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Research Article

Evaluation of Effectiveness of Fungicides against *Fusarium oxysporum* f. Sp. Lentis in Laboratory

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Abstract

Almost all types of soil have the soil-transmitted pathogen Fusarium, which significantly reduces the output of lentils. This research was carried out in Plant Pathology Division, NARC, Khumaltar to study the in-vitro efficacy of fungicides against *Fusarium oxysporum* f. sp. *lentis* using the poisoned food technique. Nine treatments with three replications were used in the experiment arranged in a complete randomized design (CRD). Eight fungicides Azoxystrobin (50% WP), Bavistin (50%WP), Carbendazim (50% WP), Chlorothalonil (75% WP), Copper oxychloride (50% WP), Nativo (75%WG), Saaf (75%WP), Propiconazole (25% WP) and Triazole (70% WP) were tested at four different concentrations (50ppm, 100 ppm, 150 ppm and 200 ppm. After 24 hours, the diameter of the fungal mycelium was measured for every 6 days until the pathogen fully covered the control plate and chemical inhibitory percentage was computed. All of the fungicides considerably reduced pathogen growth, however, Carbendazim 50%WP was particularly effective in reducing mycelial growth to zero at all concentrations, followed by Tebuconazole 50% +Trifloxystrobin 25%WG and Tilt 25%EC at 150 ppm and 200 ppm. Protector Zn 75%WP showed the least inhibition in all the concentrations after 168 hrs of incubation. With higher concentrations, the chemicals showed an increased potential to inhibit.

Keywords: *Fusarium*; Fungicides; Poisoned Food Technique; Inhibition Percent

Background

In Nepal, lentils (*Lens culinaris*) are the primary pulse crop, accounting for 62% of the total acreage and 64% of the total production [12]. Nepal leads the world fifth in exports to international markets and sixth worldwide regarding the production of lentils [5,13]. Nepal Trade Integration Strategy has recognized lentils as one of the primary agricultural crops among 12 items with high export and medium social effect potential.

Lentil is prone to several pathological issues, such as lentil wilt, root rot, collar rot, and stemphylium blight [19]. Fusarium wilt, a vascular fungal disease, is the most devastating of all lentil diseases globally, causing severe production losses of up to 100% in lengthy periods of good weather. The disease's severity, the pathogen strain's aggressiveness, and the impacted crop stage all have a role in the damage [8]. In general, *Fusarium oxysporum* causes symptoms including stunting, damping-off, wilting, chlorosis, necrosis, and browning of the leaves. In a field, wilt signs include drooping of older leaves, stunting of plants, and curling of lower leaves that gradually travel up to the afflicted plant's stems. Finally, plants become yellow and perish [13]. Crop rotation as a management approach is inadequate because the pathogen can persist in the soil as chlamydospores that can live for many years [2]. The disease thrives at 22–25°C temperatures in warm and dry soil conditions [3].

The alternative interventions, in accordance with Bendre and Barhate [4], should comprise changed cultural practices, advantageous biocontrol agents, minimal chemical usage, and resistant cultivars. Under high inoculum conditions, suitable seed dressing fungicides are also the most effective besides biocontrol agents [6]. The most extensively used and recommended strategy for eradicating infections is chemical control. Despite all the health risks associated with fungicides, they have been shown to be an effective

control approach [11]. On the other hand, synthetic pesticides are commonly used to manage crop diseases effectively and efficiently.

Materials and Methods

The study was performed in the Plant Pathology Division's laboratory at NARC Khumaltar, Lalitpur location. A completely randomized design (CRD) was used in the experiment.

Isolation and identification of the pathogen from diseased plant sample

The diseased plant sample of lentils was collected from the field of Agronomy Division, NARC and plating of infected parts (stem and roots) was performed on moist paper and in water agar (WA) media. Cultures were incubated for 24- 48 hours at 26 ± 2^0C. After 3- 4 days, sporulation and mycelial growth in Petri plates were observed under a compound light microscope by slide preparation.

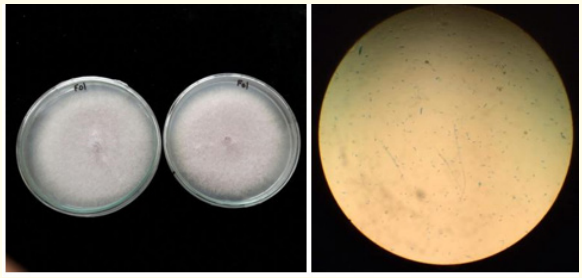

Figure 1: *Fusarium* isolated from diseased sample Microscopic view of Fol.

Assessment of fungicides against *fusarium OXYSPORUM* F.SP. LENTIS using poisoned food method

Eight fungicides were tested at four different concentrations (50ppm, 100 ppm, 150 ppm and 200 ppm) in a complete randomized design (CRD) and replicated three times. The amount of chemicals to be added were calculated as per a.i of the chemicals.

Fungicides	Trade Name (a.i.,)	50ppm	100 ppm	150 ppm	200 ppm
Azoxystrobin	Topcare 50%WP	6mg	12mg	18mg	24mg
Bavistin	Carbendazim 50%WP	6mg	12mg	18mg	24mg
Chlorothalonil	Curex 50%WP	4mg	8mg	12mg	16mg
Copper oxychloride	Protector Zn 75%WP	6mg	12mg	18mg	24mg
Nativo	Tebuconazole 50% +Trifloxystrobin 25%WG	4mg	8mg	12mg	16mg
Saaf	Carbendazim12% + Mancozeb 63%WP	4mg	8mg	12mg	16mg
Propiconazole	Tilt 25%EC	20µL	40µL	60µL	80µL
Triazole	Tricyclazole 70%WP	4.28mg	8.57mg	12.85mg	17.14mg
Control		0	0	0	0

Table 1: Details of fungicides and their concentrations used.

Stock solution (60 mL) of PDA was prepared for each treatment and autoclaved. Each treatment was added to 60 mL of the media before being poured into a Petri dish individually. Each Petri plate received 20 ml of the poisoned medium, which was then allowed to settle for 24 hours. A five mm-diameter disc of mycelia plug was placed at the center of plates containing the media and incubated for seven days was preserved. At 26 ± 2^0C., pretreated plates were incubated in the incubator. After 48 hours, mycelium growth was measured daily using a vernier caliper at intervals of 24 hours until the fungus almost completely covered the plate. By comparing the mycelial growth of the fungus in the treated plates with the control, the pathogen's mycelial growth was measured, and the percent inhibition was computed.

The equation was used to compute the percentage of fungal growth inhibition: (Vincent, 1947).

Percent growth inhibition (%) = $\frac{C-T}{C} \times 100$

Where

- C = Colony growth of the *Fol* in control plate (mm)
- T= Colony growth of the *Fol* in a treated plate (mm)

GENSTAT 15th edition was used for data analysis, ANOVA was used to analyze the data, and then Duncan's Multiple Range Test (DMRT) was performed at a significance level of 0.05.

Result and Discussion

Antagonistic impact of fungicides against *f. OXYSPORUM* F. SP. *LENTIS* using a poisoned food approach

When compared to the control, mycelial growth was considerably suppressed by all eight fungicides (Azoxystrobin, Bavistin, Chlorothalonil, Copper oxychloride, Nativo, Saff, Propiconazole, and Triazole) at P = 0.05 (Table 2). After 168 hrs of incubation, the highest inhibition i.e., 100% was shown by Bavistin in all the concentrations (50ppm, 100 ppm, 150 ppm, and 200ppm) followed by 150 ppm and 200 ppm of Saaf and Propiconazole showing 100% inhibition growth. The lowest inhibition percentage was shown by Copper oxychloride in all the concentrations.

It was confirmed that Bavistin was substantially better, displaying complete growth suppression in all doses during the whole experiment. This finding was consistent with Maheshwari., *et al*. [9] research, which found that in *in-vitro* conditions, carbendazim was the most efficient fungitoxicant for inhibiting pathogen growth against Fol. Similarly, Singh., *et al*. [15] found that carbendazim and carboxin entirely prevented Fol from growing when they examined the efficiency of six fungicides toward *Fusarium oxysporum* f. sp. *lentis*. Consequently, systemic fungicide (Carbendazim) has been shown in research findings by Taskeen-Un-Nisa., *et al*. [16] and Khola., *et al*. [7] to be more beneficial than non-systemic fungicide (Mancozeb).

While Propiconazole and Saaf's inhibitory effect raised with concentrations as they showed 100% inhibition at 150 and 200 ppm, this was in accordance with the findings of Maitlo., *et al*. [11] and Khola., *et al*. [7] who found that an increasing concentration was inversely related to an increment in the potency for inhibition.

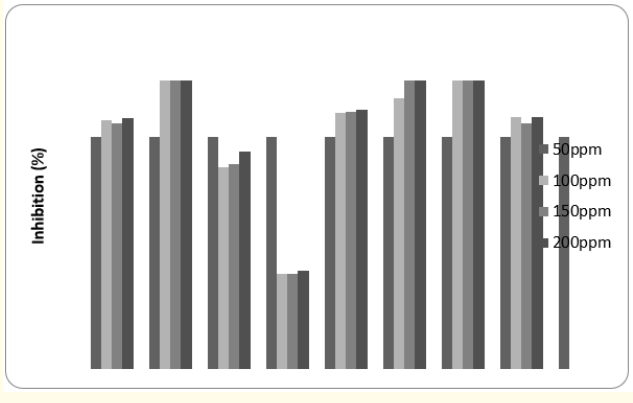

Figure 2: Growth of pathogen showing inhibition percentage after 168 hrs of incubation

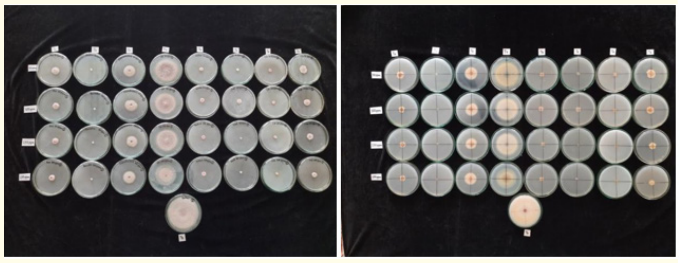

Figure 3: Inhibitory effect of fungicides on radial growth of *Fol* after 168 hrs of incubation

Conclusion

The results of this study conclude that all eight fungicides tested in the laboratory using the poisoned food approach at four different doses (50ppm, 100 ppm, 150 ppm, and 200 ppm) were successful in reducing fungal growth. Bavistin, Nativo, and Propiconazole at 150 and 200 ppm were shown to be the most potent fungicides tested, totally inhibiting fungal growth at all dosages. Azoxystrobin and Trip were moderately effective along with Chlorothalonil while Copper oxychloride ranked last among these fungicides in all the concentrations. As a result, this paper can be useful for expanding our understanding of compounds that are effective against the Fusarium wilt pathogen and for future research in this field. Additional field and greenhouse tests for screening these compounds against the disease are required for validation.

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