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FUNGITOXICITY OF DIFFERENT CHEMICAL FUNGICIDES TO SEED BORNE AND ROOT INFECTING FUNGI ASSOCIATED WITH *SOLANUM MELONGENA* L.

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ABSTRACT

Eggplants showing stunting and wilting appearance and seeds were collected from Hyderabad and Mirpurkhas districts. Nine fungi including four predominant pathogenic fungi *Fusarium solani, Rhizoctonia solani Macrophomina phaseolina* and *Fusarium oxysporum*, were found associated with these samples. Eight fungicides *viz*., Thiophanate-methyl, Fosetyl-aluminium, Propineb, Carbendazim, Copper oxychloride, Mendipropomide, Difenoconazole and Metriam were evaluated with five different concentrations *viz*., 1, 10, 100, 1000 and 10000 ppm by food poisoning method. Variation in growth reduction observed with changing concentrations of fungicides. Higher concentrations of tested fungicides were more effective than medium and lower ones. Difenoconazole at its all doses (1-10,000 ppm) caused more than 50% inhibition of *F. solani*. Among 40 different treatments only Thiophanate-methyl at 10,000 ppm and Carbendazim 10,000 ppm and 1000 ppm causes more than 50% growth reduction of *R. solani* as compared to control. Thiophanate-methyl, Carbendazim and Difenoconazole 1-10,000 ppm dose cause more than 50% growth reduction of *M. phaseolina.* Mendipropomide at its highest used dose (10000 ppm) cause only 43% inhibition of *Fusarium oxysporum.* Carbendazim and Thiophanate-methyl found equally effective against above mentioned pathogens.

Keywords: Eggplant; fungicides; chemical control; *Fusarium solani; Rhizoctonia solani*; *Macrophomina phaseolina; Fusarium oxysporum*

INTRODUCTION

Eggplant (*Solanum melongena* L.) is considered as one of the most popular vegetables throughout the world. It is widely cultivated in the tropical and subtropical regions of the world including parts of Asia, Africa and Central America (Harish *et al.,* 2011). It is also very popular vegetable in China, India, Pakistan, Bangladesh, Philippine, USA, France, Italy and Egypt (Tindall, 1983; Mbadianya *et al.*, 2013). Worldwide area under eggplant was about 1,600,000 hectares in 2010 (FAO, 2012).

Eggplant suffers from number of fungal, bacterial, viral and nematodes diseases, such as early blight (*Alterneria solani*), leaf spot (*Cercospora melongenae, Phytophathora nicotianae, Septoria lycopersici*), charcoal rot (*Macrophomina phaseolina*), fruit rot (*Phomopsis vexans, Phoma exigua, Phoma* sp.), root rot (*Pythium* spp., *Rhizoctonia solani*, *Fusarium* spp.), pink rot (*Sclerotinia sclerotiorum*), southern blight (*Sclerotina rolfsii*), black heart or soft rot (*Erwinia amylovora*), bacterial wilt (*Pseudomonas solanacearum*), root knot (*Meloidogyne* spp.), reniform nematode (*Rotylenchus reniformis*), cucumber mosaic virus, spotted wilt virus, etc (Pandey, 2010; Singh, 1998). Among these pathogens, root-infecting fungi cause severe damages to root system of plants, consequently resulting in the death of plants. There are number of reports of association of pathogenic fungi with the roots and seeds of the eggplant. Such as *Rhizoctonia solani* (Trabulsi *et al.*, 1998), *Sclerotium* (Iqbal *et al.*, 2003), *Alterneria alternata*, *Aspergillus flavus*, *Curvularia lunata*, *Fusarium oxysporum* (Habib *et al.*, 2007; Ismael, 2010; Al-Kassim and Monawar, 2000; Nishikawa *et al.*, 2006).

Infected seeds reduce the production and play key role in survival and spreading to new areas (Agarwal, 1981). Global losses due to biotic plant diseases are reported to be more than 14 percent, where in Asian countries it is more than 30 percent (Bhutta, 2010). Pathogen free soil and seed is the prerequisite in managing diseases. Germination, growth and overall plant health primarily depend on disease free seed.

Soil borne diseases are usually difficult to control due to persistent nature of inoculum in the soil even with conventional strategies. Although, many methods such as biological, cultural, physical, chemical etc. have been developed to control plant pathogens, but only few have provided satisfactory control and moreover these methods are effective only when adopted as preventative measure (Sharma, 1996; Kata, 2000) but after disease appearance they become impractical/ineffective (Ramezani, 2008). The chemical control based on the use of fungicides is most effective, quick and reliable method. The aim of this study is to identify the fungi associated with the roots and seeds of the eggplant and to find out the most effective chemical fungicides under *in-vitro* conditions.

MATERIALS AND METHODS

Collection, isolation and identification of mycoflora: The roots of eggplants showing stunting and wilting symptoms and seeds were collected from different areas of Tandojam, Hyderabad and Mirpurkhas. Samples transported to the laboratory for isolation and identification of associated organisms.

The roots of affected plants were washed with running tap water to remove adhering soil particles and cut into small pieces (1 cm) with the help of sterilized scissors. These pieces and seeds were surface sterilized with 5% commercial bleach (Sodium hypochlorite) for 2-3 minutes and placed on sterilized Petri dishes containing PDA medium amended with Streptomycin sulphate at 1 ml L^{-1} and Penicillin at 10^6 units L^{-1} to avoid bacterial contamination. Five root pieces or seeds were placed in each Petri dish. These Petri dishes were incubated at 25°C for 5-7 days. Later on, the appearing fungal colonies were purified and multiplied on PDA medium for further study. The isolated fungi were identified on the basis of morphological characteristics by using the keys developed by Booth (1971), Ellis (1971), Barnett and Hunter (1972) and Singh (1987).

Effect of different fungicides on colony growth of fungi: Eight fungicides *viz*., Difenoconazole, Carbendazim, Propineb, Mendipropomide, Thiophanate-methyl, Fosetyl-aluminium, Copper oxychloride and Metriam at 1, 10, 100, 1000 and 10,000 ppm were evaluated against *Fusarium oxysporum, Fusarium solani*, *Rhizoctonia solani* and *Macrophomina phaseolina* by food poisoned technique (Borum and Sinclair, 1968). The detail of fungicides including their trade name, chemical name, active ingredient, formulation and chemical group are given in Table 1.

The required concentrations of the fungicides were added in the PDA medium before pouring, the medium was also amended with Streptomycin sulphate at 1ml L^{-1} and Penicillin at 10^6 units L^{-1} to avoid bacterial contamination. Medium without fungicide served as control. 5 mm diameter agar disk of test fungus were cut from 8-10 days old culture plate by using sterile cork borer and placed in the centre of the solidified PDA plate. There were five replications of each treatment. The inoculated plates were incubated at 30ºC. The radial colony growth of test fungus was recorded by drawing two perpendicular lines on the back of the Petri plates crossed each other in the centre of the plate. The data on colony growth was recorded along with these lines in millimeter after each 24 hours until the plates were filled in any treatment and calculated as inhibition (%) by using the following formula:

$$\text{Inhibition} = (A-B)/A \times 100$$

Where;

A: Colony growth of the pathogen in control plates.

B: Colony growth of the pathogen in treated plates.

RESULTS

Isolation of fungi from roots of eggplant: Ten fungi namely *Aspergillus flavus*, *Aspergillus niger, Fusarium oxysporum, Fusarium solani, Macrophomina phaseolina*, *Pencillium notatum, Pythium aphanidermatum, Pythium* sp., *Rhizoctonia solani* and *Rhizopus oryzae* were isolated from the roots with varying frequency from location to location. On overall basis, *F. oxysporum* with 25% frequency appeared as most predominant fungus isolated from eggplant roots, followed by *A. niger* (18%), *R. oryzae* (17.3%), *A. flavus* (12%), *R. solani* (9.3%), *F. solani* (8.6%), *P. notatum* and *M. phaseolina* (6.6%) (Fig. 1). As compared to the roots, lesser number of fungi were isolated from the seeds of the eggplant. In total, five fungi *viz*., *A. flavus*, *A. niger*, *F. oxysporum*, *P. notatum*, *R. oryzae* and one unidentified fungus were isolated from the eggplant seed. The *A. flavus* appeared as the predominant fungus isolated from eggplant seeds (25%), followed by *A. niger* (23%), *P. notatum* (22%), *R. oryzae* (20%) and *F. oxysporum* (16%) (Fig. 2).

Effect of different fungicides on colony growth of fungi: Eight fungicides *viz.*, Thiophanate-methyl, Fosetyl-aluminium, Propineb, Carbendazim, Copper oxychloride, Mendipropomide, Difenoconazole and Polyram were evaluated against *Fusarium solani, Rhizoctonia solani, Macrophomina phaseolina* and *Fusarium oxysporum* under *in-vitro* conditions. Each fungicide was tested with 5 concentrations *viz*., 1, 10, 100, 1,000 and 10,000 ppm. Higher dose 10,000 ppm of most fungicide appears 100% lethal at which pathogen could not produce any growth.

Difenoconazole at its all doses 1-10,000 ppm caused more than 50% inhibition of *Fusarium solani*. 10,000 ppm of Thiophanate-methyl, Fosetyl-aluminium, Propineb and Carbendazim causes 100% inhibition. Thiophanate-methyl 100-1000 ppm, Propineb 1,000 ppm, Carbendazim 10-1,000 ppm, Copper oxychloride 1,000 ppm, Mendipropomide 10,000, Metriam10,000 ppm cause more than 50% growth reduction as compared to control (Fig. 3).

Table 1. Details of fungicides used in the experiment.

Chemical name	Trade name	Active ingredient	Chemical group
Thiophanate-methyl	Topsin-M	70% Thiophanate-methyl	Thiophanate-methyl
Difenoconazole	Score	24.51% Difenoconazole	Triazole
Fosetyl-aluminium	Fosetyl-aluminium	80% Fosetyl-aluminium	Phosphonate
Propineb	Antracol	70 % Propineb	Dithiocarbamate
Carbendazim	Bavistan DF	50% Carbendazim	Benzimidazole
Copper oxychloride	Copper oxychloride	50% Copper oxychloride	Copper compound
Metriam	Polyram	70% Metriam	Metriam
Mendipropomide	Revus	23.3% Mendipropomide	Mendipropomide

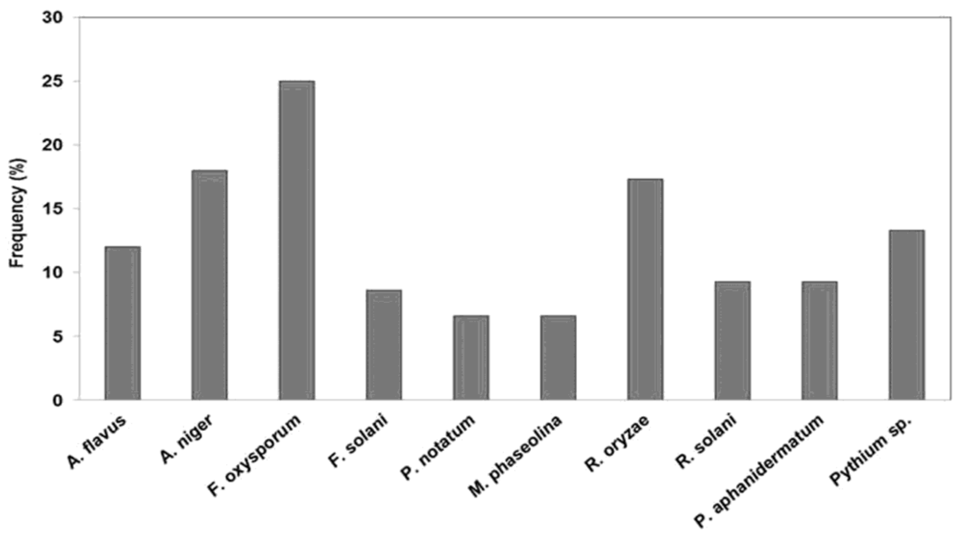

Fig. 1. Fungi isolated from eggplant roots collected from different locations.

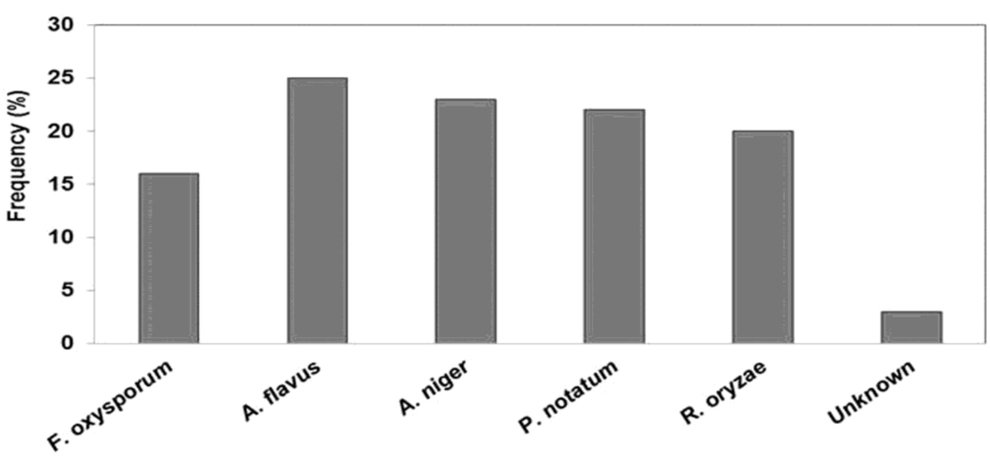

Fig. 2. Fungi isolated from eggplant seeds collected from different locations.

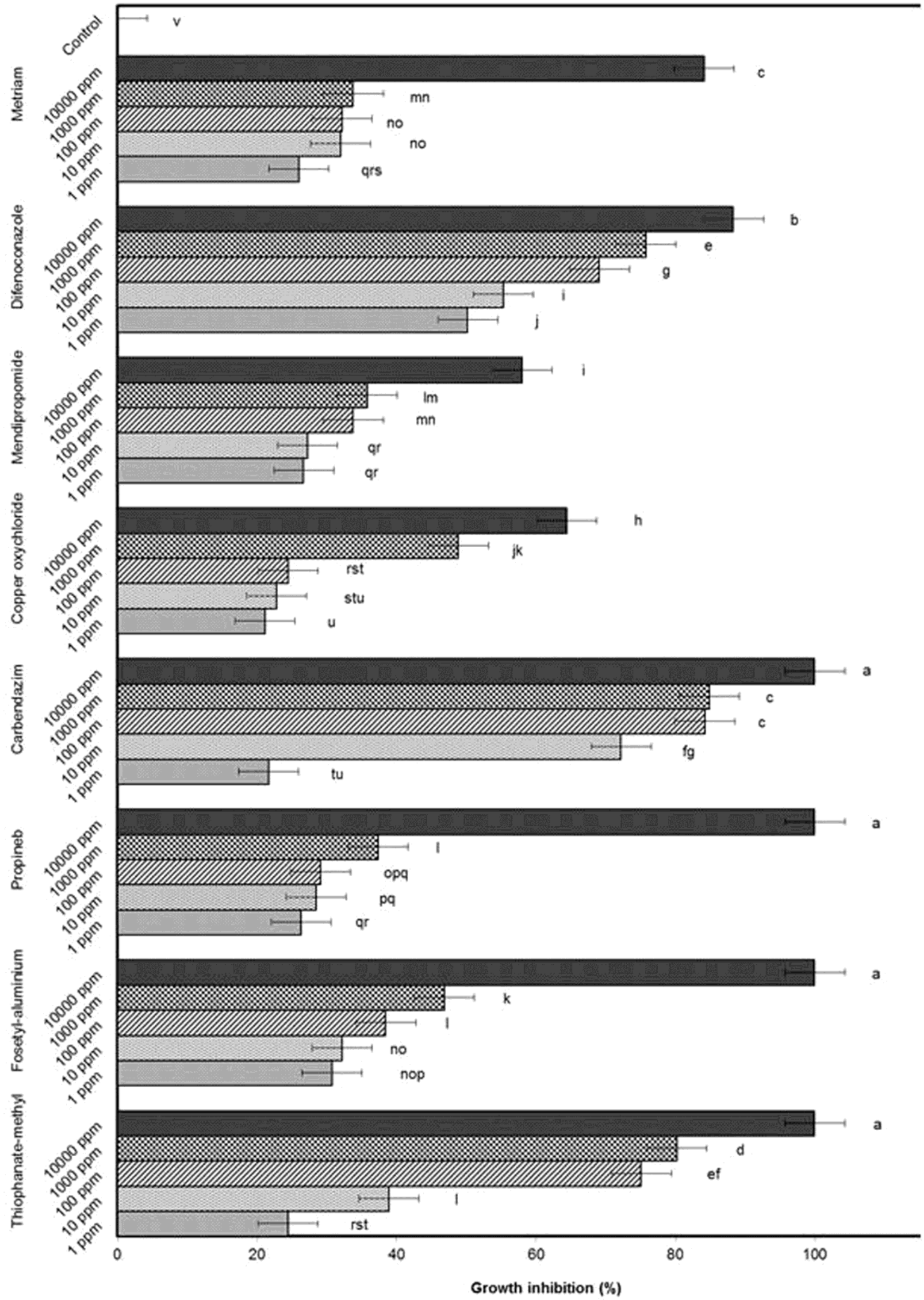

Fig. 3. Effect of different fungicides on growth inhibition of *Fusarium solani.* There were six time independent replications of each treatment. Bars labelled by the same letter denotes that values are statistically not different at $p<0.05$.

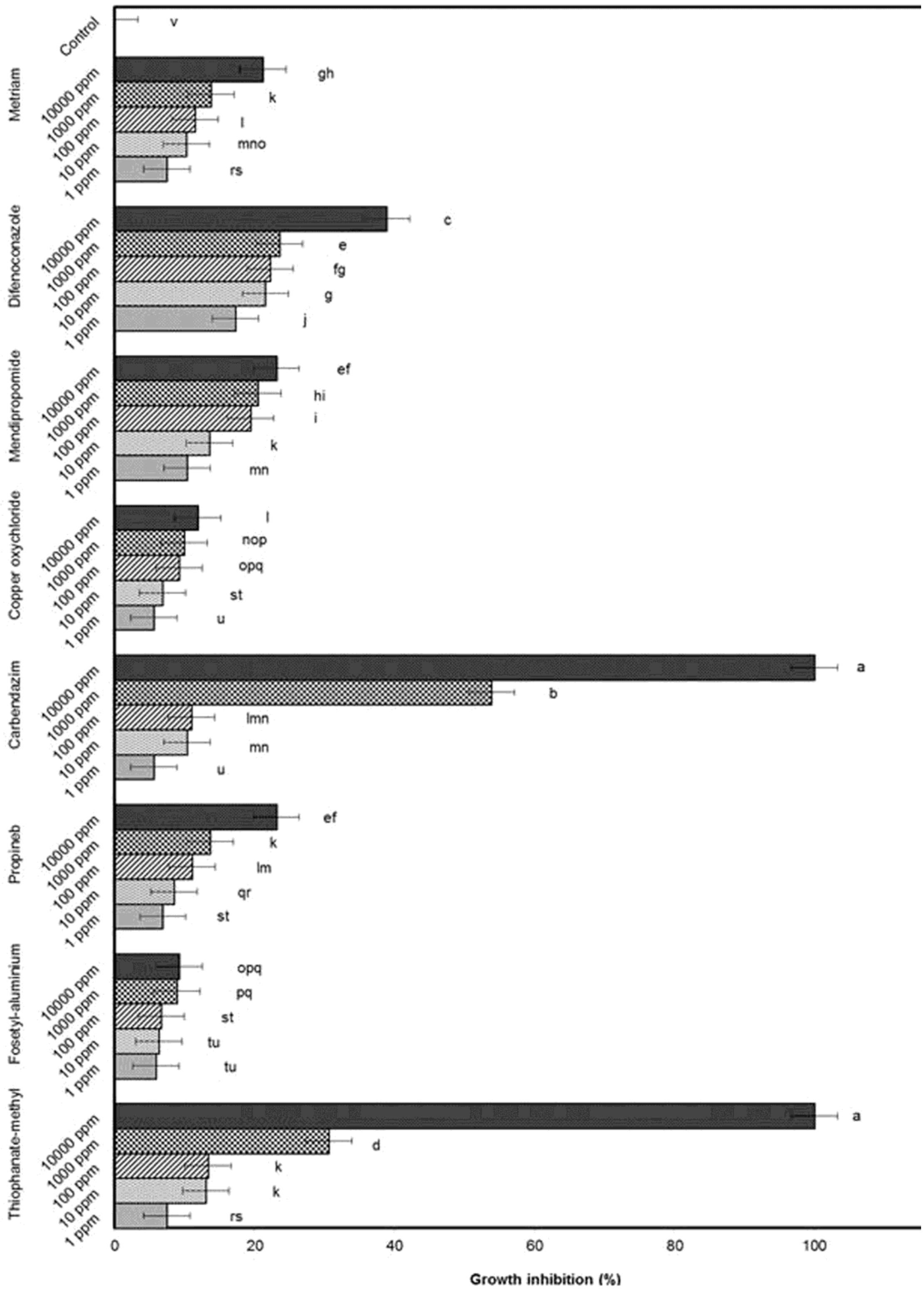

Fig. 4. Effect of different fungicides on growth inhibition of *Rhizoctonia solani.* There were six time independent replications of each treatment. Bars labelled by the same letter denotes that values are statistically not different at p<0.05.

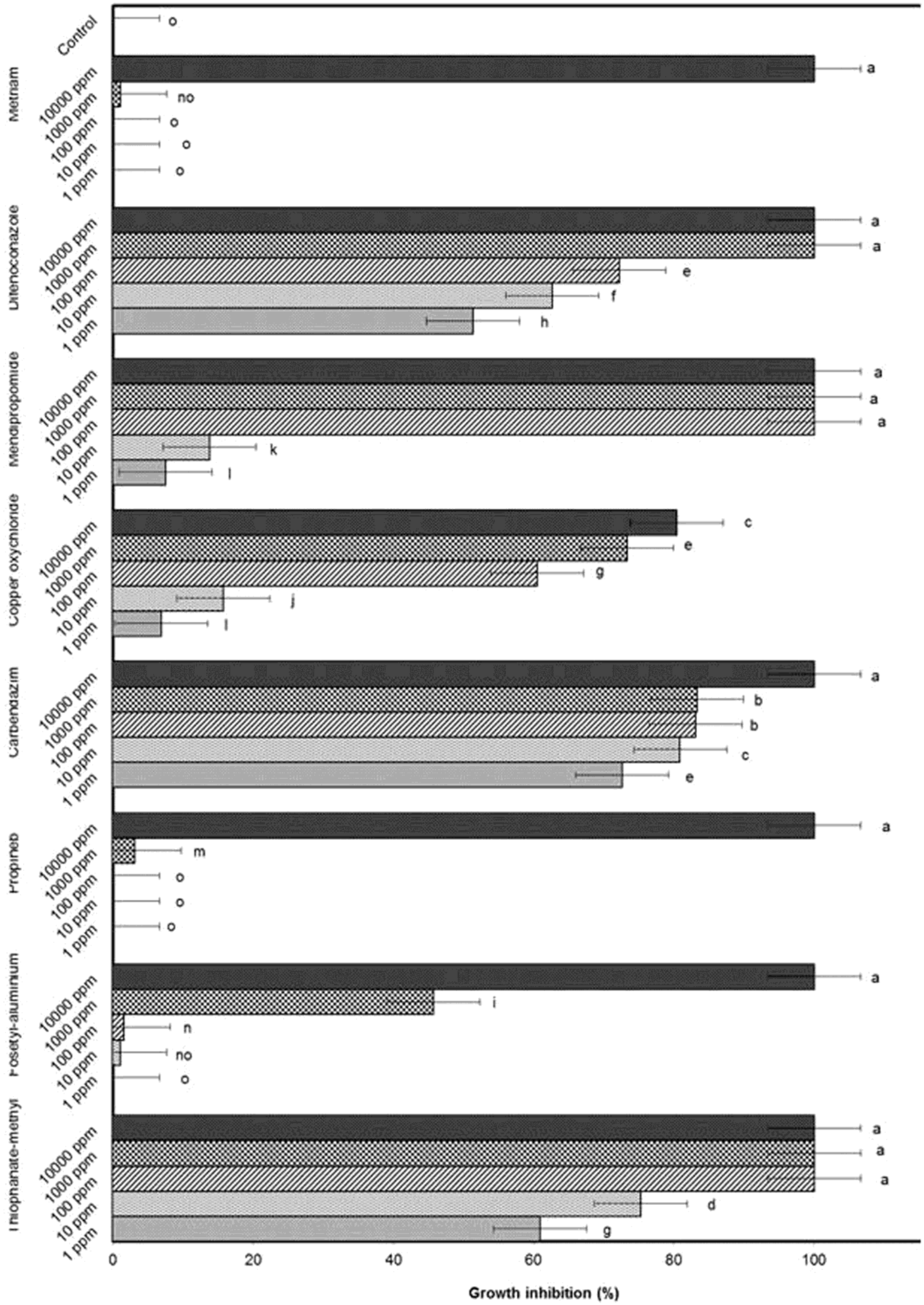

Fig. 5. Effect of different fungicides on growth inhibition of *Macrophomina phaseolina.* There were six time independent replications of each treatment. Bars labelled by the same letter denotes that values are statistically not different at $p<0.05$.

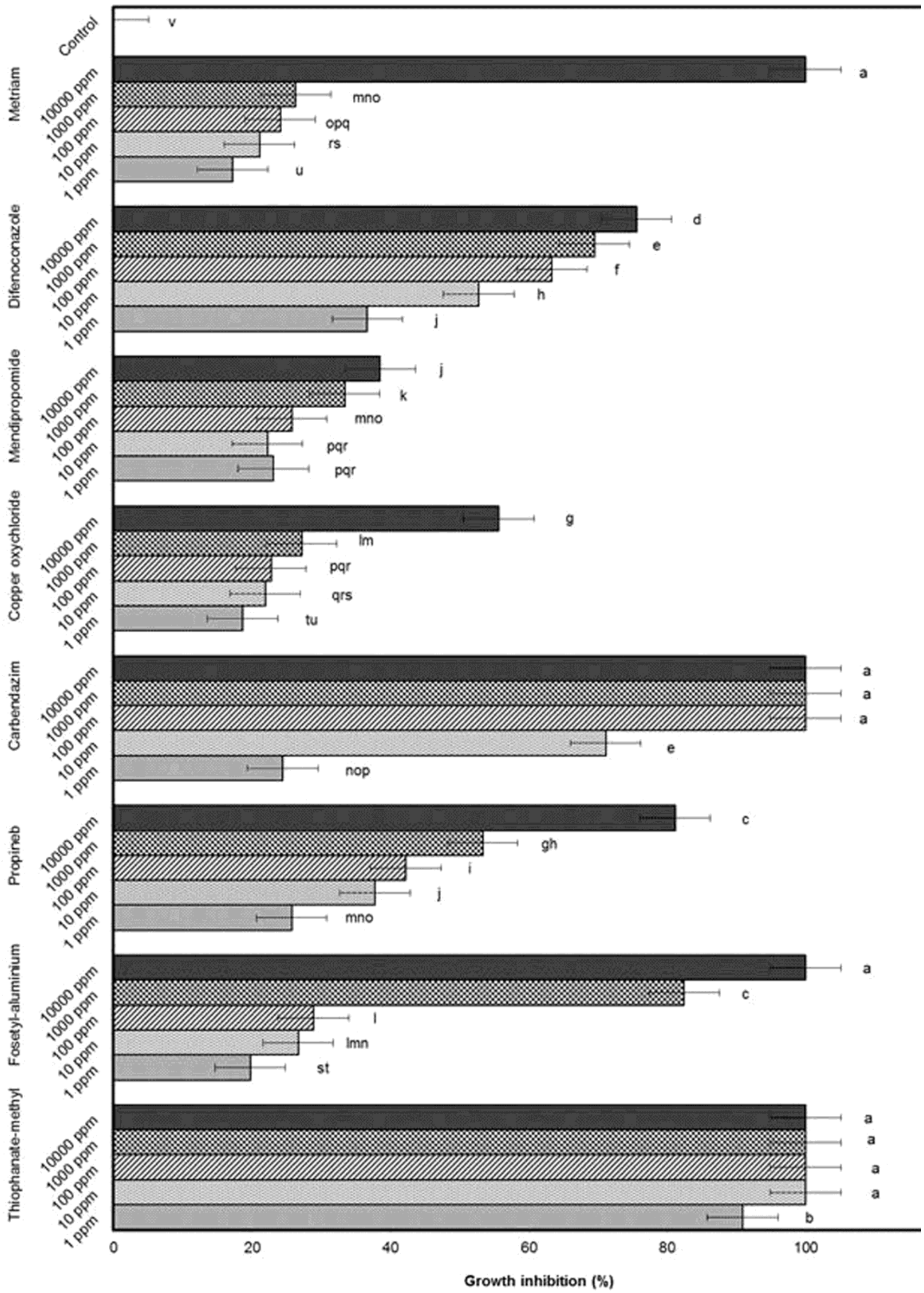

Fig. 6. Effect of different fungicides on growth inhibition of *Fusarium oxysporum.* There were six time independent replications of each treatment. Bars labelled by the same letter denotes that values are statistically not different at $p<0.05$.

Rhizoctonia solani appear resistant at all the used doses of fungicides, only two fungicides out of eight could cause more than 50% inhibition. Thiophanate-methyl at 10,000 ppm and Carbendazim 10,000 ppm causes 100% inhibition while Carbendazim at 1000 ppm causes 53% inhibition of *R. solani.* All the remaining treatments produce either no or less than 50% reduction in colony growth as compared to control (Fig. 4).

Macrophomina phaseolina at all the doses of Thiophanate-methyl, Carbendazim and Difenoconazole showed more than 50% growth reduction. Thiophanate-methyl 100-10,000 ppm, Fosetyl-aluminium 10,000 ppm, Propineb 10,000 ppm, Carbendazim 10,000 ppm, Mendipropomide100-10,000 ppm, Difenoconazole 1,000-10,000 ppm and Metriam10,000 ppm found lethal and cause 100% inhibition of *M. Phaseolina* (Fig. 5).

Fusarium oxysporum appear somewhat resistant to used doses of Mendipropomide and less than 40% inhibition was observed even with 10,000 ppm. Thiophanate-methyl 10-10,000 ppm, Fosetyl-aluminium 10,000 ppm, Carbendazim 100-10,000 ppm and Metriam10,000 ppm appears as lethal dose and no growth of *F. oxysporum* observed with these treatments (Fig. 4). The treatments such as Thiophanate-methyl 1 ppm, Fosetyl-aluminium 1,000 ppm, Antracol 1,000-10,000 ppm, Carbendazim 10 ppm, Copper oxychloride 10,000 ppm and Difenoconazole 10-10,000 ppm cause more than 50% reduction. The inhibition provided by these treatments is ranging from 52-91% (Fig. 6).

DISCUSSION

Several fungi were found to be associated with wilted, rotted roots and seeds of eggplant including most important soil borne pathogens i.e., *F. oxysporum, M. phaseolina, F. solani, R. solani*. Other workers also isolated *Fusarium solani* f. sp. *melongenae, M. phaseolina, Sclerotium rolfsii* and *Verticillium dahliae* from brinjal roots (Rangaswami and Mahadevan, 2006; Joseph *et al.*, 2008). Kuri *et al.* (2011) identified *Phomopsis vexans*, *Fusarium oxysporum, Aspergillus flavus*, *Aspergillus niger*, *Curvularia lunata, Penicillium* spp. from brinjal seeds. The prevalence and frequency of fungi varied with different regions.

During *in-vitro* chemical control of predominantly isolated fungal pathogens (*F. oxysporum*, *F. solani*, *R. solani* and *M. phaseolina*) and eight fungicides were used with different concentrations. *F. solani* found sensitive against all the used fungicide. Variation in growth reduction observed with changing concentrations of fungicides. Patel *et al.* (2004) observed that seed treatment with Carbendazim provide more effective control of okra wilt caused by *F. solani* followed by Agrozin, Derosel and Thiophanate-methyl. Similarly, Sultana and Ghaffar (2010) observed complete inhibition of *F. solani* with Fosetyl-aluminium, Benlate and Carbendazim at 100 ppm. Our results are in confirmation to those reported by Maitlo *et al.* (2013) that complete suppression or *F. solani* with Carbendazim followed by Thiophanate-methyl, Fosetyl-aluminium and Ridomil. They found copper oxychloride as completely ineffective against *F. solani.* In present study, we found contradictory result with 10000 ppm copper oxychloride could cause 64% growth reduction of *F. solani.*

Only Thiophanate-methyl and Carbendazim found effective against *R. solani,* remaining fungicide *viz.,* Fosetyl-aluminium, Propineb, Copper oxychloride, Mendipropomide, Difenoconazole and Polyram produce either no or less than 50% reduction in colony growth as compared to control. Our findings are in agreement to those reported by Soma *et al.* **(**2008), that out of four fungicides tested, Carbendazim proved to be most effective in reducing mycelial growth of *R. solani* as compared to Ridomil, Mass-M-45 and Vitavax.

Macrophomina phaseolina appears very sensitive to Thiophanate-methyl, Carbendazim and Difenoconazole, even their lowest (1 ppm) dose could cause more than 50% growth reduction. Arora *et al.* (2013) also reported that Bravistin and Thiophanate-methyl at lower concentration checked the growth of *M. phaseolina* completely, Carbendazim, Quinted and Tricylazole (Suryawanshi *et al.* 2008). Whereas, Iqbal *et al.* (2004) evaluated the sensitivity of *M. phaseolina* to Antracol (Propineb), Benomyl (Benlate), Orthocide (Captan), Mancozeb (Ridomil) and Copper compounds + Mancozeb (Trimiltox forte) at various concentrations (50, 100 and 200 ppm) and observed that mycelial growth markedly decreased with the increase in the concentration of the fungicides. They also reported that Benlate and Antracol were the most effective in the inhibition of *M. phaseolina* growth.

However, in case of *F. oxysporum* relatively more treatments completely inhibited the growth of targeted pathogen but appear somewhat resistant to used doses of Mendipropomide, which causes less than 40% inhibition even at 10000 ppm. Song *et al.* (2004) evaluated seven fungicides against *F. oxysporum* and found that Procholoraz and Carbendazim were the most effective fungicides in inhibiting the mycelial growth. Similarly Akram *et al.* (2004) reported that Benlate followed by Dithane M-45, Sencozeb, Ridomil and Antracol were the most effective fungicides in inhibiting the growth of *F. oxysporum*. Rajput *et al.* (2006) also found that Carbendazim at all the used doses (50-150mg/ml) significantly inhibited the colony growth of *F. oxysporum* followed by Thiophanate-methyl and Dithen M-45. Mukhtar (2007) found Benomyl and Carbendazim as most effective against *F. oxysporum*, whereas, Amini and Sidovich (2010) found Prochlorez and Bromucmezup highly effective against *F. oxysporum* f. sp. *lycopersici* followed by Benomyl and Carbendazim.

The present study revealed that *F. solani*, *R. solani*, *M. phaseolina* and *F. oxysporum* are frequently associated with roots and seeds of eggplants. Carbendazim and Thiophanate-methyl found equally effective against above mentioned pathogens. The identified fungicides can be used as seed treatment and further studies should be conducted to optimize the doses and schedule of fungicides for field application.

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