Ethiop. J. Biol. Sci. **21(2): 171–190, 2022**

ISSN: 1819-8678

RESEACH ARTICLE

CHARACTERIZATION OF *ALTERNARIA* SPECIES CAUSING TARO (*COLOCASIA ESCULENTA* (L.) SCHOTT) LEAF BLIGHT AND EVALUATION OF THEIR SUSCEPTIBILITY TO FUNGICIDES AND *TRICHODERMA* BIOCONTROL AGENTS

Winta Berhie[1] and Tesfaye Alemu[1]*

ABSTRACT: The output of taro in Ethiopia's main growing regions has recently suffered significantly from *Alternaria* species infection, both in terms of quantity and quality. In the present study, *Alternaria* species that cause taro leaf blight were isolated, characterized, and their susceptibility to *Trichoderma* biocontrol agents and chemical fungicides were assessed under *in vitro* condition. Using food poisoning procedures and the dual culture method, the isolates' susceptibility to fungicides and biocontrol agents was examined. The conidial length and breadth of the *Alternaria* isolates were 26.10–42.50 µm and 10.20–18.50 µm, respectively. The maximum mycelial growth of the isolates was supported by the host leaf extract medium. The pathogenic isolates grew best in optimum growth conditions with an incubation temperature of 25°C and a pH level of 6.5. Even at low doses, sancozeb was more effective than curzate against *Alternaria* isolates. Additionally, compared to *T. harzianum*, the biocontrol agent *T. viride* showed a greater mycelial growth inhibition of the test pathogen. This suggests that *Alternaria* isolates that cause taro leaf blight are susceptible to biocontrol agents. Therefore, it was advised that *Trichoderma* species be tested for effectiveness against the test pathogens in both greenhouse and field conditions.

Key words/phrases: *Alternaria* species, Chemical fungicides, Susceptibility, Taro, *Trichoderma* species.

INTRODUCTION

The taro (*Colocasia esculenta* (L.) Schott), a perennial root and tuber crop farmed for a variety of uses, is an important socioeconomic factor in tropical regions. It is a member of the Araceae family (often known as aroids). Taro has a primary corm that lies just below the soil's surface and develops to a height of 1–2 meters. From this corm, leaves and roots grow upward and downward, while daughter corms and runners develop laterally (Ubalua *et*

[1]Department of Microbial, Cellular, and Molecular Biology, College of Natural and Computational Sciences, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia. E-mail: tesfaye.alemu@aau.edu.et
*Author to whom all correspondence should be addressed

al., 2016). It has long petioles, fibrous roots, and cylindrical or frequently irregular nutrition storage organs (corms) in addition to heart-shaped green or purple leaves (Matthews *et al*., 2012).

Taro is one of the world's oldest food crops grown primarily for its edible corms to maintain food security. Ethiopia, Hawaii, the Caribbean, Papua New Guinea, Southern Africa, Malaysia, and Bangladesh are just a few of the countries where it has been grown internationally (Wang *et al*., 2017). In Ethiopia, smallholder farmers have grown taro as a staple food crop primarily and extensively in the country's densely populated and high-rainfall south, southwest, and western regions (Tewodros Mulualem, 2013). Taro contains a lot of nutrients, fiber, and carbohydrates in its petiole, leaves, and corms (Mandal *et al*., 2013).

Despite its economic significance, biotic and abiotic factors pose a threat to taro production. Leaf blight caused by numerous fungal pathogens is one of the significant biotic factors affecting crop outputs (Ruisi *et al*., 2017). In recent years, the main taro-growing regions of Ethiopia have experienced significant production reduction and quality deterioration due to *Alternaria* species' taro leaf and stem diseases (Mandal *et al*., 2013). Because *Alternaria* species has a short life cycle, the disease develops and spread quickly (Alvarenga *et al*., 2016). It spreads from one area to another through various pathways, such as airborne conidia and soil adhesion to seedlings, farm equipment, or animals (Nayyar *et al*., 2017).

Chemical fungicides are typically used to combat the *Alternaria* disease of crops (El-Gali, 2015). Fungicides, however, pose risks to both human health and the environment (Dahal and Shrestha, 2018). A highly essential and effective strategy against *Alternaria* species which causes diseases on a variety of crops, is the use of fungal biocontrol agents for plant disease management. *Trichoderma* species due to its several mechanisms of action that prevent the growth of other fungi, is an excellent candidate for the control of crop diseases (Mendoza *et al*., 2015). Therefore, the objective of the present study was to isolate and characterize *Alternaria* species from taro infected stem and leaves, as well as to evaluate their susceptibility to *Trichoderma* species and chemical fungicides under *in vitro* condition.

MATERIALS AND METHODS

Sample collection and sampling techniques

A total of 22 samples of infected taro leaves and stem with typical symptoms were randomly chosen from different locations in the

southwestern Ethiopian zones of Jimma, Keffa, and Benchi Maji. The samples were carried to the College of Natural and Computational Sciences at Addis Ababa University's Mycology laboratory in plastic bags in order to isolate the *Alternaria* species that infected Taro. It was stored at 4°C for further use. Host leaf extract were washed, air dried, and pulverized using a Retsch electrical grinding mill (Hulme-Martin Ltd, UK). Powder (20 g) of each sample specimen was macerated separately in 200 ml of analytical grade methanol, and sterilized distilled water for three days.

Isolation of *Alternaria* species

The excised diseased taro leaves and stems were first cleaned in sterile distilled water in small (1 cm) pieces. The items were then surface-sterilized by submerging them in 2% sodium hypochlorite (NaOCl) for one minute, washing them in 70% alcohol for one minute, rinsing them in sterile distilled water for one minute, and allowing them to dry on sterile tissue paper in a biosafety cabinet (Mandal *et al*., 2013). On 90 mm Petri dishes containing sterilized potato dextrose agar (PDA) media amended with streptomycin sulfate, the sterilized fragments of infected taro leave and stems were inoculated and incubated at 25°C for three days. As a control, 0.1 ml of the most recent washes was spread out over PDA medium to evaluate the effectiveness of surface sterilization. Using single hyphal tip techniques recommended by Ahmed and El-Fiki (2017), the isolates' actively growing portions were purified on the PDA, and the pure isolates were then cultivated on the PDA slant as stock cultures.

Morphological characterization and identification

The isolates' macroscopic cultural morphology examinations were carried out on PDA using the standardized microbiological methods. Throughout the incubation phase, colony form and other cultural traits were visible with the naked eye. Two-day intervals were used to measure colony expansion. Ten days following incubation, the colours and textures of the colonies were evaluated (Ahmed and El-Fiki, 2017). The microscopic structures of the isolates were also studied using slide cultures of pure *Alternaria* isolates. Using an Olympus microscope, the slides were examined at low power (10x) and medium power (40x). To display the typical spore morphology of the fungal isolates, microphotography was done (Sangdee *et al*., 2011). Then, the fungal isolates were identified as *Alternaria* species based on the macroscopic and microscopic features. The *Alternaria* isolates that were identified were given the designations AUT2, AUAT3, AUAT4, AUAT7, AUAT20, and AUAT21. The prefix 'AU' stands for Addis Ababa

University, the letters 'AT' stand for the *Alternaria* from taro plant, and the numbers were used to distinguish various isolates.

Study of different growth conditions

In this study, parameters such as culture medium, temperature, pH, moisture, and nutritional requirements were examined.

The effect of different culture medium on the mycelial growth of the test pathogen

To evaluate their effects on the isolates' mycelial growth, host extract agar (HEA), PDA, malt extract agar (MEA), and Czapek Dox agar (CDA) media were utilized. Each sterilized agar plate was centred with a 5 mm diameter agar disc that had been cut from the edge of the pure culture of each *Alternaria* isolate. At 25°C, the plates were incubated. After six days of incubation, the mycelial growth diameter for each of the three replications was measured (Smita and Dhutraj, 2017).

The effect of temperature on the mycelial growth of the test pathogen

Cultures were grown on PDA media and incubated at 15, 20, 25, 30, 35, and 40°C until full growth of the mycelia in a 90 mm plate (You *et al*., 2016) to evaluate the ability of *Alternaria* isolates to grow at restrictive temperatures. For each treatment, three replications were kept. Each isolate's colony diameter was measured in millimeters every two days, with the actual measurements being taken on the eighth day of incubation.

The effect of pH on the mycelial growth of the test pathogen

To achieve the ideal and adequate pH value for the growth of the test pathogens, potato dextrose broth (PDB) (200 g potato, 20 g dextrose per liter of sterilized water) was made and adjusted to 3.5, 4.5, 5.5, 6.50, and 7.50 pH values (You *et al*., 2016). Five mm diameter inoculum discs were placed in each of five 250 ml flasks containing 100 ml PDB. These discs were collected from the edge of a 7-day-old culture cultured on PDA. For each treatment, triplicates were kept and incubated for 14 days at 25 to 30°C. A sensitive digital balance was used to measure the biomass after filtering (Sartorius, Germany).

Carbon source utilization of the test pathogen

Richard's agar media had 20 g of agar-agar, 50 g of sucrose, 10 g KNO_3, 5 g KH_2PO_4, 2.5 g $MgSO_4$, 0.02 g $FeCl_3$, and 2.5 g of sucrose. In this medium, the fungus was grown, and sucrose was individually replaced by different

carbon sources (such as glucose, maltose, and fructose) in equal amounts. After sterilization, the medium was added to Petri plates. Five millimeter mycelial discs of *Alternaria* isolates were inoculated in triplicate on each plate containing 25 ml of basal media that had been replaced with various carbon sources, and the plates were then incubated at 25°C. Carbon sources were absent from the control plates. Up to 10 days, mycelial development was noted every 2 days (Ramjegathesh and Ebenezer, 2012).

Nitrogen source utilization of the test pathogen

We investigated the impact of various nitrogen sources on the fungus's mycelial growth using Richard's basal media. By substituting 10 g of KNO_3 with alternative nitrogen sources, the same amount of nitrogen was added. The basal medium was solely used as a control. For each treatment, duplicates were kept. Petri plates containing the sterilized media were then infected with 5 mm mycelial discs of the *Alternaria* isolates. All treatments were cultured at 25°C, and the test fungus's mycelial growth was monitored every two days until it reached 10 days (Shaikh *et al*., 2018).

***In vitro* pathogenicity test**

Taro plant stem fragments and detached leaves were used in Erlenmeyer flasks and plates for the *in vitro* pathogenicity test in growth chambers. To reduce surface contaminants, healthy leaflets and stems were disinfected with 70% ethanol and then washed with distilled water before being rinsed. The disinfected taro leaves and stems were cut into 1 cm square pieces and thoroughly dried in a laminar airflow hood before being utilized as a host for the test (Kayim *et al*., 2018). Using a sterile cork-borer, 4 mm discs of *Alternaria* isolates were extracted. A 250 ml flask containing 100 ml of PDB and three discs of the isolate were infected, and the mixture was then cultured at 25°C for 10 days. Whatman No. 42 filter papers were used to filter the mycelial mats, which were then properly cleaned. A haemocytometer was used to regulate the conidia suspension's concentration to 1×10^6 conidia/ml (Khaledi and Taheri, 2016).

Inoculation of the leaves

The *Alternaria* isolates were inoculated into surface-sterilized sections of leaves and stems using a 0.1 ml conidia suspension (1×10^6 conidia/ml). They were then put on sterilized slides on sterilized Petri plates and flasks that contained filter paper that had been impregnated with sterilized distilled water. Sterile distilled water was used to inoculate the control leaf and stem fragments. For each treatment, triplicates were kept and cultured for 20 days

at 25°C. Every two days, sterile distilled water was added to the plates and flasks to keep the atmosphere moist. Based on the colonization and mortality of the leaves and stems as well as their symptoms, virulence was established. According to the method given by Kayim *et al*. (2018), the measurement of pathogenicity was displayed as the scaling percent of tissue infections and sporulation beneath the microscopic field.

The effect of fungicides on the mycelial growth of the test pathogen

Sancozeb (mancozeb 80%) and curzate, two fungicides with different active components that are readily available on the market, were purchased from the Addis Ababa market. Curzate is a systemic fungicide made up of Copper Oxychloride 39.75% + Cymoxanil 4.2%. Under *in vitro* testing circumstances, the potency of these fungicides to inhibit mycelial growth against *Alternaria* isolates was evaluated. A stock solution with a concentration of 1,000 ppm was created for each fungicide before the commercial formulation (Gautam *et al*., 2017). To obtain the final concentrations of 100, 200, 400, 600, 800, and 1000 ppm in food poisoning procedures, sterilized PDA medium and sulfate streptomycin were added to the calculated amount of the stock solution of a fungicide. In the control set, the PDA medium received the requisite dosage of sterile water rather than fungicide. For 14 days, plates with *Alternaria* inoculations were incubated at 25 to 30°C. For each treatment, duplicates were kept. Colony diameter measurements were used to gauge the growth of isolates at each concentration.

Dual culture antagonistic evaluation of *Trichoderma* species

Trichoderma harzianum and *T. viride* were collected from Addis Ababa University's Mycology laboratory, which is part of the department of Microbial, Cellular and Molecular Biology in the College of Natural and Computational Sciences. In a dual culture, a 4 mm-diameter disc of a seven-day-old *Trichoderma* species was placed on PDA plates 10 mm from the edge, and a disc of the same size and a seven-day-old test pathogen was placed on the *Trichoderma* species' opposite side (Islam *et al*., 2018). As a control, a seven-day-old *Alternaria* isolate culture on an agar disc was positioned in the middle of the Petri dish. Triplicates were also kept. All of the plates were incubated at 25°C until the control had fully grown. Every two days, visual observations of growth inhibition were noted, and the final measurements were taken on the tenth day following inoculation. According to Koka *et al*. (2017), radial growth reduction was estimated as the percent inhibition (decrease in radial mycelial growth) and computed below.

$$PI = (C - T /C)/C \times 100$$

Where, C denotes the pathogen's radial growth measurement in the control plates and T represents the pathogen's radial growth in the experimental plates.

Statistical data analysis

One-way ANOVA was used in the data analysis with SPSS statistical software version 20 to compare means at a 5% confidence level ($P \leq 0.05$).

RESULTS

Morphological characterization and identification of the fungal pathogen

By using a regular tissue isolation procedure, 22 *Alternaria* isolates were isolated from the taro diseased leaves and stem. Based on the fungus's physical and cultural characteristics, the isolates were identified. The *Alternaria* cultures ranged in colour from grey to light brown, with some having dark brown reverses. On PDA media plates, they produced mycelia with smooth, elevated, puffy, and regular to irregular borders. Conidial length varied, reaching a maximum of 42.5 µm in isolate AUAT4 and a minimum of 26.10 µm in AUAT2. Conidial breadth also varied from 10.20 to 18.50 µm, with isolate AUAT2 having the smallest breadth and AUAT20 having the most. Five to seven septation were present in all of the isolates (Table 1 and Fig. 1).

Table 1. Variability in the conidia of *Alternaria* isolates.

Fungal isolates	Length (µm)		Breadth (µm)		Conidial features		Identified as
	Range	Mean ± sd	Range	Mean ± sd	Shape	Septation	
AUAT2	26.1–38.3	31.9 ± 6.9^{c}	10.2–15.8	12.9 ± 1.5^{c}	Muriform	5–7	*Alternaria* spp
AUAT3	30.5–40.5	36.2 ± 4.1^{b}	10.9–17.3	13.8 ± 2.5^{b}	Muriform	5–7	*Alternaria* spp
AUAT4	27.3–42.5	36.4 ± 7.1^{b}	11.6–16.9	14.4 ± 2.1^{b}	Muriform	5–7	*Alternaria* spp
AUAT7	32.3–39.4	36.0 ± 3.4^{b}	11.3–17.9	14.5 ± 1.7^{b}	Muriform	5–7	*Alternaria* spp
AUAT20	31.72–42.3	37.4 ± 3.9^{a}	12.2–18.5	15.4 ± 1.8^{a}	Muriform	5–7	*Alternaria* spp
AUAT21	28.7–41.2	34.5 ± 6.5^{c}	11.3–15.6	13.3 ± 1.2^{b}	Muriform	5–7	*Alternaria* spp
Average	26.1–42.5	35.4 ± 1.9	10.2–18.5	14.1 ± 0.9			
CV (%)		5.5		6.4			
P-value		0.0001		0.0001			

Key: Means followed by the same letter within a column are not significantly different ($P<0.05$), sd: standard deviation

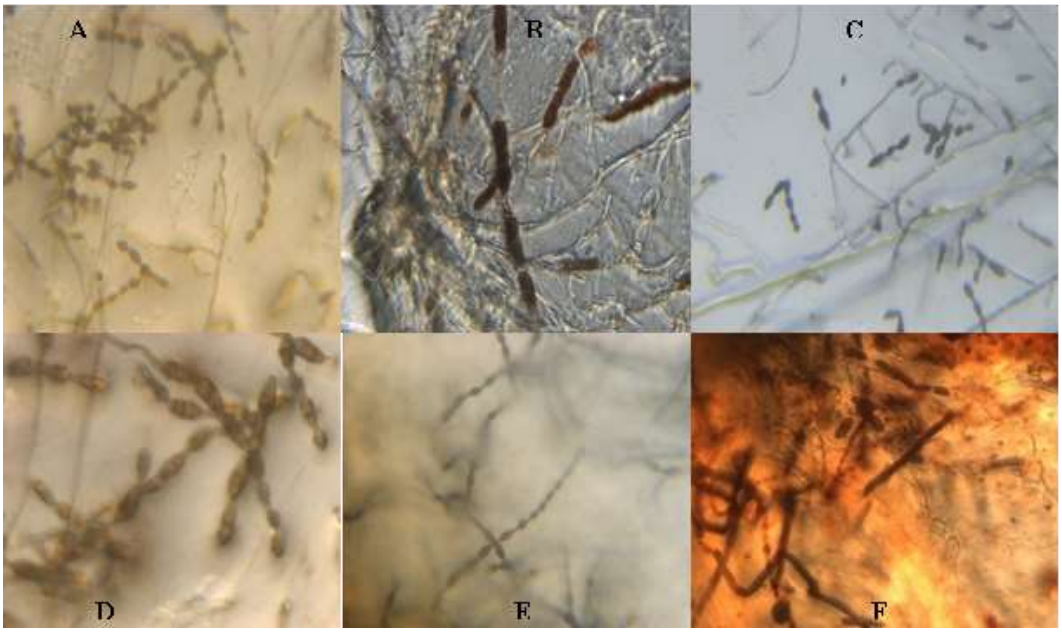

Fig. 1. Conidial morphology of *Alternaria* isolates, A: AUAT21, B: AUAT3, C: AUAT2, D: AUAT20, E: AUAT7, F: AUAT4.

Physiological characterization of the fungal pathogen

Effect of culture media on the mycelial growth of *Alternaria* isolates

Mycelial development varied significantly ($P<0.05$) depending on the isolation, media, and interaction. Every tested media supported each isolate to grow. AUAT20 (90 mm) measured the highest mean mycelial growth across all media types. On host leaf extract compared to other medium, isolates AUAT2, AUAT3, AUAT4, AUAT7, and AUAT21 showed the best mean mycelial growth with values of 52, 66, 64, 72, and 85 mm, respectively. However, all fungal isolates except for AUAT20 displayed a stable growth diameter in all culture conditions, and the minimal growth diameter was noted on CDA for all other fungal isolates (Fig. 2).

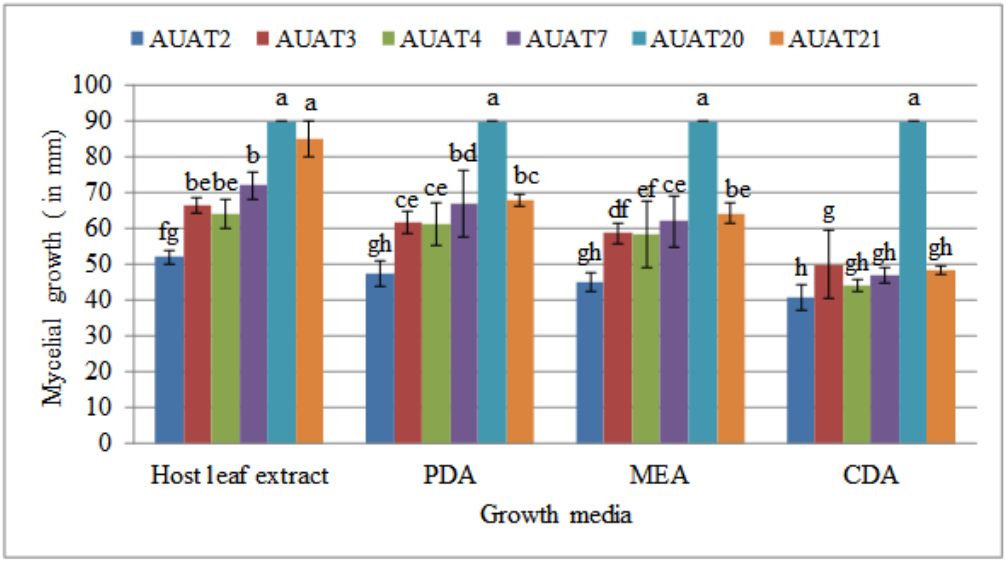

Fig. 2. The effect of different growth media on mycelial growth of *Alternaria* isolates on the six days of incubation at 25 ± 2°C. Key: A vertical line on the graphs indicates standard deviation. Graphs with the same letters are not significantly different (P>0.05). PDA – potato dextrose agar, MEA – malt extract agar, and CDA – czapex dox agar.

The effect of temperature on the mycelia growth of *Alternaria* isolates

At temperatures ranging from 15 to 40°C, the mycelial development patterns of the three *Alternaria* isolates differed. On PDA medium, all *Alternaria* isolates had maximal mean mycelial diameters between 25 and 30°C (Fig. 3). At the end of the eighth day of incubation, isolate AUAT20's mycelial growth was measured at 90 mm in a temperature range of 15 to 35°C. Additionally, at 25°C, isolates AUAT2, AUAT3, AUAT4, AUAT7, and AUAT21 showed maximum mean mycelia growth diameters of 47.33, 61.67, 61.33, 67.00, and 68.00 mm, respectively (Fig. 3).

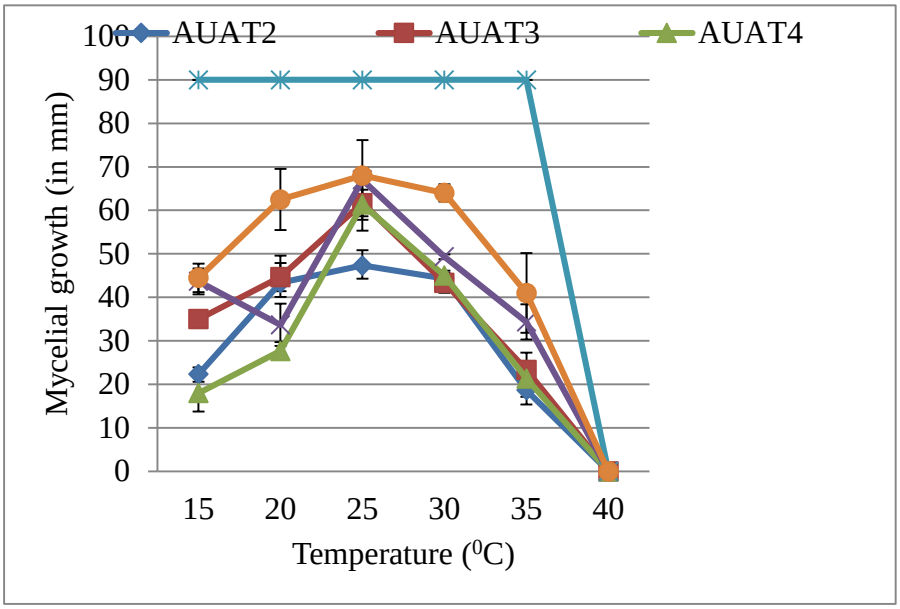

Fig. 3. The effect of temperature on the mycelial growth of *Alternaria* isolates on the eighth day of incubation in PDA medium. Key: A vertical line on the graphs indicates standard deviation.

The effect of pH values on the mycelial dry weight of *Alternaria* isolates

The distinct *Alternaria* isolates may grow over a very wide range of pH values (3.5–8.5), as shown in Fig. 4. The AUAT20 isolates, among others, were shown to have the maximum biomass in the pH ranges of 4.5 to 6.5. The AUAT2 isolate had the lowest mycelial dry weight across all of the investigated pH levels, in contrast. At pH 6.5, the AUAT20, AUAT21, AUAT7, AUAT4, AUAT3, and AUAT2 isolates produced the highest mean mycelial dry weight, with yields of 900, 820, 797, 760, 638, and 487.65 mg, respectively (Fig. 4).

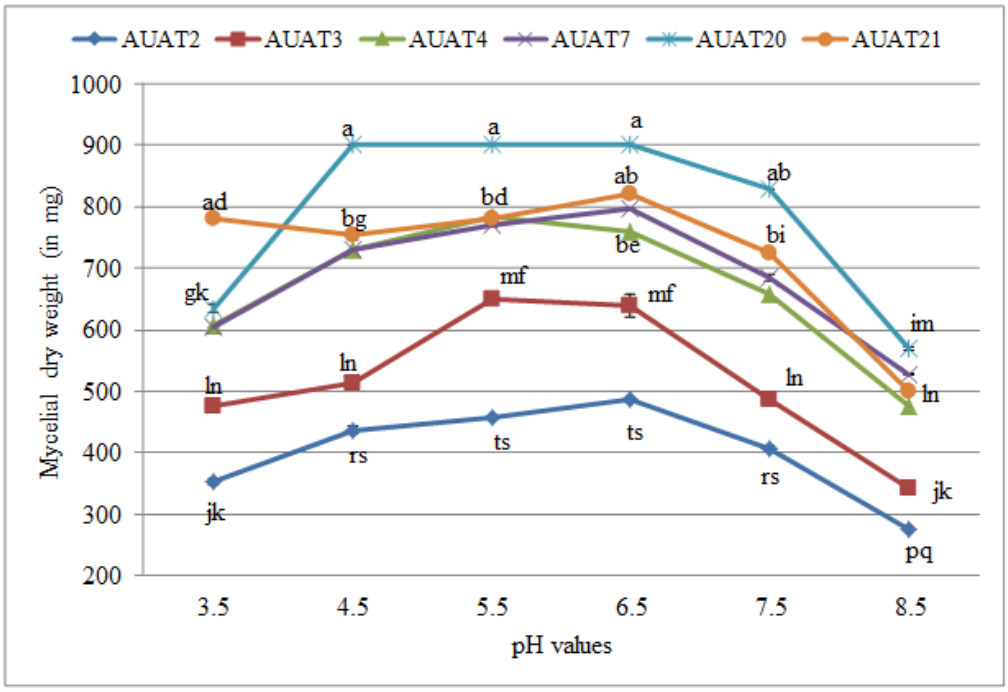

Fig. 4. The effect of pH values on mycelial dry weight of *Alternaria* isolates in potato dextrose broth on the 14th day of incubation at 25 ± 2°C. Key: Graphs with the same letters are not significantly different (P>0.05).

The effect of Carbon and Nitrogen sources on the growth of *Alternaria* isolates

As indicated in Table 2, distinct *Alternaria* isolates utilized different carbon and nitrogen sources in different ways. The maximum mean mycelial diameters obtained by the isolates AUAT2 (38.67 mm), AUAT4 (41 mm), AUAT7 (45.67 mm), and AUAT21 (55.83 mm) in the presence of sucrose show that sucrose was the most chosen carbon source by the majority of isolates. While KNO_3 supported the greatest development of the isolates AUAT2, AUAT3, AUAT4, AUAT7, AUAT20, and AUAT21 with values of 34, 46.67, 42, 48.67, and 53.67 mm, respectively, it was found to be the best source of nitrogen for the growth of *Alternaria* species.

Table 2. The effect of different Carbon and Nitrogen sources on the mycelial growth of *Alternaria* isolates on the 10th day of incubation at 25 ± 3°C.

***Alternaria* isolates**	**Mean mycelia growth diameter of *Alternaria* isolates (in mm)**							
	Carbon sources				**Nitrogen sources**			
	Fructose	**Dextrose**	**Maltose**	**Sucrose**	**KNO_3**	**$NaNO_3$**	**L. arginine mono hydrochloride**	**Urea**
	Mean ± sd	**Mean ± sd**	**Mean ± sd**	**Mean ± sd**	**Mean ± sd**	**Mean ± sd**	**Mean ± sd**	**Mean ± sd**
AUAT2	34.00 ± 1.0^{j}	37.00 ± 1.0^{hj}	35.67 ± 1.2^{ij}	38.67 ± 1.5^{fi}	34.00 ± 1.7^{gi}	33.33 ± 2.1^{hi}	31.68 ± 2.1^{hi}	30.00 ± 3.0^{i}
AUAT3	39.16 ± 0.7^{fi}	42.00 ± 3.0^{dg}	43.00 ± 2.7^{df}	42.67 ± 2.5^{df}	46.67 ± 7.6^{cd}	46.33 ± 3.5^{ce}	42.00 ± 1.0^{df}	37.8 ± 2.1^{fh}
AUAT4	36.33 ± 3.1^{hj}	40.33 ± 3.1^{eh}	39.33 ± 3.1^{fi}	41.00 ± 2.7^{dh}	42.00 ± 0.0^{df}	40.33 ± 1.5^{eg}	35.00 ± 1.7^{gi}	34.17 ± 6.4^{gi}
AUAT7	37.33 ± 4.2^{gj}	44.33 ± 3.8^{ce}	41.00 ± 3.6^{dh}	45.67 ± 1.2^{cd}	48.67 ± 1.5^{bc}	47.33 ± 4.0^{cd}	37.00 ± 2.7^{fh}	34.83 ± 7.5^{gi}
AUAT20	90.00 ± 0.0^{a}	90.00 ± 0.0^{a}	90.00 ± 0.0^{a}	90.00 ± 0.0^{a}	90.00 ± 0.0^{a}	90.00 ± 0.0^{a}	90.00 ± 0.0^{a}	90.00 ± 0.0^{a}
AUAT21	42.00 ± 2.7^{dg}	48.00 ± 1.7^{cd}	43.00 ± 3.6^{df}	55.83 ± 3.4^{b}	53.67 ± 3.2^{bc}	52.00 ± 2.7^{bc}	49.90 ± 2.8^{bc}	46.67 ± 5.5^{cd}
Average	46.47 ± 21.5	50.28 ± 19.8	48.67 ± 20.4	52.31 ± 19.4	52.50 ± 19.5	51.55 ± 19.9	47.60 ± 21.7	45.58 ± 22.5
CV (%)	46.26	39.40	41.99	37.11	37.22	38.62	45.64	49.29
P-value	0.003	0.002	0.002	0.001	0.001	0.001	0.003	0.004

Pathogenicity test

The pathogenic effect was present in all six fungal isolates (AUAT2, AUAT3, AUAT4, AUAT7, AUAT20, and AUAT21). After 20 days of incubation, they had resulted in yellowish leaf blight, stem lesions, discolouration, colonization, and mortality on healthy-appearing taro leaves and stem. With a tissue infection rate of 100%, AUAT20 was the most virulent isolate, followed by AUAT21, AUAT7, AUAT4, and AUAT3 with tissue infection rates of 80, 75, 75, and 75%, respectively. With a 60% tissue infection rate, the AUAT2 isolate was the least virulent. The re-isolated fungal pathogens had cultural and physical characteristics with the initial isolates that were taken from diseased leaves. Re-isolating the fungus from the infected stem and leaf tissue of inoculated plants was successful. The recovered isolates shared the same morphological and cultural traits as the original isolates, supporting Koch's postulates, and the symptoms on inoculated plants mirrored those seen in the field (Fig. 5).

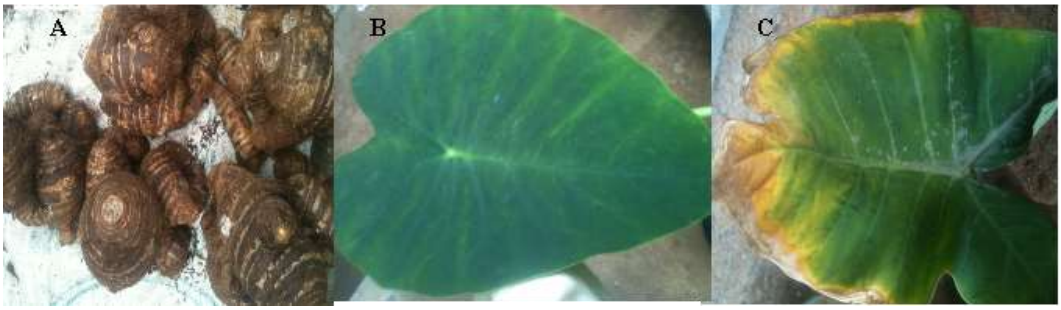

Fig. 5. Taro tubers and leaves, A: Tuber, B: Healthy leaf, C: Diseased leaf.

In vitro antagonistic evaluation of sancozeb and curzate against *Alternaria* isolates

Different levels of antifungal activity were observed between the sancozeb and curzate fungicides and the tested *Alternaria* isolates. The two fungicides completely inhibited the mycelial development of the tested pathogen at 1000 ppm concentrations, with the exception of curzate against the AUAT20 isolate. Sancozeb demonstrated more inhibition than curzate over the course of the test concentration. However, individual isolates showed varying mycelial growth responses to the fungicides (Fig. 6). In comparison to curzate, sancozeb significantly ($P<0.05$) inhibited mycelial growth against the tested fungal isolates at doses between 100 and 800 ppm. As a result, even at low concentrations, the *Alternaria* isolates were more susceptible to sancozeb than curzate (Fig. 6).

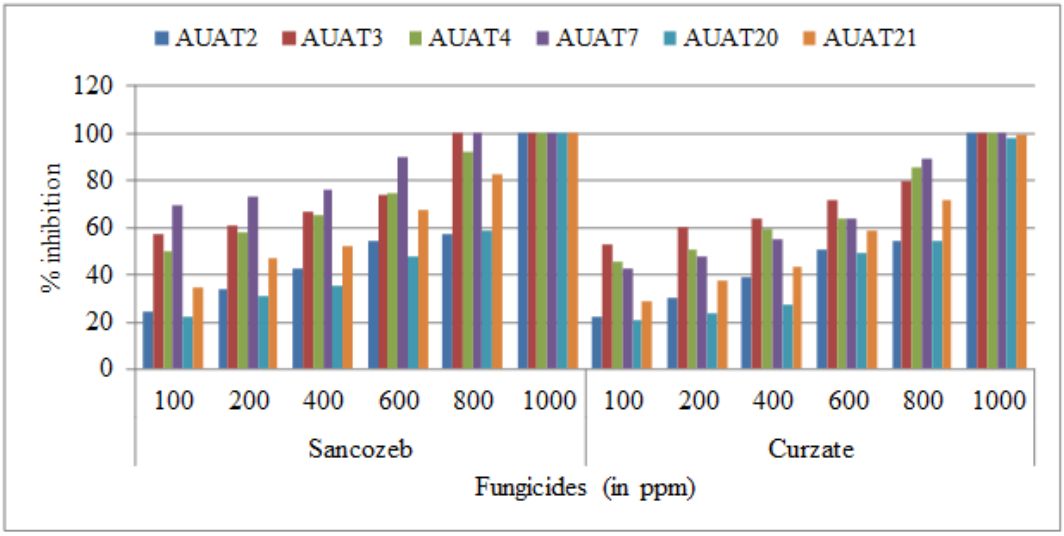

Fig. 6. *Alternaria* isolates mycelial growth percent inhibition by various concentrations of sancozeb and curzate fungicides on the 10th day of incubation at 25 ± 2°C.

In vitro antagonistic evaluation of *Trichoderma* species against the fungal isolates

On PDA medium, *Alternaria* isolates grew more slowly in double culture than *Trichoderma* species. The test pathogen's mycelial growth was considerably ($P<0.05$) inhibited by *T. viride* and *T. harzianum*. The diffusible secondary metabolites of both *T. viride* and *T. harzianum* lowered *Alternaria* isolates AUAT3 and AUAT21 to a higher degree than other isolates because they were more sensitive to them. The highest mycelial growth inhibition was recorded by *T. viride* against AUAT4, AUAT3, and AUAT21 among others with growth inhibition of 72.82, 69.19, and 64.06 %, respectively. In general, *T. viride* inhibited the tested pathogen's growth more significantly than *T. harzianum* (Fig. 7).

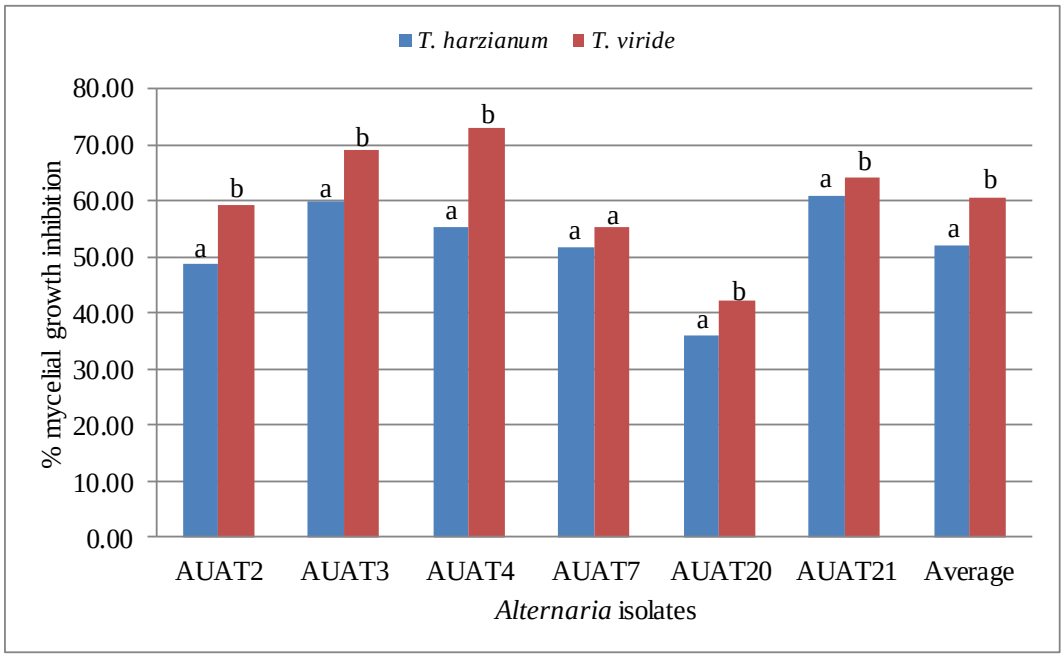

Fig. 7. *Alternaria* isolates mycelial growth percent inhibition by *T. harzianum* and *T. viride* on the 10th day of incubation at 25 ± 2°C. Key: adjacent graphs with the same letters are not significantly different (P>0.05).

DISCUSSION

In this study, *Alternaria* species that cause yellowish leaf blight were isolated from the taro infected leaves and stem on PDA media. The morphological, cultural, morphological and *in vitro* pathogenicity tests were used to identify the *Alternaria* isolates. The *Alternaria* isolates were found to produce muriform conidia with 5–7 septa that were 26.1–42.5 mm long and 10.2–18.5 mm wide. This was in agreement with Luo *et al*. (2018) who reported of *Alternaria argyranthemi* conidia, which had smooth, narrowly ellipsoid shape, 50–120 x 19–30 µm dimensions, and 4–9 transversal septa. Additionally, Sofi *et al*. (2013) discovered *Alternaria mali* conidia with 0–9 septa that were 2.35–5.62 µm wide and 27.28–68.18 µm long. *Alternaria padwickii* isolates from rice leaves had conidia that range in size from 95 to 170 × 11 to 20 µm, according to Quintana *et al*. (2017).

In this work, culture media had a significant impact on the development and generation of conidia in taro infecting *Alternaria* species. Following PDA, MEA, and CDA, the host leaf extract medium considerably supported the highest growth of *Alternaria* isolates. This might be explained by the

medium's low glucose content and its capacity to prevent other fast-growing competitors from growing. Similarly, Koley and Mahapatra (2015) found that PDA medium did not promote the mycelial development and sporulation of *A. solani* as well as oat meal agar (OMA). In contrast to the current work, Smita and Dhutraj (2017) found that *A. solani*'s mycelial development was best supported by PDA and other culture media with high sugar contents.

One of the most crucial physical elements for controlling the development and reproduction of fungi is temperature. In this study, it was shown that temperature had a substantial impact on the test pathogen's mycelia development rate, with 25°C being the optimal temperature for *Alternaria* isolates to grow. Accordingly, You *et al*. (2016) came to the conclusion that 25°C is the ideal temperature for many fungi to produce spores and grow mycelium. Similar to this, Zehra *et al*. (2017) observed that temperature has a significant impact on the fungus's radial development and sporulation. On the other hand, *Alternaria* isolates produced the most biomass at pH values of 6.5. Controlling the dissociation of the inorganic ions in the culture solution may be able to link this to the availability of vital nutrients. The pH levels of the nutrient medium control the availability of minerals and the fungus's metabolic rates (Poosapati *et al*., 2014).

Diverse *Alternaria* isolates have been found to use various carbon and nitrogen sources in noticeably different ways. The studied fungal pathogen preferred KNO_3, whereas sucrose promoted the highest development of *Alternaria* isolates. Contrarily, Ramjegathesh and Ebenezer (2012) found that maltose, followed by sucrose, was the greatest carbon source for the development of *Alternaria alternata*. They did, however, report identical outcomes to ours in terms of the use of nitrogen sources. On the other hand, Shaikh *et al*. (2018) observed that an ammonium nitrate concentration of 0.25% was an appropriate nitrogen source for growth of *Alternaria solani.*

After 20 days of incubation, the *Alternaria* isolates resulted in lesions, colouring, colonization, and mortality of the host tissue in *in vitro* pathogenicity assays. On the infected stem and leaf tissue, the isolates showed substantial levels of sporulation. This showed that the fungus is extremely virulent and the cause of the host tissue's yellowish leaf blight disease. This is consistent with reports of virulent *Alternaria* isolates to sesame and faba bean leaves under *in vitro* conditions made by Naik *et al*. (2017) and Kayim *et al*. (2018), respectively. Similar to this, *Alternaria argyranthemi* was evaluated for pathogenicity on *Chrysanthemum*

coronarium by Luo *et al*. (2018) and was found to be pathogenic on this plant. However, Sofi *et al*. (2013) also demonstrated the pathogenicity of *A. mali* isolates by inoculating detached damaged apple leaves with conidial suspension.

The current study evaluated the antagonistic activity of the fungicides sancozeb and curzate against *Alternaria* isolates. Across all tested concentrations, sancozeb showed greater mycelial growth inhibition of the studied fungal pathogen. This might be connected to the ratios of sancozeb's active components. Similar to this, Mahantesh *et al*. (2017) and Pandey *et al*. (2017) reported on the effectiveness of mancozeb 80% WP against plant pathogens that cause root rot. People prefer organically grown crops due to the harmful effects of chemical fungicides on the environment and human health. As a result, the current work employed dual culture techniques to evaluate the *in vitro* antagonistic activity of *Trichoderma* species against *Alternaria* isolates.

Trichoderma viride was more successful than *T. harzianum* at inhibiting the mycelial growth of the *Alternaria* isolates in the dual culture of *Trichoderma* species and *Alternaria* isolates. This may be due to *T. viride*'s ability to compete effectively for nutrients and space, as well as its ability to produce more readily soluble antagonistic secondary metabolites than *T. harzianum*. In concordance with this, Roy *et al*. (2019) reported that *T. viride* caused the greatest inhibition of *A. solani*'s radial development by twofold. Similar to this, Koka *et al*. (2017) found that competition and antibiosis were effective mechanisms used to demonstrate antagonistic activity of *Trichoderma* species against several fungi causing root rot disease. Additionally, Kayim *et al*. (2018) demonstrated that *T. harzianum* was an effective antagonist of *A. alternata*. Similar to this, Koka *et al*. (2017) found that competition and antibiosis were effective mechanisms used to demonstrate antagonistic activity of *Trichoderma* species against several fungi causing root rot disease. Additionally, Kayim *et al*. (2018) demonstrated that isolates of *T. harzianum* were effective antagonists against *A. alternata*. In order to handle isolates of *Alternaria* that are infected with taro, *T. viride* and *T. harzianum* may be used as possible biocontrol agents.

CONCLUSION

It is feasible to draw the conclusion from this investigation that the *Alternaria* isolates that infected taro plants had conidial dimensions with five to seven septa in varied diameters. The host leaf extract medium

promoted the test pathogen's maximum mycelial growth, and 25°C was the optimum incubation temperature. The culture media's pH levels of 6.5 were maintained in order to produce a significant amount of mycelial biomass. The preferred carbon and nitrogen sources used by *Alternaria* isolates are sucrose and KNO_3, respectively. Under *in vitro* conditions, the pure *Alternaria* isolates generated colonization, lesions, discoloration, and mortality on healthy taro stems and leaves. Even at low doses, they were more sensitive to sancozeb than curzate fungicides. In dual culture confrontation assays, *T. viride* was more successful than *T. harzianum* at inhibiting the mycelial growth of *Alternaria* isolates. Further, molecular characterization-based identification of *Alternaria* isolates infecting taro should be carried out for additional confirmation. In order to apply the effective fungal biocontrol agent concentrations, a variety of studies are required for the management of taro infecting *Alternaria* species in the field.

ACKNOWLEDGEMENT

We would like to convey our heartfelt appreciation to the Department of Microbial, Cellular and Molecular Biology (DMCMB), College of Natural and Computational Sciences, Addis Ababa University, for providing the fund and essential laboratory resources to complete this project. We appreciate the ongoing assistance of Mrs. Zenebech Aytenew and Mrs. Nigatowa Mekonen as laboratory technicians for the mycology laboratory.

REFERENCES

Ahmed, F. and El-Fiki, A. (2017). Effect of biological control of root rot diseases of strawberry using *Trichoderma* species. *Middle East J. Appl. Sci*. **7**: 482–492.

Alvarenga, T.V., Silvia, R.R., Paula, R., Elaine, A.S., Francielly, C.P., and Augusto, B.P. (2016). Characterization of *Alternaria* isolates and reaction of potato genotypes to early blight. *Ciência R. Santa Mari.* **46**(10): 1783–1789.

Dahal, N. and Shrestha, K. (2018). Evaluation of efficacy of fungicides against *Fusarium oxysporum* f. sp. *lentis in vitro* at Lamjung, Nepal. *J. Inst. Agric. Anim. Sci*. **35**: 105–112.

El-Gali, Z.I. (2015). Antagonism capability *in vitro* of *Trichoderma harzianum* against *Alternaria alternate* on *Ceratonia siliquea*. *Eur. J. Pharm. Med. Res*. **2**(2): 30–44.

Gautam, C., Prabhu, V., and Nargund, V.B. (2017). *In-vitro* evaluation of fungicides, botanicals and bioagents against *Peziotrichum corticolum* causing black banded disease of Mango. *Int. J. Curr. Microbiol. Appl. Sci.* **6**(3): 652–661.

Islam, A., Nain, Z., Alam, K., Banu, A., and Islam, R. (2018). *In vitro* study of biocontrol potential of rhizospheric *Pseudomonas aeruginosa* against *Fusarium oxysporum* f. species *cucumerinum*. *Egypt. J. Biol. Pest Control.* **28**: 90–101.

Kayım, M., Yones, A.M., and Endes, A. (2018). Biocontrol of *Alternaria alternate* causing leaf spot disease on faba bean (*Vicia faba* L.) using some *Trichoderma harzianum* isolates under *in vitro* condition. *Har. Tar. ve Gida Bilim. Derg*. **22**(2): 169–178.

Khaledi, N. and Taheri, P. (2016). Biocontrol mechanisms of *Trichoderma harzianum* against soybean charcoal rot caused by *Macrophomina phaseolina*. *J. Plant Prot. Res*. **56**(1): 22–31.

Koka, J., Wani, A., Bhat, M., and Parveen, S. (2017). *In vitro* efficacy of *Trichoderma* isolates against some fungi causing fungal rot disease of tomato. *Int. J. Adv. Res.* **5**(3): 2050–2053.

Koley, S. and Mahapatra, S. (2015). Evaluation of culture media for growth characteristics of *Alternaria solani*, causing early blight of tomato. *J. Plant Pathol. Microbiol*. **5**: 1–5.

Luo, H., Xia, Z., Chen, Y., Zhou, Y., and Deng, X. (2018). Morphology and molecular characterization of *Alternaria argyranthemi* on *Chrysanthemum coronarium* in China. *Mycobiology* **46**(3): 278–282.

Mahantesh, S.B., Karegowda, S. Kavitha, V., Kavita T.H., and Punith, K. (2017). *In vitro* evaluation of fungicides, bioagents and natural plant extracts against early blight caused by *A. solani*. *Int. J. Chem. Stud*. **5**(5): 1346–1350.

Mandal, R., Mukherjee, A., Mandal, N., Tarafdar, J., and Mukherjee, A. (2013). Assessment of genetic diversity in taro using morphometrics. *Curr. Agr. Res. J*. **1**: 79–85.

Matthews, P.J., Agoo, E.M., Madulin, D.A., and Tandang, D.N. (2012). Ethnobotany and ecology of wild taro (*Colocasia esculenta*) in the Philippines: Implications for domestication and dispersal. *Senri. Ethnol. Stud*. **78**: 307–340.

Mendoza, J.H., Pérez, M.I., Prieto, J.M., Velásquez, J.D., Olivares, J.G., and Langarica, H.R. (2015). Antibiosis of *Trichoderma* species strains native to northeastern Mexico against the pathogenic fungus *Macrophomina phaseolina*. *Braz. J. Microbiol*. **46**(4): 1093–1101.

Naik, M.K., Chennappa, G., Amaresh, Y.S., Sudha, S., Chowdappa, P., and Suresh, P. (2017). Characterization of phytotoxin producing *Alternaria* species isolated from sesame leaves and their toxicity. *Ind. J. Exp. Biol*. **55**: 36–43.

Nayyar, B.G., Steve, W., Luis, A.J., Abida, A., Muhammad, A., Saqlan, N., and Shaista, A. (2017). The incidence of *Alternaria* species associated with infected *Sesamum indicum* L. seeds from fields of the Punjab, Pakistan. *Plant Pathol. J*. **33**(6): 543–553.

Pandey, P., Kushwaha, K., and Upadhyay, V. (2017). Evaluation of potential fungicides and bioagents for the management of pea downy mildew and yield under field condition. *Int. J. Curr. Microbiol. Appl. Sci*. **6**(12): 1381–1388.

Poosapati, S., Ruvulapalli, D., Tippirishetty, N., Vishawanthaswamy, K., and Chunduri, S. (2014). Selection of high temperature and salinity tolerant *Trichoderma* isolates with antagonistic activity against *Sclerotium rolfsii*. *Springer Plus* 3: 641.

Quintana, L., Gutierrez, S., Maidana, M., Arriola, M., and Ortiz, A. (2017). Morphological characterization of *Alternaria padwickii* in rice leaves (*Oryza sativa* L.) and its prevalence in the departments of Itapúa, Misiones and Caazapa. *Int. J. Adv. Res*. **5**(5): 1109–1112.

Ramjegathesh, R. and Ebenezer, E.G. (2012). Morphological and physiological characters of *A. alternata* causing leaf blight disease of onion. *Int. J. Plant Pathol*. **3**: 34–44.

Roy, C., Nafiza, A., Mohammad, K., Sarkar, I., Moyen, U., Nadira, B., and Miskat, A. (2019). Control of early blight of tomato caused by *Alternaria solani* and screening of tomato varieties against the pathogen. *The Open Microbiol. J.* **13**: 41–

50.

Ruisi, P., Amato, G., Badagliacca, G., Salvatore, F., Giambalvo, D., and Miceli, G. (2017). Agro ecological benefits of faba bean for rainfed Mediterranean cropping systems. *Ital. J. Agron.* **12**: 233–245.

Sangdee, A., Sachan, S., and Khankhum, S. (2011). Morphological, pathological and molecular variability of *Colletotrichum capsici* causing anthracnose of chili in the northeast of Thailand. *Afr. J. Microbiol. Res*. **5**: 4368–4372.

Shaikh, F., Chavan, S., and Fatima, S. (2018). *In vitro* effect of nitrogen sources on growth of *Alternaria solani* and *Rhizoctonia solani*, causing diseases of tomato. *Int. J. Appl. Res*. **4**(11): 125–128.

Smita, C. and Dhutraj, N. (2017). Effect of culture media and temperature on growth and sporulation of *Colletotrichum truncatum* of soybean *in vitro*. *Int. J. Appl. Plant Sci. Agric*. **3**(1): 25–30.

Sofi, T.A., Muzafer, A., Beig, G., Hassan, D., Mushtaq, A., Aflaq, H., Ahangar, B.A., and Shah, D.M. (2013). Cultural, morphological, pathogenic and molecular characterization of *Alternaria mali* associated with *Alternaria* leaf blotch of apple. *Afr. J. Biotechnol.* **12**(4): 370–381.

Tewodros Mulualem (2013). Morpho-agronomical characterization of taro (*Colocasia esculenta*) accessions in Ethiopia. *Plant* **1**(1): 1–9.

Ubalua, A.O., Ewa, F., and Okeagu, O.D. (2016). Potentials and challenges of sustainable taro (*Colocasia esculenta*) production in Nigeria. *J. Appl. Biol. Biotechnol.* **4**: 053– 059.

Wang, Y.N., Hu, J.S., Borth, W.B., Hamim, I., Green, J.C., and Melzer, M.J. (2017). First report of Taro bacilliform CH Virus (TaBCHV) on taro (*Colocasia esculenta*) in Hawaii, USA. *Plant Dis*. **101**: 13–34.

You, J., Zhang, J., Wu, M., Yang, L., Chen, W., and Li, G. (2016). Multiple criteria-based screening of *Trichoderma* isolates for biological control of *Botrytis cinerea* on tomato. *Biol. Control.* **101**: 31–38.

Zehra, A., Dubey, K., Meena, M., and Upadhyay, S. (2017). Effect of different environmental conditions on growth and sporulation of some *Trichoderma* species. *J. Environ. Biol*. **38**: 197–203.