EVALUATION OF EFFICACY OF FUNGICIDES AGAINST *Fusarium oxysporum* f. sp. *lentis* IN VITRO AT LAMJUNG, NEPAL

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ABSTRACT

Fusarium is a soil borne pathogen which is common in almost all types of soil causing heavy losses in the crop production. Long term survival of pathogen in the soil as chlamydospores has increased its threat making it a highly devastating disease. High accessibility and simple application process of chemical control method has made it an effective and highly adopted approach of eliminating disease causing organisms. The present study was conducted in IAAS, Lamjung Campus to test the efficacy of fungicides in-vitro by poisoned food technique in PDA medium against Fusarium oxysporum f. sp. lentis. The fungicides tested were Carbendazim (50% WP), Chlorothalonil (75% WP) and Dithane M-45 (75% WP) at three different concentrations (100 ppm, 150 ppm and 200 ppm). The treatments were arranged in complete randomized design and replicated five times. The measurement of diameter of the fungal mycelium was taken 8 times at 48 hours interval until the fungus nearly covered the plate in control treatment and inhibition percent of the chemicals were calculated. All the fungicides inhibited the fungal growth significantly, among which carbendazim was highly effective in all the concentrations reducing 100% of mycelial growth followed by chlorothalonil. Dithane M-45 showed least inhibition i.e. 26.62% in 200 ppm (day 13). The chemicals exhibited increased tendency of inhibition with increased concentration.

Keywords: Fungicides, Mycelium growth, Percent inhibition and Poison food technique

INTRODUCTION

Lentil ranks first among the pulse crops in Nepal (Yadav *et al*., 2017). It is one of the most nutritious pulse crop ranking next to chickpea among rabi pulses having 28.5% mean value of protein (Stoilova and Pereira, 1999). In Nepal, this crop is prone to a number of pathological threats including lentil wilt, *stemphilium* blight, collar rot and root rot (Yadav, 2004). On the top of all, wilt caused by *Fusarium oxysporum* f. sp. *lentis* is one of the major disease affecting lentil all over the world (Bayaa *et al*., 1998). *Fusarium* wilt can cause complete failure of the crop, especially in warm spring and hot dry summer (Agrawal *et al*., 1993). The disease is responsible for 5-10% yield losses in lentil worldwide (Yadav *et al*., 2017).

This soil borne pathogen invades the root system of the host creating barrier for the conduction of water to the upper portion, and causes wilting resulting in death of the plants. Wilt pathogen is capable of surviving in the soils as chlamydospores that can remain viable for several years (Erskine and Bayaa, 1996) and can attack the next crop in the field even when the crop is sown after a long interval. This might be the reason for its devastating effect.

Management options are available for *Fusarium* wilt of lentil which goes from preventative to curative measures. Each control method has got its significance but none is able to work solely. Hence, different approaches for control are necessary to be applied. Few researches done in Nepal, related to control *Fusarium* wilt, have opened a wide opportunity for exploring the pathogen and finding its most effective control measure. Khare *et al*. (1975) have reported eight strains of

F. oxysporum f. sp. *lentis*. Variability of genetic characters can cause differential reactions of strains for fungicides i.e. the same fungicides might not be equally effective for all the strains. So, there is need for continuous study of the pathogen in different places and different times for developing the most effective control measure. According to Bendre and Barhate (1998), the management strategies should include modified cultural practices, resistant varieties, beneficial biocontrol agents and minimum use of chemicals. Chemical control method is the widely applicable and most preferred method for killing pathogens.

Despite of all the health hazards of fungicides, it has been proved to be the effective control strategy (Maitlo *et al*., 2014). Various chemicals are available for control of *Fusarium* wilt pathogen. Finding the most effective chemical with reliable dose against the pathogen may be a great discovery for those willing to invest their time and capital in lentil cultivation. This study was focused towards finding the effectiveness and optimum doses of fungicides that are popularly used in disease control. Therefore, the present study was carried out to compare the efficacy of various fungicides against *Fusarium oxysporum* f. sp. *lentis*, a wilt causing pathogen of lentil.

MATERIALS AND METHODS

The experiment was conducted during 2018 at the central laboratory of Institute of Agriculture and Animal Science (IAAS), Lamjung Campus, Sundarbazar. The study was designed in Complete Randomized Design (CRD) with ten treatments and five replications. Three fungicides viz. Carbendazim (50% WP), Cholorthalonil (75% WP) and Dithane M-45 (75% WP) were evaluated against *F. oxysporum* f. sp. *lentis in vitro* by poisoned food technique. The fungicides were tested at three concentrations; 100 ppm, 150 ppm and 200 ppm.

The pure culture of *F. oxysporum* f. sp. *lentis* was brought from NARC, Khumaltar and incubated for one week in bacteriological incubator at 25±2^{0}C under dark conditions. All the isolation and inoculation work were carried out in a laminar flow under aseptic condition. The platform of the laminar flow was sterilized by glowing ultraviolet light for fifteen minutes before commencement of the work. The working surface of laminar flow was surface sterilized with 75% dehydrated alcohol. The materials such as petri plates, pipettes, spatula, test tube, forceps, media, distilled water and cork borer were sterilized in autoclave at 121 ^{0}C & 15 lbs/inch^2 for 15 minutes.

Mother culture was prepared in Potato Dextrose Agar (PDA) media from the pure culture which was used as the mycelium source of fungus. 300 µl, 500 µl and 670 µl from 60000 ppm solution of each fungicides were added to 200 ml of PDA medium for obtaining 100 ppm, 150 ppm and 200 ppm of fungicidal suspension. Fifteen ml of poisoned medium was poured in each petri plates and allowed to solidify. Negative checks i.e. control plates were maintained without addition of the fungicides to the media. Six mm diameter piece of fungal mycelium was taken from seven-days old cultured plate and kept at the center of the poisoned plates. The treated plates were incubated in bacteriological incubator at 25±2 ^{0} C. Measurement of radial growth (mm) of mycelium was taken using a scale every 48 hours until the fungus nearly covered the plate. Total observations were taken eight times. Percent inhibition of fungal growth was calculated using the following formula (Vincent, 1947):

$$\text{Percent growth inhibition (\%)} = \frac{A-B}{A} \times 100$$

Where, A = Colony growth of the *Fusarium oxysporum* f. sp. *lentis* in control plate

B= Colony growth of the *Fusarium oxysporum* f. sp. *lentis* in treated plate

Data were analyzed by GENESTAT 15th Edition. The results were subjected to ANOVA followed by Duncan's Multiple Range Test (DMRT) at P= 0.05

RESULT AND DISCUSSION

All three fungicides (Carbendazim, Chlorothalonil and DM-45) significantly (P=0.05) inhibited mycelial growth compared to the control (Table 1). Among the fungicides, Carbendazim in all concentrations was found to be significantly superior showing 100% growth inhibition throughout the whole experiment. While inhibitory effect of chlorothalonil and DM-45 increased with increase in the concentrations, but decreased gradually with time.

In 5th day of experimentation, highest inhibition i.e. 100% was shown by all the concentration (100 ppm, 150 ppm and 200 ppm) of carbendazim followed by 200 ppm of chlorothalonil which showed 77.06% inhibition in radial growth of fungal colony. Lowest inhibition was observed in 100 ppm of DM-45 (40.07%) which was statistically at par with 150 ppm of DM-45.

In 9th day of experimentation, no growth of fungal mycelium was observed in all three concentrations of carbendazim which conformed 100% growth inhibition. After carbendazim, 200 ppm of chlorothalonil was effective showing inhibition of 71.49%. DM-45 (100 ppm) showed lowest inhibition among the chemicals (31.20%) which was statistically at par with 150 ppm of DM-45.

In 13th day of experimentation, the mycelial growth was inhibited 100% in each carbendazim dose, followed by 200 ppm chlorothalonil with an inhibition of 67.91% which was statistically higher than 150 ppm chlorothalonil. Lowest inhibition was seen in 100 ppm DM-45 (22.85%) which was statistically at par with 150 ppm DM- 45 (23.53%).

From the above experiment, carbendazim was found to be the most effective chemical in its every concentration against the wilt pathogen since it completely inhibited the mycelium growth of the fungus. This result was congruent with the findings of Luz *et al*. (2007), Maheshwari *et al*., (2008), Singh *et al*. (2010), and Somu *et al*. (2014). Chattannavar *et al*. (2006) and Narayanan *et al*. (2015) found complete inhibition of *F. solani* by carbendazim. Subhani *et al*. (2011) reported carbendazim to reduce the mycelial growth of *F. oxysporum* f. sp. *ciceri* while Maitlo *et al*. (2014) and Kumar (2017) reported carbendazim to completely inhibit the mycelial growth of *F. oxysporum* f. sp. *ciceri*. The main mechanism of action of benzimidazole compounds including carbendazim is primarily binding to the β- tubulin and then disturbing the microtubule dynamic (Thelingwani *et al*., 2009 and Jamieson *et al*., 2011). This binding effect of carbendazim may be the reason for inhibition of growth of the pathogen. Clemons and Sisler (1971), Davidse (1973) and Hammerschlang and Sisler (1973) reported that blockage of nuclear division was the reason for mechanism of action for benzimidazoles.

Table 1. Percentage reduction in colony growth of *Fusarium oxysporum* over control by poisoned food technique at Lamjung Campus, 2018

Chemicals	Concentration		Mycelial growth inhibition (%)		
			Day 5	Day 9	Day 13
Control	T1		0 [f]	0 [f]	0 [f]
Carbendazim	T2	100 ppm	100 [a]	100 [a]	100 [a]
	T3	150 ppm	100 [a]	100 [a]	100 [a]
	T4	200 ppm	100 [a]	100 [a]	100 [a]
Chlorothalonil	T5	100 ppm	68.60 [c]	67.35 [c]	63.47 [c]
	T6	150 ppm	69.91 [c]	68.17 [c]	64.41 [c]
	T7	200 ppm	77.06 [b]	71.49 [b]	67.91 [b]
Dithane M-45	T8	100 ppm	40.07 [e]	31.20 [e]	22.85 [e]
	T9	150 ppm	44.70 [e]	33.75 [e]	23.53 [de]
	T10	200 ppm	51.10 [d]	36.99 [d]	26.62 [d]
F-test			**	**	**
LSD			5.92	3.17	3.11
cv %			7.1 %	4.1 %	4.3 %

** *Means followed by different letters in a column are highly significantly different at P=0.05 according to DMRT.*

Chlorothalonil was found to be less effective than carbendazim but possessed more inhibition capacity than DM-45. Manasa *et al*. (2017) found out that application of chlorothalonil @0.1% decreased the fusarium population in soil which was less than that of carbendazim @ 0.2% in wilt of carnation. In the divergent, mancozeb was reported to be the best and inhibited cent percent of mycelia growth at all concentrations (1000-2500 ppm) while Chlorothalonil gave 72.52% growth inhibition of *F. solani* causing coriander root rot (Bhaliya and Jadeja, 2014). Kishore (2007) also found higher inhibition of mancozeb than chlorothalonil against *F. oxysporum* f. sp. gerberae. Long and Siegel (1975) found out amount of chlorothalonil bound to protein was related to enzyme inhibition and postulated that chlorothalonil affects catalytic activity by reacting with the 4 sulfhyldryl sites responsible for binding glyceraldehyde-3-phosphate (GAP).

The efficacy of DM-45 increased as the concentration increased from 100 ppm to 200 ppm and all the concentration of DM-45 significantly checked the colony growth but was least effective among the tested chemicals. Taskeen-Un- Nisa *et al*. (2011) and Khola *et al*. (2016) reported systemic fungicide (Carbendazim) to have higher efficacy than non-systemic fungicide (Mancozeb). Similarly, Singh *et al*. (2010) found 66% inhibition with 200 ppm concentration of mancozeb. On the contrary, Dabbas *et al*. (2008) reported complete inhibition of *F. oxysporum* f. sp. *pisi* at 200 ppm of DM-45. Singh *et al*. (2000) and Dar *et al* (2013) reported Mancozeb to be the best for growth inhibition of *F. solani* and *F. oxysporum*. Mancozeb has direct effect upon the core biochemical processes within the fungus which results in inhibition of spore germination (Wong and Wilcox, 2001). Most of the reports also concluded increase in concentration to be directly proportional to the increase in inhibition potential [Maitlo *et al*. (2014) and Khola *et al*., (2016)].

The inhibition percentage of the chemicals chlorothalonil and mancozeb were found to be decreasing along with the increase in incubation period. Kapratwar *et al*. (2016) concluded that increase in incubation period caused increased growth of *F. solani* in control as well as treated plates. This might be due to the decreased efficacy of the fungicides along with time.

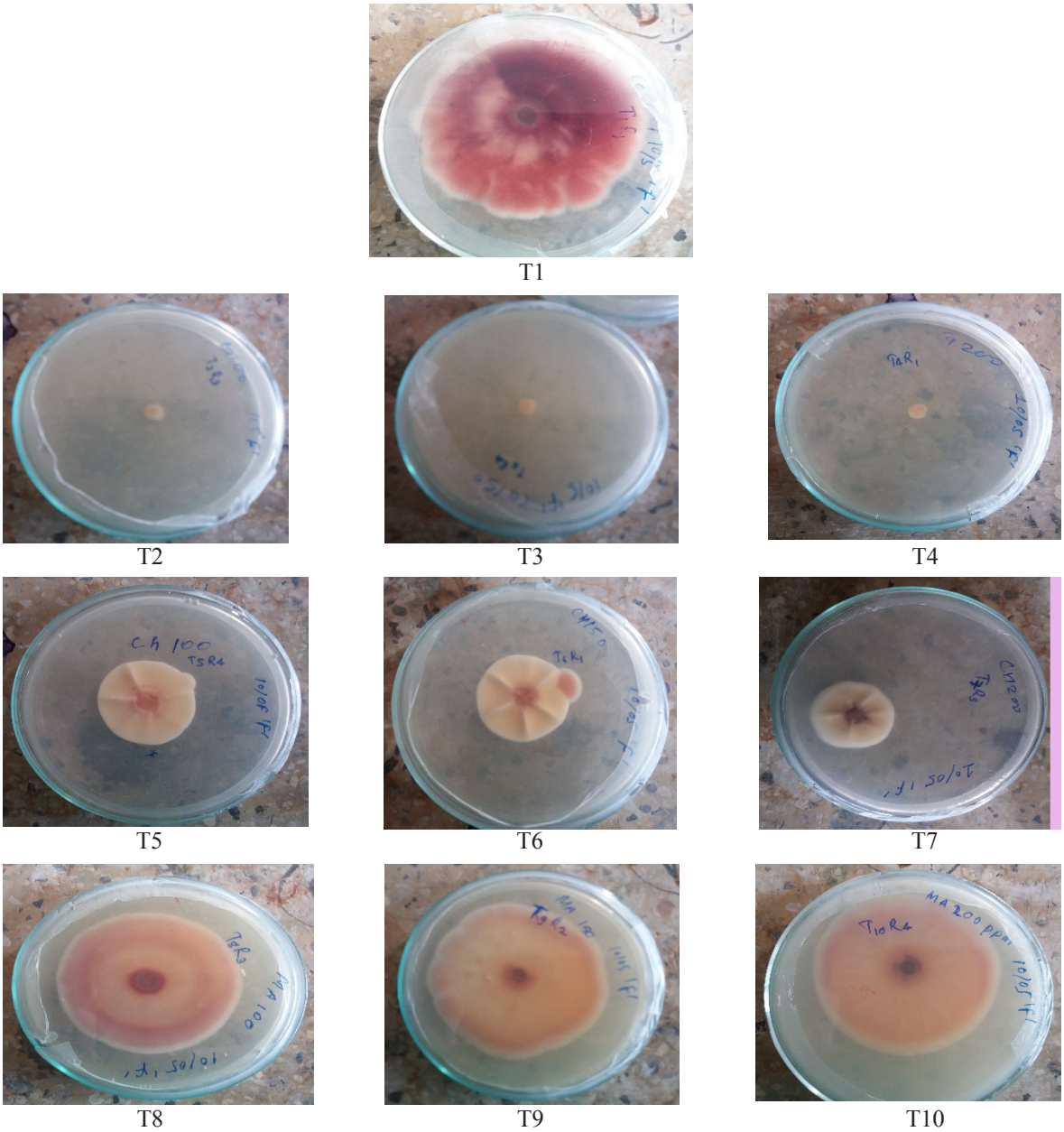

Figure 1: Inhibitory effect of fungicides on radial growth of *Fusarium oxysporum* (Day 17)

CONCLUSION

In the present study, laboratory testing of three fungicides at three different concentrations (100, 150 and 200 ppm) by food poison technique revealed that all three fungicides showed effectiveness in decreasing the fungal growth at increased concentrations. Carbendazim was proved to be the best among the tested fungicides which completely inhibited the fungal growth in all concentrations. Chlorothalonil was moderately effective while DM-45 ranked last among these fungicides.

This study can be helpful for generalizing the concept of chemicals against the wilt pathogen and for further research on this area. There is the need of further green-house and field trials for screening these chemicals against the pathogen for additional conformation.

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