In vitro Evaluation of Efficacy of Fungicides Against *Fusarium oxysporum* f. sp. *Lycopersici* (Sacc.) Synder and Hansen Causing Wilt Disease of Tomato

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Abstract

Fusarium wilt of tomato caused by *Fusarium oxysporum* f.sp. *lycopersici* is one of the most devastating and economically important fungal diseases of tomatoes. The study was conducted to assess the effectiveness of commercially available fungicides against *Fusarium oxysporum* f. sp *lycopersici*, a Nepali isolate by poisoned food technique. The experiment was carried out in a Completely Randomized Design (CRD) with five replications, in the Plant Protection Laboratory of the Ministry of Industry, Agriculture and Cooperatives, Jhumka, Sunsari. Six different fungicides: Mancozeb, Copper oxychloride, Carbendazim, Azoxystrobin, Kasugamycin + Copper oxychloride, Azoxystrobin + Difenoconazole at four different concentrations (100 ppm, 150 ppm, 200 ppm and 250 ppm) were tested for their ability to inhibit mycelial growth of the fungus, *in vitro*. The mycelial growth of the fungus was measured at 2, 4, 6, 8 and 10 days after inoculation (DAI). Among these fungicides Carbendazim, at all concentrations, showed the highest mycelial growth inhibition (100%) followed by Azoxystrobin in combination with Difenoconazole (82.79%) and Azoxystrobin alone (61.04%) at 250 ppm on 10 DAI. The results suggest that Carbendazim was the most effective at the lowest concentration (100 ppm) at *in vitro* inhibition of the fungus. Therefore, it can be a candidate to be explored for *in vivo* management of fusarium wilt of tomato caused by *Fusarium oxysporum* f.sp. *lycopersici.*

Key words: Fusarium wilt, *in vitro*, Carbendazim, inhibition

Introduction

Tomato (*Solanum lycopersicum* L.), also known as “Love apples,” “Golden apples,” and “Poor man's apples”, belongs to the "Solanaceae" family (also known as nightshade family). It is one of the most important vegetable crops in the world next to potato (Ayyar, 2019). Around 186.821 million metric tonnes of tomatoes were produced on 5,051,983 hectares globally in 2020, achieving an average yield of 37.1 metric tonnes/hectare, according to data from FAOSTAT

(Branthôme, 2022). Similarly, tomato is the most important vegetable crop having high market potentialities in Nepal (N. Ghimire et al., 2017), with total area and production of 22,600 hectares and 432,616 metric tonnes, respectively with an average yield of 19.14 metric tonnes/ha (MOALD, 2020/2021).

There are several diseases of tomato caused by many plant pathogens such as fungi, bacteria, nematode, phytoplasmas and viruses. The major fungal diseases observed in tomato are Early blight, Septoria leaf spot, Fusarium wilt, Anthracnose, Verticillium wilt, Damping off and Late blight (Tsitsigiannis et al., 2008). Among them, Fusarium wilt caused by *Fusarium oxysporum f. sp. lycopersici* is one of the most prevalent, serious diseases of tomato and causes significant losses in tomato production both in greenhouse and field – grown tomatoes (Amini & Sidovich, 2010). *Fusarium oxysporum* f. sp. *lycopersici* is soil borne fungus and it can survive up to ten years in the infected soil. The fungus directly penetrates plant roots and colonizes in vascular tissue (Inoue et al., 2002), leading to yellowing, wilting, and dying of the tomato plant. Gkobally, tomato yield is reduced to 30 to 40% due to *F. oxysporum* (Njiru, 2012) and losses can reach up to 80% under adverse weather conditions (Nirmaladevi et al., 2016).

Several plant disease management strategies such as cultural technique, biological control, growing resistant cultivars, crop rotation and chemical control are available nowadays (Abo-Elyousr & Mohamed, 2009). The use of resistant varieties is an important strategy that is effective and cheap for the management of plant diseases, however these varieties are prone to the development of new races of the pathogen over the time and the resistant varieties become susceptible (R. Singh et al., 2015). Ultimately, the safe use of fungicides is the most effective, reliable and easy methods for the management of the plant diseases (Bakhsh & Iqbal, 2007). Patiyal et al. (2020) evaluated six fungicides (Cabriotop, Chlorothalonil, Custodia, Difenoconazole, Azoxistrobin and Azoxistrobin + Difenoconazole) to evaluate the *in vitro* effect against *Fusarium oxysporum* f. sp. *lycopersici* and found that Custodia fungicide showed the best result. Similarly, V. K. Singh et al., (2010) found that Carbendazim and carboxin completely inhibited the growth *Fusarium oxysporum*. Based on that information, our study was designed to check the *in vitro* effectiveness of different fungicides available in the market against the *Fusarium oxysporum* f. sp. *Lycopersici* as the *in vitro* evaluation of fungicides offers preliminary information on their effectiveness against pathogens in a short amount of time, guiding future field testing (Somu et al., 2014).

Materials and methods

The experiment was conducted in the Plant Protection Laboratory of the Ministry of Industry, Agriculture and Cooperatives, Jhumka, Sunsari, Nepal from 7th of April 2023 to 27th April 2023. Six chemical fungicides (Mancozeb, Copper oxychloride, Carbendazim, Azoxystrobin, Kasugamycin + Copper oxychloride and Azoxystrobin + Difenoconazole) were evaluated for their efficacy on inhibition of mycelial growth of *F. oxysporum* f. sp. *lycopersici* by poison food technique *in vitro* condition (Table 1). The experiment was done in Completely Randomized Design (CRD) having a total of 25 treatments with 5 replications. The effect of six fungicides each with four different concentrations i.e., 100 ppm, 150 ppm, 200 ppm and 250 ppm were observed along with control treatment.

Table 1: List of fungicides along with their trade name, available form, active ingredients and mode of action.

S.N	Trade Name	Chemical Name	Available Form	Active Ingredients (a.i)	Mode of Action
1	INDOFIL M-45	Mancozeb	Wettable Powder	75%	Contact
2	NAGCOPER	Copper oxychloride	Wettable Powder	50%	Contact
3	BAVISTIN	Carbendazim	Wettable Powder	50%	Systemic
4	TENDEX	Azoxystrobin	Suspension Concentrate	23%	Systemic
5	CONIKA	Kasugamycin + Copper oxychloride	Wettable Powder	5% + 45%	Systemic + Contact
6	GODIWA SUPER	Azoxystrobin + Difenoconazole	Suspension Concentrate	18.2% + 11.4%	Systemic + Systemic

Bioassay of fungicides by poisoned food technique and Inoculation of pathogen

All the activities of the experiment were carried out in laminar flow aseptically. The laminar airflow was sterilized using UV light for fifteen minutes and the surface of laminar flow was surface sterilized with 99.9% Ethyl Alcohol. All the needed materials such as spatula, petri plates, cork borer, PDA, forceps, distilled water were sterilized in autoclave at 121ºC and 15 lbs/inch^2 for 15 minutes. The stock solution was prepared by mixing the required quantity of fungicides in sterile distilled water. From 10000 ppm stock solutions of each fungicide (Mancozeb, Copper oxychloride, Carbendazim, Azoxystrobin, Kasugamycin + Copper oxychloride and Azoxystrobin

+ Difenoconazole), 1000µl, 1500µl, 2000µl and 2500µl were added in 100 ml of sterilized PDA medium for obtaining four different concentrations i.e., 100 ppm, 150 ppm, 200 ppm, 250 ppm of fungicidal suspension. The poison food technique (Sharvelle, 1961) was followed to evaluate the efficiency of fungicides at different concentrations (100 ppm, 150 ppm, 200 ppm and 250 ppm) on the mycelial growth inhibition of *F*. *oxysporum* f.sp. *lycopersici.*

Then, approximately 20 ml of poisoned melted PDA medium was poured into each 8.5 cm diameter petri plate and allowed to solidify. The petri plates without amending fungicides to the media served as control plates. The inoculums were then extracted from the edge of fully grown 10 days old cultured plate of *F*. *oxysporum* using sterilized cork borer with 5mm diameter and inoculated at the center of the petri plate aseptically with the help of sterilized inoculation loop. All the inoculated petri plates were labeled before being air tight with parafilm and incubated at 25±2ºC temperature in a BOD incubator. The growth of mycelium was assessed using a scale to measure the diameter of mycelium in each treatment at 48 hours intervals for 10 days until the colony in the control plates reached the rim of petri plates. Percent growth inhibition of mycelial growth over control was calculated using the following formula (Vincent, 1947):

Percent Growth Inhibition (%) = $\frac{C-T}{C} \times 100$

where,

C = colony growth of the *Fusarium oxysporum* f. sp. *lycopersici* in control plate.

T = colony growth of the *Fusarium oxysporum* f. sp. *lycopersici* in treated plate.

The *in-vitro* test data were tabulated in Microsoft-excel data sheet. All the recorded data were subjected to analysis by using the reference (Gomez & Gomez, 1984). The data were processed to fit into R-studio and analysis were conducted using R 4.3.1. The data were analyzed through ANOVA table and different treatments were compared by multiple range test.

Results and discussion

Inhibition percentage of mycelial growth of *Fusarium oxysporum* f.sp. *lycopersici*

In the present study, the mycelial growth of *Fusarium oxysporum* f.sp. *lycopersici* on PDA plates containing six fungicides (Mancozeb, Copper oxychloride, Carbendazim, Azoxystrobin,

Kasugamycin + Copper oxychloride, Azoxystrobin + Difenoconazole) of four different concentrations (100 ppm, 150 ppm, 200 ppm and 250 ppm) were measured based on *in vitro* poisoned food technique. Among all fungicides, Carbendazim in all concentrations was found to be significantly superior with 100% mycelial growth inhibition throughout the whole experiment.

On 2 DAI, the highest inhibition percentage of mycelial growth i.e., 100% was shown by all the concentration of Carbendazim (100, 150, 200 & 250 ppm), Azoxystrobin + Difenoconazole (150 ppm, 200 ppm & 250 ppm) i.e., 100% and Mancozeb 250 ppm (Figure 1). Azoxystrobin + Difenoconazole 100 ppm followed the order and inhibited 89.98% mycelial growth. The lowest inhibition percentage was observed in Copper oxychloride 100 ppm (4.70%) followed by Kasugamycin + Copper oxychloride 100 ppm (13.64%).

On 4 DAI, 100% inhibition was obtained in all concentrations of Carbendazim. After Carbendazim, 250 ppm of Mancozeb was observed with 85.39% of growth inhibition which was statistically at par with Azoxystrobin + Difenoconazole (250 ppm, 200 ppm, 150 ppm &100 ppm) i.e., 82.46%, 82.46%, 81.87% & 80.05% respectively. Copper oxychloride 100 ppm was least effective which helped in growth of the fungus and negative growth inhibition percentage was observed with 7.34% (Figure 2) followed by Mancozeb 100 ppm (9.10%).

On 6 DAI, the highest inhibition percentage (100%) of mycelial growth was found in Carbendazim at all concentrations. Azoxystrobin + Difenoconazole 250 ppm was less effective than Carbendazim and inhibited 85.73% which was statistically at par with Azoxystrobin + Difenoconazole 200 ppm, 150 ppm & 100 ppm (85.27%, 84.91% & 80.07% respectively). Again, Copper oxychloride 100 ppm showed the lowest and negative growth inhibition percentage with 4.77% (Figure 3).

On 8 DAI, the highest inhibition percentage (100%) of mycelial growth was found in Carbendazim at all concentration followed by Azoxystrobin + Difenoconazole 250 ppm (84.62%) which was statistically at par with Azoxystrobin + Difenoconazole at 200 ppm, 150 ppm and 100 ppm. The lowest inhibition percentage of mycelial growth was recorded the same as treatment on 6 DAI i.e., Copper oxychloride 100 ppm and gave negative growth inhibition percentage (0.41%) (Figure 4).

On 10 DAI, the highest inhibition percentage (100%) of mycelial growth was found in all concentrations of Carbendazim. After Carbendazim, Azoxystrobin + Difenoconazole (250 ppm) showed the highest inhibition percentage (82.79%) which was statistically at par with Azoxystrobin + Difenoconazole 200 ppm (82.73%), 150 ppm (82.48%), 100 ppm (80.20%). The lowest inhibition percentage was observed in Mancozeb 100 ppm and negative growth inhibition percentage was found with 2.88% (Figure 5).

Table 2: Inhibition percentage in colony growth of *Fusarium oxysporum* f.sp. *lycopersici* over control by poisoned food technique.

Treatment	**Concentration (ppm)**	**Mycelial growth inhibition percentage (%)**				
		2 DAI	**4 DAI**	**6 DAI**	**8 DAI**	**10 DAI**
Mancozeb	100	37.35 ^{e}	9.10 ^{gh}	7.52 ^{ij}	0.75 ^{i}	-2.88 ^{i}
	150	37.21 ^{e}	16.58 ^{fg}	9.67 ^{hi}	8.45 ^{i}	3.97 ^{hi}
	200	47.93 ^{d}	16.71 ^{fg}	18.50 ^{gh}	14.14 ^{gh}	5.37 ^{hi}
	250	100 ^{a}	85.39 ^{b}	72.09 ^{c}	61.50 ^{c}	52.92 ^{cd}
Copper oxychloride	100	4.70 ^{h}	-7.34 ^{i}	-4.77 ^{k}	-0.41 ^{i}	1.21 ^{i}
	150	26.63 ^{f}	18.61 ^{efg}	21.90 ^{g}	22.61 ^{fg}	15.10 ^{gh}
	200	45.49 ^{d}	38.50 ^{d}	40.28 ^{def}	40.36 ^{de}	35.26 ^{ef}
	250	45.39 ^{d}	39.59 ^{d}	42.94 ^{de}	47.07 ^{d}	43.50 ^{de}
Carbendazim	100	100 ^{a}	100 ^{a}	100 ^{a}	100 ^{a}	100 ^{a}
	150	100 ^{a}	100 ^{a}	100 ^{a}	100 ^{a}	100 ^{a}
	200	100 ^{a}	100 ^{a}	100 ^{a}	100 ^{a}	100 ^{a}
	250	100 ^{a}	100 ^{a}	100 ^{a}	100 ^{a}	100 ^{a}
Azoxystrobin	100	71.50 ^{c}	64.45 ^{c}	66.91 ^{c}	62.69 ^{c}	56.19 ^{cd}
	150	72.39 ^{c}	65.15 ^{c}	68.92 ^{c}	66,47 ^{c}	60.86 ^{c}
	200	73.19 ^{c}	66.40 ^{c}	69.12 ^{c}	66.72 ^{c}	60.96 ^{c}
	250	73.96 ^{c}	66.75 ^{c}	69.70 ^{c}	66.83 ^{c}	61.04 ^{c}
Kasugamycin+ Copper oxychloride	100	13.64 ^{g}	11.47 ^{g}	21.15 ^{g}	14.70 ^{gh}	6.35 ^{hi}
	150	29.93 ^{f}	25.23 ^{ef}	33.13 ^{f}	30.49 ^{ef}	22.43 ^{g}
	200	30.73 ^{f}	28.19 ^{e}	34.35 ^{ef}	30.93 ^{ef}	23.78 ^{fg}
	250	42.15 ^{de}	41.12 ^{d}	44.11 ^{d}	40.92 ^{de}	36.27 ^{ef}
Azoxystrobin+ Difenoconazole	100	89.98 ^{b}	80.05 ^{b}	83.07 ^{b}	82.58 ^{b}	80.20 ^{b}
	150	100 ^{a}	81.87 ^{b}	84.91 ^{b}	84.29 ^{b}	82.48 ^{b}
	200	100 ^{a}	82.46 ^{b}	85.27 ^{b}	84.30 ^{b}	82.73 ^{b}
	250	100 ^{a}	82.46 ^{b}	85.73 ^{b}	84.62 ^{b}	82.79 ^{b}
Control	-	0.00 ^{h}	0.00 ^{hi}	0.00 ^{jk}	0.00 ^{i}	0.00 ^{i}
Mean	-	61.69	52.51	54.18	52.40	48.42

Treatment	Concentration (ppm)	Mycelial growth inhibition percentage (%)				
		2 DAI	4 DAI	6 DAI	8 DAI	10 DAI
CV	-	7.62	14.90	14.18	16.31	20.98
LSD	-	5.90***	9.82***	9.64***	10.73***	12.75***
SEM (±)	-	22.09	61.25	59.03	73.09	103.17

CV: Coefficient of variation, LSD: Least significant difference, Means followed by same letter in a column are not significantly different by DMRT AT 1% level of significance, SEM (±) indicates standard error of mean and *** means very highly significantly different at P≤0.001.

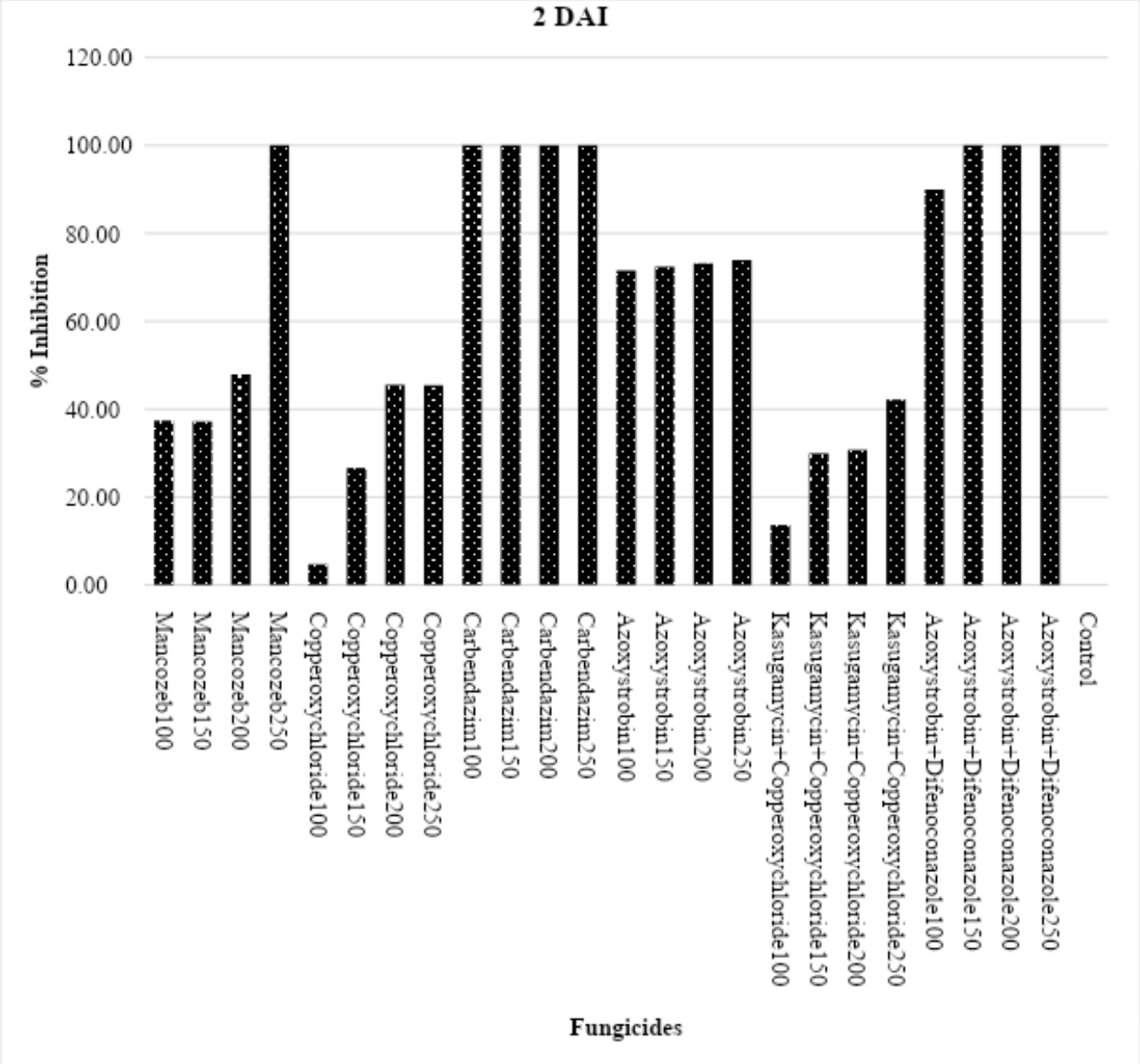


Figure 1: Effect of different fungicides on percentage inhibition of mycelial growth of *Fusarium oxysporum* on 2 DAI

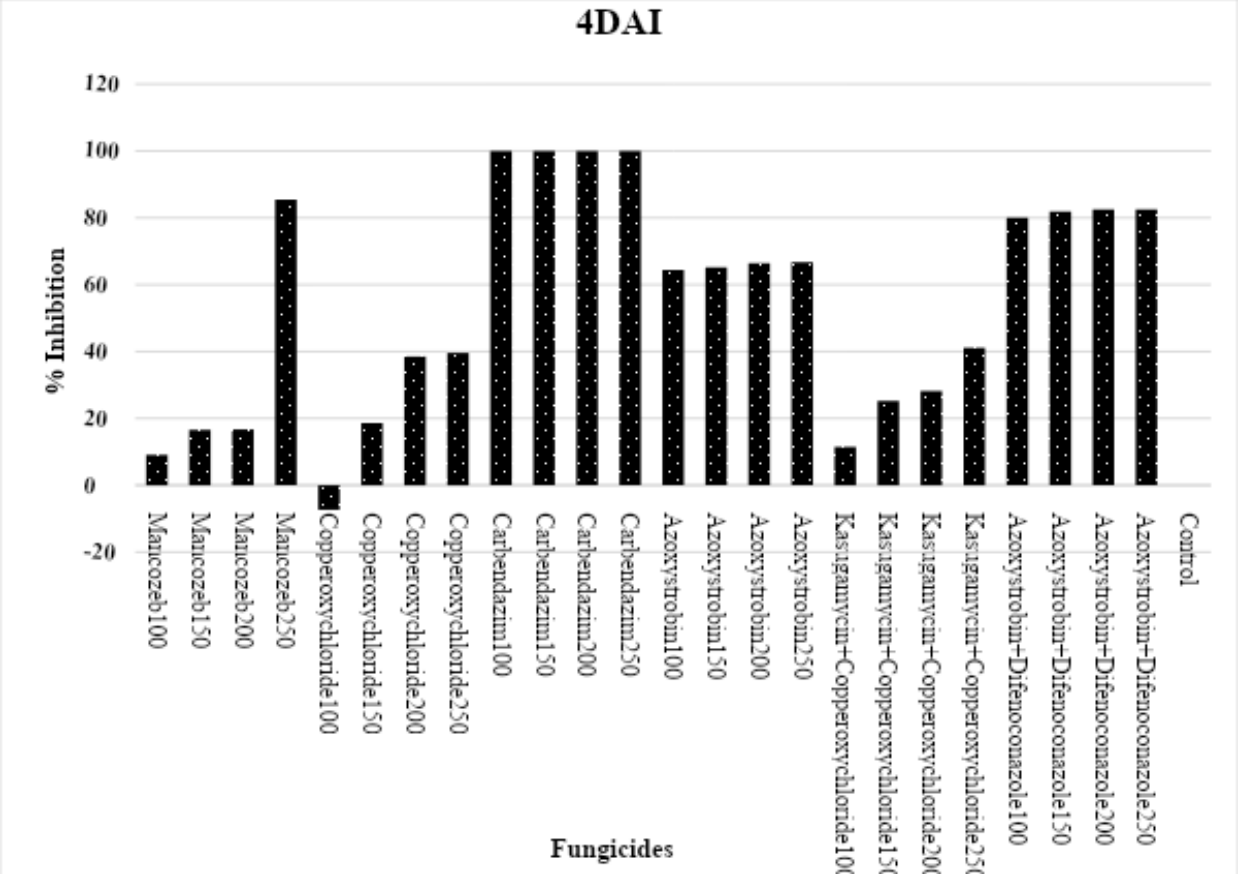


Figure 2: Effect of different fungicides on percentage inhibition of mycelial growth of *Fusarium oxysporum* on 4 DAI

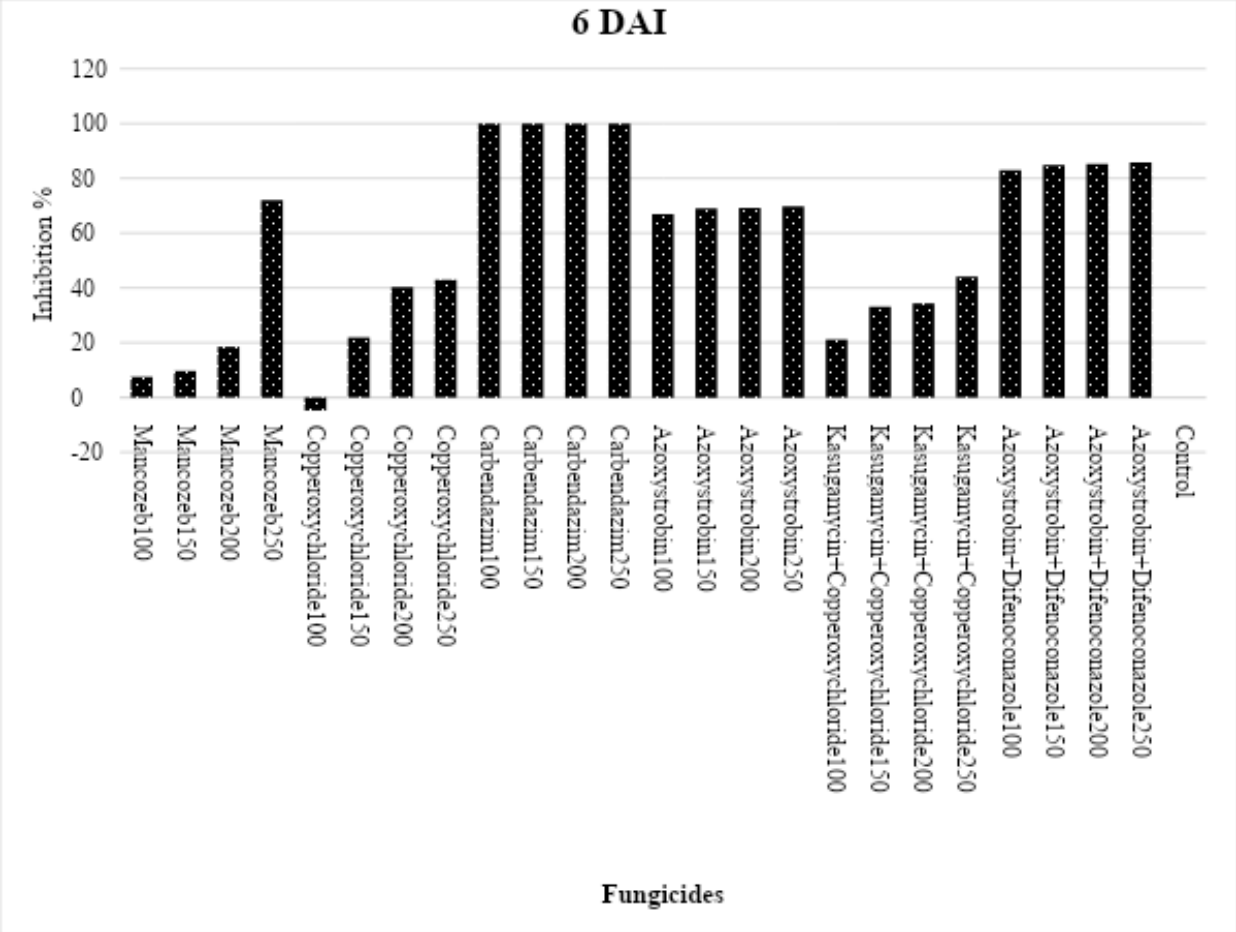


Figure 3: Effect of different fungicides on percentage inhibition of mycelial growth of *Fusarium oxysporum* on 6 DAI

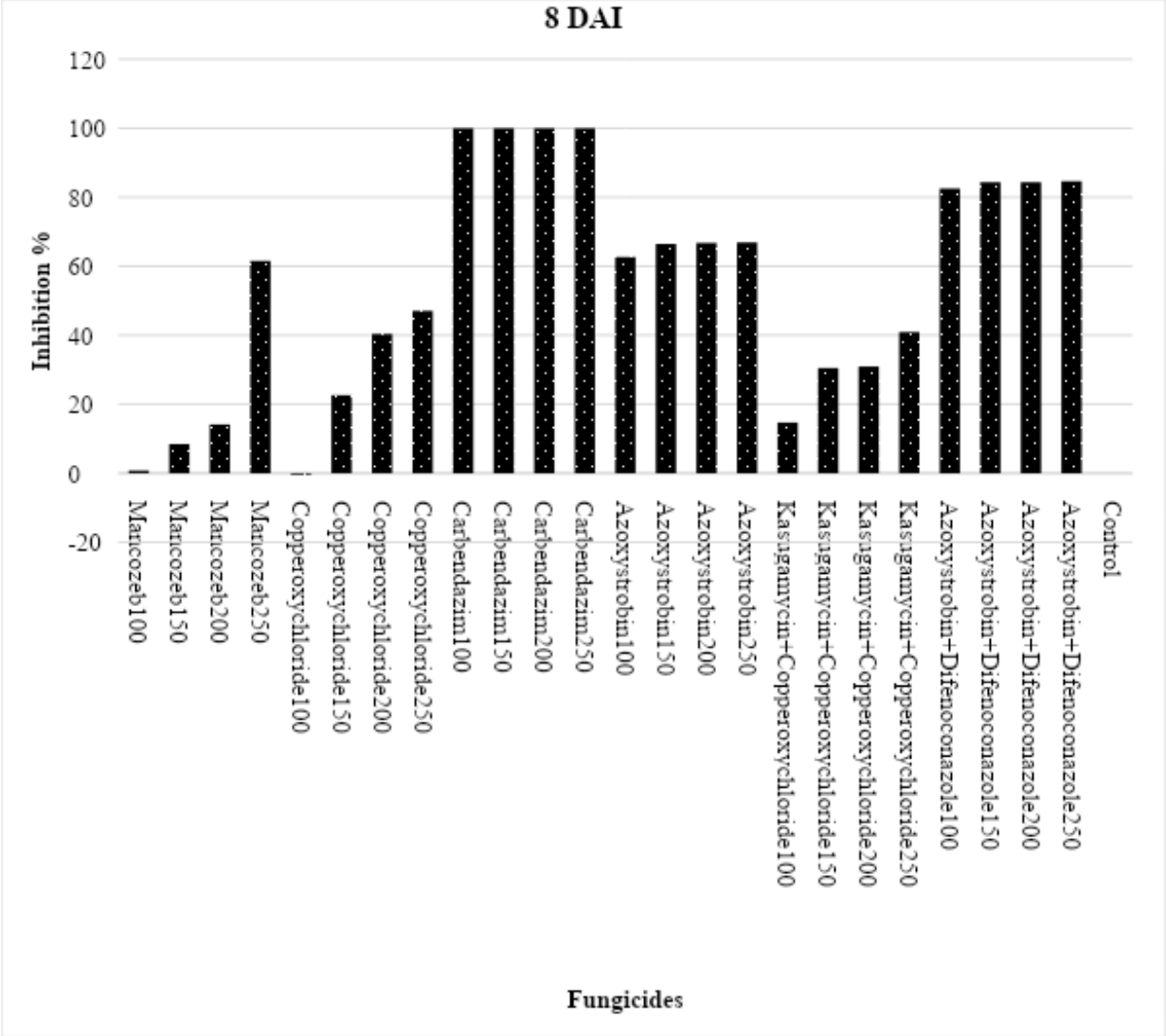


Figure 4: Effect of different fungicides on percentage inhibition of mycelial growth of *Fusarium oxysporum* on 8 DAI

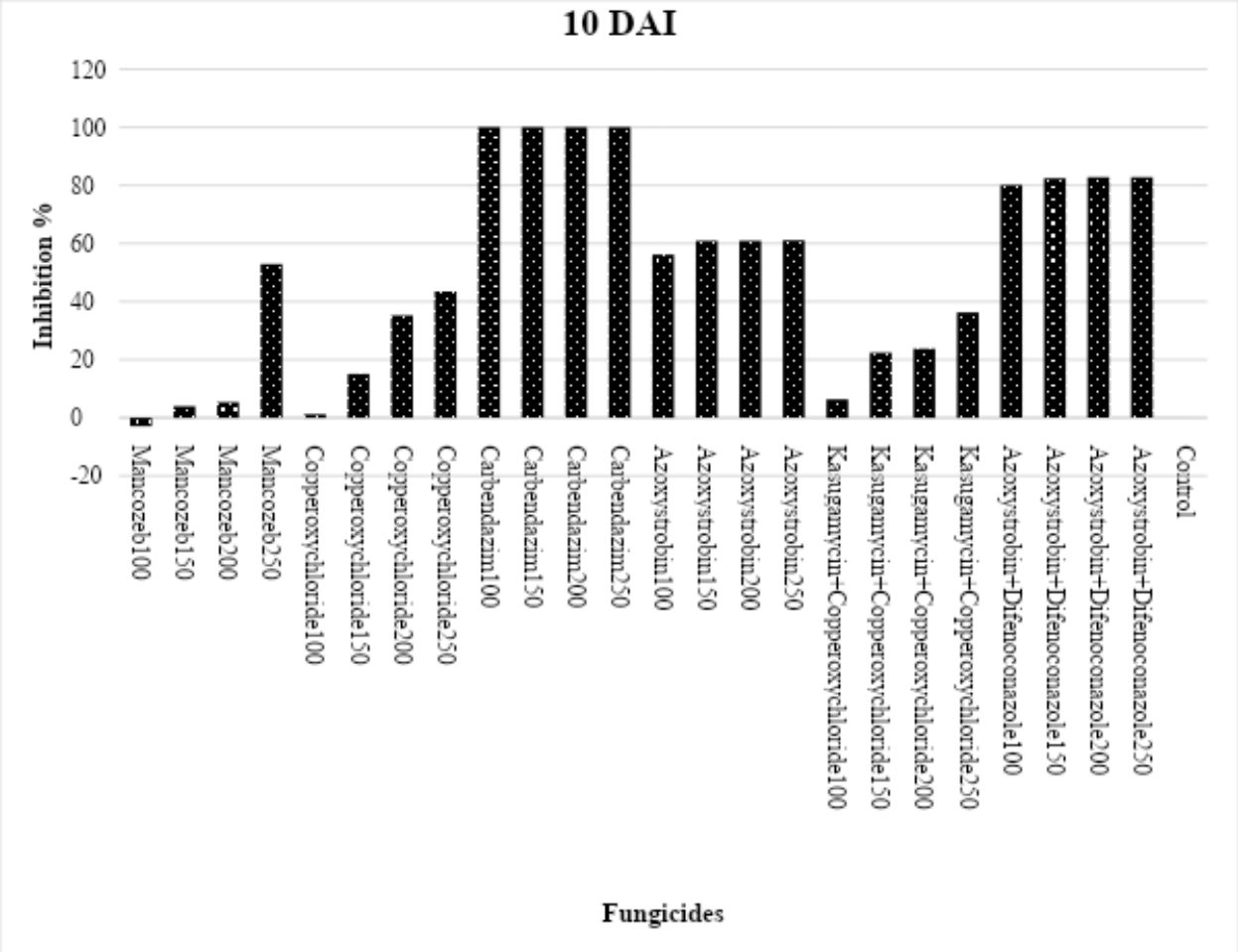


Figure 5: Effect of different fungicides on percentage inhibition of mycelial growth of *Fusarium oxysporum* on 10 DAI

In this study, Carbendazim at all concentrations i.e.,100 ppm, 150 ppm, 200 ppm and 250 ppm found to be the most effective fungicide against the *Fusarium oxysporum* with 100% mycelial growth inhibition. Similar results were found by Somu et al. (2014) as they reported total inhibition of the fungal growth at the concentrations of 500, 1000 and 2000 ppm of Carbendazim against *Fusarium oxysporum* f. sp. *cubense*. Maitlo et al. (2014) reported complete inhibition of mycelial growth of *Fusarium oxysporum* f. sp. *ciceris* by Carbendazim at almost all concentration (1 to 10000 ppm), except only 1 ppm, which produced negligible growth. Dahal & Shrestha, (2018) reported that Carbendazim was extremely effective in all concentrations, inhibiting 100% of mycelial development against *Fusarium oxysporum* f.sp. *lentis*. Devi et al. (2008) reported that Carbendazim showed 100% inhibition against *Fusarium oxysporum* at 100 ppm and 200 ppm concentrations. Ahmad et al. (2021) reported that Carbendazim at 750 and 1000 ppm proved to be

effective in laboratory condition against *Fusarium oxysporum* f.sp. *lycopercisi*. Ghimire et al., (2021) found that Carbendazim at 100 ppm gave the highest inhibition action against mycelium growth (100%) against *Fusarium solani.* Carbendazim (CBZ, methyl 2-benzimidazolecarbamate), a systemic broad-spectrum benzimidazole fungicide, is widely used to manage fungal diseases (Buch et al., 2013). The main mechanism of action of benzimidazole is that it binds to the B-*tubulin* subunit of fungal microtubules and inhibits nuclear division (Zhou et al., 2016). The pathogen's growth may have been inhibited by carbendazim in our study due to its binding effect.

Patel et al. (2021) reported that Azoxystrobin + Difenoconazole as best combination fungicides which completely inhibited the radial growth and sporulation of *Fusarium udum*. Similarly in our study, Azoxystrobin + Difenoconazole was also found to be significantly effective against the growth of the mycelium after Carbendazim. Azoxystrobin + Difenoconazole inhibited 82.79%, 82.73%, 82.48% and 80.20% on 10 DAI at 250 ppm, 200 ppm, 150 ppm and 100 ppm respectively.

In our study, Azoxystrobin showed moderate effect and resulted in 61.04% and 60.96% inhibition of mycelial growth of *F. oxysporum* at 250 ppm and 200 ppm on 10 DAI which was comparatively less effective than Carbendazim and Azoxystrobin + Difenoconazole. Similar result was observed by Niwas et al. (2020). Somu et al. (2014) reported that Azoxystrobin showed moderate inhibitive effective at 2000 ppm.

In this study, Mancozeb was found to be less effective at lower concentrations and supported the growth of fungus and gave negative inhibition growth percentage at lower concentration (100 ppm) on 10 DAI (Figure 5) but significantly inhibited the mycelial growth at high concentration (250 ppm) i.e., 100% on 2 DAI, 85.39% on 4 DAI, 72.09% on 6 DAI, 61.50% on 8 DAI & 52.92% on 10 DAI. Bhaliya & Jadeja (2014) reported mancozeb inhibited cent per cent mycelia growth at higher concentrations (1000 to 2500 ppm). Rafique et al. (2016) reported that Mancozeb was least efficient in reducing the fungal growth compared to the systemic fungicides like Carbendazim. Dahal & Shrestha, (2018) reported Mancozeb showed least effective among the tested chemicals at all concentrations i.e., 100 ppm, 150 ppm and 200 ppm. On the contrary, Dabbas et al. (2008) reported complete inhibition *of F. oxysporum* f. sp. *pisi* at 200 ppm of Mancozeb.

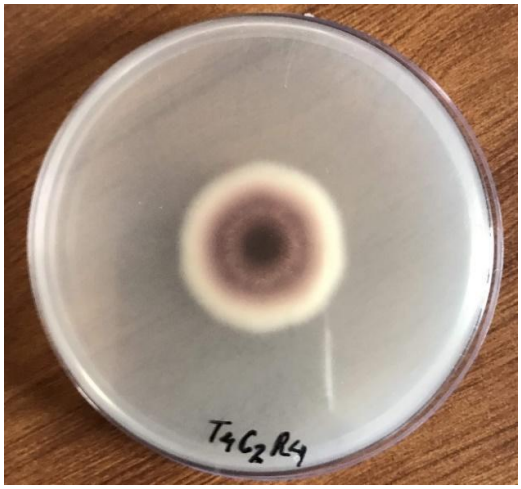
Similarly, the efficacy of copper oxychloride against the fungus increased as the concentration increased and was found to be less effective and supported the growth of the fungus and gave

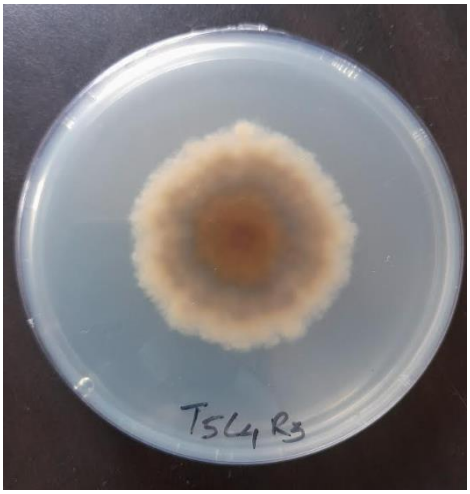
negative inhibition growth percentage at lower concentration (100 ppm) on 4 DAI, 6 DAI and 8 DAI readings (Figure 2, 3 and 4). Similar result was observed by Ghimire et al. (2021) and Baturo-Ciesniewska et al. (2015). As per them lower concentration of copper oxychloride stimulates the growth of fungus. 43.50% inhibition of colony growth was recorded even at high concentration (250 ppm) on 10 DAI in this experiment. Similarly, Maitlo et al. (2014) reported that copper oxychloride completely inhibited the colony growth of *Fusarium oxysporum* f. sp. *ciceris* at only 10000 ppm. The lower doses of fungicide appeared completely or partially ineffective to check the colony growth of the fungus.

Kasugamycin + Copper oxychloride significantly checked the colony growth but was less effective than above discussed fungicides and resulted in only 36.27% mycelial growth inhibition at 250 ppm on 10 DAI. The inhibitory effect increased with increased doses.

Carbendazim (100 ppm)

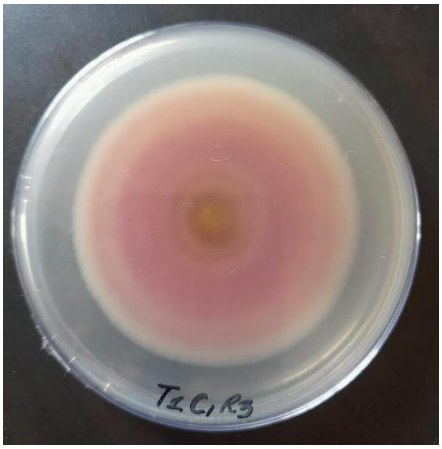
Azoxystrobin + Difenoconazole (150 ppm)





Azoxystrobin (150 ppm)

Kasugamycin + Copperoxychloride (250 ppm)



Copperoxychloride (200 ppm)

Mancozeb (100 ppm)

Control

Figure 1: The growth of *Fusarium oxysporum* f.sp. *lycopersici* at ten days after inoculation (10 DAI).

Conclusion

From the above experiment, it can be concluded that Carbendazim proved to be most effective among the tested fungicides which completely inhibited (100%) the mycelial growth of *Fusarium oxysporum* in all concentrations *in vitro*. Azoxystrobin + Difenoconazole showed satisfactory result after Carbendazim with 82.79% inhibition on mycelial growth at 250 ppm on 10 DAI. Azoxystrobin was moderately effective while Mancozeb, Copper oxychloride and Kasugamycin + Copper oxychloride were less effective among other fungicides. This result is highly

applicable to the researchers for further screening of chemicals in the field and greenhouse trial.

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References

Abo-Elyousr, K. A., & Mohamed, H. M. (2009). Note biological control of Fusarium wilt in tomato by plant growth-promoti ng yeasts and rhizobacteria. *The Plant Pathology Journal, 25*(2), 199–204.

Ahmad, S., Yousaf, M., Anjum, R., Raza, W., Ali, Y., & Rehman, M. A. (2021). Evaluation of fungicides against *Fusarium oxysporum* f. Sp. *Lycopersici* the cause of fusarium wilt of tomato. *Journal of Plant and Environment, 3*(2), 125–135.

Amini, J., & Sidovich, D. (2010). The effects of fungicides on *Fusarium oxysporum* f. Sp. *Lycopersici* associated with Fusarium wilt of tomato. *Journal of Plant Protection Research.*

Ayyar, S. (2019). Mulching and fertigation on the yield and quality of tomato. *IJCS, 7*(4), 2539–2541.

Bakhsh, A., & Iqbal, S. M. (2007). *Evaluation of chickpea germplasm for wilt resistance.*

Baturo-Ciesniewska, A., Lenc, L., Grabowski, A., & Lukanowski, A. (2015). Characteristics of Polish isolates of *Fusarium sambucinum*: Molecular identification, pathogenicity, diversity and reaction to control agents. *American Journal of Potato Research, 92*, 49–61.

Bhaliya, C., & Jadeja, K. (2014). Efficacy of different fungicides against *Fusarium solani* causing coriander root rot. *The Bioscan, 9*(3), 1225–1227.

Branthôme, F.-X. (2022). Worldwide (total fresh) tomato production exceeds 187 million tonnes in 2020. *Tomato News*. Available online: https://www. tomatonews.com/en/worldwide-total-fresh-tomato-production-exceeds-187-million-tonnes-in-2020_2_1565.html (accessed on 12 October 2022).

Buch, A. C., Brown, G. G., Niva, C. C., Sautter, K. D., & Sousa, J. P. (2013). Toxicity of three pesticides commonly used in Brazil to Pontoscolex corethrurus (Müller, 1857) and Eisenia andrei (Bouché, 1972). *Applied Soil Ecology*, *69*, 32–38.

Dabbas, M., Srivastava, J., & Rai, M. (2008). IDM for wilt disease of table pea. *Annals of Plant Protection Sciences*, *16*(1), 156–158.

Dahal, N., & Shrestha, R. (2018). Evaluation of efficacy of fungicides against *Fusarium oxysporum* f. Sp. *Lentis* in vitro at Lamjung, Nepal. *Journal of the Institute of Agriculture and Animal Science*, *35*(1), 105–112.

Devi, S., Sharma, S., & Aggarwal, A. (2008). Efficacy of fungicides on mycelial growth and enzyme production on *Rhizoctonia solani* and *Fusarium oxysporum*. *Annals of Plant Protection Sciences*, *16*(1), 135–138.

Ghimire, N., Kandel, M., Aryal, M., & Bhattarai, D. (2017). Assessment of tomato consumption and demand in Nepal. *The Journal of Agriculture and Environment*, *18*, 83.

Ghimire, R., Shrestha, R. K., & Shrestha, J. (2021). In Vitro Evaluation of Fungicides against *Fusarium solani*, the Causative Agent of Brinjal Root Rot. *Indonesian Journal of Agricultural Research*, *4*(3), 187–193.

Gomez, K. A., & Gomez, A. A. (1984). *Statistical procedures for agricultural research*. John wiley & sons.

Inoue, I., Namiki, F., & Tsuge, T. (2002). Plant colonization by the vascular wilt fungus *Fusarium oxysporum* requires FOW1, a gene encoding a mitochondrial protein. *The Plant Cell*, *14*(8), 1869–1883.

Maitlo, S., Syed, R., Rustamani, M., Khuhro, R., & Lodhi, A. (2014). Comparative efficacy of different fungicides against fusarium wilt of chickpea (*Cicer arietinum* L.). *Pakistan Journal of Botany*, *46*(6), 2305–2312.

MOALD, 2020/2021. *Statistical information on Nepalese Agriculture*. Khumaltar, Nepal: Government of Nepal; Ministry of Agriculture and Livestock Development.

Nirmaladevi, D., Venkataramana, M., Srivastava, R. K., Uppalapati, S., Gupta, V. K., Yli-Mattila, T., Clement Tsui, K., Srinivas, C., Niranjana, S., & Chandra, N. S. (2016). Molecular phylogeny, pathogenicity and toxigenicity of *Fusarium oxysporum* f. Sp. *Lycopersici*. *Scientific Reports*, *6*(1), 21367.

Niwas, R., Chand, G., & Azad, C. (2020). *In vitro* evaluation of fungicides against growth of *Fusarium oxysporum* f. Sp. *Cubense* causing panama wilt disease of Banana. *Int J Chem Stud*, *8*, 130–133.

Njiru, M. (2012). *Integrated management of Fusarium wilt of tomatoes using fungicides, organic matter and neem extract*.

Patel, M., Kumar, S., & Mishra, S. (2021). Comparative efficacy of combi fungicides and solo fungicides against *Fusarium udum* causing wilt of pigeonpea. *Pharma Innovation*, *10*(5), 1310–1314.

Patiyal, A., Mishra, J., & Prassad, R. (2020). *In vitro* evaluation of fungicides against *Fusarium oxysporum* f. Sp. Wilt of tomato. *Journal of Pharmacognosy and Phytochemistry*, *9*(3), 1670–1673.

Rafique, K., Rauf, C. A., Naz, F., & Shabbir, G. (2016). Management of vascular wilt of lentil through host plant resistance, biological control agents and chemicals. *Pakistan Journal of Botany*, *48*(5), 2085–2092.

Sharvelle, E.G. (1961) 'The nature and uses of modern fungicides.', *The nature and uses of modern fungicides.* [Preprint].

Singh, R., Biswas, S., Nagar, D., Singh, J., Singh, M., & Mishra, Y. K. (2015). Sustainable integrated approach for management of Fusarium wilt of tomato caused by *Fusarium oxysporum* f. Sp. *Lycopersici* (Sacc.) Synder and Hansen. *Sustainable Agriculture Research*, *4*(526-2016–37870).

Singh, V. K., Naresh, P., Biswas, S., & Singh, G. P. (2010). Efficacy of fungicides for management of Wilt disease of Lentil caused by *Fusarium oxysporum* f sp. *Lends*. *Annals of Plant Protection Sciences*, *18*(2), 411–414.

Somu, R., Thammaiah, N., Swamy, G., Kulkarni, M., & Devappa, V. (2014). *In vitro* evaluation of fungicides against *Fusarium oxysporum* f. Sp. *Cubense*. *Int. J. Plant Prot*, *7*, 221–224.

Tsitsigiannis, D. I., Antoniou, P. P., Tjamos, S. E., & Paplomatas, E. J. (2008). Major diseases of tomato, pepper and egg plant in green houses. *Eur. J. Plant Sci. Biotechnol*, *2*(1), 106–124.

Vincent, J. (1947). Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*, *159*(4051), 850–850.

Zhou, Y., Xu, J., Zhu, Y., Duan, Y., & Zhou, M. (2016). Mechanism of action of the benzimidazole fungicide on *Fusarium graminearum*: Interfering with polymerization of monomeric tubulin but not polymerized microtubule. *Phytopathology*, *106*(8), 807–813.