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RESEARCH ARTICLE

IN-VITRO EVALUATION OF CHEMICAL FUNGICIDES AGAINST *RHIZOCTONIA SOLANI* CAUSING DAMPING OFF OF TOMATO

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ABSTRACT

Tomato (*Lycopersicum esculentum*), despite having thousands of commercial growers and millions of tons of production, has different soil borne fungal diseases as its major setback, and *Rhizoctonia solani* causing damping off is a major one. To evaluate the potential inhibition effect of different chemical fungicides, an experiment was carried out at the Plant Pathology laboratory of Lamjung campus. Five chemical fungicides viz. Carbendazim 50% WP (Bavistin), Mancozeb 75 % WP (All M-45), Metalaxyl 8 % + Mancozeb 64 % WP (Redomill), Copper oxychloride 50 % WP (Blitox), Carbendazim 12 % + Mancozeb 63 % WP (SAAF) of each at concentrations of 50PPM, 100PPM, and 150PPM and unamended media as a control were used with 3 replications. Poisoned food technique method was followed using Completely Randomized Design in lab conditions. Carbendazim 50% WP was found to be the best among five fungicides showing mycelial inhibition above 99% at all of the concentrations used. Copper oxychloride 50 %WP on the other hand, showed minimum growth inhibition barely crossing 10% of inhibition at all of the concentration used. Mycelial growth inhibition increased on increasing the concentration of chemical fungicides used, whereas some chemicals could be effectively used under lower concentration to minimize hazardous effect on soil and environment as their performance against the pathogen was quite impressive.

KEYWORDS

Completely Randomized Design, Concentrations, Growth inhibition, Poisoned food technique

1. INTRODUCTION

Tomato (*Lycopersicon esculentum*) is an herbaceous plant that belongs to the Solanaceae family and has bisexual flowers, tap roots, and berry-like fruit (Siddiqui et al., 2019). This nutritionally rich vegetable crop possess minerals, vitamins, essential amino acids, carotenoids, monounsaturated fatty acids, and fiber proteins and has multiple health benefits, including chronic diseases such as cardiovascular and neurodegenerative ailments (Vats et al., 2022; Ali et al., 2020; Elbadrawy and Sello, 2016; Salehi et al., 2019). It is consumed raw as salad, served as a cooked vegetable, used as pickles, or even used as a processed product that reflects its versatile usage (Ali et al., 2020). Due to its immense health benefits and worldwide consumption, the global market size has reached $174.7 billion and is projected to grow to $233.13 billion in 2028 (Tomato Global Market Report, 2024).

Despite the production of huge value, there still exists a challenge of approximately 200 diseases, and soil-borne disease caused by different pathogens (Pythium, Fusarium, Rhizoctonia, Sclerotium, and Phytophthora), being the major ones (Ma et al., 2023; Shafique et al., 2016). Among those soil-borne diseases, damping off is one of the major diseases in tomatoes, which has the potential to affect 5 to 80% seedlings, causing heavy economic loss (Lamichhane et al., 2017). One of the significant pathogens of damping off is *Rhizoctonia solani*, a non-sporulating mycelial form having typical septation and a distinctive branching with essentially multinucleate cells, whose growth is favored by high soil moisture, compaction, overcrowding, poor ventilation, and cool, damp, cloudy weather (Cerkauskas, n.d.; Gill et al., 2001; Jiskani et al., 2007). It generates chains or clusters of monilioid cells, also known as barrel-shaped chlamydospore cells or sporodochia, from which sclerotia is formed. This sclerotia's hard and resilient nature makes the pathogen survive in the soil for a long-time facilitating the initiation of infection and spreading disease (Lin et al., 2023).

Tomatoes are particularly vulnerable to this pathogen in humid conditions, especially if the soil is cold and wet. It disrupts the early stage of the crop establishment, causing poor emergence of the seeds and the death of seedlings (Jiskani et al., 2007; Lamichhane et al., 2017). Its symptoms can be seen in two phases: pre-emergence damping, where seeds decay prior to emergence. Whereas in the post-emergence phase, stems near the soil surface of seedlings become weak, develop water soaking, eventually browning and shriveling of the stem, and ultimately topple down by being unable to bear the load of the upper parts (Abdelghany et al., 2022; Massawe et al., 2013). This results in huge economic losses, including the cost of repairing damaged seed or seedlings and replacing the damaged ones with new ones (Lamichhane et al., 2017). A group researcher reported that it can cause 25-75% losses annually, depending on the host variety and environmental factors (Adhikari et al., 2015).

It can be controlled through different methods, including both proper cultural practices (good drainage beds, optimum dose of nitrogenous fertilizers, removal and replacement of top soil with fresh healthy soil every five years, steam sterilization of the seedbeds) and chemical method (application of fungicides) (Gibson, n.d.; Kataria and Gisi, 1996; Lumsden and Locke, 1989). Chemical control is the most widely used method with various formulations and trade names on the market (Karima and Nadia, 2012; Mohamed et al., 2015). For instance, Mancozeb, a Dithiocarbamate fungicide with a contact-based action, disrupts the lipid metabolism, respiration, and the production of adenosine triphosphate in fungal cell

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(Anita et al., 2024). Another fungicide, carbendazim, is a benomyl metabolite and a widely used broad-spectrum benzimidazole fungicide (Bai et al., 2024). Whereas Saaf and Krilaxyl Gold are the mixture of mancozeb with carbendazim, and Metalaxyl, respectively (Subedi et al., 2022). Beside this, other fungicides, such as Copper oxychloride are used (Dhami and Maharjan, 2023).

Primary method of controlling pest disease in crops and after harvest for quality assurance has been the use of pesticides (Anita et al., 2024). Furthermore, fungicides are often used haphazardly; however, their lower concentration can control disease effectively. Despite the number of fungicides used for this disease, there is limited research focused on the comparative effectiveness of fungicides along with concentration. Hence, we tend to evaluate those lower concentration of fungicides at laboratory conditions, which will provide a base for in vivo tests, ultimately supporting the disease management systems for tomato farming.

2. MATERIAL AND METHODOLOGY

2.1 Site Selection

The experiment was carried out in the Plant Pathology Laboratory of the Institute of Agriculture and Animal Science (IAAS), Sundarbazar, Lamjung in 2022. The study was designed in a fully controlled condition inside the laboratory. The aseptic condition was maintained for the culture of the pathogen avoiding any sort of contamination.

2.2 Isolate Collection

Pure culture of *R. solani* was brought into the lab from NARC. It was kept in a refrigerator at 4°C.

2.3 Equipment

The equipment and apparatus which have been used in this study were laminar flow, BOD incubator, refrigerator, autoclave, glassware, microscope, hot air oven, electronic balance, forceps, inoculation needle, cork borer, blade, etc

2.4 Fungicides

Five currently available fungicides on the Nepalese market were tested against the pathogen at three concentrations viz. 50 ppm, 100 ppm, 150 ppm. The experiment was carried out in a completely randomized design (CRD) using 3 replications at each concentration using the poisoned food technique.

Table 1: List of fungicides used in experiment along with their trade name and mode of contact

S. N	Chemical name	Trade Name	Active ingredients	Mode of contact
1	Carbendazim 50% WP	Bavistin	50% WP	Systemic
2	Mancozeb 75 % WP	All M-45	75% WP	Contact
3	Metalaxyl 8 % + Mancozeb 64 % WP	Redomill	72% WP	Systemic + Contact
4	Copper oxychloride 50 % WP	Blitox	50% WP	Contact
5	Carbendazim 12 % + Mancozeb 63 % WP	SAAF	75% WP	Systemic + Contact

2.5 General laboratory Procedure

2.5.1 Sterilization of Equipment

Major experimental apparatus used were petri plates, conical flask, vessels, test tube, beaker, pipette, inoculation loop, spirit burner, centrifuge machine, autoclave, incubator and hot-air oven. All the necessary equipment was washed with a detergent solution in running tap water and dried. Then the glassware was sterilized in a hot air oven at 170 °C for one hour and the heat resistant plastic beaker was sterilized in an autoclave at 15 psi, 121 °C for 20 minutes.

2.5.2 Disinfection of the Inoculation Chamber

All tests including isolation, subculturing, and other research, were carried out in an aseptic laminar airflow chamber. The chamber was sterilized by exposing a UV light on it for 15 minutes and wiping it down with ethanol (75%). The blades, forceps, inoculation loop, inoculation needle, and other instruments were disinfected by heating them over a spirit lamp.

2.5.3 Preparation of Media

As a growing medium for fungal pathogens, Potato Dextrose Agar (PDA) containing 2% agar was utilized. The appropriate amount of media was prepared according to the prescription by mixing PDA powder at a rate of 39 gm per 1000 ml distilled water. The media was then sterilized in an autoclave at 15 psi and 121 °C for 20 minutes before being allowed to cool. When the media temperature reached around 40 °C, it was placed into sterile petri dishes inside laminar flow and allowed to solidify. After solidification, the media was employed for pathogen inoculation.

2.5.4 Preparation of Mother Culture

Pure culture refrigerated was used to make mother culture, which was then utilized for sub-culturing and treatment. A small mycelial thread was excised from pure culture and transferred to fresh culture media using a sterile inoculation loop. Those mycelial inoculated petri plates were called mother cultures which were incubated in an incubator at 27 °C for 7 days.

2.5.5 Preparation of fungicidal solution

2.5.5.1 Preparation of stock solution

The stock solution of each fungicide to be used was made based on their concentration. 1 gram of each solid fungicide was mixed in 10 ml of distilled water to prepare a stock solution with a concentration of 50,000 ppm of Carbendazim, 50,000 ppm of copper oxychloride, 75,000 ppm of Mancozeb. 75,000 ppm of Carbendazim + Mancozeb and 72,000 ppm of Metalaxyl +Mancozeb.

2.5.5.2 Dilution of stock solution

To get the required ppm of fungicides (50, 100 and 150 ppm), the prepared stock solution was diluted by using the following formula:

$C1\ V1 = C2\ V2$

Where,

C1 = Concentration of stock solution (ppm)

V1 = Volume of stock solution (ml)

C2 = Desired concentration of fungicide solution (ppm)

V2 = Measured volume of PDA (ml)

With the help of micropipettes, the desired volume of each fungicide with their diluted concentration was poured in a sterilized beaker containing PDA media of 60 ml and was thoroughly shaken for uniform mixing of fungicides before pouring into petri plates to get desired concentrations of active ingredients of each fungicide. In a sterilized and cooled media streptomycin (0.25 g/l) was added to check bacterial growth. 20 ml of amended media was poured into a 90 mm sterilized petri plate and was allowed to solidify.

2.5.6 Method of inoculation

Amended solid media (poisoned media with fungicides) in the petri plate was inoculated with seven days old mother culture. Mycelial disc from culture was inoculated with a sterilized cork borer of 5 mm diameter and placed at the center of the petri plate in an inverted position so that it came in direct contact with the surface of the medium. Unamended PDA plates with test pathogen served as control. Prepared plates were marked with treatment details by using a marker. Each treatment was similarly replicated three times. The inoculated petri plates were sealed tightly with help of para film and were incubated at 27°C in an incubator for seven days. After 7 days, data was collected.

2.6 Growth Inhibition Test

The observations on mycelial growth were recorded in 7th days of incubation in each treatment using the vernier caliper scale. The percent growth inhibition of mycelial growth over control was calculated by using the formula of (Vincent, 1947).

$PGI = (C-T) / C \times 100$

Where,

PGI = Percent growth inhibition; C = Average diameter of colony in control treatment; T = Average diameter of colony in fungicidal treatment

2.7 Statistical Analysis

All the data were entered in Ms. Excel and analysis of variance was done using R Studio software. Mean comparison was done using Least Significant Difference (LSD) test at 0.05 level of significance.

3. Results and Discussion

The growth of R. solani was found to decrease with the increase in concentrations of all fungicides, as shown in Tables 1 and 2. Among the tested fungicides, Bavistin (Carbendazem) was found to be the best among five fungicides, showing mycelial inhibition above 99% at all of the concentrations used. Carbendazim, a systematically active benomyl metabolite, acts on the nucleus of pathogenic fungi and inhibits or disrupts mitosis by forming sub-units of microtubule, effectively controlling R. solani (Bai et al., 2024). Some researcher evaluating fungicidal efficiency against the particular pathogen, also found that bavistin has 100% growth inhibition (Sigdel et al., 2022). Similar results were found by most of researchers (Babli et al., 2022; Maharjan et al., 2023; Karkee et al., 2020; Sriraj et al., 2014). However, a group researcher claimed that 100% inhibition was observed only at 1000 ppm, while at lower concentrations, approximately 75% inhibition was seen (Kumar et al., 2017).

Redomill, the mixture of Metalaxyl and Mancozeb, also inhibits the growth of R. solani over 94%. It disrupts nucleic acid synthesis, inhibits spore germination, and interferes with the fungal pathogen cell. Besides, inhibition exhibited by Carbendazim 12% + Mancozeb 63% WP was above 90% at all concentrations. Some researchers observed a similar finding (Sigdel et al., 2022; Karkee and Mandal, 2020). However, in other study author revealed 100% inhibition at 0.1%, which is a higher concentration than our experiment, 0.1% (Srinivas et al., 2013). This fungicide converts into an isothiocyanate when exposed to air, inactivates the sulfhydryl groups' enzymes, and disturbs the functioning of fungal enzymes.

While Mancozeb has the inhibition of approximately 82% to 98% from 50 ppm to 150 ppm, respectively. A group researcher also observed its higher efficiency (Biljana et al., 2018). It is a contact-based fungicide with multi-site protection action and is often linked with its ability to disrupt the processes of lipid metabolism, respiration, and the production of adenosine triphosphate (Anita et al., 2024). Like Carbendazim 12% and Mancozeb 63 %WP, it also interacts with sulfhydryl groups of amino acids and enzymes and inactivates them, ultimately inhibiting the growth of fungal cells. Copper oxychloride 50% WP on the other hand, showed minimum growth inhibition, barely crossing 10% of inhibition at all of the concentrations used. At 72 hours, 16.24% and 14.94% inhibition of *R. solani* was observed at 72 hours at 100 and 200 ppm, respectively (Sigdel et al., 2022).

Table 2: Effect of fungicides and their concentration on mean diameter growth (mm) of *Rhizoctonia solani* under different replications

Replication 1			
Concentration	**50 PPM**	**100 PPM**	**150 PPM**
Carbendazim	7	6	5
Mancozeb	32	20	12.5
Metalaxyl + Mancozeb	17	18	10
Copper oxychloride	86.5	85	84
Carbendazim + Mancozeb	26	11	10
Control	86	86	86
Replication 2			
Concentration	**50 PPM**	**100 PPM**	**150 PPM**
Carbendazim	8	5	5
Mancozeb	43	12.5	10
Metalaxyl + Mancozeb	22.5	16	10.5
Copper oxychloride	88	87	83
Carbendazim + Mancozeb	27.5	15	13
Control	88	88	88
Replication 3			
Concentration	**50 PPM**	**100 PPM**	**150 PPM**
Carbendazim	7	7	5
Mancozeb	35	14	8.5
Metalaxyl + Mancozeb	23.5	16	9
Copper oxychloride	86	84	84
Carbendazim + Mancozeb	28	14	13
Control	87.5	87.5	87.5

Table 3: Significant difference between mean diameters among chemical fungicides

Chemical fungicides	**50 PPM**	**100 PPM**	**150 PPM**
Carbendazim	7.33^{e}	6^{c}	5^{d}
Mancozeb	36.67^{b}	15.5^{b}	10.33^{c}
Metalaxyl + Mancozeb	21^{d}	16.67^{b}	9.83^{c}
Copper oxychloride	86.83^{a}	85.33^{a}	83.67^{b}
Carbendazim + Mancozeb	27.17^{c}	13.33^{b}	12^{c}
Control	87.17^{a}	87.17^{a}	87.17^{a}
Mean	44.36	44.36	34.67
CV (%)	6.38	4.67	3.53
LSD	5.03	3.69	2.18

Table 4: Effect of fungicides and their concentration on mean growth inhibition percentage of *Rhizoctonia solani*

Chemical fungicides	50 PPM	100 PPM	150 PPM
Carbendazim	99.63[a]	99.85[a]	100[a]
Mancozeb	82.69[c]	97.09[a]	98.91[ab]
Metalaxyl + Mancozeb	94.53[ab]	96.73[a]	99.07[ab]
Copper oxychloride	3.01[d]	6.33[b]	9.98[c]
Carbendazim + Mancozeb	90.79[b]	97.99[a]	98.44[b]
Mean	74.13	79.60	81.28
CV	3.82	2.18	0.78
LSD	5.16	3.15	1.15

3.1 Comparison of inhibition percentage of *R. solani* at different concentrations of fungicides

As illustrated in Table 3, there was a significant difference between the treatments used over control. At 50 PPM, Carbendazim showed the highest inhibition of fungus (99.63%), which is on par with Redomill (94.53%), followed by SAAF (90.79%) and Mancozeb (82.69%). While the lowest inhibition was shown by copper oxychloride (3.01%). When chemicals were used at 100 PPM, Carbendazim showed an inhibition percentage of 99.85%, which is at par with SAAF (97.99%), Mancozeb (97.09%), and Redomill (96.73%). The lowest inhibition percentage was shown by copper oxychloride (6.33%). When concentration is increased to 150 ppm, Carbendazim still exhibits the highest level of inhibition (100%), which is on par with Redomill (99.07%) and Mancozeb (98.91%), followed by SAAF (98.44%). Like other concentrations, Copper oxychloride showed the lowest inhibition of 9.98% at 150 ppm. The result also clarifies the effectiveness of fungicides such as Carbendazim, Redomill, and SAAF even at a lower concentration of 50 PPM.

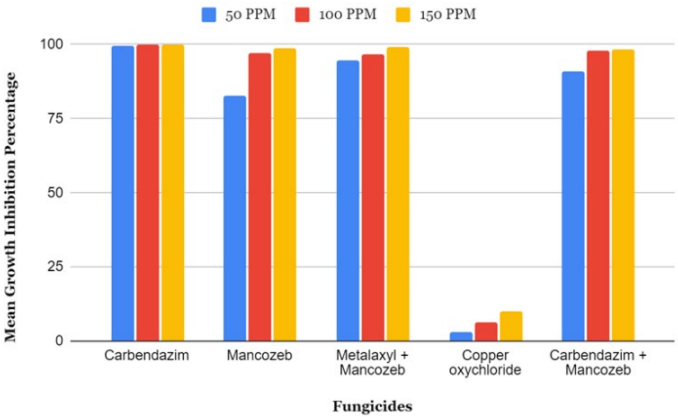

Figure 1: Effect of different fungicides and their concentrations in mean growth inhibition percentage of *Rhizoctonia solani*

4. Conclusion

Testing the efficacies of fungicides against *Rhizoctonia solani*, which causes serious plant diseases including damping off of tomatoes, revealed that Carbendazim is found to be effective at all concentrations, significantly par with Metalaxyl + Mancozeb. While SAAF at 100 and 150 ppm shows the higher inhibition as compared to 50 ppm. Similarly, Mancozeb also shows no significant difference at 100 ppm and 150 ppm concentrations with Carbendazim. Among five fungicides, copper oxychloride was found to be least effective, with inhibition percentages of 3.01%, 6.33%, and 9.98% at 50 ppm, 100 ppm, and 150 ppm, respectively. Mycelial growth inhibition increased with increasing the concentration of chemical fungicides used, whereas some chemicals could be effectively used under lower concentrations to minimize hazardous effects on soil and the environment, as their performance against the pathogen was quite impressive. However, these in vitro research findings should be verified in the field conditions before taking for field application.

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