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In vitro Study on Effect of Some Fungicides viz., Carbendazim, Mancozeb, Conjoint Carbendazim Mancozeb and Sulphur against *F. oxysporum**

[1]M.I. Shah, [1]Phalisteen Sultan, [2]A. Nasier,
[3]P. Williams, [1]Arif Jan, [4]M. Sajad, [1]Suriya Rehman and [1]A.S. Shawl
[1]Department of Microbiology and Mycology, RRL-Sanatnagar, Srinagar-190005
[2]Biotechnology Division, Regional Research Laboratory, Canal Road, Jammu Tawi-180001
[3]Department of Plant Protection and Plant Pathology, AAI-DU-Allahabad-211007
[4]Department of Entomology, SKUAST-K, Shalimar, Srinagar, Jammu and Kashmir India-190004

Abstract: The present study was conducted to see the effect of some fungicides viz., Carbendazim, Mancozeb, Sulphur and conjoint Carbendazim and Mancozeb against *Fusarium oxysporum* in the division of Microbiology and Mycology of this laboratory. Poisoned food technique was adopted for carrying out this experiment for measuring radial growth of the test fungus in cm. All the treatments significantly reduced the growth of the *F. oxysporum* udum as compared to control but it was observed that the growth of the fungus were significantly less in 10,000 ppm concentration as compared to 10, 100 and 1000 ppm concentration of fungicides. On comparative analysis of different fungicides tested Mancozeb showed maximum inhibition of *F. oxysporum*, udum as compared to other fungicides.

Key words: Fungicides, carbendazim, *Fusarium*, conjoint, fungus

Introduction

India is an agricultural based country and agriculture is the main source of income for more than 65% of population. The agricultural sector contributes 35% of the natural products and account for a sizeable share to total value of country's exports, besides supplying raw material to large number of industries. The cultivation of cereals, pulses, oil seeds, fibre crops, fruit crops and vegetables play an important role in agriculture. Agricultural production mainly depends on various factors like rainfall, fertilizers and quality of seeds, use of right methods of cultivation and production. According to Pesticides Association of India (1996), the total loss due to pests and disease damage is approximately 18% of total production. In India crop diseases alone are destroying crops to the tune of Rs. 5000 crores evey year. The important methods of plant disease management are cultural, biological, physical and chemical (Panday and Upadhyay, 1997). The chemical control is the most extensively used method for controlling plant diseases. Pesticides have been extensively used to control different kinds of pests inimical to the interests of man both in agriculture and public health. Pesticides have dramatically reduced the morbidity of human beings and livestock. It has also played a pivotal role in boosting agricultural productivity.

Fusarium is one of the most common soil inhabiting plant pathogenic fungi which causes wilt of pigeon pea, wilt of guava and gram etc. and is responsible for huge losses to other respective host crops. *F. oxysporum* f.sp.udum was isolated from diseased Pigeon pea plants, laboratory tests have shown that plant extracts were shown to have inhibitory effects on the pathogen (Verma *et al*., 1998).As reported by Das and Sengupta (1998) variability among six isolates of *F. udum* was observed in relation to cultural characteristics, morphology and pathogenicity on 6 cultivars of Pigeon pea. In

Corresponding Author: Phalisteen Sultan, Department of Microbiology and Mycology, RRL-Sanatnagar, Srinagar-190005, India

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the present study *F. oxysporum* f.sp.udum was isolated from diseased Pigeon pea. Besides being the cause of wilt diseases, species of this genus are also found associated with seedling height and damping off diseases. The history, distribution and economic importance, symptoms and disease cycle and management of the disease caused by *Fusarium udum* was reported earlier (Upadhyay *et al*., 1992). In view of the importance of *Fusarium* in causing wilt disease and huge losses in different crops, an experiment *in vitro* study on effect of some fungicides viz. carbendazim, mancozeb, sulphur and conjoint carbendazim, mancozeb against *F. oxysporum* "udum" was under taken with the following objectives, To isolate and identify the test fungi from infected plant parts of Pigeon pea and to evaluate the efficiency of the fungicides used against *Fusarium oxysporum* f.sp. udum by using poisoned food technique.

Materials and Methods

The material and the methods followed in the present study are based on poisoned food technique. This study was conducted in the year 2005 in Division of Microbiology, Regional Research Laboratory, CSIR, Sanatnagar, Srinagar, India. The petridishes; pipettes, conical flasks, etc. were thoroughly washed with the help of detergent powder and running tap water and were dried in a hot oven. The petridishes and pipettes were wrapped in clean paper and sterilized in an oven at a temp. of 180°C for 2 h. For isolation and growing of fungi, the Potato Dextrose Agar (PDA) medium was used. Dextrose at the rate of 2% and Agar-Agar (1.5 -2.0%) was added in the medium and pH of the medium was adjusted to 6.0-6.5. The medium was sterilized at 15 Ibs pressure for 15 min in an autoclave.

Fusarium oxysporum was isolated from roots of infected Arhar plant showing characteristic symptoms of wilt disease in the field. These roots were examined under microscope to confirm the presence of *Fusarium udum* inside them. The infected root parts were cut into small pieces (2-3 cm), surface sterilized with 0.1% mercuric chloride solution for 20 sec washed thrice with sterilized water and transferred aseptically on a PDA media contained in petridishes. The inoculated petridishes were incubated at 22-25°C for 4-6 days and the pathogen was identified as *Fusarium oxysporum*. The cultures of *Fusarium* were purified from isolation dishes and maintained by periodic sub-culturing on PDA slants after every 15 days. The genus *Fusarium* was identified by Link (1809) with fusiform spores borne on a stream, extensive mycelium and cottony conidiophores variable, slender and simple or more stout short branched, single or grounded, variable conidia, principally of two kinds, macro conidia, boat shaped with a well marked foot at the attachment end of the spore. Aerial mycelium is either absent or usually with profuse development of sporangia.

Selection of Test Fungicides

Four fungicides namely Carbendazim, Mancozeb, Sulphur and conjoint Carbendazim and Mancozeb were selected to evaluate their effect on *Fusarium oxysporum* (Table 1). The required dilutions of fungicides (10, 100, 1000 and 10,000 ppm) were prepared by taking the active ingredients. The actual quantity of various formulae products used in the study was calculated by considering

Table 1: List of fungicides used in the present study

S.No.	Trade name of fungicide	Common name	Active ingredient (%)	Formulated chemical required for 10000 ppm
1	Bavistin	Carbendazim	50	B.A.S.F, 2.0 g Mumbai-India
2	Indofil-M-45	Mancozeb	75	Indofil chemical 1.33 Company Mumbai-India
3	Companion	Carbendazin and Mancozeb	75	Mancozeb 63% + Carbendazim 12% 1.33 Indofil chemical Company Mumbai-India
4	Sulphur	Sulphur	80	Anusulf product company limited 25 g Haryana.

percentage of active ingredient in the product. The Poison food technique was adopted in the experiment. The principle involved in this technique is to supplement the nutrient medium with a toxic chemical and then allowing a test fungus to grow on the medium and evaluate the effect of such chemicals by measuring the growth of the fungus.

Inoculation with Test Organism

Required amount of fungicides were added to medium to get the required concentration of the test fungicide with the help of a sterilized pipette. The chemically amended medium was poured in sterilized petridishes and allowed to set. With the help of a sterile cork borer, dishes of 0.5 cm diameter were cut from actively growing fungus culture and transferred aseptically in the center of petridish containing the test medium. Three replicates for each concentration (10,000, 1000, 100 and 10 ppm) for each chemical including control (without chemical) were maintained. The inoculated Petri dishes were incubated at 26 -28°C and the fungal diameter was measured after 3rd and 5th day. The data was analyzed statistically by "F" test and at 5% level of significance to determine the significant difference between different treatments. The F test as suggested by Fisher and Yates was used to determine the significant difference.

Results and Discussion

The present study was conducted to study the effect of fungicides against soil born fungal pathogen. The fungus was isolated from diseased plant material of Pigeon pea and the effect of fungicide was studied by poison food technique on solid media. Four fungicides namely Mancozeb, Carbendazim, conjoint Carbendazim, Mancozeb and Sulphur were used in the present study. All the four fungicides were used against *Fusarium oxysporum*. Sharma *et al*. (1998) has found that an alkaloid isolated from *Fumora indica* was shown to posses inhibitory effect on spore germination of some plant parasitic and saprophytic fungi. Effect of different concentrations of Mancozeb on *F. oxysporum* f sp. udum was observed at different time intervals after inoculation. The radial growth of colony after 72 h of incubation was significantly different at all the four concentrations (T_1, T_2, T_3 and T_4). The effect of Mancozeb against *Fusarium oxysporum* was recorded in increasing order. T_4 (0.60) < T_3 (0.80) < T_2 (0.90) <T_1 (1.30) < T_0 (2.73) (Table 2). After 120 h of incubation, the growth of colony was significantly less in T_4 (10,000 ppm) as compared to T_1 (10 ppm), T_2 (100 ppm) and T_3 (1000 ppm). There was significant difference between T_1, T_2 and T_3 (Table 3). The effect of conjoint carbendazim mancozeb was also recorded in increasing order after 120 h T4 (1.60) < T_3 (1.87) <T_2 (2.12) < T_1 (2.90) < T_0 (4.82) (Table 6).

Effect of Different Concentrations of Sulphur on Fusarium oxysporium f.sp. *udum*

The radial growth of colony after 72 h of incubation showed significant difference in all four concentration (T_1, T_2, T_3 and T_4). The effect of different concentrations of sulphur against *Fusarium oxysporum*, f.sp.udum was recorded in increasing order T_4 (1.62) < T_3 (2.00) < T_2 (2.40) < T_1 (2.85) <T_0 (3.20) (Table 7). After 120 h of incubation the growth of colony was significantly less in T_4 (10,000 ppm) as compared to T_1 (10 ppm) T_2 (100 ppm) and T_3 (100 ppm). There was significant difference between T_1, T_2 and T_3. The effect of sulphur was also recorded in increasing order after 120 h T_4 (1.77) < T_3 (2.22) < T_2 (2.95) < T_1 (3.62) <T_0 (4.25) (Table 8).

Discussion

The effect of four selected fungicides viz., Mancozeb, carbendazim, conjoint Carbendazim Mancozeb and Sulphur against *Fusarium oxysporum* f.sp. udum was studied. The present study indicated that mancozeb inhibited the growth of *Fusarium oxysporum* f.sp.udum in treatment after 72 h and 120 h of incubation. Sumetha and Gaekwad (1998) reported that linear growth of pathogen

in culture was completely inhibited by Bavistin (Carbendazim) (Table 2 and 3) Topsin M-70 (Thiophanate methyl) and Thiram each at 0.18%, Captan at 0.15% and Diathane Z-78 (Zineb) at 0.3% (Bezbaruah *et al*., 1996). Pradhan *et al*. (1998) have found that different concentrations of Zinc, Iron and Boron have significant inhibitory effect on incidence of wilt. The guidelines on disease management of Soyabean point to the use of pesticides in integrated pest management (Sinclair and Mc Glamery, 1997). The widespread use of pesticides for the control of Soyabean seed borne diseases (Allam *et al*., 1969; Sundresh and Hirnath, 1982; Shah *et al*., 1992; Horn *et al*., 1975; Gupta *et al*., 1993; Sinclair and McGlamery, 1997) it was considered worthwhile to use systematic fungicides viz., Mancozeb, Canbendazim, conjoint carbendazim mancozeb and sulphur against *Fusarium oxysporum*. In seed tests as seed treatments in Pigeon pea, best reduction of wilt was given by Benomyl and Thiram (Haware and Kannayian, 1994).

The results obtained in the present study on the effect of Carbendazim on *Fusarium oxysporum* f.sp.udum after 72 h and 120 h of incubation inhibited the growth of fungus at different concentrations of treatment, T_1 (10 ppm), T_2 (100 ppm), T_3 (1000 ppm) and T_4 (10,000 ppm) as compared to control (Table 4 and 5). Malati and Sonam (1987) reported that in general, the three

Table 2: Effect of different concentrations of Mancozeb on *Fusarium oxysporum* f. sp. udum after 72 h of incubation

		Replication					
Treatments	Conc.(ppm)	R_1	R_2	R_3	R_4	Total	Mean
T_0	0	2.50	3.00	2.75	2.70	10.95	2.73
T_1	10	1.60	1.40	1.00	1.20	5.20	1.30
T_2	100	1.10	0.90	0.80	0.70	3.50	0.90
T_3	1000	0.90	0.80	0.70	0.60	3.00	0.80
T_4	10,000	0.60	0.60	0.55	0.55	2.30	0.60

S.E = 0.128, C.D = 0.274

Table 3: Effect of different concentrations of Mancozeb on *Fusarium oxysporum* f.sp. udum after 120 h of incubation

		Replication					
Treatments	Conc.(ppm)	R_1	R_2	R_3	R_4	Total	Mean
T_0	0	4.30	4.20	4.40	4.30	17.20	4.30
T_1	10	2.40	2.10	2.20	2.10	8.80	2.20
T_2	100	1.40	1.50	1.40	1.30	5.60	1.40
T_3	1000	1.10	1.10	1.00	1.00	4.20	1.05
T_4	10,000	0.90	0.80	0.90	0.80	3.40	0.85

S.E = 0.0666, C.D = 0.143

Table 4: Effect of different concentrations of Carbendazim on *Fusarium oxysporum* f.sp. udum after 120 h of incubation

		Replication					
Treatments	Conc. (ppm)	R_1	R_2	R_3	R_4	Total	Mean
T_0	0	4.6	4.5	4.8	4.3	18.2	4.55
T_1	10	3.2	3.0	2.8	5.1	12.2	3.02
T_2	100	2.3	2.7	2.0	2.1	8.6	2.15
T_3	1000	2.0	1.6	1.8	1.5	6.9	1.72
T_4	10,000	1.6	1.4	1.5	1.2	5.7	1.42

S.E = 0.1222, C.D = 0.2622

Table 5: Effect of different concentrations of conjoint mancozeb carbendazim on *Fusarium oxysporum* f.sp. udum after 72 h of incubation

		Replication					
Treatments	Conc.(ppm)	R_1	R_2	R_3	R_4	Total	Mean
T_0	0	3	3.2	3.1	2.8	12.1	3.02
T_1	10	1.8	1.6	1.7	1.6	6.7	1.66
T_2	100	1.5	1.3	1.4	1.4	5.6	1.40
T_3	1000	1.2	1.1	1.2	1.2	4.7	1.17
T_4	10,000	0.9	0.8	0.8	0.9	3.4	0.85

S.E = 0.082, C.D = 0.177

systematic fungicides tested were more effective than the 3 non systematic fungicides against 9 pathogens (*Rhizoctonia batatiola*, *Marphomia phaseolina*, *Diplodia corchri*, *Botrydiplodia theobromae*, *Colletotrichum gloeosprides Glomalla cirrgulati*, *Phytopthera Nicotianae var. Parrastica*, *Phoma sabdariffae*, *Myrothecium roridium*, *Fusarium udum* and *Alternaria limi* in *in vitro* tests. Carbendazim was the most effective followed by Calixin. The results obtained in the present study on the effect of conjoint carbendazim, mancozeb on *F.oxysporum* f.sp. udum after 72 h and 120 h of incubation inhibited the growth of fungus at different concentrations of treatment of T1(10 ppm), T2(100 ppm), T3(1000 ppm) and T4(10,000 ppm) as compared to control. The growth of fungus in T4 (10,000 ppm) is significantly less as compared to T1 (10 ppm), T2 (100 ppm) and T3 (1000 ppm).

It is thus obvious from the present study that some fungicides, which cause inhibition in growth of fungi could be tried under field conditions. In our laboratory studies, some fungicides control the growth of *Fusarium oxysporum* f.sp udum".The results obtained in present study on the effect of sulphur on *Fusarium oxysporum* f.sp. udum" after 72 h and 120 h of incubation inhibited the growth of fungus in different concentrations of treatment T1(10 ppm), T2(100 ppm), T3(1000 ppm) and T4(10,000 ppm) as compared to control (Table 7 and 8). The growth of fungus in T4(10,000 ppm) is significantly less as compared to T1(10 ppm), T2(100 ppm) and T3(1000 ppm). The results thus obtained are in confirmation of Maliti and Sonam (1987). Panday and Upadhyay (1997) conducted experiments to determine the comparative performance of chemical, biological methods for the control of wilt of Pigeon pea, where Bavistin was found highly effective. But in the present study Mancozeb was found more effective as compared to Bavistin.

The mancozeb decreased the growth of *F. oxysporum* f.sp.udum in all treatments compared to control after 72 h and 120 h. The T4 (10,000 ppm) is most effective against *F. oxysporum* f.sp. udum

Table 6: Effect of different concentrations of conjoint Mancozeb, Carbendazim on *Fusarium oxysporum* f.sp. udum after 120 h of incubation

		Replications					
Treatments	Conc.(ppm)	R_1	R_2	R_3	R_4	Total	Mean
T_0	0	4.7	4.9	4.8	4.9	19.3	4.82
T_1	10	3.2	2.8	2.9	2.7	11.6	2.90
T_2	100	2.2	2.1	2.0	2.2	8.5	2.12
T_3	1000	2.6	1.9	1.8	1.8	7.5	1.87
T_4	10,000	1.6	1.5	1.6	1.7	6.4	1.60

S.E = 0.105, C.D = 0.226

Table 7: Effect of different concentration of sulphur on *Fusarium oxysporum* f.sp. udum after 72 h of incubation

		Replication					
Treatments	Conc.(ppm)	R_1	R_2	R_3	R_4	Total	Mean
T_0	0	3.3	3.4	3.1	3.0	12.8	3.20
T_1	10	2.9	3.0	2.8	2.7	11.4	2.85
T_2	100	2.5	2.6	2.2	2.3	9.6	2.40
T_3	1000	2.0	2.1	1.9	2.0	8.0	2.00
T_4	10,000	1.7	1.6	1.5	1.7	6.5	1.62

S.E = 0. 0760, C.D = 0.1630

Table 8: Effect of different concentrations of sulphur on *Fusarium oxysporum* f.sp. udum after 120 h of incubation

		Replication					
Treatments	Conc.(ppm)	R_1	R_2	R_3	R_4	Total	Mean
T_0	0	4.3	4.4	4.2	4.1	17.0	4.25
T_1	10	3.6	3.8	3.6	3.5	14.5	3.62
T_2	100	2.8	2.9	3.0	3.1	11.8	2.95
T_3	1000	2.3	2.2	2.1	2.3	8.9	2.22
T_4	10,000	1.8	1.9	1.6	1.8	7.1	1.77

S.E = 0.0980, C.D = 0.2102.

followed by T3(1000 ppm) and T2(100 ppm) and T1(10 ppm) as compared to control (T0). The Bavistin decreased the growth of *F. oxysporum* f.spudum in all treatments compared to control after 72 h and 120 h. The T4 (10000 ppm) is most effective against *F. oxysporum* f.sp. udum followed by T3 (1000 ppm), T2 (100 ppm) and T1 (10 ppm) as compared to control (T0). The conjoint carbendazim and mancozeb decreased the growth of *F. oxysporum* f.sp udum in all treatments compared to control after 72 h and 120 h. The sulphur decreased the growth of *F. oxysporum* f.sp. udum in all treatments compared to control after 72 h and 120 h.

It is thus obvious from the results summarized above that the best used fungicide was Mancozeb which showed maximum inhibition of *F. oxysporum* f.sp. udum (0.60 cm) followed by conjoint Carbendazim and Mancozeb (1.20 cm), Carbendazim (1.25 cm) and Sulphur (1.62 cm). The growth rate of *Fusarium oxysporum* evaluated on PDA medium amended with different concentrations of test fungicides showed significant reduction ($p < 0.05$) in growth.

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