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## RESEARCH ARTICLE

### Isolation, Identification and *In Vitro* Test for the Biocontrol Potential of *Trichoderma viride* on *Fusarium oxysporum* f. sp. *Lycopersici*

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#### Abstract:

#### Introduction:

Tomato production in Ethiopia is challenged by many pests and diseases. *Fusarium* wilt is one of the most important diseases of tomato affecting its productivity.

#### Methods:

Tomato tissue and soil samples were collected from tomato farmlands around Aksum town to isolate and identify pathogenic *Fusarium* species and *Trichoderma* species with biocontrol efficacy. Samples were processed in the Aksum University Biotechnology laboratory following standard procedures.

#### Results and Discussion:

Eight *Fusarium* and five *Trichoderma* isolates were obtained. Six of the *Fusarium* isolates were identified as *Fusarium oxysporum*, whereas the remaining two were *Fusarium equiseti* and *Fusarium circinatum*. Detached leaf bioassay of the *F. oxysporum* on tomato leaves showed leaf lesion on the tomato variety, Melka oda. The isolated *Trichoderma* strains were screened for biocontrol potential against virulent *F. oxysporum* *in vitro*. The *Trichoderma* isolate showing the highest biocontrol efficacy against the virulent *Fusarium* was morphologically identified as *Trichoderma viride*. *in vitro* *F. oxysporum*-*T. viride* dual culture assay demonstrated that *T. viride* inhibits the growth of *F. oxysporum* f.sp. *lycopersici* with 76.94% growth inhibition.

#### Conclusion:

*Fusarium oxysporum* is prevalent in tomato growing farmlands covered in this study. *T. viride* identified in this study is an effective biocontrol agent for the identified *F. oxysporum* f.sp. *lycopersici* *in vitro*.

**Keywords:** Antagonist, Detached-leaf bioassay, Dual culture, Growth inhibition, Morphological, Rhizosphere, Axum.

#### Article History

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## 1. INTRODUCTION

Tomato is one of the major economically important vegetables worldwide [1, 2]. In particular, it is a widely grown vegetable cash crop in Ethiopia [3]. It has been found to be the most profitable crop to small farmers in the country [4]. While the productivity of tomato at demonstration sites and research plots in the country reached levels of 250 and 400 qt/ha, respectively, productivity in the agricultural farms is very low (90 qt/ha,) [4] as compared to the productivity level in the rest

of the world, which is between 600-1000 qt/ha [5]. This is because tomato production in Ethiopia faces a variety of constraints, among which are pests and diseases.

*Fusarium* wilt is one of the most important diseases of tomatoes worldwide. It is a devastating disease that affects many important food and vegetable crops and a major source of loss to farmers worldwide [6]. The disease is characterized by wilted plants, yellowed leaves and reduced or even total loss of crop yield [7]. There are more than 100 *Fusarium* vascular wilt diseases worldwide [8, 9]. *Fusarium* wilt of tomato is commonly caused by the fungal pathogens, *F. oxysporum* or *Fusarium solani*. *Fusarium equiseti* is also becoming an emerging tomato wilt pathogen [10]. The most familiar formae species to tomato is *Fusarium oxysporum* f.sp. *lycopersici* [2,

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10]. The pathogen penetrates the plant, colonizing and leaving the vascular tissue dark brown and the discoloration extends to the apex, leading to the plants wilting, collapsing and dying. The initial symptoms of the disease appear in the lower leaves gradually, trailing to wilting of the plants.

Ethiopian farmers apply chemical spray to protect their crops from pests and diseases. In Axum areas, farmers apply fungicides; Ridomil and Mancozeb. But the use of chemical sprays on edible crops is unacceptable for human health concern and from an environmental point of view [11] and more importantly, as *Fusarium* wilt disease is soil-borne in nature, application of fungicides to control this pathogen is not practical [12, 13]. Ethiopian GTP II has planned to establish stringent testing and certification services regarding chemical use and resulting residues on agricultural commodities with a view to providing chemical free organic products for the market [13]. As a result, there is a need for some eco-friendly biocontrol systems that help to mitigate these problems. Biological control of plant pathogens by microorganisms has been considered a more natural and environmentally acceptable alternative to the chemical treatment methods [14, 15]. Various types of biological control agents such as bacteria and fungi are involved in biocontrol activity [16, 17]. Among them, the fungal genus *Trichoderma* plays a major role in controlling plant diseases [18]. It is one of the best known and well described biocontrol fungi for its antagonistic properties against phytopathogens [19]. Several strains of *Trichoderma* have been developed as biocontrol agents against fungal diseases of plants [20, 21]. Most biocontrol agents are from the species of *T. harzianum*, *T. viride* and *T. hamatum* [20, 15].

Farmers in the surroundings of Axum town cultivate tomato. But their tomato cultivation is affected by fungal infections both on the field and after harvesting, the consequences of which are loss of a significant quantity of the product (Pers. Com, local agricultural expert). *Fusarium* species alone accounted for 25% postharvest deterioration of tomato fruits [unpublished]. Therefore, the current research presented the isolation, identification and development of the fungal biocontrol agent, *Trichoderma* species against the fungal pathogen of tomato, *Fusarium oxysporum* f.sp. *lycopersici*. The objective sought to evaluate the biocontrol efficacy of *Trichoderma* species against *F. oxysporum* *in vitro* by dual culture assay and *in vivo* by detached leaf bioassay.

## 2. MATERIALS AND METHODS

### 2.1. Study Area and Study Period

The areas considered in this study were the farmlands within a 5 km radius from Axum town; Hatsebo in the east, Dura in the west and Medoge in the south through the altitudinal range of 1834-2096 m.a.s.l. Plots of lands cultivated with tomato were investigated for the occurrence of *Fusarium* wilt disease and 20 tomato and 26 soil samples were collected from the plots using sterile polyethylene plastic sampling bags. The collected samples were transported to Aksum University, Biotechnology Laboratory for isolation and identification of plant pathogenic microfungi and fungi with biocontrol potential. The study was conducted through the period between March 2018 to April 2019.

### 2.2. Isolation of *Fusarium* Pathogens from Tomato Vegetative Parts

The *Fusarium* wilt pathogens were isolated following the tissue transplanting technique [22]. The tomato plant parts were washed with running tap water to remove soil and dust from the tissues; followed by the stem and root samples cut into 5mm-10mm sized slices using sterile scissors and surface sterilised using 96% ethanol for 1 minute. The surface disinfected tissues are then washed with three successive changes of sterile distilled water to washout the ethanol and were blot dried using Whatman Number 1 filter papers. Blot dried tissues were placed onto 10cm glass Petri dishes containing the solidified Potato Dextrose Agar (PDA) medium. The plates were then incubated at 25±2 °C for seven days with periodic checks for the emergence of fungal mycelia from the transplanted sample tomato tissues. Fungal hyphae emerging on the transplanted tissues were transferred onto the fresh PDA medium by means of the hyphal tip transfer technique [23]. The cultured fungal isolates were purified by three subsequent sub-culturing on to PDA. Eight pure fungal cultures inspected to be *Fusarium* were maintained in the form of slant culture at 4°C for further study.

### 2.3. Cultural Characters of *Fusarium* Isolates

The cultural characters of the isolates on the PDA medium were studied by culturing each isolate in three replicates at 25±2°C. Cultural characteristics (colony appearance, growth pattern, marginal shape, surface colony colour, reverse colony colour/pigmentation, radial growth) were examined seven days after inoculation (7DAI) and the growth rate was measured. Radial growth was evaluated by measuring the radius of the petri dish covered by the colony culture using an mm-calibrated glass ruler. Growth rate data was recorded by measuring the radius that the colony covered 24-hour intervals for seven days. The growth rate value was then calculated by subtracting the radial growth value on the first day from the radial growth value of the second day and so on for the seven days; finally tabulating the mean of all the differences thereby giving the growth rate of that specific isolate [23].

### 2.4. Morphological Identification of *Fusarium* Isolates

A slide culture was prepared for each of the eight isolates by taking a 5mm bit from a seven-day old pure culture and inoculating on to a rectangular cut of the PDA medium. It was then placed on sterilized glass slides and covered with coverslips. The slides were then placed inside a sterilized Petri dish underlay with moistened filter paper to provide moisture for the fungi, and incubated at 25± 2° C [22]. The mycelia developed on the slide culture are then subjected to microscopic examination to morphologically differentiate the identity of the isolates to species level at 40X magnification [23] using an Olympus Microscope (Carl Zeiss Microscopy, Germany).

### 2.5. Isolation of *Trichoderma* Species

Soil samples were collected from rhizosphere areas of referenced garden trees (Mango, Lemon, Orange, Guava and Avocado) within 100cm cardinal points and 10-15cm deep [24]. *Trichoderma* species were isolated by a soil serial

dilution- agar plating method and stored at 4°C for later use (Fig. 1). Culture purification was attained by repeated sub-culturing through the hyphal tip transfer technique [23].

## 2.6. Identification of the Trichoderma Isolate

The identification of the *Trichoderma* was made based on in-depth observation of cultural characters and Microscopic examination of morphological features with reference to the literature [24]. To capture a photo of the conidia, a digital camera (SONY Cyber-shot, 16.1 Mega pixels) was used by attaching to the eyepiece of the microscope. The photograph was captured by focussing the zoom adjustment to obtain a magnified image.

## 2.7. Radial growth and Growth Rate of Isolates

The radial growth and growth rate evaluation was done for both *Fusarium* and *Trichoderma* isolates. Radial growth is recorded by measuring growth seven-days post-inoculation using an mm calibrated glass ruler. The growth rate is also recorded by measurements taken every 24 hrs and calculating the difference. The tabular value was obtained by subtracting the radial growth of the isolate on day one from the radial growth of the isolate on day two. This is taken as the growth rate value, *i.e.* how fast does it grow within 24 hours of duration [25].

## 2.8. Pathogenicity Test for Fusarium Isolates

The pathogenicity of the isolated *Fusarium* species was

tested through bioassay on detached leaves of the Tomato cultivar-Melka oda grown on pots following standard procedure [26]. Healthy looking vigorous tomato leaves cultivated on pots with autoclave sterilized soil were used for the bioassay. Sixty (60) days old leaves were cut from the parent plant and transported to the laboratory. The leaves were surface sterilised with 70% ethanol for 30 seconds, rinsed with three successive changes of sterile distilled water and blot dried. The leaves were placed in sterile plates and were inoculated with 500µl spore suspension of *Fusarium oxysporum*. The control leaves were inoculated with the same amount of sterile distilled water and the treatments were in three replicates. The plates were incubated at 25± 2° C until the emergence of mould growth was observed on the surface of the leaves. Observations were taken starting from the second day where the lesion diameter was recorded.

## 2.9. Biocontrol Potential of T. Viride against F. Oxysporum by in vitro Biocontrol Test

Five *Trichoderma* isolates initially obtained from soil samples taken from garden areas of Hatsebo and Medoge kebeles were screened through preliminary *in vitro* bioassay of dual culture with one of the pathogenic *Fusarium* isolates. *Trichoderma* with the better biocontrol potential was used for the main experiment. The biocontrol potential of *T. viride* was evaluated using a dual culture assay. The percentage growth inhibition (PGI) was calculated using the following formula.



Fig. (1). Procedure of isolating and culturing Trichoderma species from rhizosphere soil region.

$$PGI = \frac{(C-T)}{C} \times 100\% \quad \text{Where: } C = \text{radial growth of } F. \text{ oxysporum \textit{ on the control plate}}$$

$$T = \text{radial growth of } F. \text{ oxysporum \textit{ on the dual culture}}$$

### 2.10. Data Collection

Qualitative data such as aerial mycelium, colony colour, reverse colour and marginal shapes were recorded. Quantitative data such as radial growth and growth rate, percent growth inhibition (PGI) were recorded by measurement using metric system units.

## 3. RESULTS AND DISCUSSION

### 3.1. Isolation

Upon incubation at 25± 2° C for three to seven days, mycelial masses of fungi emerged on top of the inoculated specimen (Fig. 2). After three subsequent sub-culturing and microscopic examinations, eight pure cultures of *Fusarium* were identified. 87.5% (7 isolates) of the *Fusarium* were found from the tomato samples collected from Dura plots. 12.5% (1 isolate) was found from the tomato samples collected from the Hatsebo plots. Five *Trichoderma* isolates are found from soil samples taken from garden areas of Hatsebo and Medoge kebeles. To the best of our knowledge, this is the first investigation conducted on *Fusarium* and *Trichoderma* species from the farmlands around Axum town, the centre of ancient Axumite civilization, where scientific studies are emerging

following the establishment of Aksum University as a governmental higher learning institution. It also begins to fill gaps at the country level with information on the importance of *Fusarium* species. That hitherto, has largely been lacking [27].

### 3.2. Detached Leaf Bioassay Test of *Fusarium* Species on Tomato Leaves

Pathogenicity tests of the isolated *Fusarium* species on detached leaves of tomato are presented in Table 1. As indicated in the table, two of the *Fusarium* isolates (DT1 and DT2) caused 100% leaf damage on the tomato leaves. The control leaves that received sterile distilled water were free from any mould development, whereas the leaves that received *Fusarium* spore suspension showed mould growth on their surface and finally became wounded (Fig. 3). This implies that the *Fusarium* species isolated from the tomato tissues are pathogenic to the tomato variety, Melka oda used for this test. *F. oxysporum*, *F. solani*, *F. equiseti* and *F. striatum* have been reported to affect tomato [10]. But the finding that *F. circinatum* causes injury on tomato leaves is a first-time report by the current investigation. This also strengthens reports that indicate new pathogenic *Fusarium* species are continually evolving [10].

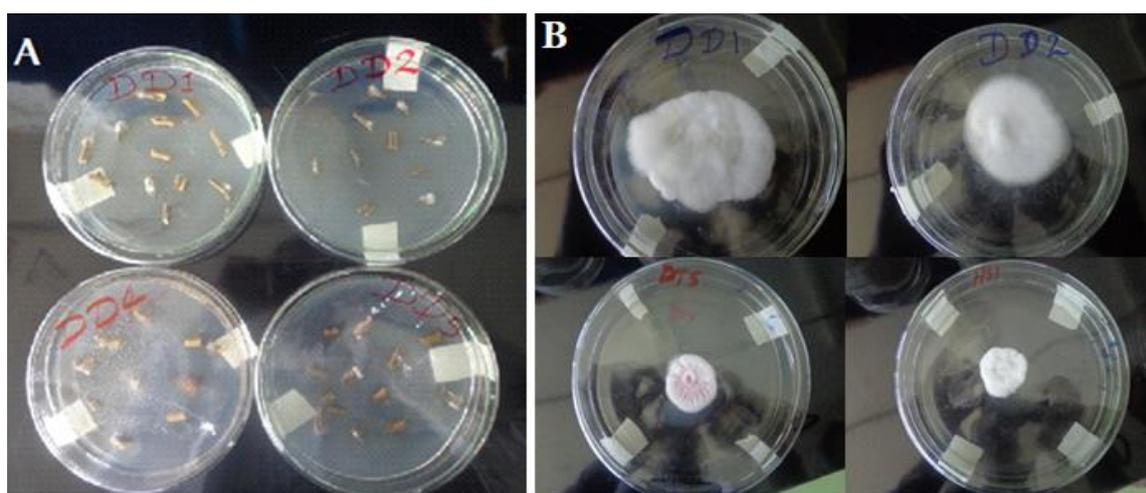


Fig. (2). Fungal hyphae emerged from tomato tissues transplanted onto PDA medium (A), cultures after primary subculture (B).

Table 1. Pathogenicity test of the *Fusarium* isolates by detached leaf bioassay.

No.	Treatments	Isolate code/species	Percent leaf damage value
1	Sterile distilled water	control	0%
2	Fusarium spore suspension-1	DD1( <i>F. circinatum</i> )	56.56%
3	Fusarium spore suspension-2	DD2w ( <i>F. equiseti</i> )	60.46%
4	Fusarium spore suspension-3	DD2 ( <i>F. oxysporum</i> )	70.27%
5	Fusarium spore suspension-4	DD5 ( <i>F. oxysporum</i> )	68.48%
6	Fusarium spore suspension-5	DT1 ( <i>F. oxysporum</i> )	100%
7	Fusarium spore suspension-6	DT2 ( <i>F. oxysporum</i> )	100%
8	Fusarium spore suspension-7	DT5 ( <i>F. oxysporum</i> )	90.5%
9	Fusarium spore suspension-8	HS1 ( <i>F. oxysporum</i> )	92.5%

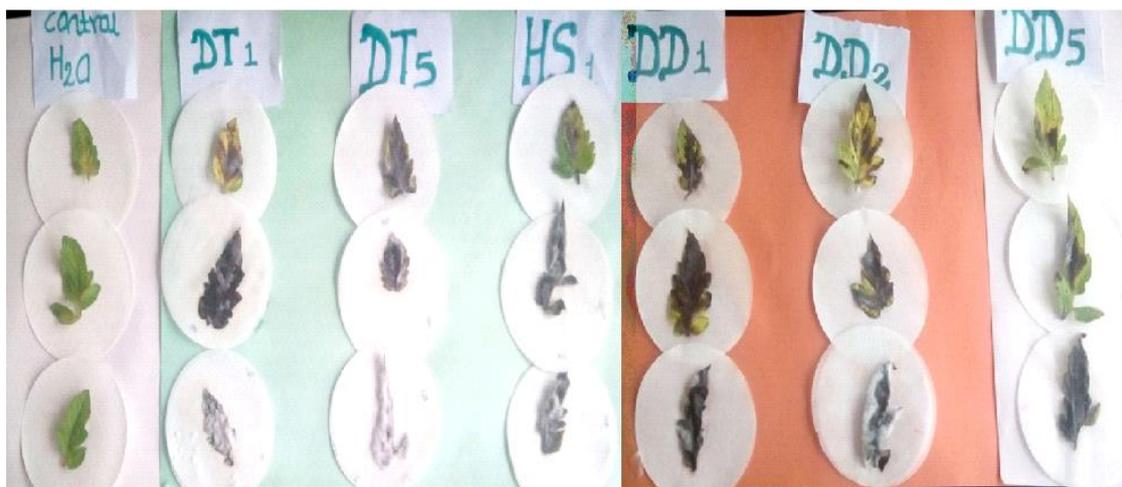


Fig. (3). Bioassay of *F. oxysporum* f. sp. *lycopersici* on detached tomato leaves.

### 3.3. Cultural Characteristics of Fusarium Isolates on PDA

The cultural characters of the eight *Fusarium* isolates are presented in Table 2. The isolates showed purple tinged white, white cottony and full pink colony colours (Table 3). On the reverse side, the isolates showed various pigmentations (light reddish purple, gray with a light purple tinge, yellow stained

white, normal purple and intense dark reddish purple) (Fig. 4), as reported in a previous study [10]. The growth rate of the isolates ranged from 4.66mm to 7.28mm which is far less than that of FOL isolates from Mexico-7cm-12cm [10]. Two of the isolates are rated as fast growing (DD1 and DT1) and the remaining were medium growing (DD2, DD2w, DD5, DT2, DT5, HS1).

Table 2. Radial growth and growth rate of *Fusarium* isolates on PDA at 25± 2° C.

No.	Isolate	Radial growth 7DAI (mm)	Mean Growth rate in 24 hrs (mm)	Ratings: Fast/medium/slow	Species identity	Percentage occurrence
1	DD1	52.13	7.28	Fast growing	<i>Fusarium circinatum</i>	12.5%
3	DD2w	40.5	5.98	medium	<i>Fusarium equiseti</i>	12.5%
2	DD2	43.13	6.44	medium	<i>Fusarium oxysporum</i>	75%
4	DD5	46.16	6.83	medium	<i>Fusarium oxysporum</i>	
5	DT1	54.25	8.27	Fast growing	<i>Fusarium oxysporum</i>	
6	DT2	41.75	5.98	medium	<i>Fusarium oxysporum</i>	
7	DT5	33.38	4.66	medium	<i>Fusarium oxysporum</i>	
8	HS1	41.50	6.10	medium	<i>Fusarium oxysporum</i>	

Abbreviation: DAI= Days after inoculation and the categorization is according to Agriculture and Agrifood Canada, 1996.

Table 3. Cultural and Morphological characters of the identified *F. oxysporum*.

Character	Character descriptor		Description
Cultural characters	colony color	surface	Pink floccose mycelium -Regularly circular -flattened spreading with concentric circles
		reverse	reddish purple
	pigmentation	Produced Intense reddish purple pigment	
Morphological characters	macroconidia	Single, short, hyaline, appeared in single on PDA medium.	
	microconidia	Ellipsoidal shape and non septated	
	chlamydo-spore	Abundant pseudo-chlamydo-spores	

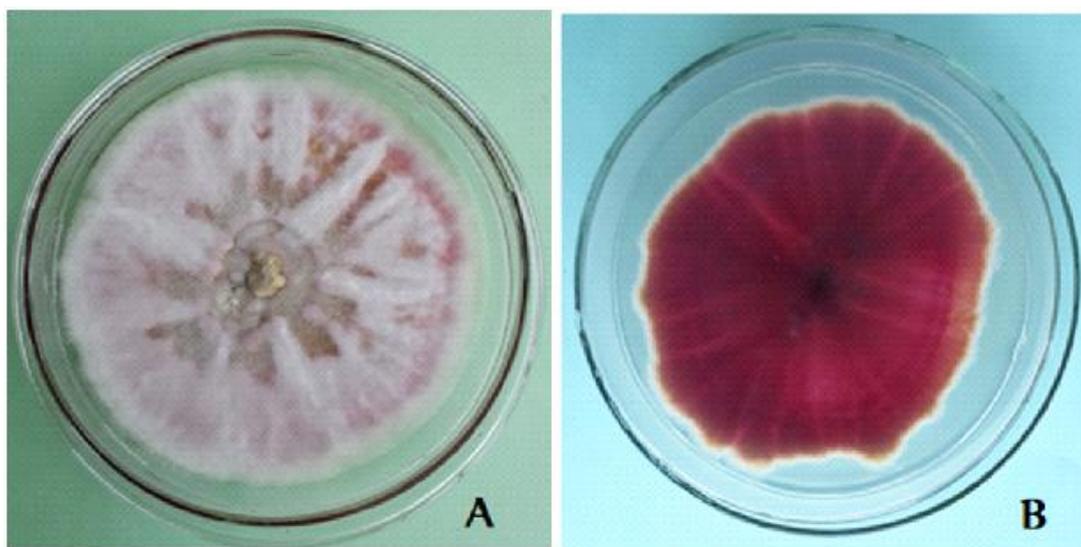


Fig. (4). colony appearance of *F. oxysporum* (A) front side, (B) back side/pigmentation.

### 3.4. Morphological Characteristics of *Fusarium* Isolates Grown on PDA

The morphological character of the *Fusarium* isolates is presented in appendix 1. Generally, macroconidia are more or less abundant, 3-5 septate, straight and with more ventral curvature than dorsal in *F. oxysporum* (Fig. 5). In *F. circinatum*, macroconidia with 3 septa, thinner and slender occasionally found in bunch and chlamydo spores are absent. In *F. equiseti*, macroconidia were with prominent foot shape, abundant on the aerial mycelium with the interwoven state. These morphological features are similar to the descriptions of

the respective *Fusarium* species [23, 25]. *Fusarium oxysporum* and *Fusarium equiseti* are commonly isolated from soil and plant tissue samples in Ethiopia [27]. As to our information, the isolation of *Fusarium circinatum* from diseased tomato tissues in Ethiopia is the first-time report by this investigation. Similarly, researchers from Mexico also identified *Fusarium circinatum* from diseased tomato that is confirmed by a molecular method [10]. From the isolated *Fusarium* cultures, *F. oxysporum* accounted for 75% of occurrence, whereas the *Fusarium equiseti* and *Fusarium circinatum* occurred each with 12.5%. The occurrence rate is also similar to a previous report [10].

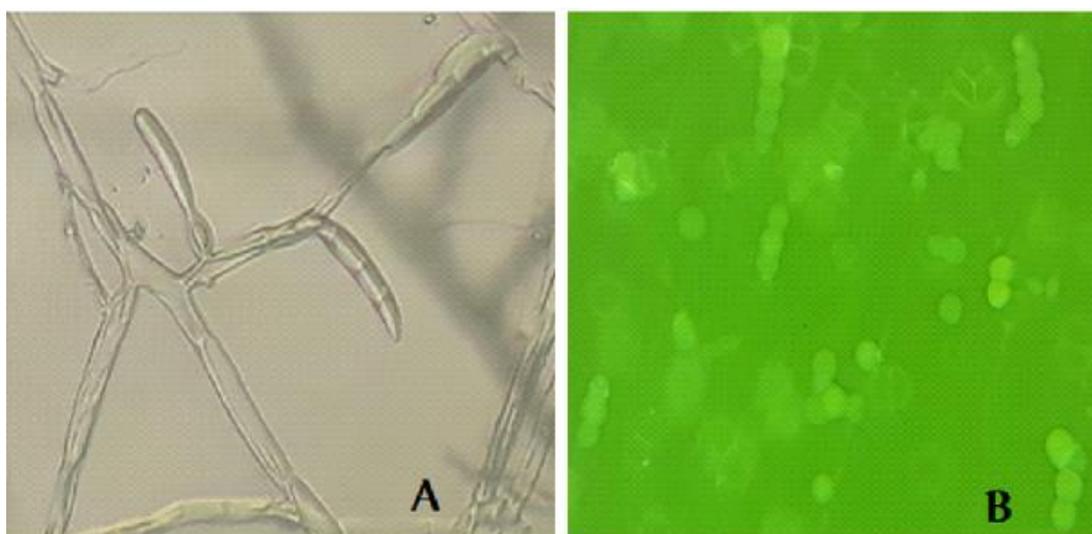
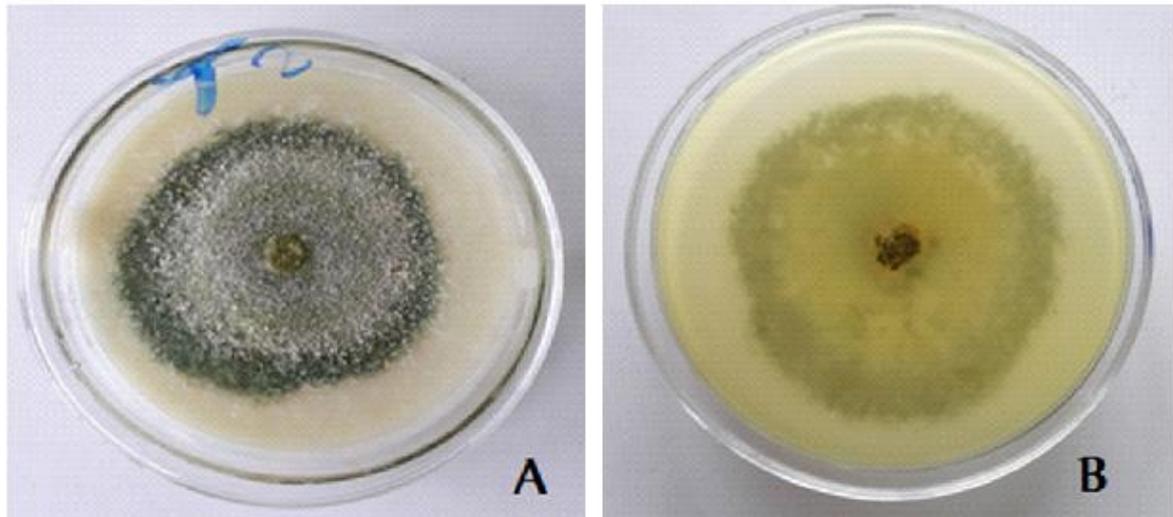


Fig. (5). Banana shaped Macroconidia with 3 septa (A) and fluorescent microscopy of microconidia of *F. oxysporum* in chains and in clusters (B) at 400x magnification.



**Fig. (6).** front culture appearance of *Trichoderma viride* with concentric circles (A), back side (B).

### 3.5. Cultural Characteristics of the *Trichoderma* Isolate with Biocontrol Potential

White scanty mycelia in the early 1-3 days; Warty green concentric rings in 4-5 days; whole warty green culture in 5-7 days and yellowish green pigment were observed on the reverse side [28] (Fig. 6).

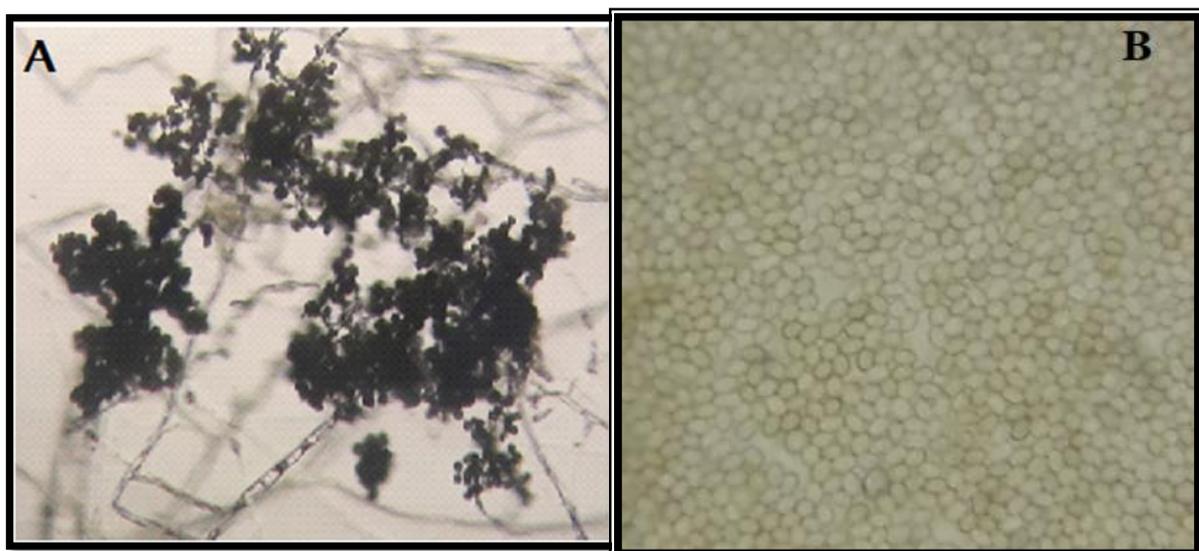
### 3.6. Radial Growth and Growth Rate Data of the *Trichoderma* Isolate

The isolated *Trichoderma* species has a mean radial growth of 100mm 3days after inoculation and a mean growth rate of 31.70mm per 24 hours. The mean radial growth value of the current *Trichoderma* isolate is very high as compared to a previous report from Ethiopia, which was 57.67mm, 7DAI on

PDA and with similar growth conditions [29]. The recorded growth rate is also higher than a previous report where the measurements were 28mm-30mm [30]. The implication of this is that the current *Trichoderma* isolate has better biocontrol potential (Table 4).

### 3.7. Microscopic Features of the Isolated *Trichoderma*

Microscopic examination of the morphology of *Trichoderma* isolates revealed dark green grossly warty conidia conspicuously observed with irregular ornamentations and oval conidia (Fig. 7). The microscopic features of this *Trichoderma* isolate as examined using the Olympus system microscope is similar to the description of *Trichoderma viride* [31]. Accordingly, the *Trichoderma* isolate is identified as *Trichoderma viride*.



**Fig. (7).** dark green conidiophores (A) and oval conidia of *T. viride* (B) at 400x magnification.

**Table 4. Radial growth and Growth rate data of the *Trichoderma* isolate.**

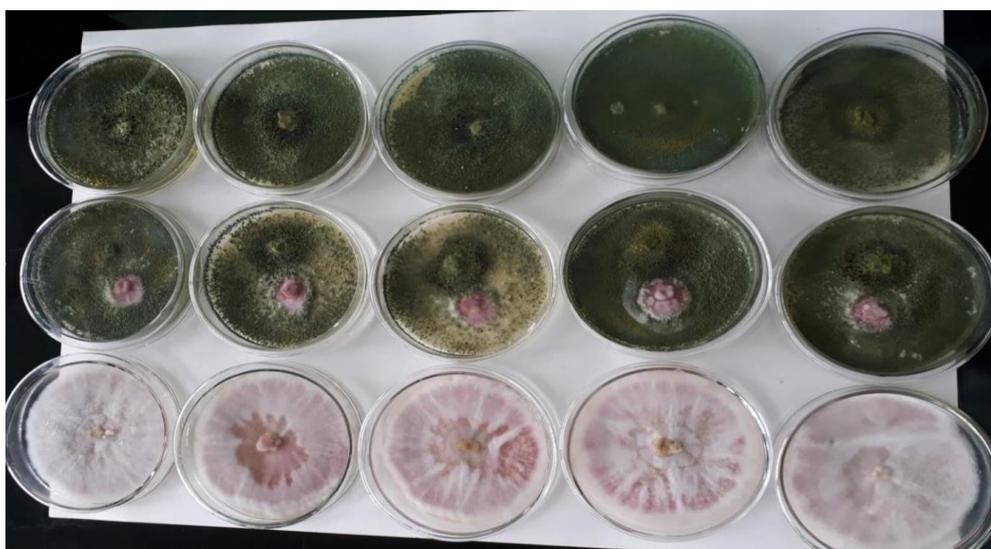
Isolate	Mean radial growth record (mm)			Mean growth rate/24 hrs (mm)		
	1DAI	2DAI	3DAI	GR-1	GR-2	Mean GR
Trichoderma	36.70	65.90	100	29.20	34.10	31.70

Note: These data are means of five replicates, DAI= Days after inoculation, GR= Growth rate

**Table 5. Percent growth inhibition value of *T. viride* on *F. oxysporum*.**

Mean radial growth of <i>Trichoderma</i> species in control plate (mm)	Mean radial growth of <i>F. oxysporum</i> in control plate (mm)	Mean radial growth of <i>F. oxysporum</i> in dual culture (mm)	Mean %GI
100	100	22.00	76.94%

Abbreviation: %GI= Percent growth inhibition, the data is mean of five replicates



**Fig. (8).** *F. oxysporum* fsp *lycopersici*. vs *T. viride* dual culture assay: the top row plates are: *T. viride* control cultures; the bottom row plates are *F. oxysporum* fsp *lycopersici* control culture and the middle row of plates is the dual culture assay showing *T. viride* dominated and invaded *F. oxysporum*.

**3.8. In Vitro Biocontrol Experiment**

*in vitro* dual culture assay of the pathogenic *F. oxysporum* and the biological control agent, *Trichoderma* species, is presented (Table 5). In the *in vitro* dual culture experiment, the *Trichoderma* isolate showed high biocontrol efficacy (76.94%). This PGI value is higher than a previous report (61.80% - 72.05%) [32]. The *Trichoderma* showed a very fast spread to cover the entire space on the medium during the first three days, restricting the growth of the *F. oxysporum* fsp. *lycopersici* within a narrow cleft. Later, the *Trichoderma* started shuttering its conidia on top of its own colonized space. Finally, the *Trichoderma* species also shuttered its conidia on top of the encapsulated *F. oxysporum*. *F. oxysporum* showed colony expansion until the *Trichoderma* approached near its vicinity; then, the *Fusarium* was totally under control and colonized by the shots of *T. viride* conidia resting and germinating on its colony, leading to a mycoparasitic activity on the *Fusarium oxysporum*. f. sp. *lycopersici* (Fig. 8).

**CONCLUSION**

Isolation and morphological identification revealed *Fusarium oxysporum*, *Fusarium equiseti*, and *Fusarium*

*circinatum* in the tomato growing farmlands around Axum town. *Fusarium oxysporum* fsp. *lycopersici* is the dominant isolate in its occurrence. The isolates were consistent in their cultural characters across different batch cultures on the PDA medium. Detached leaves bioassay is a good indicator for *in vivo* pathogenicity of *Fusarium oxysporum* on tomato. The *Trichoderma viride* identified in this study is an effective antagonist for identified *F. oxysporum* fsp. *lycopersici* *in vitro*. It showed competition for space and nutrients as well as mycoparasitism against the phytopathogen, *Fusarium oxysporum*.

**ETHICS APPROVAL AND CONSENT TO PARTICIPATE**

Not applicable.

**HUMAN AND ANIMAL RIGHTS**

No human or animal were used in the study.

**CONSENT FOR PUBLICATION**

Not applicable.

**AVAILABILITY OF DATA AND MATERIALS**

All the data and materials pertaining to this research article are within the manuscript.

**FUNDING**

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**CONFLICT OF INTEREST**

The authors declare no conflict of interest, financial or otherwise.

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The authors have agreed upon the publication of this research article in The Open Agriculture Journal. The authors duly acknowledge Aksum University for funding the research project.

**APPENDIX****Appendix 1. Cultural and Morphological characteristics of the *Fusarium* isolates on PDA medium.**

No	Isolate code	Cultural characters of isolates on PDA			Morphological characters on PDA			Species identity identified as
		Colony color		Pigmentation	macroconidia	microconidia	chlamydo spores	
		surface color	reverse color					
1	DD1	Purple tinged white with furry aerial mycelium. Regularly circular radial growth	Light reddish purple	Light reddish purple	Mostly 3-septated, slender narrow Arranged in bunch formed on the aerial mycelium. Not abundant	Berry looking phialides arranged in clusters, chains and in single are abundantly observed	Not seen	<i>Fusarium circinatum</i>
2	DD2	-Purple tinged white aerial mycelium -Regularly circular radial growth -no concentric circles	Gray with light purple tinge	light red tinged pigment	Short stout macroconidia with 3-5 septa arranged in bunch were Abundant	Short monophialides were more frequently observed. Ellipsoidal Microconidia	Pseudo chlamydo spores were abundantly observed	<i>Fusarium oxysporum</i>
3	DD2w	-White cottony aerial mycelium -irregular in marginal growth with in and out curvatures on the outside -no concentric circles	Yellow stained white	light	Highly abundant macroconidia formed on the aerial mycelium in bunch form. Highly twisted and interwoven	absent	Chlamydo spores rarely observed	<i>Fusarium equiseti</i>
4	DD5	-Purple tinged white colony with furry mycelium _regularly circular radial growth pattern	Gray with light purple tinge	-No pigmentation	Abundant macroconidia in bunch; 3-5 septated, shorter and straight	Elliptical microconidia in rare presence Short phialides	present	<i>Fusarium oxysporum</i>
5	DT1	Whitish purple, furry aerial mycelium Dense and spreading growth -Regularly circular Radial growth With no concentric circles	normal purple	Pigment: light red tinged purple	Long non septated hyaline; straight and pointed on both ends	Large ball looking monophialides formed on the aerial mycelium	present	<i>Fusarium oxysporum</i>

(CRRGF/FZ/ Table 3) contd....

No.	Cultural characters of isolates on PDA				Morphological characters on PDA			Species identity
	Isolate code	Colony color		Pigmentation	macroconidia	microconidia	chlamydo spores	identified as
		surface color	reverse color					
6	DT2	-Purple tinged white aerial mycelium with furry appearance -Regularly circular growth Spreading growth	Gray with light purple tinge	No	macroconidia with 3-5 septa arranged in bunch abundantly seen	Ellipsoidal microconidia	Abundant pseudo chlamydo spores	<i>Fusarium oxysporum</i>
7	DT5	-Pink floccose mycelium -Regularly circular -flattened spreading with concentric circles	Intense dark reddish purple; yellowish bright at the margin Golden bright on the margin	Intense reddish purple pigment	macroconidia with 3-5 septa arranged in bunch abundantly seen	Mostly ellipsoidal in shape; non septated, hyaline; inside the agar. Dark ballus and branched short phialides.	Abundant pseudo chlamydo spores	<i>Fusarium oxysporum</i>
8	HS1	White fluffy aerial mycelium Irregular lobed radial growth No concentric circles	Yellow tinged white	light yellowish pigment	Septated macroconidia curved from both ends. Narrow in the middle. Formed on the mycelium and also detached. Mostly in single; fusiform.	Spherical to elliptical microconidia	Abundantly seen in pairs, in chains and in cluster at intercalary and apex position.	<i>Fusarium oxysporum</i>

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