academicresearchJournals

Vol. 8(3), pp. 156-163, April 2020
DOI: 10.14662/ARJASR2020.130

ISSN: 2360-7874
http://www.academicresearchjournals.org/ARJASR/Index.htm

**Academic Research Journal of Agricultural Science and Research**

*Research article*

# Prospects of host resistance and biocontrol agent for the management of hot pepper Fusarium wilt (*Fusarium oxysporum* f.sp. *capsici*) in the Central Rift valley of Ethiopia

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Accepted 30 April 2020

**Hot pepper (Capsicum annum L.) is one of the important cash crops to Ethiopian smallholder farmers and an important agricultural commodity which contribute to export earnings. Fusarium wilt caused by *Fusarium oxysporum f.sp. capsici*(FOC) is one of the major pathogen that constrained production and productivity of hot pepper in Ethiopia. The present study was conducted to evaluate the potentials of host resistance and biological control 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 disease resistance in 21 varieties/accessions. Results obtained from wilt incidence and vascular disease index data analysis revealed significant differences in disease resistance. Nevertheless, Oda Haro and ACC80061 were the only promising materials that showed moderately resistant reaction with wilt magnitude of 33.3 and 25% respectively. Regarding the efficacy of the biocontrol agents, in vitro growth of FOC, significant variation has been observed and from the tested six bioagents, the highest mycelial growth inhibition (85.2%) was obtained from FOC treated by *Trichoderma asperellum*. In general, Fusarium wilt of hot pepper can be managed by using host resistance and biological control. Nevertheless, the efficacy and economic validity of these methods should be verified under multi-location field studies.**

**Key Words:** Hot pepper, Fusarium wilt, Resistant evaluation, Bioagent

**Cite this article as:** Endriyas G., Daniel T., Getachew A(2020). Prospects of host resistance and biocontrol agent for the management of hot pepper Fusarium wilt(*Fusarium oxysporum f.sp. capsici*) in the Central Rift valley of Ethiopia. Acad. Res. J. Agri. Sci. Res. 8(3): 156-163

## INTRODUCTION

Hot pepper (*Capsicum annum* L.) is an important crop grown as vegetable and spice in all the continents of the world (Berke, 2002). In Ethiopian, hot pepper is an important agricultural commodity which contributes to export earnings (Beyene and David, 2007). It is rich source of vitamin A, E and C, making it an ideal more than any other vegetable crop (Boselad and Votava, 2000). According to FAOSTAT (2017), a world average yield of 32.3 t/ha green and 3.8 t/ha dry pepper have been reported. In Ethiopia, average dry and green hot pepper production during the 2014 production year was 1.6 t/ha and 10.7t/ha, respectively (Abraham *et al.*, 2016),

this is far below the world's average productivity.
However, wilt disease caused by *Fusarium oxysporum* are becoming the leading problems of pepper producing countries worldwide. For example, up to 35% fusarium wilt incidence has been reported in many states of USA (Naqvi, 2004). Recent report on distribution and intensity of Fusarium wilt, with 15.1, 30.9, 40.0, 42.9, 46.0 and 46.5% wilt incidence in Adami Tullu Jiddo Kombolcha, Dugda, Adama, Meskan, Alaba and Mareko districts, respectively(Gabrekiristos E. *et al*., 2020). Recently, Assefa *et al*. (2015) reported that, 86.4% wilt incidence caused by Fusarium wilt in Bako, Ethiopia. More importantly, a study on yield loss assessment due to fusarium wilt in one of the major growing areas of Ethiopia revealed a high yield loss ranging between 68 and 71% (Teshome *et al.,* 2012). Another study on assessment of hot pepper diseases in South Nation, Nationalities and Peoples Region (SNNPR) of Ethiopia by Shiferaw and Alemayehu (2014) showed the occurrence of 30-55% fusarium wilt incidence and confirmed fusarium wilt as the leading fungal disease of pepper in the area.

In plant disease 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 (EARO, 2004). Nevertheless, these management options should be evaluated to be applicable and bring tangible changes to the end users.

The use of resistant varieties is the best strategy for disease control for soil-borne pathogens (Sheu *et al*., 2006). In Ethiopia Significantly, ($P \leq 0.05$) lowest wilt (4.66%) incidence and AUDPC (279%-day) were obtained by integration of the moderately resistant variety Oda-haro with solarization-fungicide-flat seedbed (Teshome *et al.,* 2012).

Use of biological agents is another alternative for the management of such disease in an environmentally friendly manner (Lugtenberg and Kamilova, 2009; Akram *et al.,* 2013). Bio-control agents such as *Trichoderma* spp are known to secrete extracellular lytic enzymes and other compounds which enhance their antagonistic activity against Fusarium wilt of pepper (Ozbay& Newman, 2004). Keeping in view the importance of the crop and the damage caused by this disease, the present study was undertaken to manage hot pepper fusarium wilt by variety/accessions evaluation and biocontrol agents.

## MATERIALS AND METHODS

### Evaluation of host resistance against *Fusarium oxysporum* f.sp.*capsici*

This study was carried out to evaluate fusarium wilt resistance reaction of 11 released varieties and 10 accessions, which are currently under screening trials for various traits. Of these, the 6 released varieties and all the accessions were obtained from Melkassa Agricultural Research Center. The remaining 5 released varieties were obtained from Bako Agricultural Research Center. Variety Oda Haro and Mareko Fana were used as tolerant and susceptible controls, respectively (Table 1).

**Table 1**. List of hot-pepper varieties and accessions evaluated for fusarium wilt resistance

| No. | Name | Source | No. | Name | Source |
|---|---|---|---|---|---|
| 1 | Mareko fana[a,S] | MARC | 12 | ACC 229334[b] | MARC |
| 2 | Melka Shote[a] | MARC | 13 | ACC 223648[b] | MARC |
| 3 | Melka Awaze[a] | MARC | 14 | ACC 212587[b] | MARC |
| 4 | Melka Zala[a] | MARC | 15 | ACC 24047[b] | MARC |
| 5 | Melka Oli[a] | MARC | 16 | ACC 80061[b] | MARC |
| 6 | Melka Dera[a] | MARC | 17 | Bako Local[a] | BARC |
| 7 | ACC 211470[b] | MARC | 18 | Oda Haro[a,T] | BARC |
| 8 | ACC 230798[b] | MARC | 19 | Kume[a] | BARC |
| 9 | ACC 212679[b] | MARC | 20 | Dinsire[a] | BARC |
| 10 | NJP[b] | MARC | 21 | Dame[a] | BARC |
| 11 | ACC 80[b] | MARC | | | |

Type of hot-pepper plant materials are indicated as[a] (released variety) and [b] (accession/pipe line). [S] and [T] refer to tolerant and susceptible variety, respectively, MARC: Melkassa Agricultural Research Center, BARC: Bako Agricultural Research Center

For resistant reaction, the FOC isolate 4DGK identified as most virulent in the pathogenesity test in Melkassa Agricultural research center in 2019 was used and inoculation was performed at five-leaf stage(Gabrekiristos E *et al*., 2020). After inoculation, the plantlets were briefly dried on a paper towel and transplanted to pots with the area of 26cm in diameter and 22cm in height containing top soil and sandy soil in 2:1 ratio and grown under greenhouse conditions.

The experiment was arranged in a completely randomized design with five replications (i.e. three plants per pot which is considered as one replication). Accordingly, 15 plants were used for each variety or accession (i.e. a total of 315 plants were used for the whole experiment). The level of resistance to FOC was assessed on the basis of the number of diseased plants or plant deaths due to fusarium wilt (wilt incidence). Accordingly, fusarium wilt incidence was monitored at 50 days post inoculation (DPI). Using these data, hot-pepper varieties/accessions were grouped in to five classes: immune, resistant, moderately resistant, susceptible and highly susceptible (Table 2).

**Table 2.**Assigning of hot-pepper resistance to fusarium wilt into different classes (Monaim and Ismail, 2010)

| Wilt incidence range | Resistance class | Designation |
|---|---|---|
| 0 | Immune | I |
| 1-20 % | Resistant | R |
| 21-40 % | Moderately resistant | MR |
| 41-50 % | Moderately susceptible | MS |
| 51-70 % | Susceptible | S |
| 71-100 % | Highly Susceptible | HS |

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

To explore biocontrol as an alternative potential option in the management of hot-pepper fusarium wilt, the antagonistic effect of selected fungal and bacterial biocontrol agents (BCAs), previously known for their efficacy against other fungal pathogens such as *Colletotrichum capsici* and *Colletotrichum gleosporides* (Aswini *et al*., 2016) were used. Accordingly, the *in vitro* antagonistic potentials of five fungal BCAs (*Trichoderma harzianum, T. viride, T. longibiracher and T. asperellum*) obtained from Ambo Plant Protection Research Center and one bacterial BCA (*Bacillus subtilis s*train QST 713) and the commercial bioagent MYTECH *(Paciliomus lilicinus)* obtained from Ziway AQ rose was evaluated *via* dual culture assay according to Irfan and Khalid (2007). Briefly, a 6 mm diameter agar plug of the 4DGK isolate and the fungal BCAs were taken from seven days old cultures with the help of sterilized cork borer. Agar plugs of the FOC and fungal BCAs were placed at the opposite sides of the PDA plates (9cm diameter) 1cm from the edge, and incubated at 28°C for one week. For the bacterial BCA, 24 hours old culture was point inoculated in the same way as the fungal BCAs. Finally, percent mycelial growth inhibition was calculated by;

$$I = \frac{C-T}{C} \times 100,$$

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.

## RESULTS AND DISCUSSION

**Evaluation of host resistance against *Fusarium oxysporum* f.sp.*capsici* isolate**

The response of 11 released varieties and 10 accessions of hot pepper to *F. oxysporum* f.sp*. capsici* infection was studied under greenhouse conditions. Results showed that, most of the tested varieties/accessions were highly susceptible to the pathogen while few exhibited moderately resistant reactions (Table 3). In general, it takes about three weeks for inoculated plants to show wilting symptoms. Unlike vascular discoloration which is considered as a primary symptom (Figure 1), it is known that wilting is a secondary symptom of FOC infection in hot pepper (Ferniah *et al.,* 2014). Therefore, the response of hot pepper varieties/accessions to FOC infection in the present study was further investigated by assessing disease severity (vascular discoloration). Interestingly*,* inoculated plants typical symptom of the isolate 4DGK (Figure1) and none of the tested varieties and/or accessions showed a complete resistance to FOC. Results showed on this assessment key, varieties/accessions were grouped in to five classes, moderately resistant, moderately susceptible, susceptible, highly susceptible and resistance (Table 3).

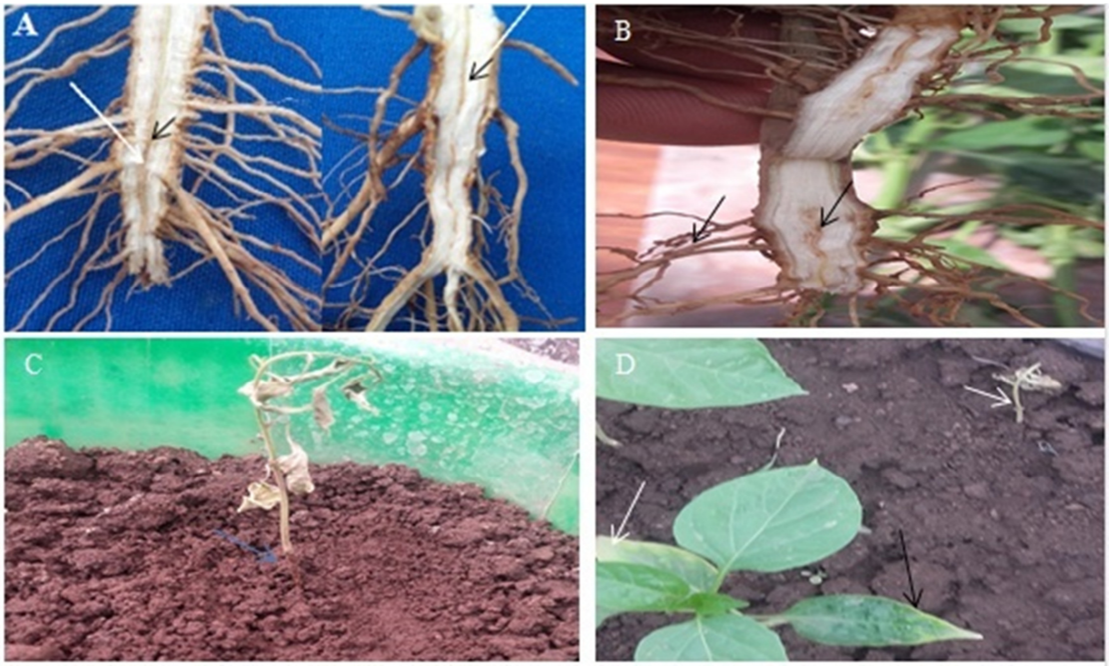


**Figure 1.**Symptoms of fusarium wilt caused by *Fusarium oxysporum* f.sp.*capsici* isolate 4DGK one susceptible hot pepper Mareko fana variety under greenhouse conditions. A and B: Vascular discoloration on tap and lateral roots. C: wilted (dead) plant sixty days after root dip inoculation. D: leaf yellowing (arrow) twenty days after inoculation.

**Table 3.**Response of hot pepper varieties/accessions to *Fusarium oxysporum* f.sp.*capsici* infection under greenhouse conditions

| Var/Acc | FWI (%) | VDI (0-5 scale) | RC |
|---|---|---|---|
| MelkaOli[RV] | 75.0[bc] | 4.8[a] | HS |
| Marekofana[RV;S] | 100.0[a] | 4.7[ab] | HS |
| Dinsire[RV] | 100.0[a] | 4.5[b] | HS |
| BakoLocal[RV] | 100.0[a] | 4.2[c] | HS |
| ACC 211470[Acc] | 50.0d[e] | 4.1[cd] | MS |
| ACC 223648[Acc] | 83.3[ab] | 4.0[de] | HS |
| NJP[Acc] | 100.0[a] | 3.9[def] | HS |
| Kume[RV] | 100.0[a] | 3.9[def] | HS |
| ACC 212587[Acc] | 75.0[bc] | 3.8[ef] | HS |
| Dame[RV] | 75.0[bc] | 3.8[ef] | HS |
| Melka Zala[RV] | 75.0[bc] | 3.7[f] | HS |
| ACC 230798[Acc] | 75.0[bc] | 3.5[g] | HS |
| ACC 229334[Acc] | 75.0[bc] | 3.2[hi] | HS |

**Table 3.** Continues

| | | | |
|---|---|---|---|
| Melka Dera[RV] | 75.0[bc] | 3.0[j] | HS |
| ACC 24047[Acc] | 58.3[cd] | 3.4[hg] | S |
| Melka Awaze[RV] | 58.3[cd] | 3.3[gh] | S |
| Melka Shote[RV] | 50.0[de] | 3.0[ij] | MS |
| ACC 80[Acc] | 50.0[de] | 2.2[k] | MS |
| ACC 212679[Acc] | 41.7[ef] | 2.1[kl] | MS |
| Oda Haro[RV;T] | 33.3[ef] | 2.0[l] | MR |
| ACC 80061[Acc] | 25.0[f] | 1.5[m] | MR |
| **CV (%)** | **21.3** | **4.9** | **-** |
| **LSD (5%)** | **21.3** | **0.2** | **-** |

Varieties/Accessions were grouped into resistance classes based on the level of vascular disease index (Ulloa *et al.,* 2006). Varieties/accessions are sorted in increasing level of disease resistance (decreasing vascular disease index).**[RV]**: released variety.**[Acc]**: accession/pipe line. [T] and [S] refer to tolerant and susceptible reference varieties, respectively. **VAR**: Variety.**ACC**: accession.**FWI**: fusarium wilt incidence **VDI**: vascular disease index.**RC**: resistance class.**HS**: Highly susceptible.**S**:susceptible.**MS**: Moderately susceptible.**MR**: moderately resistant. For each column, different letters indicate significant differences between treatments at P≤0.05 (LSD test).

Among the tested plant materials, Oda Haro and ACC 80061 were the only better plants that showed moderately resistant reaction with 33.3 and 25.0% wilt incidence, respectively, perhaps promising materials for future resistance breeding works. Two accessions (ACC 80 and ACC 211470) and one variety (Melka Shote) were moderately susceptible with a wilt incidence value of 41.7%, 50.0% and 50.0%, respectively. The remaining all varieties/accessions were identified either as susceptible or highly susceptible to *F.oxysporum* f.sp. *capsici*(Table 3).

Based on the two disease assessment results (vascular discoloration and wilting), the performance of ACC 80061 and Oda Haro under infection with the highly potent isolate 4DGK was promising. The possible explanation for such a response might be the production of antifungal compounds such as phenolics produced which are known as a major resistance mechanism against fusarium wilt (Iftikhar *et al*., 2005). In greenhouse experiment 33.3% and 25% fusarium wilt incidence on Oda Haro and ACC 80061 were recorded, but, Teshome *et al.* (2012); reported that, when Oda haro variety was integrated with solarization and fungicide treatments, significant reduction of wilt incidence (4.66%) was obtained.

Identification and evaluating pepper genotype for their reaction to fusarium wilt is pre-requisite to a disease resistance-breeding. The use of resistant variety is beneficial not only to reduce the losses due to diseases but also to minimize the toxicity due to application of fungicides (Parey *et al*., 2013; Manu *et al*., 2014). The results of present study coincide with previous work done by Teshome *et al*.(2012) who reported that Oda Haro was moderately resistant by combining compatible integrated management options and Mareko Fana were reported as susceptible variety. The recent work by Jaywant *et al*., (2017) also showed that among 22 cultivars screened for resistance against *F. oxysporum*, none was found fully resistant/immune; only one cultivar (CO-4) has been found resistant with wilt incidence of 6.67%.

## In *vitro* efficacy of biocontrol agents against *Fusarium oxysporum* f.sp. *capsici*

The *in vitro* mycelial growth inhibition efficacy of six (five fungal and one bacterial) biocontrol agents (BCAs) against the virulent FOC isolate 4DGK (isolated from infected hot pepper fields in Dugda district and identified as the most aggressive isolates(see pathogenesity test) was evaluated in a dual culture assay(Gabrekiristos E. *et al*.,2020). Statistical analysis revealed that, all the evaluated BCAs significantly reduced *in vitro* mycelial growth of *F. oxysporum* f.sp. *capsici* 4DGK (Table 4). More importantly, there was significant difference among BCAs regarding mycelia growth inhibition. From the fungal BCAs, the *Trichoderma* spp. were the most effective and provided up to 85.2% mycelial growth inhibition. Interestingly, even the *Trichoderma spp.* significantly differs in efficacy. *T. Asperellum* provided the highest growth inhibition (85.2%) followed by *T.viride, T.longibiracher* and *T. harzianum,* which reduced mycelial growth up to 80.4%, 77.6% and 77.3%, respectively. Compared to *Trichoderma spp.,* the efficacy of *Paciliomus lilicinus* and *Bacillus subtilis* was significantly lower but still their efficacy is not negligible (Table 4).

**Table 4.**Effect of fungal and bacterial biocontrol agents on *in vitro* mycelial growth of *Fusarium oxysporum* f.sp.*capsici* 4DGK in dual culture assay nine days after incubation.

| Biocontrol agents | Mycelial growth inhibition (%) |
|---|---|
| *Trichoderma asperellum* | $85.2^{a}$ |
| *Trichoderma viride* | $80.4^{b}$ |
| *Trichoderma longibiracher* | $77.6^{c}$ |
| *Trichoderma harzianum* | $77.3^{c}$ |
| *Paciliomus lilicinus* | $64.4^{d}$ |
| *Bacillus subtilis* | $61.4^{e}$ |
| Control (fungal growth without BCA) | $0.0^{f}$ |
| CV % | 2.8 |
| LSD(0.05) | 1.8 |

Means followed by same letter indicate no significant difference between treatments LSD test (P ≤ 0.05; p= 0.05).

In most pepper producing countries much emphasis has given to bioagents in order to reduce fungicide use, to avoid environmental pollution, reduce cost of management and occurrence of lethal, aggressive and virulent strains of FOC (Sultan *et al*., 2012). Because of the nature of the pathogen (as it survive in the soil) methods such as the use of fungicide is limited. Several studies on biocontrol agents have been found to contain antifungal potential against FOC (Heydari and Pessarakli, 2010). These antagonistic organisms adopted numerous types of mechanisms such as antibiosis, parasitism, induced resistance, and competition for nutrients and lytic enzymes to inhibit the growth of Foc (Suprapta, 2012; Segarra *et al*., 2013). *Trichoderma* spp. inhibits *in vitro* fungal plant pathogens by producing diffusible, volatile and nonvolatile secondary metabolites such as mycotoxins (Reino *et al*. 2008).

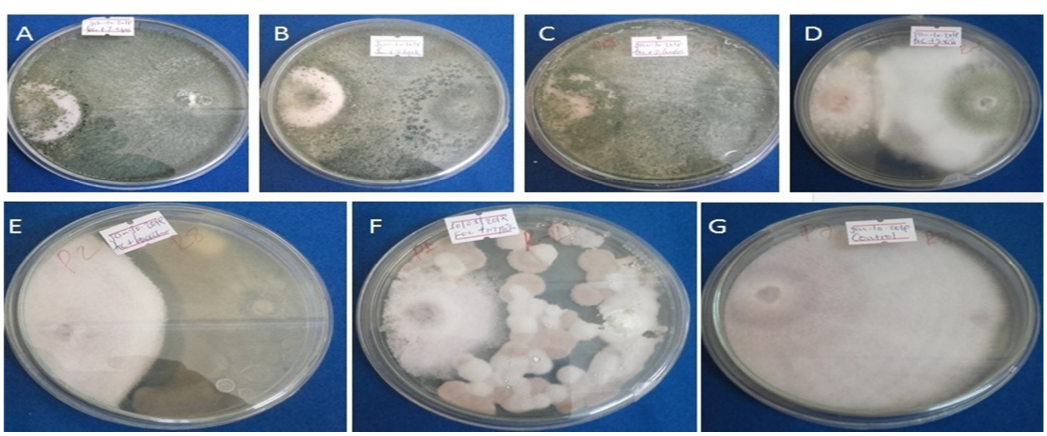


**Figure 2.**Dual culture assay on potato dextrose agar showing mycelial growth inhibition of *Fusarium oxysporum* f.sp.*capsici* 4DGK by *Trichoderma asperellum* (A), *T.harzianum*(B), *T.longibiracher*(C), *T.viride*(D), *Bacillus subtilis*(E), *Paciliomus lilicinus*(F) and *F. oxysporum* f.sp. *capsici* 4DGKgrowth without BCA (G)

It has been also visualized that *Trichoderma* spp. hinder pathogenic invasion through the release of organic metabolite such as chitinase, pachybasin and volatile inhibitory compounds i.e. acetaldehyde (Bunker and Kusum, 2001). Since *Trichoderma spp*. is also known for its fast growth rate (Suprapta, 2012). In the present study, such a scenario seems to be observed in case of *Trichoderma asperellum* and *Trichoderma harzianum* combinations (Fig 2A, C). The antagonists significantly reduced the growth of *F. oxysporum* either by overgrowing or by restricting growth which forms an inhibition zones.

Similar results were also observed where *T. viride* and *T. harzianum* as the most significant antagonistic organisms due to secretion of extracellular lytic enzymes and other compounds like harzianien and viridian which enhance their antagonistic activity against fusarium wilt of pepper (Ozbay& Newman , 2004). The use of *Trichoderma species* have been successfully employed as biological control agents due to their high reproductive capacity, efficient utilization of nutrients, strong aggressiveness against other pathogens as well as rapid and effective colonization of wound sites against the invading pathogens (Arya A, 2010; Okigbo, 2004 and Suprapta, 2012).

## SUMMARY AND CONCLUSION

Hot pepper (*Capsicum annuum* L.), is an important vegetable crop grown worldwide for its economic and nutritive importance. The production of this crop is affected by many biotic and abiotic factors and among the biotic factors diseases has been identified as a major limiting factor. 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.

In the host-resistance study, 21 materials (11 released varieties and 10 accessions) were tested. Of these, most plants showed different levels of susceptible reactions. The only exceptions were ACC 80061 and Oda Haro that showed moderately resistant reaction based on wilt incidence and vascular disease index assessments. Regarding the efficacy of antagonist, the results of the *in vitro* mycelial growth inhibition revealed significant effect of the bioagents and the level of mycelium growth significantly vary among antagonists with the highest inhibition (85.2%) caused by *Trichoderma asperellum*. The disease management study also identified potential hot pepper variety/accession that can be used in future studies focusing of disease resistance. The observed significant *in vitro* efficacy of antagonists also gives a hope of combating this disease with the help of biological agents.

Finally, from host resistance study, accession ACC 80061 is recommended for further research in pepper disease resistance breeding program, which can be used as a source of moderately resistant gene to FOC. In addition, *T. asperellum* and *T.viride* were effective in inhibiting FOC *in vitro.* Nevertheless, further research has to be conducted in order to verify in vivo efficacy both under controlled (greenhouse) and field conditions.

To early combat hot pepper fusarium wilt, Varietal screening should be intensively conducted to replace susceptible one and Country wide epidemiological survey is important for early detection. Bioagent evaluation should have to be intensively conducted both at green house and field condition. Integrated fusarium wilt management approach including root deep of recommended fungicide should be practiced and made ready for end users.

## ACKNOWLEDGMENTS

I would like to thank Melkassa Agricultural Research Center (Horticulture and Plant protection staff member) and Korean project on international Agriculture for the support in budget for undertaking the survey and laboratory work. Similarly, I would like to acknowledge Plant science and Horticulture Staff members of Jimma University, college of Agriculture and Veterinary medicine for their contributions.

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