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Chemical Control of *Fusarium* Wilt of Pigeonpea

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Pigeonpea의 *Fusarium* 시들음병에 대한 화학적 방제

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ABSTRACT: The fungicidal effects of four commercial fungicides, two herbicides and two insecticides have been examined on *Fusarium udum*, causing wilt disease of pigeonpea *in vitro* and *in vivo*. The fungicides Bavistin and MeMc inhibited the growth of the test pathogen completely at 8 and 30 ppm. The herbicide Butachlore inhibited the growth of the test pathogen up to 80.4%. The insecticides, Ekalux and Thiodane partially inhibited the radial growth at 1000 ppm. In unsterilized and sterilized soil MeMc was most effective in controlling the disease in comparison to Bavistin and Ekalux. Maximum rhizosphere fungal population was recorded in MeMc amended soil and minimum in case of Bavistin.

KEYWORDS: Fungicides, Herbicides, Insecticides, *Fusarium* wilt, Pigeonpea

Pigeonpea (*Cajanus cajan* (L) Mill sp) is one of the most extensively cultivated pulse crops in India and several other tropical and sub-tropical countries. It is exposed to wilt disease caused by *Fusarium udum*, a soil-borne plant pathogen, causing great loss in yield. There are several examples of use of pesticides and other chemicals for the control of diseases. In recent years, wealth of information is available on the effect of fungicides on the control of soil-borne plant pathogens and their activities in soil (Kannaiyan and Prasad, 1983; Ray and Das, 1987; Singh and Dwivedi, 1988). Some common herbicides have been reported to inhibit the growth of plant pathogens in soil, although the concentrations have been much higher than to the employed in the field. Altman and Campbell (1977) have summarized the effect of herbicides on plant diseases. When insecticides are applied to soil they may affect soil-borne pathogens in addition to other microorganisms and ultimately plant diseases (Bollen, 1961; Cole and Batson, 1975). The present investigation deals with the effect of a few pesticides on *Fusarium udum* causing wilt disease of pigeonpea *in vitro* and *in vivo* to compare the inhibitory effect of the said fungicides with herbicides and insecticides which may also be used as potential fungicides.

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Materials and Methods

In vitro study

Four fungicides viz., MeMc, Bavistin, Blitox, Indofil M-45 and two herbicides viz., Butachlore and 2,4-D and two insecticides viz., Ekalux and Thiodane were evaluated in laboratory in terms of growth of the test pathogen, *Fusarium udum* by poisoned food technique (Flack, 1907). Different concent-

rations of each pesticide were prepared on double strength on active ingredients basis in sterilized distilled water under aseptic conditions and double strength of Czapek-Dox Agar medium was also prepared. Ten ml of each concentration was added to 10 m*l* of CDA medium in a sterilized petri plate and was mixed thoroughly and was allowed to solidify. Thus, the desired concentration of the pesticides to be tested was obtained. Each petri plate containing 20 m*l* treated solid medium was inoculated centrally with a 5 mm agar block of 5 days old culture of the pathogen. For the control treatment, 10 m*l* of sterilized distilled water was added with 10 m*l* of melted CDA medium instead of pesticides solution. Three replicates for each concentration were prepared at the same time. The radial growth of colony was measured after 7 days of incubation. The percent growth was calculated with the following formula:

$$\frac{dc-dt}{dc} \times 100$$

dc=diameter of control set, dt=diameter of treated set

***In vivo* study**

For *in vivo* study three most effective pesticides in inhibiting the growth of test pathogen, viz. MeMc, Bavistin and Ekalux were selected.

Preparation of mass culture of the test pathogen Mass culture of the test pathogen was prepared on wheat grains following the method of Singh *et al.* (1966). The grains were prevetted by boiling them in water for 20-30 minutes. This raised the moisture content of grains to 40~50 per cent. Excess water was drained off by spreading the grains on wire mesh. Boiled grains were mixed with Gypsum (calcium sulphate) and chalk powder (calcium carbonate) at the rate of 2% and 0.5% respectively on dry weight basis. This helped to neutralise the pH of the medium and prevented the grains from sticking to one another. The grains were placed in bottles with non-absorbent cotton plugs and steam sterilized. The bottles were cooled and inoculated separately with the test pathogen by adding 10 disc of 5 mm diameter cut from the margin of the actively growing cultures. After inoculation the bottles were incubated at 25±2°C for 10~15 days for complete growth of the pathogen. During the period of incubation the bottle were shaken twice to ensure rapid and uniform colonization.

Preparation of the pots Sufficient soil samples (50 kg) from a pigeonpea field were collected and brought into the laboratory. The soil was air-dried at room temperature (30°C) for 24 h and then ground in a pestle and mortar and sieved through a 2 mm pore size sieve. The sterilized soil was prepared by filling field (unsterilized) soil in containers and sterilizing them in an autoclave at 15 lb pressure for 30 minutes. The unsterilized and sterilized soils samples were well mixed separately with 1% (w/w) pure inoculum of the pathogen prepared on wheat grains and was taken in platic pots (25×20 cm) and kept at room temperature for one week to allowthe pathogen to etablish well in soil.

Each pot containing soil inoculated with pathogen was added with aqueous solution of pesticides so as that its final concentration becomes 0.01, 0.05 and 0.1%. Three replicates were taken for each concentration for unsterilized and sterilized soil respectively. Seeds of susceptible cultivar variety of pigeonpea namely bahar were surface sterilized by soaking them in 0.1% aqueous solution of NaOCl for 1 min and washed thoroughly several times with sterilized distilled water to remove every trace of NaOCl. The surface sterilized seeds were

sown in each pot and 10 seedlings were allowed to grow per pot. Visual observation of disease development was made regularly and the final per cent wilting of the plants was noted after 45 days of sowing. The per cent seedling mortality and per cent disease control were calculated using the following formula:

$$\text{Per cent mortality} = \frac{\text{No. of seedlings in un-inoculated pot soil} - \text{No. of seedlings in inoculated pot soil}}{\text{No. of seedlings in un-inoculated pot soil}} \times 100$$

$$\text{Per cent disease control} = \frac{\text{Mortality \% in check} - \text{Mortality \% in treatment}}{\text{Mortality \% in check}} \times 100$$

To check the rhizosphere mycoflora amended with pesticides, the soil samples from root region of plants with complete symptoms of wilting and grown in unsterilized and sterilized soil inoculated with the pathogen (1% w/w) and amended with pesticides were collected for initial (when plants were in seedling stage) and for final samplings (when plants were 45 days old showing complete symptoms of wilting). Rhizosphere microflora were isolated by dilution plate technique (Warcup, 1950).

Statistical analyses

The data from all the test were analysed statistically by applying Two-Way Analysis of Variance (ANOVA), and Critical Difference (CD) were calculated by standard methods as described by Goon *et al.* (1986).

Results

All the fungicides showed inhibitory effect on radial colony growth of the pathogen at 50, 100, 200, 500 and 1,000 ppm. It was noticed that Bavistin completely inhibited the growth of *F. udum* even at 8 ppm concentration while MeMc at 30 ppm concentration. Blitox and Indofil M-45 caused 46 and 31% inhibition at 50 ppm and 92.2 and 69.60% at 1,000 ppm, respectively. The per cent growth inhibition of *F. udum* was increased with increase in concentration of the fungicides. The toxicity of these fungicides was noted in descending order such as Bavistin, MeMc, Blitox and Indofil M-45 (Table 1).

Table 1. Effect of some fungicides on colony growth of *F. udum* (% inhibition)[a]

Concentrations (ppm)	Bavistin	Blitox	Indofil M-45	MeMc
1	61.1	-[b]	-	63.2
2	65.5	-	-	66.6
4	78.8	-	-	68.8
6	81.1	-	-	71.1
8	100	-	-	73.3
10	100	-	-	80.0
20	100	-	-	85.5
30	100	-	-	100
40	100	-	-	100
50	100	46	31.1	100
100	100	86	38.8	100
200	100	87.4	58.2	100
500	100	89.3	62.2	100
1000	100	92.2	69.6	100

[a]Data are means of three replicatss (P=0.01).
[b]Not detected.

Table 2. Effect of herbicides and insecticides on radial colony growth of *F. udum* (% inhibition)[a]

Concentrations (ppm)	Butachlore	2,4-D	Ekalux	Thiodane
50	56.6	5.5	65.5	60.0
100	61.1	11.1	67.7	66.7
200	68.8	13.3	73.3	68.8
500	77.7	27.7	77.7	73.3
1000	80.4	29.3	79.3	76.6

[a]Data are means of three replicatss (P=0.01).

The herbicide Butachlore caused minimum (56.6%) inhibition at 50 ppm and miximum (80.4%) at 1,000 ppm, whereas 2,4-D was least effective and it showed minimum (5.5%) inhibition at 50 ppm and maximum (29.3%) at 1,000 ppm concentration (Table 2). The

insecticides Ekalux and Thiodane, partially inhibited the radial colony growth of *F. udum* at each concentration. Both the insecticides at 50 ppm inhibited the growth of the pathogen upto 65.5 and 60%, respectively. At 1,000 ppm concentration, the growth inhibition just increased marginally when they inhibited the growth only upto 79.3 and 76.6%, respectively (Table 2). Statistically significant mean values for fungicides, herbicides and insecticides concentration against radial colony growth of *F. udum* were observed (P=0.01).

In both unsterilized and sterilized soils, MeMc was most effective to control the disease in comparison to Bavistin and Ekalux. It was noticed that at 0.1% of concentration, the disease control was 50% in case of MeMc followed by 45% by Bavistin and 33% by Ekalux in unsterilized soil (Fig. 1(A)). However, in case of sterilized soil amended with pesticides, MeMc, Bavistin and Ekalux reduced the disease marginally upto 55, 48 and

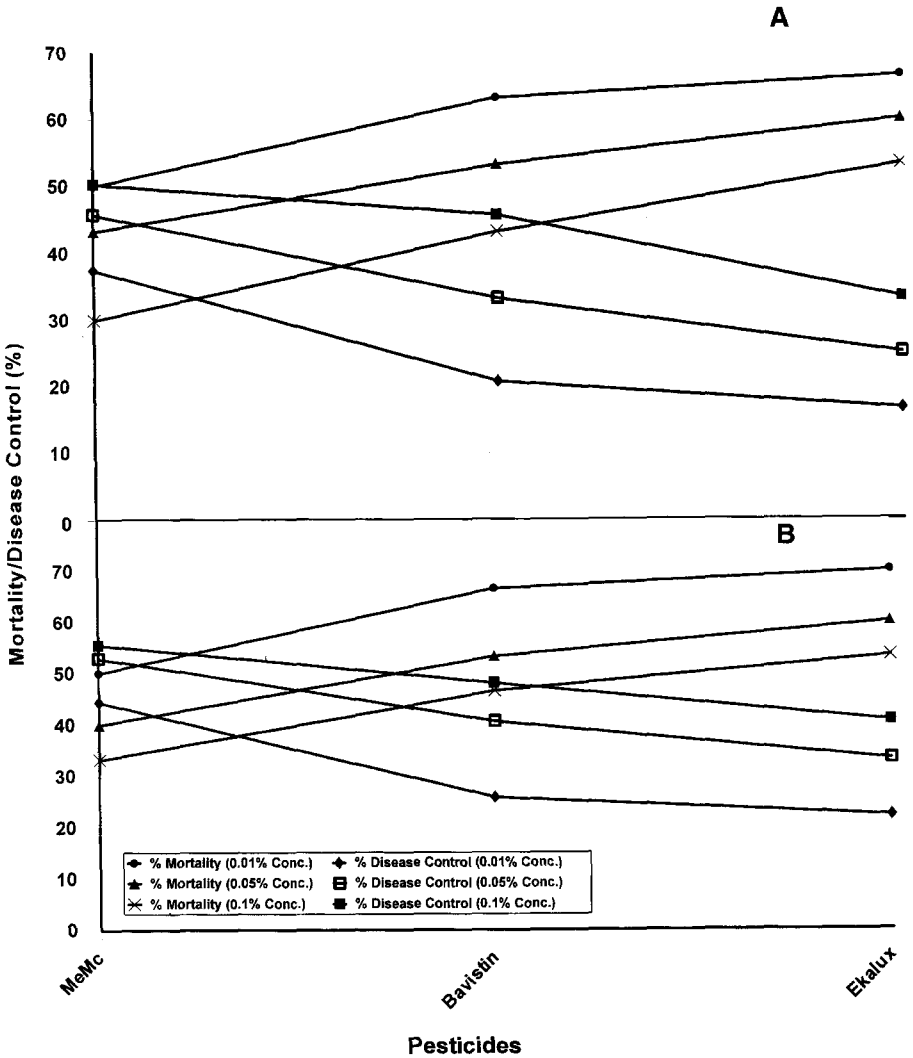

Fig. 1. Effect of pesticides on per cent mortality and per cent disease control in potted unsterilized (A) and sterilized (b) soils. Data are means of three replicates (P=0.01).

40%, respectively at 0.1% concentration (Fig. 1(B)). In general, agradual decline in wilting was observed in case of each pesticide with increase in concentration. The disease control was more pronounced in sterilized soil. The data was found to be highly significant (P=0.01).

It was also recorded that maximum rhizosphere fungal population was found in MeMc amended soil and minimum in Bavistin amended soil. However, in both the soils (unsterilized and sterilized), the maximum population of the pathogen was observed with Ekalux and minimum with MeMc. Increase in concentration of pesticide decreased the population of the pathogen (Fig. 2). The mean values of rhizosphere fungal population at differentconcentrations and samplings were statistically significant in unsterilized soil (P= 0.01). The reduction in population of the pathogen, however, showed highly significantvalue in pesticides amended unsterilized and sterilized soil (P=0.01).

Discussion

In vitro evaluation of some fungicides

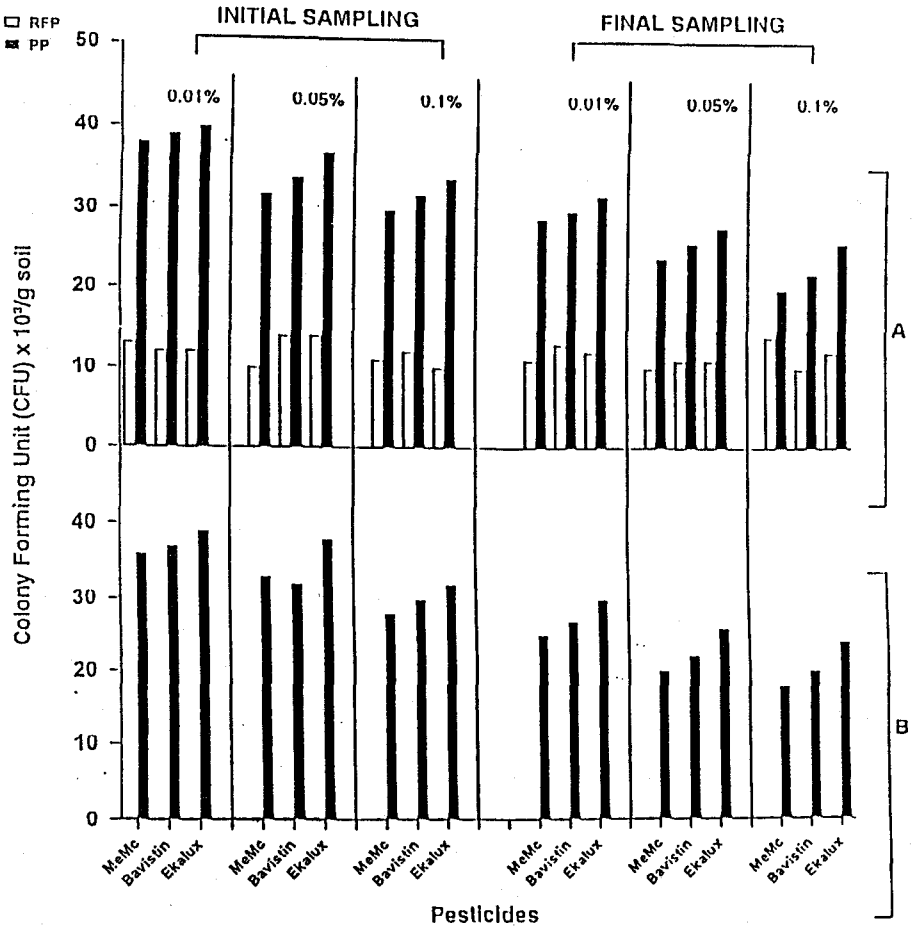

Fig. 2. Effect of soil amendments with pesticides on population dynamics of *F. udum* and rhizosphere microllora in two consecutive samplings of pigeonpea in unsterilized (A) and sterilized (B) soils. 0.01, 0.05 and 0.1% are conc. of pesticides (w/w) amended in soils. Data are means of three replicates (P=0.01).

revealed that all of them caused complete or partial inhibition of *F. udum* at their used concentration. Similar observations have also been reported for other fungicides by Verma and Vyas (1977), Kothasthane and Agrawal (1978), Goyal and Mehrotra (1981), Vishwakarma and Basu Chaudhary (1982), Bashar (1990), Vinay Kumari (1992), and Jariwala *et al.* (1993).

Blitox and Indofil M-45 were also found to be partially effective against *F. udum* at higher concentrations (1,000 ppm). Inhibitory effect of Dithane M-45, Blue Copper, Bavistin, Mancojeb, MeMc, Thiram and Foltaf were reported against *Fusarium oxysporum* f. sp. *ciceri* by Bashar (1990). Vinay Kumari (1992) reported that Dithane M-45 was nost effective in inhibiting radial colony growth of *Drechslera oryzae*. Blitox and Indofil M-45 were found to be less effective against *F. udum* which might by due to tolerant capacity of the test pathogen (Dekker, 1976). Singh and Singh (1970) observed that reaction of *Fusarium* to fungicides varies from species to species and sometimes even isolate to isolate of the same species.

It is evident from the result that the effect of Butachlore on colony growth of *F. udum* was less effective and ineffective due to 2,4-D. Several herbicides have been used for control of plant disease (Jacobson and Hopen, 1976). These herbicides are commonly biocidal and they impose non-target effect (Altman and Campbell, 1977). Chappel and Muller (1956) showed that Dinoseb was effective against a range of peanut pathogens and it reduced the severity of stem rot (Backman *et al.*, 1977). The effect of 2,4-D on fungi have been studied by Szegi (1970) and reported that higher concentrations of 2,4-D are inhibitory against fungi but lower concentrations may be stimulatory or may have no effect. Similarly, Vyas *et al.* (1986) studied the effect of ten soil incarporated herbicides on the root diseases complex of soyabean caused by three pathogens namely *Fusarium oxysporum, Sclerotinia rolfsii* and *Rhizoctonia bataticola* and found that all the herbicides significantly reduced the hyphal growth of the fungi. Dhiman *et al.* (1992) have seen the effect of herbicides on the development of *Alternaria* diseases in vegetable crops and have found that herbicides treatment suppress the rate of infection in bulb crop of onion.

Unlike fungicides, the insecticides have considerable effects at higher concentrations only. Nevertheless, none of them arrested the growth of the pathogen completely. During the present study, the maximum inhibition in colony growth of *F. udum* was recorded with Ekalux. The effect of different insecticides on soil-borne pathogens has been reported by several workers. Altman and Campbell (1977) reported that insecticides may affect soil-borne pathogens in addition in insecticidal properties. Siddiqui *et al.* (1987) reported the efficacy of Metasystox-R against some common fungi and found that *R. solani* showed greatest sensitivity against the insecticides. Bashar (1990) reported the effect of B.H.C., Ekalux, Monocil and Thiodane on radial colony growth of *Fusarium oxysporum* f. sp. *ciceri* and found that these were partially effective at higher concentration.

The least effectiveness of the above herbicides and insecticides might be due to tolerant capacity of the pathogen and residual effect of pesticides. The tolerance of fungi towards chemicals may be due to changes in biochemistry of fungal cell walls that inhibit the entry of pesticides inside cells to a greater or lesser extent and there by not reaching the site of action. Such changes may result in the decrease of the permeability of cell membrane and pesticidal detoxication even before the site of action (Dekker, 1976). Conversion of a chemical in an active form may also be responsible for detoxication mechanism (Nakanishi and Oku, 1969).

It is revealed from the result that significant reduction in per cent disease control was observed with MeMc and Bavistin when amended in unsterilized and sterilized soil, whereas the insecticides Ekalux was not found much effective. In recent years, several workers have reported control of soil-borne plant pathogens by use of pesticides (Kannaiyan and Prasad, 1983; Kotasthane *et al.*, 1987; Siddiqui, 1987; Singh, 1992).

Bashar (1990) reported that MeMc and Bavistin, amended in soil, significantly reduced the wilt incidence of chickpea. Shugha *et al.* (1995) reported that Bavistin and Thiram alone and in combination were found to be highly effective in reducing wilt incidence of chickpea under glass house and field conditions. The varying degree of reduction in per cent disease control of the *Fusarium* wilt of pigeonpea in MeMc, Bavistin and Ekalux amended soil at different concentration in the present study clearly indicates their direct as well as selective toxicity. Nene and Thapliyal (1979) reported that the organo mercurials are more toxic than the inorganic ones because of lipid solubility of organo mercurials are more toxic than the inorganic ones because of lipid solubility of organo mercurials which facilitates diffusion. This may probably explain the high toxicity of MeMc against test pathogen observed in present case also.

The result indicates that the rhizosphere fungal population was significantly affected due to increasing concentrations of the pesticide MeMc, Bavistin and Ekalux either in initial or in final sampling but there was inhibitory effect on the pathogen's population. The maximum effect was observed in final sampling at the highest concentrations for all the above pesticides amended in the soil. The varying degree of reduction in the number of propagules of *F. udum* in pesticide amended soil at different concentrations and at different times of samplings clearly indicates their direct toxicity.

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