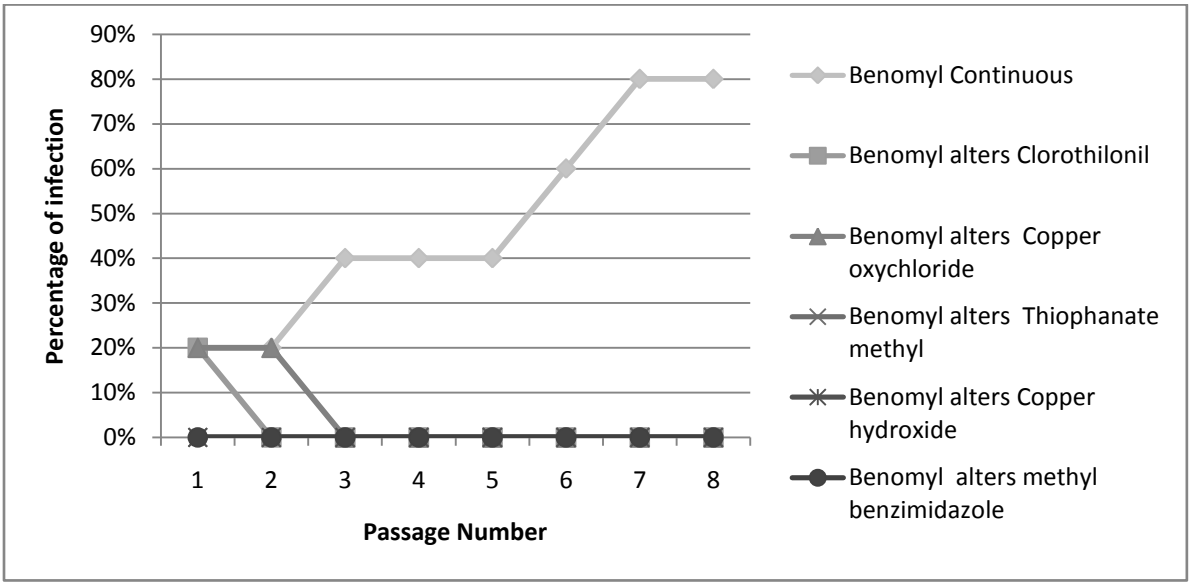
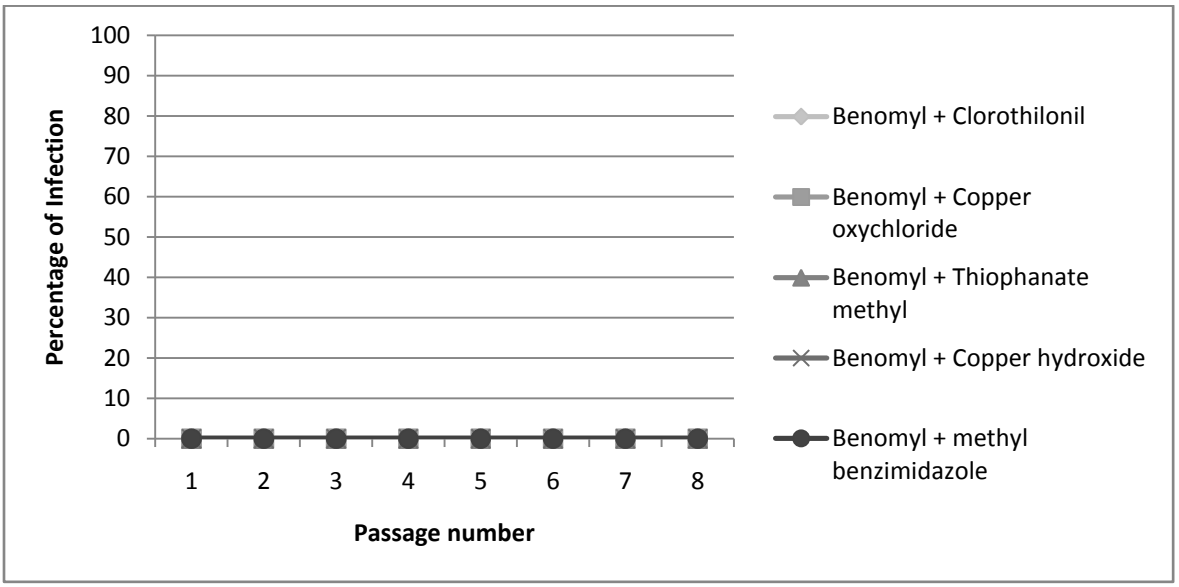


Fig: 4. Effect of exposure of Fusarium udum (*in vivo*) to the mixture of benomyl with other fungicides on the development of resistance during eight successive passages



When sensitive isolate of *Fusarium udum* was cultured on benomyl for eight successive passages continuously, increase in resistance was observed. But when benomyl was used in alteration with kavach, blitox, roko, kocide and bavistin it helped in reducing benomyl resistance significantly in *Fusarium udum*.

When benomyl was used in mixture with kavach, kocide, blitox, roko and bavistin for *in vitro* study it helped in complete inhibition of the pathogen at 1st stage itself. Similar results were obtained for *in vivo* study when benomyl was used in mixture with the above mentioned fungicides.

Pigeonpea plays a major role in providing the solution for the global food security challenge, balanced diet and alleviation of poverty because it can be utilized in many different ways as a source of food, fodder and feed, (Rao *et al*. 2002). Inspite being an important commercially and neutraceutical valued crop, very less effort has been taken for its improvement. For the low yield of the crop many factors are responsible. One factor where it raises a serious concern is the attack by various fungal organisms which is responsible for low yield worldwide. Wilting of the plant caused by *Fusarium udum* is very much prevalent in India which account upto 70 % of the total loss caused. Though the severity of disease varies in different climatic zones globally, wilt caused by *Fusarium udum* is one of the severest diseases affecting the crop. Many chemical practices help to a larger extent in containing the spread of the disease. Chemical practices involve use of various systemic and contact fungicides. By using proper chemical formulation one can easily determine the effectiveness of the fungicide for that particular fungal pathogen. In present context benomyl proved to be very effective when it was used in alteration and in mixed proportion. Result show when benomyl was used continuously the pathogen tend to develop resistance. But, when benomyl was used in alteration with other systemic fungicides the pathogen showed gradual decrease in growth but, as it approached the second passage the growth got totally

arrested. Similarly, when benomyl was used in mixture with other fungicides a very surprising result was obtained, the growth of pathogen was totally arrested at first passage itself showing a complete inhibition of the pathogen. Hence, chemical fungicides show a very impressive result giving a very less time to pathogen to express itself and ultimately arresting the total growth of the pathogen before it spreads. *in vivo* results were very much similar to as of *in vitro*. So, alternate and mixed mode of action of fungicides on *Fusarium udum* is found to be very effective.

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