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In Vitro Evaluation of Some Fungicides against *Fusarium oxysporum* the Causal of Wilt Disease of Hot Pepper (*Capsicum annum* L.) in Ethiopia

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Abstract

Fusarium wilt caused by *Fusarium oxysporum* f.sp. capsici (FOC) of hot pepper is one of the major pathogens that constrained production and productivity of hot pepper in Ethiopia. The present study was conducted to evaluate the efficacy of some selected fungicides for the management of this disease in one of the major hot pepper production regions in Ethiopia, the Central Rift Valley. Isolate 4DGK was used as the most virulent isolate with 100% wilt incidence to Mareko fana variety. As a result, it was used to evaluate the level of fungicides in combating the isolate. Results obtained from radial growth on petri-dish having 9 cm revealed significant differences in mycelial growth inhibition. Among tested 5 fungicides, URGI 75% WP, Nativo SC 300, Twinstar 75 WG led to 98.8%, 94.0% and 92.3% mycelia growth inhibition, respectively. Mancodex Super 72 WP (2.9%) and Agro Laxyl MZ 63.5 WP (6.5%), that was not effective in inhibiting the mycelial growth of hot pepper fusarium wilt. In general, Fusarium wilt of hot pepper can be managed by fungicides. Nevertheless, the efficacy and economic validity of fungicides should be verified under multi-location field studies.

Keywords: Radial growth; 4DGK; Fungicides; Management

Introduction

Wilt diseases caused by soil borne pathogen(s) are among the most serious diseases affecting several cultivated plants worldwide. Among wilt diseases Fusarium wilt of hot pepper induced by *Fusarium* spp. is reported by many investigators to cause great losses in pepper production in different countries in the world [1]. Conversely wilt causing disease on hot pepper is the leading production constraint in Ethiopia. Furthermore, a yield loss study in Ethiopia due to fusarium wilt in one of the major growing areas revealed yield loss ranging between 68% and 71% [2]. Its occurrences in Ethiopia with varying distribution and incidences of 15.1%, 30.9%, 40.0%, 42.9%, 46.0% and 46.5% in Adami Tullu Jiddo Kombolcha, Dugda, Adama, Meskan, Alaba and Mareko districts were recorded respectively [3].

Hot pepper producing farmers in general and farmers engaged to produce Mareko Fana variety in Ethiopia has experienced a severe decline in production and productivity due to the problem described above. Therefore, there is a high demand from producer's side to focus on disease management options. The management options so far recommended for the control of fusarium wilt of pepper in Ethiopia include the use of crop rotation, fallowing, resistant varieties, and in the severe cases application of chemicals [4]. Nevertheless, these management options should be evaluated to be applicable and bring tangible changes to the end users. In vitro Fusarium wilt mycelial growth inhibition by Trichoderma asperellum revealed 85.2% growth reduction in 7th date [5]. However, there has been little information generated through research on management options towards the diseases. So, to support the smallholder farmers availing different management options are must. Wilt causing pathogens can be managed also by chemical means and by Screening of germplasms/ lines for resistance [6]. Carbendazim recorded complete inhibition of mycelial growth followed by benomyl at 5 cm depth as soil-drench [7]. The objective of the present study was to evaluate the in-hibitory activity of some fungicide's alternatives on the growth of *F. oxysporum* the causal agent of Pepper wilt in vitro. Keeping in view the importance of the crop and the damage caused by this pathogen, the present study was undertaken to evaluate different fungicides in-vitro condition.

Material and Methods

Source of tested microorganisms and fungicides

Isolate of *Fusarium oxysporum* was obtained from the study made by Gabrekiristos E, et al. where it was identified as the most pathogenic isolate which can induce 100% wilt incidence on popular and commonly growing hot pepper variety Mareko fana in Ethiopia. The isolate was originated from Dugda district and designated as 4DGK [3]. This isolate was maintained at Melkassa Agricultural Research Center in Plant Protection division. Furthermore, the isolate 4DGK was further tested for its pathogenicity and confirmed for its ability to induce the wilt symptoms to all inoculated susceptible Mareko Fana seedlings.

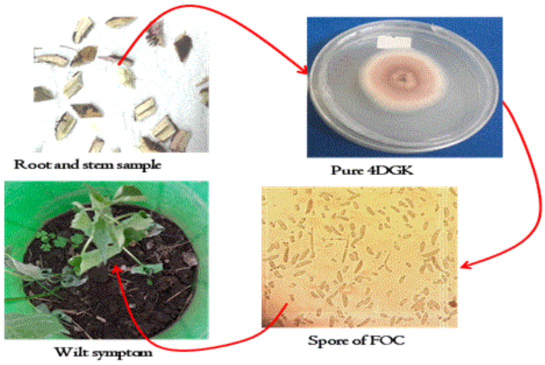

Figure 1: *Fusarium oxysporum* and the symptom, Source: Endriyas G. 2019.

Efficacy of fungicides against *F.oxysporum* f. sp. capsici in vitro

To explore fungicides as an alternative option in the management of hot pepper fusarium wilt, the antagonistic effect of selected fungicides, previously known for their efficacy against other fungal pathogens such as Powdery mildew, Downy mildew, wheat rust, early blight was evaluated. Accordingly, the in vitro antagonistic potentials of five fungicides (URGI 75% WP, Twinstar75 WG, Agro Laxyl MZ 63.5 WP, Nativo SC 300, Mancodex Super 72 WP) obtained from Melkassa Agricultural Research Center (MARC) and the virulent isolate, 4DGK was also obtained from MARC plant pathology program (Table 1). The selected fungicides were evaluated via dual culture assay according to Irfan and Khalid [8].

No	Trade name	Common name	A.I. content	Mode of action
1	Mancodex Super 72 WP	Metalaxyl +Mancozeb	80:640 g/kg	Systemic +Contact
2	Nativo SC 300	Trifloxystrobin +Tebuconazole	100:200 g/l	Systemic +Contact
3	Twinstar 75 WG	Trifloxystrobin +Tebuconazole	50:25% w/w	Systemic +Contact
4	URGI 75% WP	Carbendazim +Mancozeb	120+640 g/Kg	Systemic +Contact
5	Agro Laxyl MZ 63.5 WP	Mancozeb +Metalaxyl	56:7.5%	Systemic +Contact
6	Mock	Control	None	-

Table 1: List of evaluated fungicide and their description.

Laboratory Tests

The inhibitory effect of fungicides on the growth of *F. oxysporum* isolate was evaluated using the culture technique. Tested chemicals were added to conical flasks containing sterilized PDA medium before its solidifying to obtain the proposed concentrations and rotated gently to ensure equal distribution of added fungicides. A separate PDA flask free of tested fungicides was used as mock (control) treatment. The supplemented media were poured into sterilized Petri-dishes (9 cm) approximately 15 ml per each. Mycelial disc (6 mm) taken from the periphery of an actively growing PDA culture of tested fungus *F. oxysporum* was placed at the centre of the prepared Petri dishes, then incubated for ten days at 28 ± 2 °C. Four replicates were used for each treatment. The average linear growth diameter of colonies was measured and reduction in fungal growth was calculated in relative to mock treatment. The mycelial growth inhibition by fungicides were observed and measured in 12hr intervals. The inhibition of FOC mycelial growth was calculated by; I=C-T/C×100, Where, I, C and T refers to percent mycelial growth inhibition, FOC colony growth in control plates and FOC colony growth in BCA plates, respectively.

Statistical analysis

The experiment was set up in a complete randomized design (CRD) having four replication and each treatment were repeated two times. One-way ANOVA was used to analyze differences between antagonistic inhibitor effect and linear growth of pathogenic fungi in vitro. A general linear model option of the analysis system SAS 9.3 was used to perform the ANOVA. Duncan's multiple range test at $P \leq 0.05$ level was used for means separation [9].

Result and Discussion

In vitro efficacy of selected fungicides against *Fusarium oxysporum* f.sp. capsici

The inhibitory effect of selected fungicides on the growth of *Fusarium oxysporum* isolate indicate that, URGI 75% WP, Twinstar 75 WG and Nativo SC 300 was best performing in inhibiting mycelial growth. Three fungicides having the nature of both systemic and contact action strongly inhibited the growth of the test fungi (Table 2, Figure 2). URGI 75% WP (Carbendazim+Mancozeb; 120+640 g/Kg), Nativo SC 300 (Trifloxystrobin+Tebuconazole; 100:200 g/l), Twinstar 75 WG (Trifloxystrobin+Tebuconazole; 50:25% w/w) led to 98.8%, 94.0% and 92.3% inhibition of mycelia, respectively. However, the minimum inhibition in mycelial growth was recorded in Mancodex Super 72 WP (Metalaxyl+Mancozeb 80:640 g/kg), 2.9% and Agro Laxyl MZ 63.5 WP (Mancozeb+Metalaxyl 56:7.5%), 6.5% that was significantly lower than rest of the treatments (Table 2, Figure 2).

Treatments	Means of radial growth	%Inhibition
URGI 75% WP	0.05^{a}	98.8^{a}
Nativo 75WG	0.25^{b}	94.0^{b}
Twinstar75 WG	0.3^{b}	92.3^{b}
Agro Laxyl MZ 63.5 WP	3.9^{c}	6.5^{c}
Mancodex Super 72 WP	4.1^{d}	2.9^{d}
Mock	4.2^{e}	0.0^{e}
CV (%)	2.64	2.88
LSD (0.5)	0.08	2.13
Note: Means followed by same letter indicate no significant difference between treatments LSD test ($P \leq 0.05$; p=0.05).		

Table 2: Effect of fungicides on mycelial growth of *Fusarium oxysporum* f.sp capsici after ten days.

Interestingly, URGI 75% WP (Carbendazim+Mancozeb; 120+640 g/Kg) is best performing fungicide in this study. In a study by Chavan (2007) reported complete inhibition of the F. solani mycelial growth in vitro with carbendazim and carbendazim 12%+mancozeb 63%. Nikam et al. reported carbendazim (1000 ppm) alone and in combination with thiram was most effective against growth inhibition of *Fusarium oxysporum* f.sp. ciceris [10].

Mancodex Super 72 WP having Metalaxyl+Mancozeb (80:640 g/kg) and Agro Laxyl MZ 63.5 WP having Mancozeb+Metalaxyl (56:7.5%) active ingredients do not have an effect on growth and sporulation of hot pepper fusarium wilt in vitro condition (Figure 2d,e). Almost the result of Mancodex Super 72 WP, Agro Laxyl MZ 63.5 WP and untreated check is similar. Figure 2a, shows the mycelial death of test pathogen is clearly observed resulting the outstanding performance for the management of hot pepper fusarium wilt. The present result also confirm the finding of Madhavi and Bhattiprolu have reported that the integration of different treatments, including seedling root dip with carbendazim (0.1%), addition of vermicompost (100 g/kg soil), drenching with fungicide carbendazim+mancozeb (0.2%) and soil application of Trichoderma viride (10 g/pot) was highly effective against Fusarium wilt disease in chilli, which showed 89.8 per cent reduction in the wilt incidence [11].

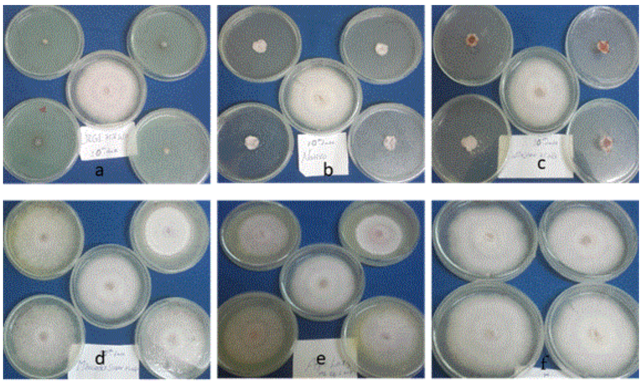

Figure 2: Mycelial growth inhibition of 4DGK with different (a. URGI 75% WP, b. Nativo SC 300, c. Twinstar 75 WG, d. Mancodex Super 72 WP, e. Agro Laxyl MZ 63.5 WP, f. Mock) after 10-day post incubation.

Carbendazim recorded complete inhibition of mycelial growth at 2000 and 3000 ppm at all depths, followed by benomyl at 1000, 2000 and 3000 ppm only at 5 cm depth as soil drench [12].

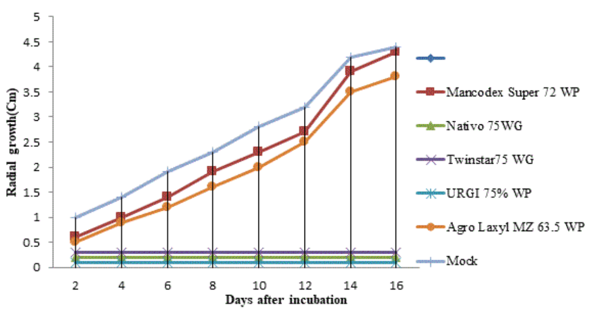

Figure 3: Progression of radial growth in two days interval.

Summary and Conclusion

Among various diseases of hot pepper caused by fungi, bacteria and viruses, fusarium wilt caused by *Fusarium oxysporum* f.sp. capsici (FOC) is the most common and causes qualitative and yield damages. This pathogen is identified in most of hot pepper producing areas in Oromiya, South Nation Nationalities and peoples and Amhara regions.

For immediate remediation evaluating fungicides and availing for the users is the option to easily reduce the epidemics of the pathogen. In this experiment five fungicides were tested in vitro. Of these, three (URGI 75% WP, Twinstar75 WG and Nativo SC 300), fungicides were effective in vitro mycelial growth inhibition and two (Mancodex Super 72 WP, Agro Laxyl MZ 63.5 WP), of them were not effective in controlling the test organism. URGI 75% WP, Nativo SC 300 and Twinstar75 WG led to 98.8%, 94.0% and 92.3% mycelia growth inhibition, respectively.

Finally, from tested fungicides to manage hot pepper fusarium wilt, URGI 75% WP, Twinstar75 WG and Nativo SC 300 are recommended for further research. Nevertheless, further research has to be conducted in order to verify in vivo efficacy both under controlled (greenhouse) and field conditions. Integrated fusarium wilt management approach including root deep of recommended fungicide should be practiced and made ready for end users.

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References

1. Abdel-Monaim MF, Ismail ME (2010) The Use of Antioxidants to Control Root Rot and Wilt Diseases of Pepper. Not Sci Biol 2: 46-55.
2. Soboka TB, Fininsa C, Gorfu D (2012) Integrated Approach and Plant Extract Management Options against Pepper Wilt (*Fusarium oxysporum* Var. Vasinfectum) at Bako, Western Ethiopia, MSc. Thesis in Plant Pathology, Haramya University, Ethiopia.
3. Gabrekiristos E, Teshome D, Ayana G (2020) Distribution and Relative Importance of Hot Pepper Fusarium Wilt (*Fusarium oxysporum* f.sp. capsici) and Associated Agronomic Factors in the Central Rift Valley of Ethiopia. Adv Crop Sci Tech 8: 437.
4. (EARO) Ethiopian Agricultural Research Organization (2004) Ethiopian Agricultural Research organization, Annual report, Addis Ababa, Ethiopia.
5. Gabrekiristos E (2019) Distribution of Hot Pepper Fusarium Wilt (*Fusarium oxysporum* F.sp.capsici) and Evaluation of Host Resistance and Biocontrol Agents against the Pathogen in the Central Rift Valley of Ethiopia. MSc thesis.
6. Joshi M, et al. (2012) Screening of Resistant Varieties and Antagonistic *Fusarium oxysporum* for Biocontrol of *Fusarium* Wilt of Chilli. J Plant Pathol Microbiol 3: 5.
7. Jaywant A (2016) Pathogenic Variability and Management of *Fusarium* wilt of Chilli (*Capsicum annuum* L.). PhD Thesis in plant pathology in College of Agriculture CCS Haryana Agricultural University, Hisar. pp: 95.
8. Irfan YS, Khalid AN (2007) In-vitro biological controls of *Fusarium oxysporum* causing wilt in *Capsicum annuum*. Mycopathology 5: 85-88.
9. Winer BJ (1971) Statistical Principles in Experimental De-sign. 2nd edn, MiGraw-Hil Kogakusha, LTD. pp: 596.

10. Chavan SS (2007) Studies on fungal diseases of patchouli with special reference to wilt caused by *Fusarium solani* (Mart.) sacc. M.Sc (Agri) Thesis, Univ Agri Sci, Dharwad. 98.
11. Nikam PS, Jagtap GP, Sontakke PL (2007) Management of chickpea wilt caused by *Fusarium oxysporum* f.sp. ciceris. African J Agri Res 2: 692-697.
12. Madhavi GB, Bhattiprolu SL (2011) Evaluation of fungicides, soil amendment practices and bioagents against *Fusarium solani*-causal agent of wilt disease in chilli. J Horti Sci 6: 141-144.