Prevalence of Diseases Incidenceand Mycoflora of Rhizosphereat Distinct Phenological Stages in Different Treatments of Okra in Hamelmalo, Eritrea

Abstract

Okra (Abelmoschus esculents L. Moench.) is a vegetable widely grown in Eritrea and cultivated in 514 ha of land with an average yield of 10t/ha. However, the production of this crop is highly affected by nematodes and other diseases. There is very less information on disease occurrence and soil mycoflora, hence, this research was carried out in the field of okra, in Hamelmalo Agricultural College, to identify the occurrence of diseases; percentage occurrence of disease incidence and identification of field mycoflora at various phenological stages of okra in various treatments such as control (T1), farm yard manure (T2), intercropping with a legume (T3) and chemical fertilizer (T4). This experiment was conducted in a completely randomized block design. During the experiment, powdery mildew and blight were the main diseases that occurred, and diseases like damping off, wilt, and leaf spot were also observed. In addition to that various soil mycoflora were found including, two nematode predacious fungi *Arthrobotrisoligospora* and *Drechmeria*sps.which were identified for the first time in the rhizosphere soils of okra, in Eritrea. The study revealed that the flowering stage was found significantly different at the incidence of powdery mildew but all growth stages of okra were non-significant at blights. Chemical fertilizer was found to be the best in cases of disease prevalence and intercropping was in cases of pests' infestation. Based on the results of the current study it can be concluded that different phenological stages were found to be significantly influenced by all the treatments. But in T3 the cultivation which was with cowpea attracted the insect pests like African bollworms. However, the incidence of blight on all the treatments at different phonological stages was non-significant.

Keywords: *Blight; Disease Incidence; Diseases; Okra; Pest, Powdery Mildew.*

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INTRODUCTION

Okra *(Abelmoschus esculents*L. Moench, (Family: Malvaceae) is one of the important vegetable crops and is called '*bamia'* in Eritrean local language.Okra is a warm-season crop that is considered to have originated in India (Rao, 1985) and later, probably,came from Ethiopia in Africa (Khalid *et al.,* 2005) and is cultivated in many parts of the world, including West Africa, India, Brazil and the United States (Kemble et al., 1995; ECHO, 2003; Alimi, 2004).It is a perennial herbaceous plant treated as an annual that has a high nutritional value and grows very quickly with high temperatures which lends its production to more tropical parts of the world (Costa et al., 1981). In Eritrea, this crop is produced in zobaAnseba, Gash Barka, and Northern Red Sea, both under rain-fed and irrigation during the dry season, accounting for the largest area under cultivation (514 ha) with an average yield of 10t/ha (MoA, 2015). This herb grows to 2m tall the alternate three-lobed leaves may reach 30cm in length and are generally hairy. The leaves are 10-20 centimeters (3.9-7.9in) long and broad, palmately lobed with 5-7 lobes. The flowers are 4-8 centimeters (1.6-3.1in) in diameter, with five white to yellow petals, often with red or purple spots at the base of each petal. The fruit is a capsule up to 18 centimeters (7.1in) long with a pentagonal cross-section, containing numerous seeds. (Incalcaterra et al, 2000) (Fig. 1).

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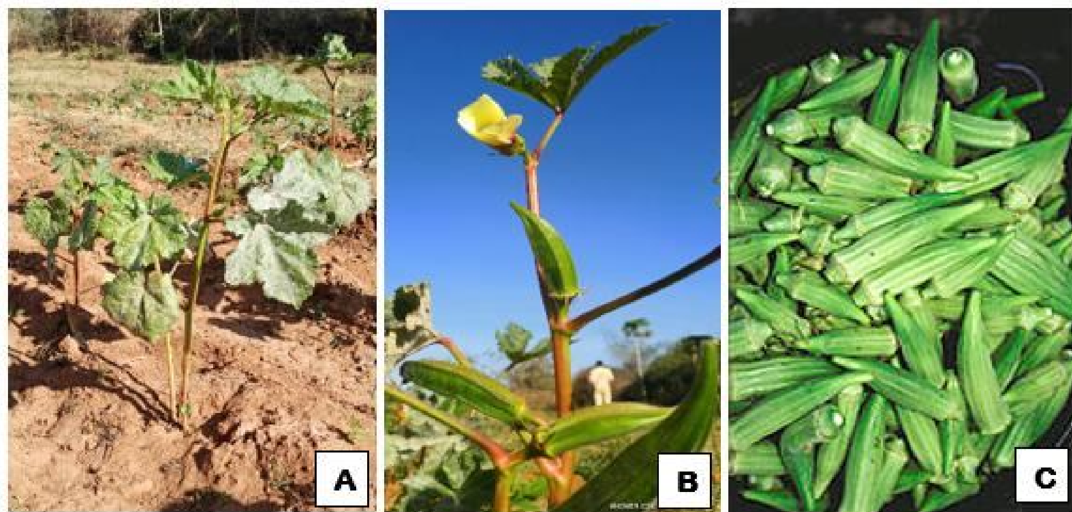

Figure 1. Whole plant (A), flower (B), and fruits (C).

Okra yield can be affected by different cultivating factors,for instance, sowing date, application of fertilizer, and plant spacing; abiotic factors such as temperature, humidity, and rainfall; and biotic factors that cause losses in okra production like diseases caused by different pathogens and pest infestation. Fertilization, with phosphorus, is considered one of

the major factors that greatly affect the seed yield and quality of okra (Gupta et al., 1981; Mohanta, 1998; Sadat, 2000).The highest significant mean values of leaf area and leaf numbers were reported with the application of phosphorus and Potassium fertilizer 200kg/ha was the best for getting the best economic returns (Bahran and Sethumadhava, 2021). The improper use of these factors may cause the development of different kinds of diseases.However, the production of this crop is highly affected by nematodes, powdery mildew, and other diseases (Adugna and Sethumadhava, 2023). Since farmers in Eritrea do not have enough knowledge ofthe time of harvesting and diseases that attack okra yield loss occurs in great economic damage (Shaha et al.,1989). There is no or very little information on disease occurrence and soil mycoflora which can cause diseases on okra. It is, therefore, this research is perceived to identify the occurrence of diseases; isolation,and identification of rhizosphere mycoflora at various phenological stages in various treatments of okra.

MATERIALS AND METHODS

Experimental site: This experiment was carried out in the fields of Hamelmalo Agricultural College (HAC), Anseba region, which is located 13 km North of Keren on the side of the Keren–Nakfa road line, from August to December 2020. The altitude of the area is about 1330 m above sea level and the average rainfall and annual temperature of the area are 436 mm and 24°C respectively (MoA, 2008). The experimental field was well ploughed and harrowed to a fine tilth. The cultivating field was ripped along with the previous crop residues, and organic matter and was destroyed all young weeds thoroughly before okra planted. After two weeks, it was leveled and prepared 3m × 4m sized plots with the provision of channels and sub-channels for irrigation (Fig. 2).

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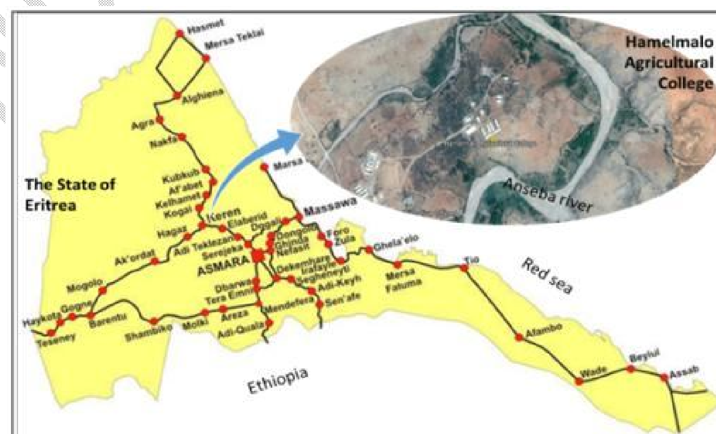

Figure 2. Hamelmalo is located on the Northern side of Keren.

Cultivation: Seeds were sown in rows on the flat beds at 2-3 cm depth on ridges or beds in rows 70cm apart with 40cm between plants.***Weeding*** was done frequently to control the growth of weeds in okra and earthing up in the rows was done. The first weeding was done after 20 days of sowing and the second weeding was done after 40 days of sowing. Pre-sowing ***irrigation*** was given to ensure good germination when sufficient moisture was not present in the soil. The next irrigation was given after seed germination by using furrow irrigation.

Sample Collection: Every week,**disease observation**wasobserved whether any pests or diseases were developed. Diseases were identified based on the symptoms observed on the plant. The samples of infected plant partswere collected for the identification of the causal organism.

In each plot about 11 **rhizospheresoil samples** were collected randomly for the assessment of soil-borne fungi and analyzed its percentage occurrence. Fungal identifications were carried out based onthe characterization of symptoms and also examined under a compound microscope with 40x magnification power isolating the minute colonies and from infected parts of plants (Aneja, 2004; Barnet and Hunter, 1972;Gilman, 2001; Nagamani et al., 2006 and Rifai, 1969).The data was recorded and analyzed the disease incidence and the soil samples were taken into consideration for identifying the pathogens.

Isolation of Fungi: All materials used in this experiment were sterilized using ethyl alcohol and boiled water except for the leaf samples and field soils. For the isolation of pathogens, the moist blotter paper method, Soil Plate Method (SPM), Serial Dilution Method (SDM),and Potato Dextrose Agar (PDA) media were used. The diseased leaf parts were cut into small pieces of 3 mm diameter starting from the healthy part. Thesesamples were washed with tap water and surface sterilized with 10% NaOCl for 30 to 60 seconds, again rinsed with sterile water, and blotted to dry on clean tissue paper. After drying, three pieces were aseptically placed on Petri dishes containing moistened blotter paper and PDA medium. Next, the inoculated Petri dishes were marked with the name of the sample and were incubated at a temperature of 25°C for 5 days, until pathogen proliferation on the blotter paper surface and PDAmedium (ISTA, 1976).

Potato Dextrose Agar: For the preparation of a liter of PDA media, 200g of peeled and sliced potatoes were boiled and filtered the extract by a muslin cloth. Dextrose 20g and 20g of Agar

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dissolved in water separately in two beakersthen mixed with the potato filtrate. Later, brought the volume up to 1000ml and autoclaved at 121 °C, 15 lbs. pressure for 15 minutes. The medium was poured into the Petri dishes quickly under aseptic conditions for the isolation of soil mycoflora. The pH of the medium was maintained at 5.5 being optimal for the growth and sporulation of fungi and it was acidified with 25% lactic acid (added 3 to 5 drops per 100 ml melted agar).

***War cupMethod*:**The Soil Plate Method (SPM) was used as an isolation technique from root-associated soils. A small amount (about 0.15g) of rhizosphere soil was scattered on the bottom of the sterile Petri dish and molten cooled (40-45° C) PDA medium was added, and then rotated gently to disperse the soil particles in the medium. The plates were then incubated at room temperature for 4-6 days. One re-isolate from each soil sample of each fungal colony was selected at random for further sub-culturing and maintained on PDA slants (Warcup, 1950).

Serial Dilution Method:Rhizosphere soil dilutions were made by suspending 1 g of the soil of each sample in 9 ml of sterile distilled water in the Serial Dilution Method (SDM) (Waksman, 1922). Dilutions of 10^{-3}, $10^{-4,}$ and 10^{-5} were used to isolate fungi to get individual fungal colonies. One ml of the suspension of each concentration was added to sterile Petri dishes, in triplicates of each dilution, containing sterilized PDA medium. Before pouring into Petri dishes, streptomycin solution (1%) was added to the medium to prevent bacterial growth. The plates were then incubated at 28±2 °C for 4-6 days. Fungal colonies that were grown and well dispersed at higher dilutions were easily isolated for staining.

Identification of Fungi:Colony characteristic features of fungi were studied macroscopically by observing their growth which includes length and width, color, the presence or absence of aerial mycelium, margin, wrinkles, and furrows as texture and also observed microscopically (Diba et al., 2007 and Gilman, 2001). With the help of a sterilized inoculating needle, a small margin young portion of the growth from the culture plate was transferred onto the slide. The specimen was teased carefully using an inoculating wire loop to avoid squashing and overcrowding of the mycelium.

Experimental Design: The experiment was conducted witha completely randomized block design (RCBD) in which there were four treatments i.e., T-1: control, T-2:farm yard manure, T-3: intercropping with –legume (cowpea - *Vigna unguiculata* L.)and T-4:chemical fertilizer

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(urea)and each treatment was replicated three times.The infected plant parts were collected at three growth stages (seedling stage –20-25 days after sowing; vegetative stage – adult stage;and fruiting stage – before harvesting) and the soil samples were analyzed for fungi from each treatment (Table1).

Table1. Design of the Experiment

Number of treatments	4
Number of replications	3
Total number of experimental plots	12
Individual plot size	3×4 sq. m.
Plot to plot distance	0.70 m
Plant-to-plant distance	0.40 m
Number of plants per row	9
Number of rows in each plot	3
Distance between replication	0.85 m
Total area of experimental field	206.25 sq.m.
Number of plants for observation per plot	9

After identifying the diseases, the percentage occurrence was recorded at different growth stages in all treatments by using the following formula:

$$\text{Percent Disease Incidence} = \frac{\text{number of plants infected}}{\text{total number of plant observed}} \times 100$$

Statistical Data Analysis: Data on disease incidence and rhizosphere fungi at various phenological stages of okra from the different treatments were recorded and analyzedusing MS Office Excel. Data on percentage occurrence of fungi were subjected to analysis of variance by using GENSTAT software, (version 2012).

RESULTS AND DISCUSSION:

There were six diseases and seven fungi thatwere responsible for these diseases, and five insect pests were noticed during the three growth stages of okra. Seventeen fungi were isolated and identifiedfrom the rhizosphere soil.

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Diseases Identified at distinct phenological stages in four treatments of Okra

Damping off, early blight, leaf spot, wilt, powdery mildew, and viral infections were noticed in different growth seedling stages. Some leaf minor infestations were also recorded in all treatments; those have played a vital role in the viral transmission. Damping off was only observed in T3 at a seedling stagewhich was caused by species of *Rhizoctonia* (Table 2). The reason why damping off was seen in T3 is that intercropping with cowpeasdecreases air circulation within plants and enhances the development of damping off. However early blight and powdery mildewwere found at all treatments enhanced by different environmental conditions. In addition to this all treatments, at the seedling stage, are affected by wilt because it is caused by four different fungal species such as *Fusarium*, *Pythium*, *Rhizoctonia,* and *Verticillium.*

At the vegetative stage, leaf spot diseases were observed, only in T1; and Vein-Clearing or Yellow Vein Mosaic were noticed in all treatments.

Fusarium wilt, early blight, and powdery mildew diseases were found at the flowering stage in all treatments including control. In T2 the soil organic matter and hence increase the availability of food for the pathogen to develop the disease further, at the same time these diseases also were noticedin T4.

A decreasing trend, in the total number of diseases, from the seedling stage to the flowering stage, was observed in T1 and T3. It may be because of growing of plant growth-promoting microbes (PGPM) around the root atmosphere soils in the case of T3, or it may be due to the absence of chemical fertilizer in the case of T1.

YVMV was noticed only at the vegetative stage, it could be a reason that during this period more insect pests (aphids) were infested on okra and they can be transmitted by the virus (Table 2).

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Table 2. Occurrence of Diseases and Insect Pests in Four Treatments at Different Growth Stageson Okra in Hamelmalo

Growth stages of okra		Seedling Stage				Vegetative Stage				Flowering Stage			
Diseases Identified	**Causal Organism/s**	***T 1**	**T 2**	**T 3**	**T 4**	**T 1**	**T 2**	**T 3**	**T 4**	**T 1**	**T 2**	**T 3**	**T 4**
Dumping Off	*Rhizoctonia*sps	-	-	√	-	-	-	-	-	-	-	-	-
Early Blight	*Alternaria*sps.	√	√	√	√	√	√	√	√	√	√	√	√
Leaf Spot	*Ascochytaabelmoschi*	√	√	√	√	√	-	-	-	-	-	-	-
Wilt	*Fusarium*sps	√	√	√	√	-	-	-	-	√	√	√	√
	*Pythium*sps	√	√	√	√	-	-	-	-	-	-	-	-
	*Rhizoctonia*sps	√	√	√	√	-	-	-	-	-	√	-	√
	*Verticillium*sps	√	√	√	√	-	-	-	-	-	-	-	-
Powdery Mildews	*Erysiphecichoracearum*and*Sphaerothecafuliginea*	√	√	√	√	√	√	√	√	√	√	√	√
Vein-Clearing or Yellow Vein Mosaic	YVMV	-	-	-	-	√	√	√	√	-	-	-	-
Pests Identified		**T 1**	**T 2**	**T 3**	**T 4**	**T 1**	**T 2**	**T 3**	**T 4**	**T 1**	**T 2**	**T 3**	**T 4**
1	*Leaf minor*	√	√	√	√	√	-	√	-	-	-	-	-
2	*Aphids*	√	√	√	√	√	√	√	√	√	√	√	√
3	*African Boll Worm*	-	-	-	-	-	-	-	-	-	-	-	√
4	*Mealy Bugs*	√	-	√	-	√	√	-	-	-	-	-	-
5	*Blister Beetle*	-	√	√	-	√	-	√	-	-	-	-	-
Total insect pests		**3**	**3**	**4**	**2**	**4**	**2**	**3**	**1**	**1**	**1**	**1**	**2**

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***Treatments:** T1=Control; T2=Farm Yard Manure; T3= Intercropping with cowpea; T4= Chemical fertilizer
√: Present; -: Absent

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1. **Damping off:**

Symptoms: Damping-off is caused by *Rhizoctonia*sps. After the seedlings have emerged, the fungus attacks on stem and makes it water-soaked, soft, and incapable of supporting the seedling, then falls over and dies. On the lower part of the stem tan to reddish-brown lesions are observed. Reddish-brown lesions also appeared just below the soil line, and then lesions enlarged in all directions and increased in size on most of the roots.This disease was not noticed in further growth stages of okra. These observations were similar to Agrios, (2005) in that plants change their ontogenic resistance in their reaction (susceptibility or resistance) to disease with age.

Cultural characteristics: Soil was collected from the fields of okra at the seedling stage and isolated in the PDA medium for culture. The young myceliumgrew,initially, in colorless, but later it turned yellowish or light brown with age.

Causal organism: After incubation period colonies were identified as*Rhizoctonia*sps.It consists of long cells and producesbranches that grow at approximately right angles to the main hypha, are slightly constricted at the junction,and have a cross wall near the junction (Fig. 3).

Figure3. Damping off on the base of the stem at the seedling stage;*Rhizoctonia*sps.(40x).

2. **Early blight:**

Symptoms: Early Blight diseases appeared as leaf spots and blights at seedling, vegetative,and flowering stages. The leaf spots were generally dark brown to black, often numerous and enlarging, and usually developing in concentric rings, which give the spots a

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target-like appearance. Lower, senescent leaves are usually attacked first, but the disease progresses upward and makes affected leaves turn yellowish, become senescent, and either dry up and droop or fall off.The results are matching with the observation recorded by Syed et.al., (2016), that *Alternaria* blights were reported in okra from all the three sub zobas (Hamelmalo, Hagaz, and Adi-Tekeliezan) of Zoba Anseba.

Cultural Characteristics: The colonies appeared as concentric rings, olive-black to dark brown colored as mycelium grows on potato dextrose agar (PDA) media after incubation of 5 to 7 days. Conidia emerged on the surface of the colony.

Causal Organism:*Alternaria*sps. have dark-colored mycelium, they produce short, simple, erect conidiophores that bear single or branched chains of conidia. Conidia are large, dark, long/pear-shaped, and multicellular, with both transverse and longitudinal cross walls (Fig.4).

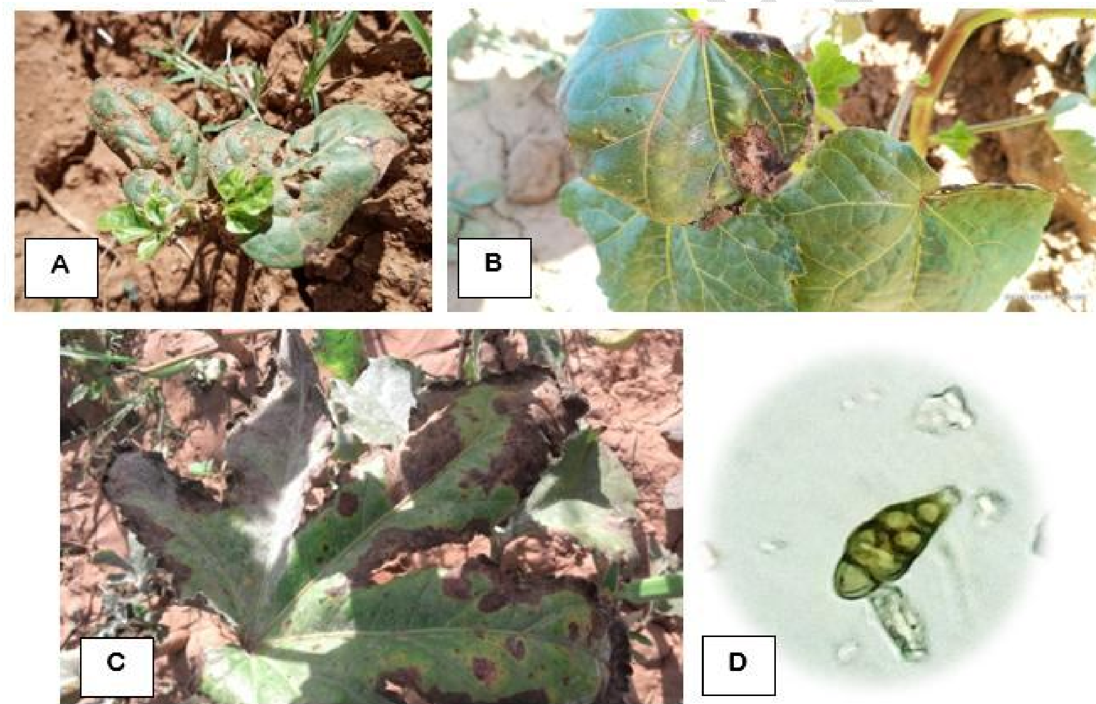

Figure 4. Early blight symptoms on a leaf at seedling stage (A), vegetative stage (B), and flowering stage (C); Conidium (D) of *Alternaria*sps. observed under a microscope (40x).

3. **Leaf Spot disease:**

Symptoms: Infection developed on the upper surface of the leaf at the seedling stage. At the vegetative stage, these symptoms were small, round to irregular in shape, and brown in color

appeared on the upper surface of the leaf. The results match with the observation recorded by Adugna and SethumadhavaRao, (2003).

Cultural characteristics: dark brown colored mycelium grown on PDA medium after 5- 7 days of incubation at 25-28 ºC.

Causal organism:*Ascochytaabelmoschi*Lib.hyphaebranched, septatebrown in color, pycnidia (fruiting bodies) globose and dark brown color. Conidia two-celled, ovoid to oblong and smooth (Fig. 5).

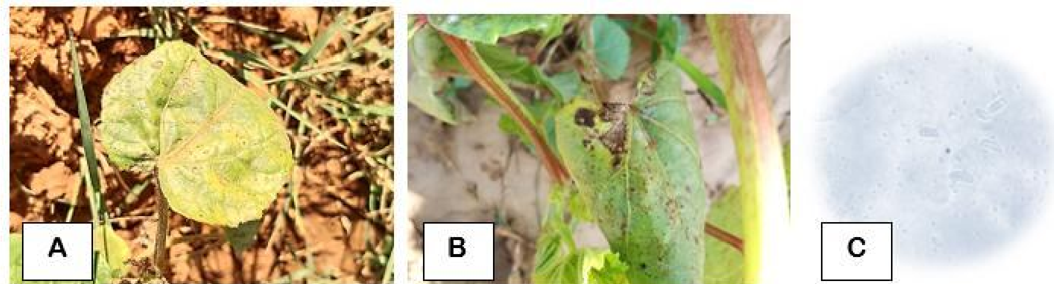

Figure 5. Leaf spot symptoms on a leaf at seedling stage(A), and at vegetative stage (B) of okra;*Ascochyta* conidia (C) observed under a microscope (40x).

4. **Wilt:**

Symptoms: Vascular wilts appeared as more or less rapid wilting, browning, and dying of leaves and succulent shoots of plants followed by death of the whole plant. Wilts occurred as a result of the presence and activities of the pathogen in the xylem vessels of the plant (Figs. 6-9). This result is in support of CABI, (2005), which reveals, that in wilt disease, initially older leaves turn yellow followed by complete wilting of the crop and it can attack the crop at any stage, but contrary, during this research work, no wilt infection was recorded at vegetative stage. It is a matter of fact that at this growth stage, the plant could have developed resistance against wilts. As long as the infected plant is alive, wilt-causing fungi remain in the vascular (xylem) tissues and a few surrounding cells. Though this infection is caused by four genera of fungi,*Fusarium*, *Pythium*, *Rhizoctonia,* and *Verticillium*, only *Fusarium* and *Rhizoctonia* were identified at the flowering stage (Fig. 10).

***4.1 Fusarium*:** Wilt is a common disease that occurs on the okra due to the conidia of *Fusarium*. The mycelium or conidia penetrates the vascular tissue and discoloration of tissue. The fungus forms hyphae which grow and fill all the junctions of the epiderma. In

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the crown region of some vegetables, the development of hyphae is more rapid (Lagopodi*et al*. 2002).

Cultural Characteristics: Brightwhitish to pinkish colonies grown on PDA medium. Aerial mycelium appeared as floccose to tufted, loosely cottony on the agar surface.

Causal Organism:*Fusarium*sps. branched and unbranched conidiophores produce micro-conidia and macro-conidia. Micro-conidia are aseptate, oval-shaped, and never from in chains. Macro-conidia are 2-5 septate, fusiform, curved, and pointed at both ends. Pedicellate conidia were found abundant on the PDA media surface (Fig. 6).

Figure 6. Wilt symptoms at the seedling stage; *Conidia of Fusarium*under a microscope (40x).

***4.2 Pythium*:**They cause damping-off and rots at the bottom of the stem or near the soil line on or on the root.

Cultural Characteristics:fast-growing, mostly colorless, white colonies reaching 4-5 cm on PDA medium. Thread-like, aseptate,and curved branches of hyphae well well-developed.

Causal organism:*Pythium* hyphae arising at right angles, sporangia spherical and blunt-ended. Conidia colorless, thick-walled. Oogonia spherical short side branches with a smooth wall (Fig. 7).

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Figure 7. Wilt symptoms at the seedling stage; *Pythium* oogonia under a microscope (40x).

***4.3 Rhizotonia*:**They may cause damping off and root rots on okra.

Cultural Characteristics:fast-growing, colorless colonies grown on PDA medium, aerial hyphae rapidly becoming brown.

Causal organism:*Rhizoctonia*sps. loosely packed, branching at right angles, barrel-shaped hyphal cells in pale brown to brown color. *Rhizoctonia* cannot produce any conidia. Asexual fruit bodies and spores lacking. Sclerotia formed in irregular size and shape and black or brown in color (Fig. 8).

Figure 8. Wilt symptoms at seedling stage; *Rhizoctonia* conidia (40x)

***4.4 Verticillium*:**Verticillium is responsible for a serious wilt. Some of the species of *Verticillium* are considered to be root inhabitants and vascular pathogens. Crown leaves initially wilt and take on a faded green color. As symptoms progress, leaf margins develop "V"-shaped chlorotic lesions, which eventually collapse and turn necrotic. Brown discoloration of root and stem tissues at the crown of the plant is visible.

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Cultural Characteristics:colonies grown moderately on PDA, flocculose, white, yellowish or orange-brown, dark mycelium.

Causal Organism:*Verticillium* septate, branched hyphae lightly colored. Conidiophores are verticillately branched and short, and conidia are small, one-celled. They produce balls at the apex of the phialides (Fig. 9).

Figure 9. Plant completely collapsed at seedling stage –encircled; *Verticillium* branched conidiophores (40x)

5. **Powdery Mildews:**

Symptoms: Symptoms appeared on tender leaves, at the seedling phase, in the form of white thread-like structures (Fig. 10).Later, duringthe vegetative growth period, they were visible as pale yellow spots on stems, petioles, and leaves.Gradually these spots turned into white mass on the upper and/or lower leaf surfaces. As the spots enlarge, conidia (clearly visible at flowering stage), are produced from affected tissue and the spots take on a powdery appearance. Infected leaves gradually turn yellow and become brown and papery. According to Varela and Seif (2004), severe infection causes the affected plant parts and leaves to roll upward and eventually scorch. Infection occurs when humidity is in the range of 52 to 75% and air temperatures are around 26 to 27 °C.These results are matched with Heffer, et al., (2006), their observation showedthatmildew causes skin cracking on fruits. Later, the diseased fruit drops.

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Figure 10. White patches of mass on the upper leaf surface at earlier growth stage (A); vegetative (B) and flowering stage (C).

Cultural characteristics: These fungi which can cause powdery mildew are obligate parasites, meaning that they require a living host to survive. Hence it is not cultured in the Laboratory.

Causal Organism:*Golovinomycescichoracearum* (synonym = *Erysiphecichoracearum*) *Podosphaeraxanthii* (synonym = *Sphaerothecafuliginea*) (Fig. 11).

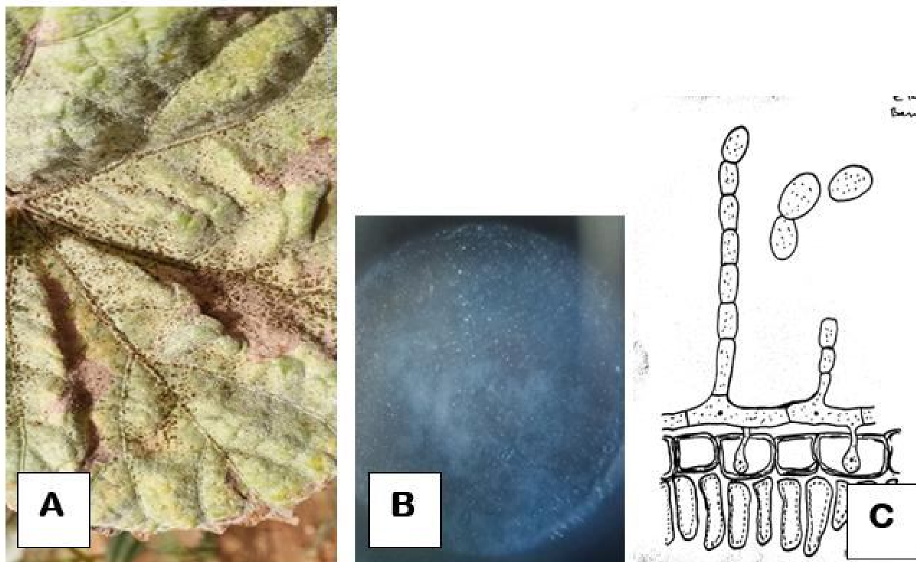

Figure 11. Powdery mildewfruiting bodies on a leaf at flowering stage (A) of okra; Conidiophore and conidia - find globular shining conidia - of *Erysiphecichoracearum*observed under 40x (B); and hand-drawn picture (C).

6. **Vein-Clearing/Yellow Vein Mosaic:**

Yellowing of the entire network of veins in the leaf blade is the characteristic symptom. Late in the season (at the vegetative growth phase), severe infections the younger leaves to turn yellow, and become reduced in size, and the plant was highly stunted. The veins of the leaves were cleared by the virus and the interveinal area became completely yellow or white (Fig. 12).

Pathogen: Yellow vein mosaic virus (YVMV).

Figure 12. Yellowing of veins in the leaf

Insect Pests at Different Phenological Stages

Leaf minor, aphids, African bollworms, mealy bugs, and *blister beetle* pests were identified during different crop growth stages. Aphids were the prominent pest observed in all treatments at three different growth stages. *Leaf minor, mealy bugs,* and *blister beetle* were not noticed in the flowering stage (Figs. 13). On the other hand, *African Boll Worm* was found only in T4 at this stage. Moreover, intercropping with cowpea (T3) and FYM (T2) showed a declining tendency in the pest population from the seedling stage to the flowering stage. This is due to the presence of two hosts (okra and cowpea) and insect pests were attracted by the cowpea to reduce the pest infestation to the targeted main crop or okra(Table 2).

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Figure 13. Insect Pests on okra

Soil Mycoflora Isolated from distinct phenological stages of Okra

Seventeen soil mycoflora (*Alternaria*sps, *Ascochyta*sps, *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Arthrobotrys*sps, *Cladosporium*sps, *Curvularia*sps, *Drechslera*sps, *Fusarium*sps, *Meria*sps, *Mucor*sps, *Penicillium*sps, *Pythium* sps, *Phtophthora*sps, *Rhizoctonia*sps and *Verticillium*sps.) were identified from different treatments at different growth stages (Table 3).

At the seedling stage 14 different fungal flora, 8 soil fungi at the vegetative stage, and 11 geo-fungi at the flowering stage were identified. In seedling stage 9 fungi in T1, T2, and T4;tenfungal species in T3 were identified which were associated with the rhizosphere of the host plant. In the vegetative stage 3 fungi in T1, 1 fungus in T2, 5 fungi in T3, and 2 fungi in T4; and flowering stage 8 fungi in T1, and T2, 3 fungi in T3, 2 fungi in T4 were identified that are associated to the rhizosphere of the host plant.

*Ascochyta*sps, *Drechslera*sps. and *Verticillium*sps fungi were isolated only at the seedling stage in T1, T3, T2, and T4 respectively.Whereas,*Arthrobotrys*sps. found in T1 and T2 at flowering stage. *Phytophthora*spswere noticed in T2 at the vegetative stage, on the other hand, *Aspergillus niger*mycoflora were noticed more in all treatments, except T1, T2, and T3 of the vegetative stage.

In the intercropping treatment (T3) maximum mycoflora(10) at the seedling stage, 5 fungal species at the vegetative stage, and only 3 fungi at the flowering stage were recorded when considering other treatments. A significant decreasing observation was noticed in the number of fungal species tremendously fromthe seedling stage to the flowering stage. This may be due to intercropping of cowpea as a legume, according to the research done by Mcdonagh and Hillyer(2003), which played a role in N2-fixation has been one of the reasons as fertilizer for improving N supply and not allowing the growth of mycoflora.

FYM treatment (T2) of the vegetativestagehas exhibited the lowest, i.e. only one fungus(*Phytophthora*sps.), was identified. This could be due to the presence of antagonistic microbes which are abundant in manure and might not permit to developmentof other fungi. At the vegetative stage, crops may grow at optimum levels of nutrients in biologically active soils, because roots can spread wide and deep, and soil organisms such as mycorrhizal fungi help plants access nutrients (Brady et al., 2008). Whereas plants growing in anearlier stage, biologically-depleted soil (seedling and floweringstages) may show deficiencies in nutrients owing to restricted root growth or lack of beneficial organisms to help the crop take up nutrients as a result, a maximum (10) mycoflora were found in other treatments and growth stages.The results are in line with the observation recorded by Marcial et al.(2003), Viebahn et al. (2005), Mougel et al. (2006), Broz et al. (2007), Singh et al. (2007) and Broeckling et al. (2008). A similar scenario was reported by Buée et al., (2009) that the plant species, plant developmental stage, and soil type have been indicated as major factors determining the composition of rhizosphere fungal communities, where mycorrhization takes place and this microbial complex likely plays a role in mineral weathering and solubilization processes.

*Meria*spp, a nematophagus fungus was identified, for the first time in Eritrea, in T1 at the vegetative stage of okra(Table 3).

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Table 3. Occurrence of Soil Fungal Flora at Different Phenological Stages of Okra in the Fields of HAC

Phenological Stages of Okra	Seedling Stage				Vegetative Stage				Flowering Stage			
Fungal sps. Identified	**T 1**	**T 2**	**T 3**	**T 4**	**T 1**	**T 2**	**T 3**	**T 4**	**T 1**	**T 2**	**T 3**	**T 4**
*Alternaria*sps.	-	√	-	√	-	-	√	-	√	-	-	-
*Ascochyta*sps.	√	-	-	-	-	-	-	-	-	-	-	-
Aspergillus flavus	√	√	√	√	-	-	-	√	√	√	-	-
Aspergillus fumigatus	√	√	√	√	-	-	-	-	√	√	√	√
Aspergillus niger	√	√	√	√	-	-	-	√	√	√	√	√
Arthrobotrysoligospora	-	-	-	-	-	-	-	-	√	√	-	-
*Cladosporium*sps.	-	√	√	-	-	-	√	-	√	√	-	-
*Curvularia*sps.	-	√	√	-	-	-	√	-	√	√	√	-
*Drechslera*sps.	-	-	√	-	-	-	-	-	-	-	-	-
*Fusarium*sps.	√	√	-	√	√	-	√	-	√	-	-	-
*Drechmeria*sps.	-	-	-	-	√	-	-	-	-	-	-	-
*Mucor*sps.	√	√	√	√	-	-	-	-	-	-	-	-
*Penicillium*sps.	√	-	√	√	-	-	-	-	-	√	-	-
Pythium sps.	√	-	√	√	-	-	-	-	-	√	-	-
*Phytophthora*sps.	-	-	-	-	-	√	-	-	-	-	-	-
*Rhizoctonia*sps.	√	-	√	-	√	-	√	-	-	-	-	-
*Verticillium*sps.	-	√	-	√	-	-	-	-	-	-	-	-
Total fungi (17)	**9**	**9**	**10**	**9**	**3**	**1**	**5**	**2**	**8**	**8**	**3**	**2**

***Treatments:** T1=control; T2=Farm Yard Manure; T3= Intercropping with cowpea; T4= Chemical Fertilizer; √**: present; -: absent**

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Alternaria*sps*:this soil fungus was one of the saprophytes and occurred at all growth stages of okra. Colonies grown on PDA media, olive black in color. Conidia are short, and simple, with transverse septa (Fig. 14). Actually, many *Alternariasps.* are mostly saprophytic, i.e., they cannot infect living plant tissues but grow only on dead or decaying plant tissues. *Alternaria* may attack, through wounds, on senescent or old tissues of petals, leaves, and ripe fruits when they approach maturity (Agrios, 2005).

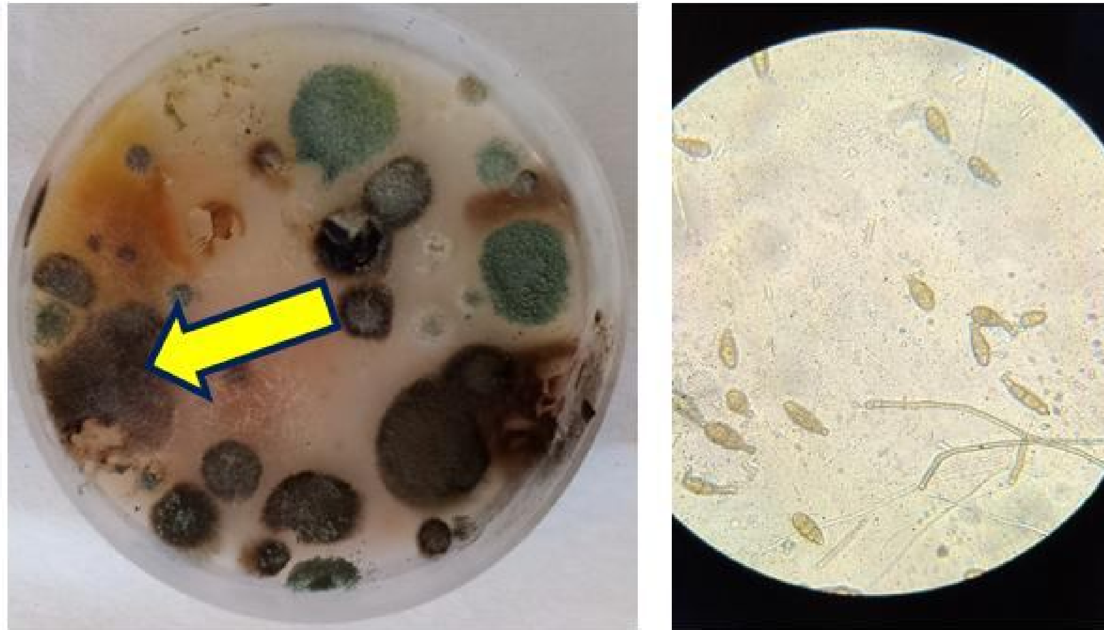

Figure14. Colony on PDA (shown with arrow); Conidium of *Alternaria* (40x)

***Ascochyta*sps:** this pathogen is found only in T1 at the seeding stage.Dark brown colonies were cultured on PDA. Conidia were 2-celled, ovoid, and smooth (Fig. 15).

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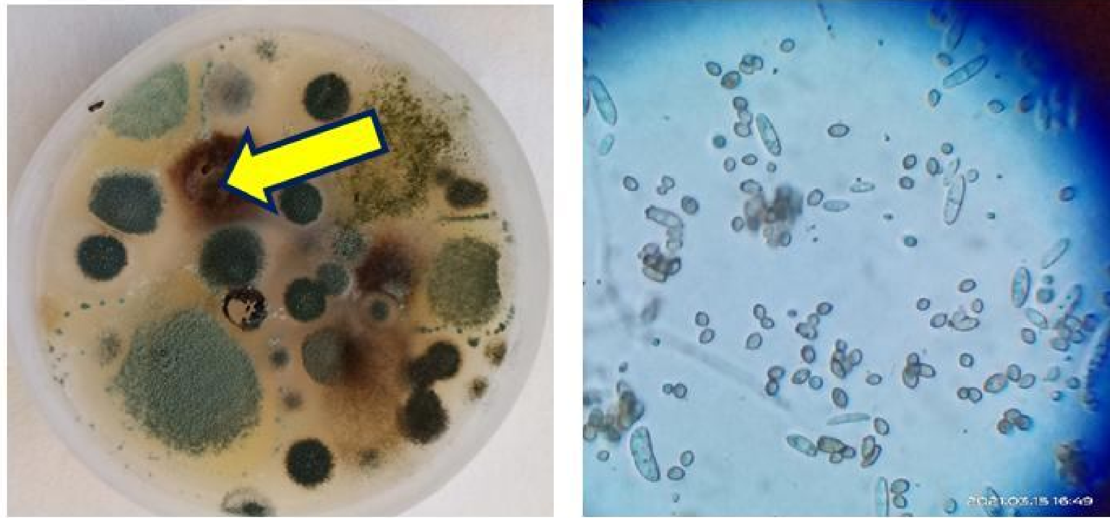


Figure 15.*Ascochyta* colonies and conidia (40x)

Aspergillus flavus: it was noted in all treatments at the seedling stage, but not identified in T3 at both vegetative and flowering stages. Colonies were dark yellow-green and turned into deep gray-green in older cultures (Fig. 16).

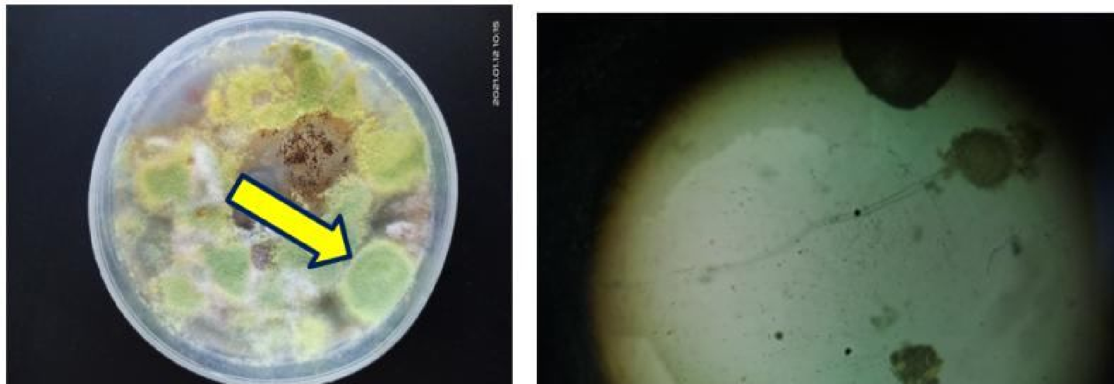


Figure 16. Colonies of *Aspergillus flavus* and conidiophore (10x)

Aspergillus fumigatus: it was identified in both growth stages except the vegetative stage.Colonies dull blue-green and velvety. Conidiophores bearing a single series of phialides (Fig. 17).

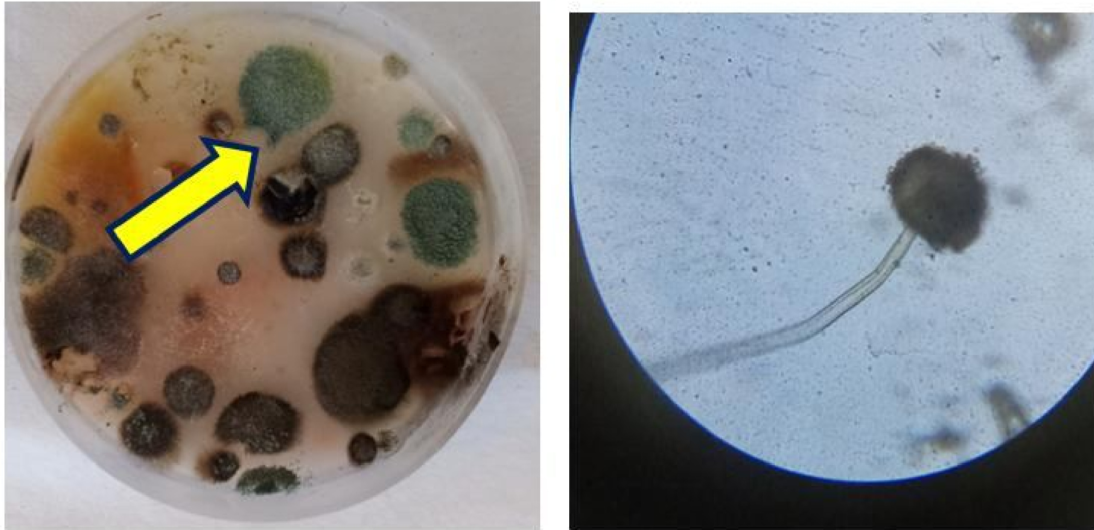


Figure 17.Colony and conidophore of *Aspergillus fumigatus*(10x)

Aspergillus niger:it was prominent in both seedling and flowering stages.Carbon black to deep brownish-black colonies with cordial heads on PDA media were observed (Fig. 18).

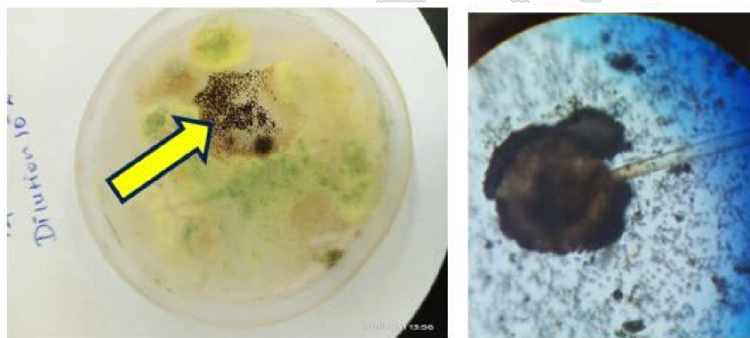


Fig.18. Carbon-black colony with conical heads and conidiophore (10x)

Arthrobotrisoligospora: It was observed in the rhizosphere soils of okra at the flowering stage only.This fungus is predacious on nematodes. Some plant parasitic nematodes, either juveniles or adults cause diseases or at least might be involved in the production of the disease on okra. Plant pathogenic nematodes,in contrast, are also parasitized by other microorganisms or sometimes, are trapped by some predatory fungi. The nematode-trapping fungi are predatory species that capture nematodes with specialized devices of various types such as adhesive nets. Such type of fungi *Arthrobotrisoligospora*wasidentified in T1 and T2 at the flowering stageand*Drechmeria*sps. was noticed at a vegetative stage in T1.*Arthrobotrys* fungus was also identified earlier by Medhanieet al., (2017) for the first time in the rhizosphere of chilli of Derato village in SubzobaHagaz. According to Mankau (1961), the nematode root-knots were reduced in tomato and okra by using *Arthrobotrysdactyloides.*

Arthrobotrisoligospora has a complex net of adhesive hyphae. This adhesive network arises by the short lateral branches that curve round and anostomose with the parent hyphae or with neighboring branches forming a complex three-dimensional network in which eelworms become entangled and held by a sticky secretion produced by the cells of a loop. This predatory organism**is** characterized by the production of large, two-celled, obovate conidia borne on node-like swellings either in a single whorl at the apex or in a series of whorls along the length of the conidiophores. The first conidium produced is apical and successions of conidia are produced in the acropetal manner(Fig. 19).

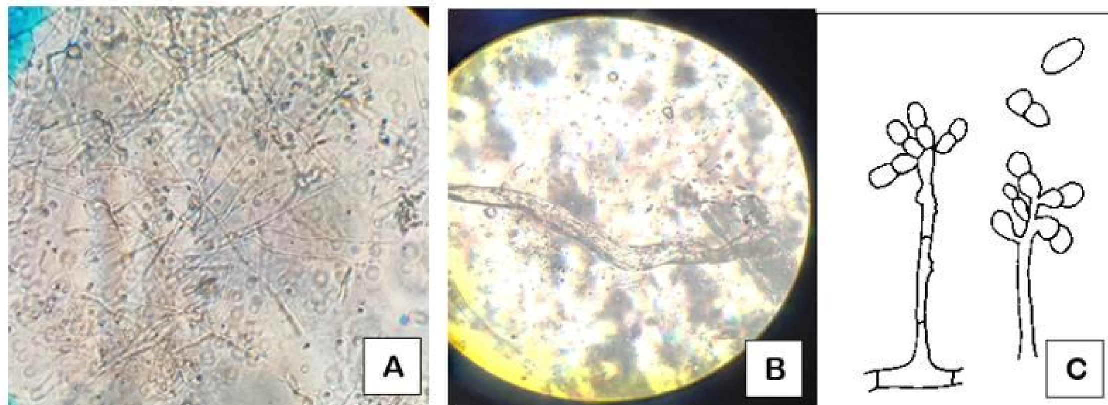


Fig.19. Adhesive network of *Arthrobotrys oligospora*(A);a nematode trapped by the mycelium of *Arthrobotrys (B);*Conidiophores and conidia.

***Cladosporium*sps**was not identified in T4 of all growth stages of okra. Colonies were light olive green and covered with pale grey aerial mycelium. Conidiophores produce 0-2 septate conidia. The conidia are rounded at the ends and thick-walled (Fig. 20).

Fig.20. Colony of *Cladosporium*; Conidia and conidiophore

***Curvularia*sps:** this pathogen was present in T3 of all phenological stages. Colonies were observed in dark grey and hairy brown. Conidia simple, culved with 3 transverse septa. Middle septum dark brown with paler end cells (Fig. 21).

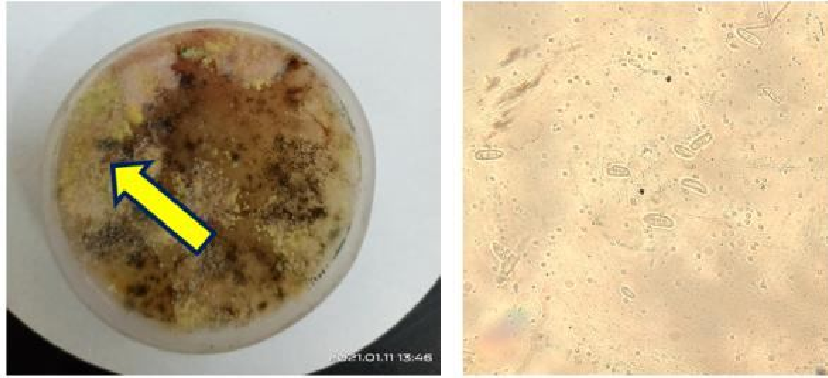


Fig.21. Colony of*Curvularia*sps. and conidia

***Drechslera*sps:** Only in treatment T3, at seedling stage of okra this pathogen was identified. Colonies on the PDA medium it was observed as velvety, dark gray with full of mycelium (Fig.22).

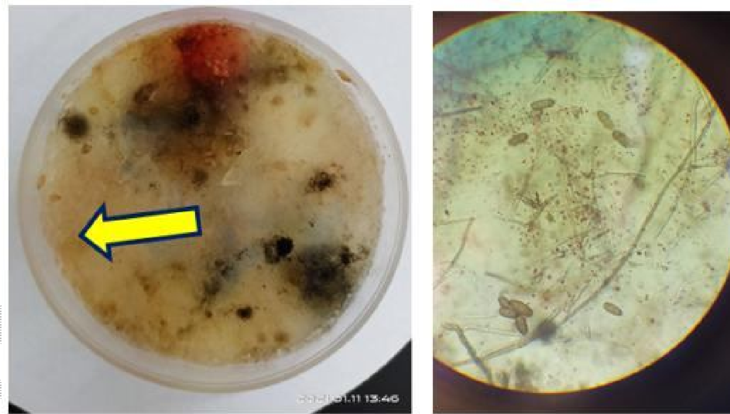
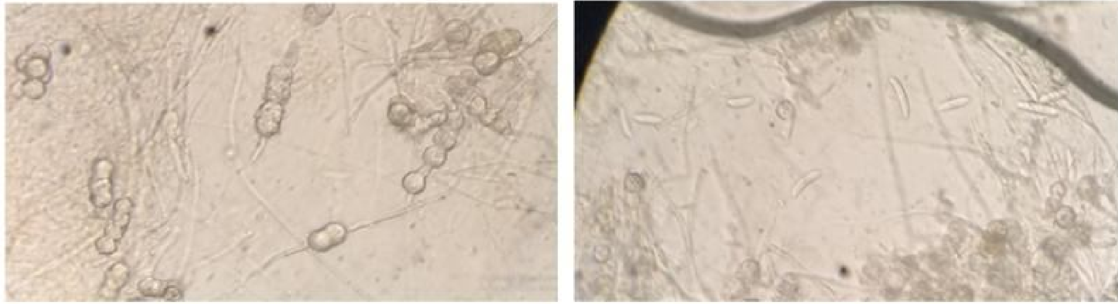


Fig.22Velvety, dark gray *Drechslera*mycelial colony, and conidia

***Fusarium*:**this is a soil fungus and easily grown on PDA with whitish to pinkish colonies appearing on PDA medium. Loosely cottony aerial mycelium appeared as floccose to tufted on the agar surface.*Fusarium*sps. Intercalary and terminally chlamydospores were noticed. Micro-conidia and macro-conidia were observed separately (Fig. 23).



***Drechmeria*:**This predacious fungus was found only in treatment T1 at a vegetative stage in the rhizosphere soil of okra. Conidia are conical, unicellular, produced on conidiophores. Colored mycelium was noticed grown on PDA media on dead nematodes. Hyphaecontainconidia thatare conical, hyaline, and unicellular, produced singly or in clusters on apical or lateral sterigmata borne on the simple conidiophores. The conidia at maturity bear a globose adhesive knob at the narrow end. Such conidia readily adhere to the cuticle of a nematode (Fig. 24).

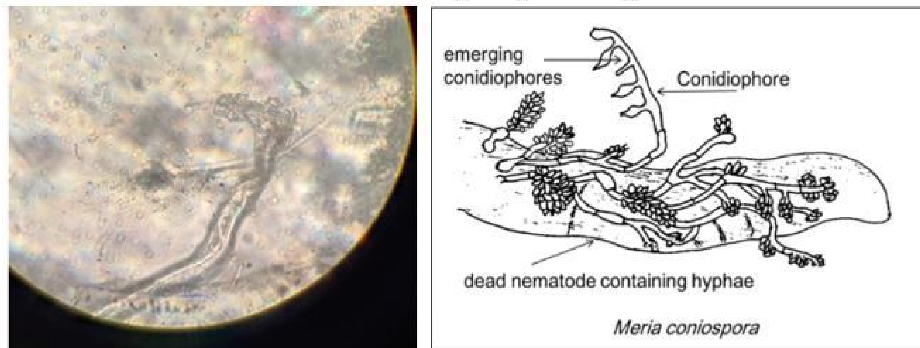


Fig. 24. Conidophores of *Drechmeria*;hand-drawn after observation

***Mucor*sps:** This saprophyte was isolated in PDA media at the seedling stage in all treatments. This fast-growing fungus appeared initially white to yellow, gradually growing into dark grey with sporangia. Mycelium without rhizoids and stolons. Unbranched with terminal sporangia are on the branch ends (Fig. 25).

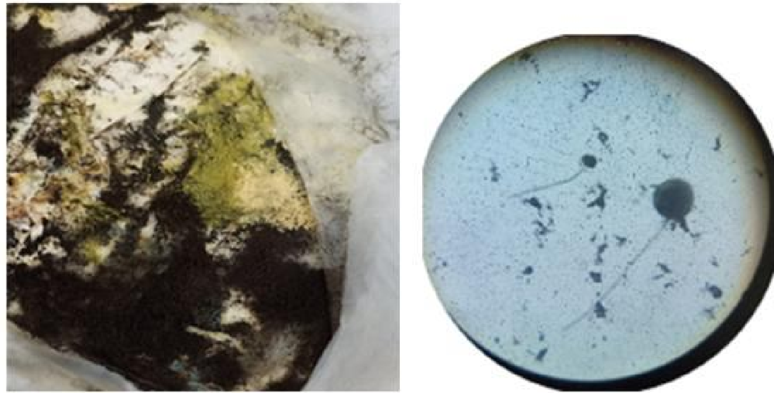


Fig.25. *Mucor* mycelium with sporangiophore and spores (40x)

Penicilliumsps: At the vegetative stage of okra, *Penicillium*sps were not appeared. Radial colonies plane, deep, smooth texture, or granular were noticed. Whitemycelium with entire margins produce grayish conidiation. Conidiophores are branched and brush-like. Conidia are borne in long chains on phialides (Fig. 26).

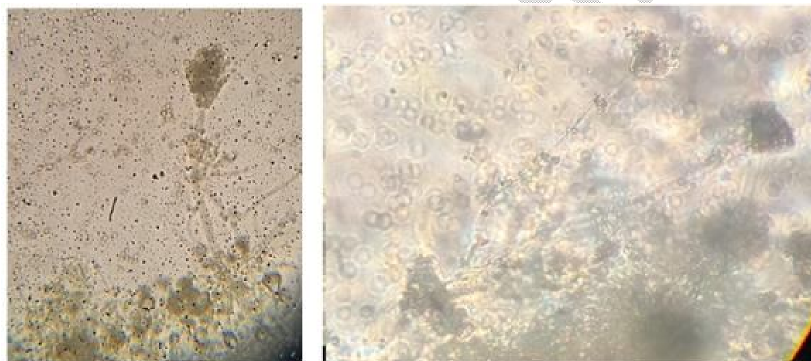


Fig.26.Aerial hypha phialides and conidia of *Penicillium*sps.

***Pythium* sps:** This fast-growing pathogen grows in colorless colonies. The mycelium shows coenocytic and thread-like hyphae. Spherical or cylindrical sporangia are terminal or intercalary. Zoospores are formed in a vesicle at the tip of the sporangium. Echinulate oogonia are spherical. (Fig. 27).

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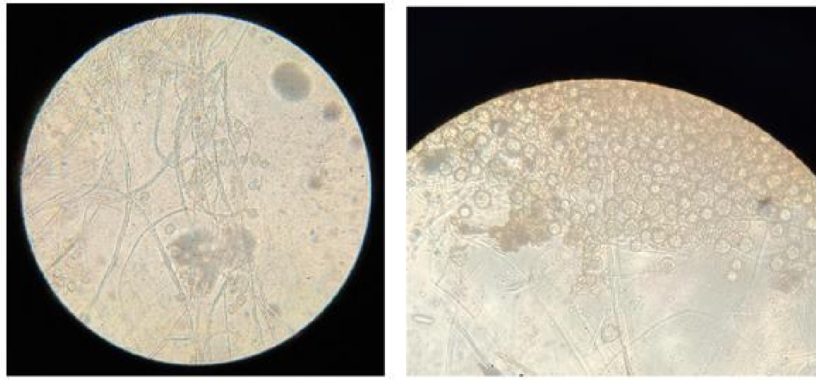

Fig.27. Mycelium and Oospores of *Pythium* (10x and 40x)

***Phytophthora*sps:**Slow-growing colonieson PDA with sporangia.Lemon-shaped papillate sporangia are borne on the sporangiophores. The zoospores release from the sporangium through the exit at the point of the papilla (Fig. 28.).

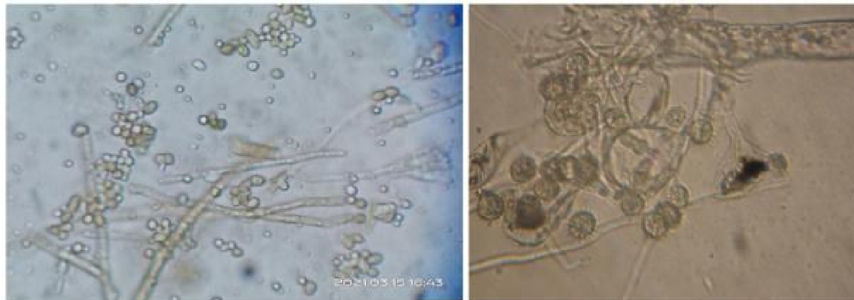

Fig.28. Sporangia of *Phytophthora*and;Zoospores release from the sporangium

***Rhizoctonia*sps:**The young colonies were some shade of brown. The cells of septate hypha are barrel-shaped, branching at right angles and pale brown in color. The lateral branches from the main hyphae are invariably constricted at the point of the origin and the septum occurs in the branch near the junction (Fig.29.)

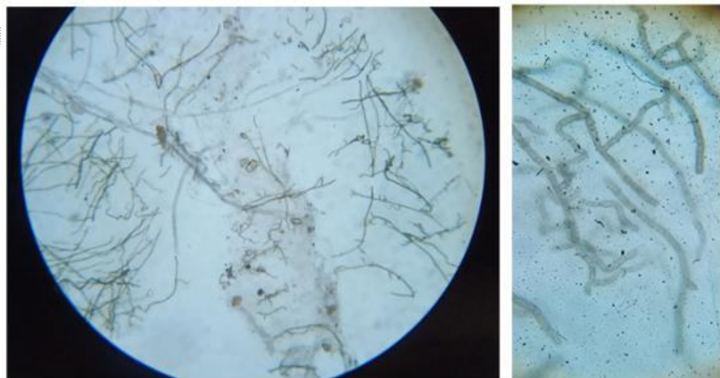

Fig.29. Mycelium of *Rhizoctonia*(10x, and 100x)

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***Verticillium*sps:** White to yellowish, orange, orange-brown colonies grown on PDA media. Septate, branched hyphae with erect and branched conidiophores. Conidia are borne on the branchlets produced at the apex of the phialides (Fig. 30). It is in line with Rawat et al., (1999) showed that Qualitative analysis of microflora present in therhizosphere of okra during all stages of plant growth showed thepresence of egg parasitizing and antagonisticfungi *Verticillium*sp.

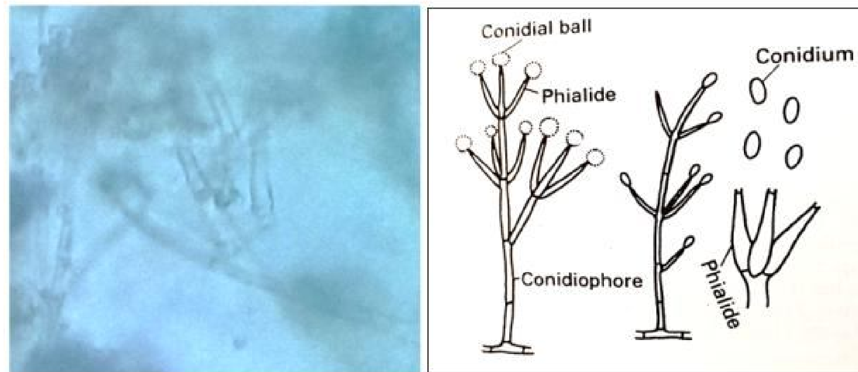

Fig.30. *Verticillium*(40x); Conidiophores and conidia dehisced from phialides

4.6. Percentage Occurrence of Disease Incidence of Powdery Mildew and Blights

This experiment showed that there was a lower disease incidence of powdery mildew recorded at T3 (38.7) of the seedling stage and the highest incidence was in T1 (95.53) of the flowering stage. This could be a reason that control treatment has no management applied, and intercropping was an asset in declining of disease. Disease incidence of blight was highly recorded in T2 (81.1) of the flowering stage which was an effect of *Alternaria* of soil fungi, and lowest was found in T1 (39.4) of the seedling stage of okra (Table 4).

The percentage occurrence of disease incidence of powdery mildew was non-significant inthe seedling stage but there was a slight difference among the treatments highest value recorded at control (95.53) and lowest at intercropping (38.7). It was non-significant at the vegetative stage but there was a slight difference among the treatments highest value recorded at intercropping (75.8) and the lowest value at chemical fertilizer (69.0). The disease incidence of powdery mildew was the highest at control (95.53) and lowest at chemical fertilizer (91.27) at the flowering stage.

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Table 4. Percentage Occurrence of Disease Incidence of Powdery Mildew and Blight in Four Treatments at Different Phenological Stages of Okra

% of Disease Incidence of Powdery Mildew								
Phenological Stages of Okra	**Treatments***				**Statistics**			
	T 1	**T 2**	**T 3**	**T 4**	**Mean**	**LSD**	**SE**	**CV (%)**
Seedling Stage	56.4	50.0	38.7	44.5	47.4	NS	10.29	21.7
Vegetative Stage	70.83	70.3	75.8	69.0	71.5	NS	6.21	10.9
Flowering Stage	95.53	93.8	92.2	91.27	93.21	1.792	1.244	1.3

% of Disease Incidence of Blight								
Phenological Stages of Okra	**Treatments***				**Statistics**			
	T 1	**T 2**	**T 3**	**T 4**	**Mean**	**LSD**	**SE**	**CV (%)**
Seedling Stage	39.4	47.2	49.6	42.67	44.7	NS	4.16	9.3
Vegetative Stage	49.0	52.1	44.7	40.62	46.6	NS	5.06	10.9
Flowering Stage	67.3	81.4	63.0	56.5	67.1	NS	3.13	4.7

***Treatments:** T1=control; T2=Farm Yard Manure; T3= Intercropping with cowpea T4=chemical fertilizer

Percentage Occurrence of Disease Incidence of Blight was found to be non-significance at all stages, however, there was a slight difference in all stages at different treatments, in the flowering stage the highest (81.4) DI was recorded at T2 and lowest (39.4) was recorded at T1(Table 4).

CONCLUSION

The study conducted on okra (*Abelmoschus esculents* L. Moench.) at Hamelmalo Agricultural College identified powdery mildew and blight as the major diseases, with additional occurrences of damping off, wilt, and leaf spot. Chemical fertilizer was one of the most effective in reducing disease prevalence while intercroppinConsentg with cowpeas attracted pests. The flowering stage showed a significant difference in powdery mildew incidence, whereas blight incidence was non-significant across all growth stages and treatments. Notably, nematode predacious fungi *Arthrobotrisoligospora* and *Drechmeria*sps. were identified for the first time in the rhizosphere soils of okra in Eritrea. The study shows the influence of different treatments on okra's different growing stages.

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Disclaimer (Artificial intelligence):

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

REFERENCES

Adugna Haile and G. Sethumadhava Rao. (2023). *Diseases of Crops in Eritrea*. Clever Fox Publishing.Chennai, India. p. 206. ISBN:978-93-56484-78-8.

Agrios, G.N. (2005). Plant Pathology, 5th Ed, Department of Plant Pathology. The University of Florida, Elsevier Academic Press 30. Corporate Drive, Suite 400, Burlington, MA 01803, USA.

Alimi T., (2004). Use of Cultural Practices and Economic Impact of Insecticide Use, Awareness and Practices of Insecticide Safety Precaution on Okra Production. *Journal of Vegetable Crop Production* 10(1): 23-36.

Aneja K.R., (2004). Experiment in microbiology, plant pathology, and biotechnology. 4th Ed, New International (P) Limited Publisher, India, 121-128.

Bahran Kinfe and G. Sethumadhava Rao. (2021). Effect of Sowing Dates, Phosphorous and Potassium Fertilizer Rates on Phenological, Growth and Yield Aspects of Okra (*Abelmoschusesculentus L*) in Hamelmalo Subzone, Eritrea. *Journal of Experimental Agriculture International. 43(7): 93-102, 2021;* Article no. JEAI.72994. ISSN: 2457-0591. DOI:10.9734/JEAI/2021/v43i730717.

Barnett H L and Hunter B B (1972). Illustrated Genera of Imperfect Fungi. Burgess Publishing Company. Minneapolis.

Brady, Nyle C., and Ray R. Weil. 2008. *The Nature and Properties of Soils, 14th ed*. Pearson Education, Inc., Upper Saddle River, NJ. 965 pp.

Broeckling CD, Broz AK, Bergelson J, Manter DK, Vivanco JM (2008). Root exudates regulate soil fungal community composition and diversity. *Appl Environ Microbiol* 74:738–744.

Broz AK, Manter DK, Vivanco JM (2007). Soil fungal abundance and diversity: another victim of the invasive plant *Centaurea maculosa*. ISME J 1:763–765.

Buée M. &W. De Boer & F. Martin & L. van Overbeek & E. Jurkevitch. (2009). The rhizosphere zoo: An overview of plant-associated communities of microorganisms, including phages, bacteria, archaea, and fungi, and of some of their structuring factors. Springer Science Business Media. *Plant Soil* (2009) 321:189–212. DOI 10.1007/s11104-009-9991-3

CAB International (2005). *Crop Protection Compendium*, 2005 Edition. Wallingford, UK. www.cabi.org

UNDER PEER REVIEW

Costa, M. C. B., G.D. Oliveira, H.P. Haag. (1981). Nutrição mineral de hortaliças - Efeito da omissão dos macronutrientes e do boro no desenvolvimento e nacomposiçãoquímica de hortaliças. Nutrição mineral emhortaliças. *Campinas:FundaçãoCargil.* 6: 257-276.

Diba K, Kordacheh P, Mirhendi S M, Rezaie S and Mahmoudi M (2007). Identification of Aspergillus species using morphological characteristics. *Pakistan Journal of Medical Sciences* 23: 867-872.

ECHO (2003). Plant Information Sheet. ECHO, North Fort Myers, Florida, USA.

Gilman J C (2001). A Manual of Soil Fungi, 2nd Indian Edition, Biotech Books, Delhi, 2001.

Gupta, A., K. Srinivas and V. Shukla. (1981). Response of okra *(Abelmoschus esculentus* L. Moench) to plant spacing and nitrogen, phosphorus fertilization. *Indian J. Hort. Sci.* **38.** (3-4): 218-222.

Heffer, V., Johnson,K.B., Powelson, M.L. and Shishkoff, N. (2006). Identification of Powdery Mildew Fungi anno 2006. *The Plant Health Instructor*. DOI: 10.1094/PHI-I-2006-0706-01 www.apsnet.org

Incalcaterra G, F.G. Vetrano, P. J. Stoffell, D. J. Canttliffe and G. Damato. (2000). Effect of two sowing dates and plastic mulch on okra production. *ActaHorticulturae*, Vol.53:329-336.

ISTA. International Seed Testing Association. (1976).

Kemble, J.M., Sikora E.J., Zehnder, G.W., and Patterson M.G., (1995). Guide to Commercial Okra Production. http://www.aces.edu/pubs/docs/A/ANR-0959/

Khalid, U., Ahmed, E. and Muhammad, U.K. (2005). Integrated weed management in okra. *Pak. Weed Sci. J.,* 11(1/2): 55-60.

Lagopodi A.L., Ram A.F.J., Lamers G.E.M., Punt P.J., Van den Hondel C.A.M.J.J., Lugtenberg B.J.J, Bloemberg G.V. 2002. Novel aspects of tomato root colonization and infection by *Fusariumoxysporum*f. sp. *radicis-lycopersiciwere* revealed by confocal laser scanning microscopic analysis using the green fluorescent protein as a marker. Mol. Plant Microbe Interact. 15 (2): 172–179.

Mankau, R. (1961). An attempt to control root-knot nematode with *Dactylariathamasia*Drechsler and *Arthrobotrysarthrobotryoides*Lindau. Plant Disease Reporter, 45: 164-166.

Marcial Gomes NC, Fagbola O, Costa R, Rumjanek NG, Buchner A, Mendona-Hagler L, Smalla K (2003) Dynamics of fungal communities in bulk and maize rhizosphere soil in the tropics. Appl Environ Microbiol 69:3758–3766.

Mcdonagh J. F. And A. E. M. Hillyer. (2003). Grain Legumes in Pearl Millet Systems in Northern Namibia: An Assessment of Potential Nitrogen Contributions. *Expl Agric.* 39 (349–

UNDER PEER REVIEW

362 C). Cambridge University Press. DOI: 10.1017/S0014479703001364. Printed in The United Kingdom

Medhanie,E., G. Mussie, A. Selamawit, G. Sethumadhava Rao and N. Syed Danish. (2017). Occurrence and Identification of Rhizosphere Mycoflora of Selected Vegetable Crops Grown in Zoba Anseba, Eritrea. *Trends in Biosciences, 10(33). ISSN 0974-8431. 7099-7104.*

MoA. (2015). Statistical report, Asmara, Eritrea.

MoA. Ministry of Agriculture Zoba Anseba. (2008).

Mohanta, H. C. (1998). Effect of phosphorus and genotypes on the yield and contributing characters in okra *(Abelmoschus esculentus* L. Moench). M S. Thesis, Dept. of Horticulture, IPSA, Salna, Gazipur.

Mougel C, Offre P, Ranjard L, Corberand T, Gamalero E, Robin C, Lemanceau P (2006). The dynamic of the genetic structure of bacterial and fungal communities at different development stages of *Medicagotruncata*Geartn. Cv. Jemalong line J5. New Phytol 170:165–175.

Nagamani A, Kumar I K and Manoharachary C (2006). Hand Book of Soil Fungi, IK International Publishing House Pvt Ltd, New Delhi, India.

Rao, P. U. (1985). Chemical composition and biological evaluation of okra (Hibiscus esculentus) seeds and their kernels. *Plant Foods for Human Nutrition*. 35 389-396.

Rawat, R., Pandey, A., Saxena, G. and Mukerji, K.G. (1999). Rhizosphere biology of root-knot diseased *Abelmoschus esculentus* about its biocontrol. In “From Ethnomycology to Fungal Biotechnology: Exploiting Fungi from Natural Resources for Novel Products”. (eds. Singh, J. and Aneja, K.R.) Kluwer Academic/Plenum. Publishers, London pp. 173-183.

Rifai, M.A. (1969). A revision of the genus Trichoderma Mycological papers.

Sadat, M. S. I. (2000). Studies on the effects of different levels of nitrogen, phosphorous, and potassium on the growth yield and seed production of okra *(Abelmoschus esculentus* L.).M S. Thesis, Dept. of Horticulture, Bangladesh Agril. Univ., Mymensingh, Bangladesh.

Shaha, P.K., Aditya, D.K. and SharfUddin,A.F.M. (1989). Effects of plant spacing and picking interval on the growth and yield of okra cv.Pusa. Sawani. *BangladeshHort*., 17(2):0-14.

Singh BK, Munro S, Potts JM, Millard P (2007). Influence of grass species and soil type on rhizosphere microbial community structure in grassland soils. Appl Soil Ecol 36:147–155.

Syed Danish Yaseen Naqvi, Adugna Haile, Sethumadhava Rao, Belay Teweldemedhin, Virendra Kumar Sharma, and Aggrey Bernard Nyende. (2016). Evaluation of husbandry,

UNDER PEER REVIEW

insect pests, diseases, and management practices of vegetables cultivated in Zoba Anseba, Eritrea. *Journal of Eco-friendly Agriculture 12(1): 47-50.*

Varela, A.M., Seif, A. (2004). A Guide to IPM and Hygiene Standards in Okra Production in Kenya. ICIPE, Kenya. ISBN: 92 9064 161 5.

Viebahn M, Veenman C, Wernars K, van Loon LC, Smit E, Bakker PAHM (2005) Assessment of differences in ascomycete communities in the rhizosphere of field-grown wheat and potato. FEMS *MicrobiolEcol* 53:245–253.

Warcup J H (1950). The Soil Plate Method for Isolation of Fungi from Soil. Nature. London, 1950. 166: 117-118.

UNDER PEER REVIEW