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In Vitro Evaluation of Fungicides against *Fusarium solani*, the Causative Agent of Brinjal Root Rot

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Abstract. Root rot in brinjal is one of the most important problems. In the present research, five different fungicides; *Mancozeb*, *Copper oxychloride*, *Hexaconazole*, *Metalaxyl+Mancozeb*, and *Carbendazim*, at 100 ppm were evaluated for their efficacy against the causative agent of this disease*, Fusarium solani, In-vitro*. The experiment was carried out in Completely Randomized Design with seven replications. Our result shows the difference in the efficacy of all tested fungicide against this pathogen. The application of *Carbendazim* at 100 ppm gave the highest inhibition of mycelium growth (100%) followed by *Mancozeb* (85.08%) and *Metalaxyl+Mancozeb* (64.3%) (at the same dose) on the 7th day of incubation suggesting *Carbendazim* (100 ppm) as a better one to use for the management of *F. solani*.

Keywords: brinjal, fungicides, fusarium, pathogen, root rot

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1. Introduction

Brinjal (*Solanum melongena L,* 2n=2x=24*),* also known as eggplant, is an annual *herbaceous* plant belonging to the family "Solanaceae". It is the second most important vegetable after tomato in the *Solanaceae* family. It is widely grown in Asian countries including Nepal. The productivity of crops is 14.48 Mt/ha in the country [1]. Brinjal plant is affected by several diseases caused by fungi, bacteria, nematodes, viruses, and phytoplasma. Fungal diseases seen in brinjal are *Alternaria* leaf spots (*Alternaria melongenae* and *A. solani*), *Cercospora* leaf spots (*Cercospora solani* and *C. melongenae*), collar rot (*Sclerotinia sclerotiorum*), damping off (*Pythium spp, Phytophthora spp*), fruit rot (*Phytophthora nicotianae*), *Phomopsis* blight (*Phomopsis vexans*), *Verticillium* wilt (*Verticillium dahliae*) and root rot (*Fusarium solani*). Among these, root rot of brinjal caused by *F. solani* is an important disease and causes a huge loss in yield. The symptoms of this disease include; yellowing, drooping, and drying of leaves followed by reddish-brown discoloration and decaying of the roots. Lesions start from the collar

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region and increase gradually downwards into the roots. The pathogen can remain in the soil for several years. Vector for this pathogen can be tools and bean straw manure which can carry spores and mycelium into the soil. Rainfall and flood can also redistribute the spores and cause infection. The fungus uses chlamydospore as the survival structure.

2. Materials and Methods

2.1. Experiment Location

The experiment was conducted at the Central Lab of Institute of Agriculture and Animal Science (IAAS), Lamjung Campus, Sundarbazar, Lamjung in 2019.

2.2. Isolation of *Fusarium solani*

Isolate of *F. solani* was collected from NARC, Khumaltar. A test tube of pure culture was brought and preserved in a refrigerator with a temperature of 4°C. Mother culture was prepared on PDA for further experimentation.

2.3. Fungicides

Five different fungicides belonging to three different categories based on their efficacy; Systemic, Contact, and Systemic + Contact fungicides were tested in the experiment. The systemic fungicides comprised of *Carbendazim* (*Carbamates*) and *Hexaconazole* (*Triazole*), Contact fungicides comprised of *Mancozeb* (*Dithiocarbamate*), and *Copper oxychloride* (*Trihdroxide*), and the mixed fungicides comprised of *Metalaxyl* + *Mancozeb* (*Acylalanines*) (Table 1).

Table 1. List of Fungicides and Their Active Ingredients Used in The Experiment

Trade Name (Active Substance)	Available Form	Active Substances Concentration	Chemical Group	Mode of Action
Dithane M-45 (*Mancozeb*)	Wettable Powder	75%	*Dithiocarbamate*	Contact
Blutoxx (*Copper Oxychloride*)	Wettable Powder	50%	*Trihydroxide*	Contact
Contaff (*Hexaconazole*)	Solution	5%	*Triazole*	Systemic
Bavistin (*Carbendazim*)	Wettable Powder	50%	*Carbamates*	Systemic
Kriloxyl Gold (*Metalaxyl* + *Mancozeb*)	Wettable Powder	8% + 64%	*Acylalanines*	Systemic+ Contact

2.4. *In vitro* Studies of Mycelial Growth Inhibition

The efficacy of five different fungicides were evaluated against *F. solani* on PDA using the poisoned food technique. An experiment was conducted in Completely Randomized Design

(CRD) with a total of 6 treatments and 7 replications. Five chemical fungicides were studied at 100 ppm concentration along with control treatment.

T1: Control (non-amended)

T2: *Blutoxx* (100 ppm)

T3: *Kriloxyl* gold (100 ppm)

T4: *Dithane* M-45 (100 ppm)

T5: *Bavistin* (100 ppm)

T6: *Contaff* (100 ppm)

The required amount of fungicide was mixed in 150 mL of distilled water for preparation of the 10X stock solution and a calculated amount of stock solution was added in 100 mL sterilized potato dextrose medium to obtain 100 ppm concentration. Then, 15 mL of amended media was poured into 90 mm sterilized Petri plates and was allowed to solidify. Control plates were prepared without amending pesticide. Mycelial disc of 3 mm radius from 7 days old mother culture was inoculated at the center of the Petri plate and incubated at 22°C for seven days. The measurements of mycelial growth were recorded in the 3rd and 7th days of incubation in each treatment. The percentage growth inhibition of mycelial growth over control was then calculated by using the formula given by Vincent [2].

$$\text{PGI} = \frac{C - T}{C} \times 100$$

Where: PGI = Percent growth inhibition; C = Average area of the colony in the control treatment; T = Area of the colony in fungicidal treatment

2.5. Data Analysis

Data were subjected to analysis of variance and separation of means among the treatments were determined using LSD test at 5% level of significance using Statistical Packages for Social Science (SPSS 16.0 version) and MS Excel (2010).

3. Results and Discussion

3.1. Growth Inhibition Percentage (Day 3)

One hundred percent inhibition was obtained in *Carbendazim* preventing complete radial growth of fungus which was highly significant ($p \leq 0.05$). *Mancozeb* followed the order and executed 84.9% inhibition. Next in order, *Mancozeb* + *Metalxyl* showed 71.24% inhibition followed by *Hexaconazole* with 55.8% growth inhibition (Figure 1). *Copper oxychloride* was found to be the least effective which instead supported the growth of fungus and a negative growth inhibition percentage was obtained with 5.6% ($p \leq 0.05$).

3.2. Growth Inhibition Percentage (day 7)

On the 7th day, we found the same trend as the *Carbendazim* was highly effective in inhibiting the growth with 100% inhibition (Figure 1). *Mancozeb* had 85.08% inhibition ($p \leq 0.05$). *Mancozeb* + *Metalxyl* executed 64.3% of growth inhibition followed by *Hexaconazole* with 35.6 percent of inhibition ($p \leq 0.05$). *Copper Oxychloride* was the least effective with 2.6 percent of growth inhibition.

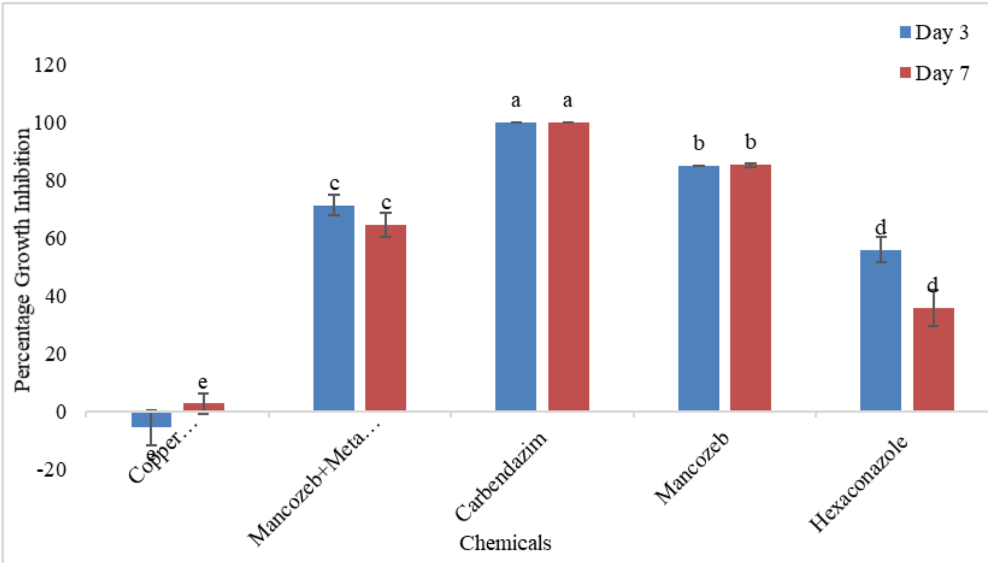

Figure 1. Graph Showing Percentage Growth Inhibition of Fungicides Against *F. solani* over Control in Poisoned Food Technique of Experiment on 3rd and 7th day. Each Alphabet Shows Statistical Differences Analyzed by Using DMRT and LSD.

The growth of *Fusarium solani* at seventh day after inoculation as can be seen in Figure 2.

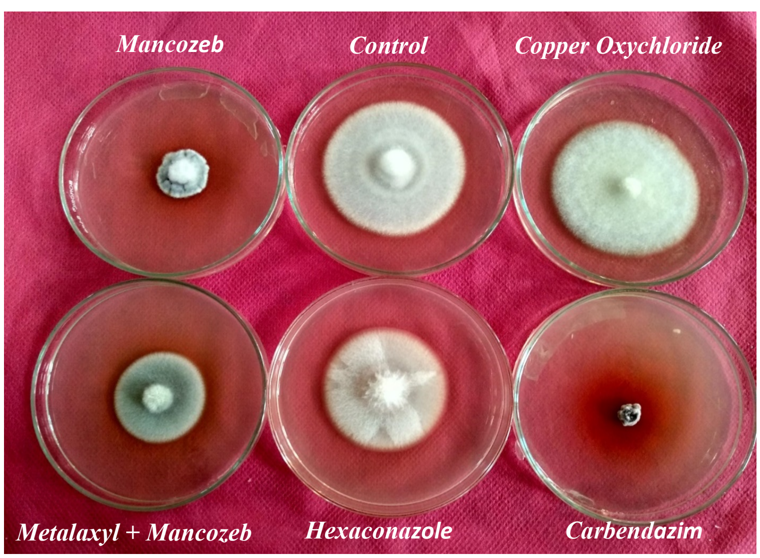

Figure 2. Growth of *Fusarium solani* at Seventh Day after Inoculation

Carbendazim, being a broad spectrum fungicide acts against *ascomycetes*, *basidiomycetes*, and *deuteromycetes*. It falls under a class of *benzimidazoles* which binds with the B-*tubulin* subunit of microtubules of fungi and block nuclear division [3]. Davidse [4] and Hammerschlag and Sisler [5] reported that *methyl*-2-*benzimidazole carbamate* (MBC) inhibits mitosis as well as DNA and RNA synthesis and arrest spindle formation by developing a complex between MBC and subunit of the microtubule. In our experiment, *Carbendazim* was the best performing fungicide among the tested fungicides. It showed 100% inhibition on the growth of *F. solani* in both of the observation dates. Our result is supported by Yadav et al.[6], Soni and Verma [7] and (Taskee- TaskeenUn-Nisa et al. [8] They found the inhibiting nature of *Carbendazim* on mycelia growth of *Fusarium* spp.

Hexaconazole has performed well in test fungus but the effectiveness was less than that of *Mancozeb*. This result is similar to the result of Chennakesavulu et al. [9] His experiment concluded 93.88% inhibition of mycelial growth of *Fusarium udum* by *Hexaconazole* after 100% inhibition by *Carbendazim*. Dibya Bharati [10] reported a cent percent inhibition of *F. solani* by using the *triazole* group of fungicides which is due to their interference with the ergosterol biosynthesis. Khilari et al. [11] also reported inhibition order of *Carbendazim* (100%) followed by *Hexaconazole* (84.23%) which is in favor of our result.

Similarly among non-systemic fungicides, *Mancozeb* has the best performance and this result is supported by the findings of Singh et al. [12]. They recorded *Mancozeb* to be best for growth inhibition of *F. solani* and *F. oxysporum*. Allen et al.[13] also recorded cent percent inhibition of all four *Fusarium* species by *Mancozeb*.

In our experiment, the *copper oxychloride* supported the growth of mycelium and gave a negative inhibition percentage on the initial reading (Figure 1). A similar result was observed by Baturo-Ciesniewska et al. [14]. According to them, the use of the low concentration of *copper oxychloride* stimulates the growth of fungus. Literature suggests some heavy metals when applied at lower concentrations (40 ppm) fall within the limits of carrying fungus which affects the growth and reproduction [15]. The cell wall of some *deuteromycetes* (*Aspergillus niger*) acts as a barrier to restrict the internalization of solutes and can retain up to 37-77% of copper [16]. Contrasting views can be seen in the literature that, Bhaliya and Jadeja [17] reported the *copper oxychloride* had a good inhibition (79.25%) on *Fusarium solani* causing coriander root rot when used at 2500 ppm concentration.

Metalaxyl shows inhibitory action on endogenous RNA polymerase activity that causes inhibition of RNA synthesis [4]. The combination of *Metalaxyl* and *Mancozeb* shows a synergistic effect. Average mycelial growth inhibition was recorded with the test combi-fungicides which are supported by the finding of Mannai et al. [18].

4. Conclusion

Among the systemic fungicides tested under in vitro conditions, *Carbendazim* (100%) was found to be the most effective in growth inhibition of *F. solani*. In the case of non-systemic fungicides, *Mancozeb* proved to be the most effective in growth inhibition. *Copper oxychloride* was least effective and some result suggests that reduction in dose in some fungicides maybe even more dangerous than not using of the fungicides.

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Conflicts of Interest

The authors declare that there is no conflict of interest.

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