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Status of Lentil (*Lens culinaris* Medicus) Seed Borne Mycoflora and Evaluation of Botanical Extracts and *Trichoderma* spp. for their Management in Ambo District, West Shewa Zone, Ethiopia

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Abstract

This study was carried out to identify and quantify seed mycoflora associated with lentil seed samples and evaluate the antimicrobial activities of seven plant extracts and three *Trichoderma* species against the seed borne fungal pathogens as management strategies. *In vitro* antifungal evaluation of plant extracts was done by food poisoning technique and dual culture method was applied for evaluation of antagonistic effect of *Trichoderma* species against test fungi. Fifty seed samples were collected from different farmers' saved seeds. A total of 5 fungal species belonging to 5 genera were recovered from seeds and identified. Among them *Fusarium oxysporium f.sp.lentis, Cladosporium spp., Aspergillus spp. and Penicillium spp.* were the most predominant fungi to all tested seed samples. *Fusarium oxysporium f. sp. lentis* was found to transmitted from seed to seedlings and affect seed germination. The maximum seed germination rate (95.00%) was observed in Dase Akililo and minimum (38.50%) in Golja- seed samples. *In vitro* evaluation of hot and cold-water extracts of botanicals and *Trichoderma* species against *Fusarium oxysporium f. sp. lentis* showed maximum inhibitory action. All the tested plant extracts at 5, 10 and 15% concentrations, significantly inhibited the mycelial growth of the test fungi. The inhibitory action of the aqueous plant extracts on mycelial growth increased with increased in concentrations and the hot water extracts gave high toxicity than cold water extracts in the test fungi. The highest inhibitory effect was recorded in *Allium sativum* hot water extracts at 15 % concentration (79.26%). *In vitro* evaluation of antagonistic effect of *Trichoderma* species, all of them exhibited the strongest antagonistic activity against the test fungi. Generally, the current results indicated that most of the plant extracts and all three *Trichoderma spp*. tested in the present study results were potentially inhibit the growth of *Fusarium oxysporium f. sp. lentis*. Hence, further screening of active ingredients and the formulation of the products is needed to recommend for the end users.

***Key words*:** Lentil, Seed borne mycoflora, *Trichoderma* species, Botanicals, Hot and cold-water extracts

Lentil (*Lens culinaris* Medikus) is one of the important high value cool season pulse crops widely grown in Ethiopia. Lentil is a short and slender annual cool-season food legume, which was domesticated early in the Fertile Crescent of the Middle East. It is classified into two groups by seed size, namely the Chilean and Persian types. The large seeded Chilean type has 1000 seed weight of 50 g or more. The small seeded Persian type has 40 g or less of an average weight per 1000 seeds [1]. In many parts of the world, lentil is the cheapest protein food and contains dietary fiber, vitamin B and minerals, iron, but among the cool season legume crops, lentils are the richest in their important amino acids (lysine, arginine, and leucine); however, there is a shortage of certain lentil amino acids, including methionine and cystine. The crop has great significance in cereal-based cropping systems because it fixes nitrogen and the straw provides animal feed [2]. The total lentil cultivated area in the world is estimated at around 4.34 million hectares with annual production and productivity of 4.95 million tons and 1260 kg ha^{-1} respectively [3]. Ethiopia is the leading producer of lentil in Africa, followed by Morocco and Tunisia and is seventh leading producer in the world [4]. It is one the dominant pulse crop and the total area and production volume are about 113,684 ha and 0.17 million tons, with an average yield of 1.5 tons ha^{-1} [5]. It is mainly grown in the highlands of the country during the main and small rainy seasons [6]. Lentil planting date varies from region to region, and it usually ranges from late June to Mid-July in both mid and high-altitude areas, depending on the amount and distribution of rainfall, temperature, topography, and elevation of the areas where there is not water logging problem [7]. In the

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waterlogged highlands, planting is done when the rain stops in late September and early October. A soil pH of 6-8 is conducive for lentil production, but it can also tolerate a moderate alkalinity. It requires a minimum of 500 mm rainfall and a maximum of 850 mm; in the higher rainfall areas, good drainage is essential since waterlogged soil will have a great negative effect on yields and can aggravate disease development [8] because the crop plant is highly susceptible to excessive moisture.

The average seed yield of lentil in farmers' fields in Ethiopia generally ranges from 0.6 to 0.8 t /ha^{-1} [7]. Not surprisingly, lentil yield of the country was restricted to about 2.0 t/ha-^{1} has been reported from experiments performed under controlled field experimental conditions [9]. This low lentil productivities are attributed to various diseases, insect pests, poor agronomic practices, and lack of improved cultivars and crop protection technologies [10]. The crop suffers from various plant diseases, which are caused by fungi, viruses, nematodes, and parasitic weeds and insect pests there by resulting in huge economic losses [11]. In Ethiopia, lentil wilt/root rot complex caused by *Fusarium spp, Rhizoctonia solani, R. bataticola, Sclerotium rolfsii, Ascochyta blight* (*Ascochyta lentis*), rust (*Uromyces viciae fabae*), *Anthracnose, Botyrits grey molds* and *Stemphylium* are the most important biotic factors causing lentil yield reductions [12]. Damages such as seed death and decreased seed vigor caused by seed borne pathogens are not always recognized by users [13]. However, research reports showed that infected seeds play a key role in the dissemination of plant pathogens and disease establishment. In general seed borne pathogens have significant influence on production and food industry because they; (i) can affect germination, growth, and crop productivity, (ii) cause seed and seedling diseases resulting in the development of systemic or local infections, (iii) cause contamination of grains with mycotoxins that represent a health risk to humans and animals [14] Planting infected seed allows for an even distribution of the disease within the crop and increases the number of initial infection sites from which the disease will spread during subsequent rain events. Indeed, Akema *et al*. [15] reported that seed borne pathogen has been implicated in the introduction of the disease into new areas or in the rapid spread of the disease within fields. Many fungal pathogens, some of which are seed transmitted, often reduce the germination ability, or kill the infected plants or substantially reduce the productive capacity. Some of these fungi produce aflatoxins which damage the liver and induce carcinogenic, mutagenic and teratogenesis [16].

Therefore, detecting the type and control of seed-borne fungi is extremely important and the damaging effects can be relieved through integrated approaches [17] and seed treatment with effective fungicides can greatly help in reducing the initial inoculum level and preventing the spread of the disease. Planting seed that is free of seed borne pathogens is the primary means to limit the introduction of the pathogen into a field and prevent early establishment of disease [18]. Hence, seed health quality is very important in lentil production since the cost of seed and potential seed treatments are a significant part of input costs. Hence, this study was undertaken to investigate the percentage incidence of major seed-borne fungi associated with lentil seeds and to evaluate the antifungal effect of *Trichoderma* spp. and some botanical extracts as management strategies for seed borne pathogens.

MATERIALS AND METHODS

Description of the study area

Ambo is located 113 km West of Addis Ababa at 8°98' North latitude and 37°83' East longitude. Ambo district has a total geographical area of 83,598.69 sq. km, with elevation of 2,068 meters above sea level. Annual rainfall ranged from 900-1100 mm and the temperature ranged from 10-27°C, with an average of 18°C. The soil type of the survey sites is vertisol [19].

Seed sample collection

Seed samples were collected from five Peasant Association's (PAS) (Golja, JijiguWeransa, Ya'i Cabo, Kure Gatira and Dase Akililo) of Ambo district, West Shewa, Ethiopia during 2020. Five PAS were purposively selected consulting with the District Agricultural office because of their importance in lentil production, which attract a number of research and development organizations to intervene with different lentil production problems. One kg of seed sample was collected from each farmer from different parts of seed lots according to International Seed Test Association [20] sampling procedure. In each PA's ten farmers were randomly selected. The samples were taken to the laboratory of Ambo University, Gudar campus for isolation and identification of seed associated fungal pathogens, seed germination and seeds to seedlings diseases transmission tests.

Botanicals used

Garlic, *Allium sativum* L. (cloves), *Vernonia amygdalina* (leaf), *Aloe vera* (leaf), *Moringa stenopetala* (leaf and seeds), *Datura stramonium* (leaf) and *Rumex minima* (roots) were used in this study which were collected from Ambo local market and the surrounding villages. The samples were separated in to its selected parts (leaf, clove, root and seed) washed thoroughly under tap water followed by sterilized distilled water, cut into smaller size of about 1-3 cm long and air-dried under shade at room temperature for 1 to 2 weeks and then pounded using sterile mortar and pistil in to fine powder and kept in refrigerator at 4 °C until use.

Bio-agents used

Purified culture of *Trichoderma* isolates (*Trichoderma harzianum, Trichoderma hamatum and Trichoderma viride* used in this study were obtained from Plant Pathology Department of Ambo University College of Agriculture and Veterinary Sciences. The stock cultures obtained were reinitiated in sucrose peptone broth (SPB) for multiplication, as recommended by Kumar [21], the propagules (colony forming unit, cfu) suspension of each *Trichoderma* isolates were prepared in sterile distilled water from 7-days-old-culture on PDA [22]. The fungal inoculum was harvested by flooding the culture with SDW and then rubbing the culture surface with a sterile glass rod. The fungal propagules concentration in each suspension was determined by counting using a hemocytometer slide and adjusted at 10^8 cfu / ml and used in dual culture test.

Determination of lentil seed borne fungi

Agar plate technique was used for fungal isolation and identification [20]. Four hundred seeds were randomly drowned from each sample. Sub samples of four of 100 were used as a replicate. Seeds were surface disinfected by soaking in 0.5% Sodium hypo chloride (NaOCl) solution for 10 minute with constant agitation and rinsed in three changes of sterile distilled water for a minute. The seeds were placed on sterile filter paper to dry and then plated hilum down wards, on potato dextrose agar. Ten seeds per plate were arranged and incubated 1-2 weeks at 20 °C under alternating cycles of 12 hours light and 12 hours darkness for sporulation of fungi and development of

fruiting bodies. After incubation the seeds from which pathogen recovered was recorded and mean present seed infection level were calculated for each replicate from infection proportion per plate. Colonies obtained from each infected seed subjected to culture, morphological and microscopic characterization for identification [20].

Determination of the effect of seed mycoflora on seed germination and seed to seedling disease transmission

To understand seed to seedling transmissions, studies were carried out under plate culture in the growth chamber. The sand used in the tests was sterilized in autoclave at 121°C and pressure of 15 lb; for one hour after which it was ready for use. One hundred lentil seeds in three replicates were randomly taken from each sample, surface sterilized and planted in sand media by using a sterilized forceps at equidistance to avoid cross contamination and placed in growth chamber at 28 ± 2 °C for 21days in alternating cycles of 12 hours darkness and 12 hours light. The seeds were kept moistened by adding sterilized distilled water every three days throughout the planting period. Seed germination and disease incidence and symptoms development were inspected every day and data were taken. Finally, the seedlings infection percentages of the tested plants were calculated with formulae:

$$\text{Seedling infection percentage} = \frac{\text{No. of seedlings affected by a pathogen}}{\text{Total number of seed sown}} \times 100$$

Seed germination percentage is also calculated as:

$$\text{Seedling germination percentage} = \frac{\text{No. of seedlings in each tray}}{\text{Total number of seed sown}} \times 100$$

The incidence of the fungi in seedlings was determined on the 7th, 14th and 21st day after plating in sterilized sand. In addition, progress of the disease and symptoms developed were inspected in the plated plants. Transmission Efficiency (TE) of fungi from seeds to seedlings in each sample was estimated from the incidence data with the following formula:

$$TE = \frac{C \times 100}{S}$$

Where; TE is transmission efficiency of Fungi from seeds to seedling

C is infection percentage of seedlings by Fungi in the transmission study

S is seed infection percentage of the sample by Fungi during laboratory assay on agar plate.

Extraction of botanicals

In vitro evaluation of antimicrobial assay of the plant extracts were done in Plant Pathology laboratory of Ambo University College of Agriculture and Veterinary Science. Botanical extractions were made in hot sterilized distilled water (SDW). Hundred grams (100g) of powder from each plant separately dissolved in 500 ml of hot SDW to have 20% crude extract. For garlic, cloves were peeled, washed with distilled water, cut into small pieces, and the pieces was ground to a thick paste. Hundred grams of the paste were transferred into a 1000 ml conical flask and filled up to 500ml with hot SDW to have 20% crude extracts [23]. The mixture shaken on rotary shaker to obtain a homogeneous suspension and covered with aluminum foils and left to stand for 24 hours at room temperature. The crude extract aseptically separated from the debris with What-man filter paper. Then the crude extracts were store in refrigerator at 4 °C for further use.

In vitro evaluation of botanical extract against test fungi

Plant extracts were evaluated against mycelial growth of seed borne test fungi, *Fusarium oxysporum f. sp. lentis* by using food poisoned technique. The plant extracts were used at 5, 10 and15% concentrations for which 5, 10 and15 ml of botanical crude extracts was mixed with 95, 90 and 85 ml of sterilized molten PDA media. The medium was thoroughly shaken for uniform mixing of plant extract. 20 ml of agar media was poured into sterile petri-plates and allowed to solidify. Five mm of agar disk of test fungi was cut off from 7days old culture plate by using sterile cork borer and placed in the center of petri-plate containing different concentration of plant extract. The experiment was conducted in Complete Randomized Design (CRD), with 8 treatments and 3 replications. The Petri plates containing only PDA medium without plant extracts consider as control. All these inoculated Petri plates were incubated at 25±2 °C and the data of mycelial growth of the fungus was observed after 24 hours of inoculation till 8 days of inoculation. The present inhibition of mycelial growth of pathogens was calculated by using the formula given by [24].

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

Where;

I = Per-cent inhibition in mycelia growth,

C = Growth of the pathogen in control plates

T = Growth of the pathogen in plant extract treated plates.

In vitro assay for the bio-agents

All the three *Trichoderma spp* were re-initiated in Sucrose Peptone Broth (SPB) for mass production as recommended by [21]. The bio-agents were screened for efficacy by dual culture method. Culture of test fungi was inoculated on PDA medium 2cm away from the center and incubated at 28°C for four days. After four days same plates were inoculated with *Trichoderma spp.* 2cm away from the center opposite periphery of the previous inoculums of test fungi and again kept for incubation at 24-28°C for four days. The plates were monitored every day and antimicrobial activity of the *Trichoderma spp* was observed and inhibition zone were measured with digital caliper. The percent inhibition of mycelial growth of pathogen was calculated by using this formula of Vincent [24].

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

Where;

I = Per-cent inhibition in mycelia growth,

C = Growth of the pathogen in control plates

T = Growth of the pathogen in dual culture plates.

Data analysis

Data of fungi associated with the seed and their effect on seed germination and disease transmission analyzed with descriptive statistics. The efficacy data were subjected to Analysis of Variance (ANOVA) using SAS software 9.4 version and when ANOVA shows significant difference between treatments mean separation were performed with Least Significant Difference (LSD) at P>0.05.

RESULTS AND DISCUSSION

Prevalence of seed borne fungi on lentil seeds

In this study, the detection of fungi from 50 seed samples showed that a total of10,365 seeds out of 20,000 seeds were found infected by different fungi. This reveals that 51.83 percent of the seed samples were infected with one seed borne and different saprophytic fungi. A total of five fungal species namely, *Fusarium oxysporium f,sp.lentis*, *Cladosporium spp.*,

Aspergillus spp. Penicillium spp., and Rhizopus spp. were isolated from lentil seed samples. Out of 10,365 fungal colonies isolated from the lentil seed samples in the present study 26.16% were *Fusarium oxysporium f,sp.lentis, 24.13% Cladosporium spp., 20.31% Aspergillus spp. 16.92%, Penecillum spp., and12.48% Rhizopus spp* (Table 1). Such similar reports have been made by [25]. These species have been reported to damage or deteriorate the seed at storage level and reduce the quality of seed and viability capacity of the crop. Despite the storage fungi like *Aspergillus* and *Pencillium* produced secondary metabolite that leads to the production of mycotoxin which cause serious problem on human health like carcinogenic and affect different organs of human during consumption.

Table 1 List of fungi isolated from 50 lentil seed samples by agar plate method

PAS	Seed infection percentage							
	Seed sample code	*Fusarium spp*	*Cladosporium spp*	*Aspergillus spp.*	*Penecillum spp*	*Rhizopus spp*	Total infection	Total infection %
Golja	GF001	66	19	21	16	20	142	35.50
	GF002	57	19	4	62	30	172	43.00
	GF003	60	60	2	29	30	181	45.25
	GF004	70	23	2	46	68	209	52.25
	GF005	45	53	62	11	40	211	52.75
	GF006	55	21	0	45	0	121	30.25
	GF007	49	55	3	53	55	215	53.75
	GF008	17	65	77	6	10	175	43.75
	GF009	49	20	2	10	10	91	22.75
	GF0010	64	56	4	59	59	242	60.50
Jijigu	JWF0011	50	63	4	0	48	165	41.25
Weransa	JWF0012	19	63	4	0	49	135	33.75
	JWF0013	41	19	78	0	59	197	49.25
	JWF0014	65	64	53	9	30	221	55.25
	JWF0015	47	23	68	41	2	181	45.25
	JWF0016	65	21	10	39	67	202	50.50
	JWF0017	70	33	10	4	66	183	45.75
	JWF0018	41	35	90	81	10	257	64.25
	JWF0019	35	67	73	8	28	211	52.75
	JWF0020	57	56	20	70	74	277	69.25
Ya'i Cabo	YCF0021	75	48	39	83	0	245	61.25
	YCF0022	57	58	45	2	6	168	42.00
	YCF0023	48	60	46	1	4	159	39.75
	YCF0024	65	63	11	23	3	165	41.25
	YCF0025	16	55	86	1	45	203	50.75
	YCF0026	54	48	62	75	28	267	66.75
	YCF0027	74	59	23	75	30	261	65.25
	YCF0028	44	49	38	4	45	180	45.00
	YCF0029	52	55	68	36	5	216	54.00
	YCF0030	51	43	86	48	0	228	57.00
Kure	KGF0031	51	64	42	15	32	204	51.00
Gatira	KGF0032	33	54	57	20	0	164	41.00
	KGF0033	76	71	3	58	0	208	52.00
	KGF0034	75	43	70	65	3	256	64.00
	KGF0035	75	86	54	6	0	221	55.25
	KGF0036	57	0	72	78	12	219	54.75
	KGF0037	78	69	55	0	29	231	57.75
	KGF0038	58	71	39	68	43	279	69.75
	KGF0039	54	48	76	4	27	209	52.25
	KGF0040	49	59	4	70	12	194	48.50
Dase	DAF0041	47	74	62	68	25	276	69.00
Akililo	DAF0042	74	75	62	60	10	281	70.25
	DAF0043	42	49	49	87	10	237	59.25
	DAF0044	50	34	90	0	0	174	43.50
	DAF0045	67	41	10	57	25	200	50.00
	DAF0046	49	78	48	0	58	233	58.25
	DAF0047	66	78	55	0	0	199	49.75
	DAF0048	53	81	46	77	58	315	78.75
	DAF0049	55	53	23	45	6	182	45.50
	DAF0050	44	0	97	39	23	203	50.75
Total infection		2711	2501	2105	1754	1294	10365	51.83
Total infection (%)		26.16	24.13	20.31	16.92	12.48	100	

*Mean values are mean of Four replicates. $GF00_{1-10}$= Sample collected from GoljaPA's, $JWF00_{11-20}$= Sample collected from JijiguWeransa, YCF0021-30= Sample collected from Ya'i Cabo, KGF0031-40= Sample collected from Kure Gatira, DAF0041-50= Sample collected from Dase Akililo

Identification of fungi based on cultural and morphological characteristics

In this study, cultural, microscopic observation and pathogen crop indexing were used to characterize the selected colony cultures. During the examination of the hyphae and spore characteristics of each fungus were noted very well (Table 2, Fig 1). Based on their colony morphology, mycelial growth, pigmentation, and microscopic observations from the total of 10,365 colonies 2711 showed distinctive features of *Fusarium spp.*, 2501 *Cladosporium* spp., 2105, *Aspergillus* spp., 1754 *Penecillum* spp., and 1294 *Rhizopus* spp.

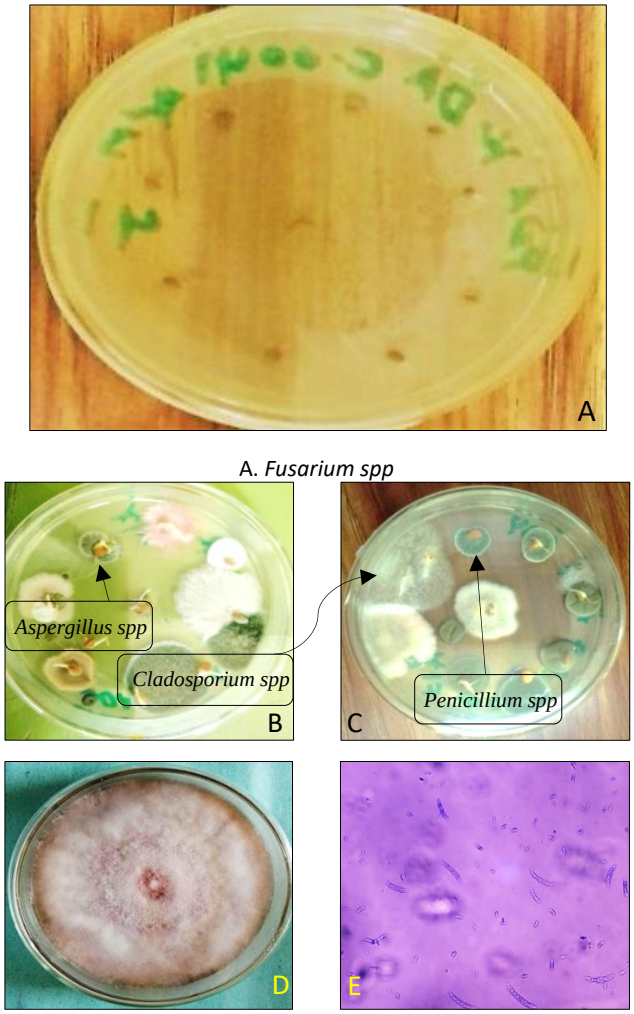


Fig 1 The protocol of pathogen identification
(A). Seed inoculation, (B-C). Growth of pathogen on seed
(D). Pure culture of pathogen, (E). Microscopic view of pathogen

Effects of seed-borne fungi on germination of seeds

The results of germination percentage of seed samples indicated that the percentage ranges from 55.63% to 73.03%. The seed samples obtained from Dase Akililo PA showed highest percentage of germination (70.43%). The germination percentage of lentil seeds differed from location to location and farmer to farmer. Seed germination of a major lentil producing kebeles, Dase Akililo was higher (73.03%) followed by Kure Gatira (69.99%) but Golja has lowest germination percentage (55.63%) as shown in (Table 2). The seed germination also differs within the same PA's. Seeds collected from Dase Akililo PA in the farmer of seed sample code DAF0048, showed the highest germination (95.00%), followed by the seed sample code DAF0042 and DAF0041 with germination (90.75% and 82.00%), respectively, but the seeds collected from Golja PA in the farmer of seed sample code GF009 showed the lowest germination (38.50%) followed by the seed sample code GF001 and GF006 with germination (45% and 46.25%) respectively. The results also reveal that sample with the highest fungal prevalence resulted in the lowest seed germination. The current result is in agreement with the findings of Hasan *et al*. [26] who reported that the common fungal seed mycoflora are mostly known to produce mycotoxins that adversely affect the seed germination, shoot and root length of all test pulses in variable quantity.

In the seed to seedling bioassay distinct symptoms of seedling wilt was observed which was related to *Fusarium* wilt. This indicated that the *Fusarium* recovered from the seed was transmitted pathogenic fungi and may serve as primary source of infection to the lentil crop. These fungi served as primary inoculum for spread of diseases and have epidemiological significance. However, those saprophytic fungi such as *Cladosporium spp.*, *Aspergillus spp.*, *Pencillium spp.* and *Rhizopus* species not showed any disease symptom on the seedlings. Seed-borne fungi are important from the economic point of view as they render losses in several ways (Table 3, Fig 2). Seed-borne infection of fungal pathogens are important not only for its association with the seeds which cause germination failure and/or causing disease to the newly emerged seedlings or growing plants but also contaminate the soil by establishing its inoculum permanently [26]. Seed transmitted pathogens considerably may cause quality losses, viz. seed abortion, seed rot, seed necrosis, reduction or elimination of germination capacity, seedling damage and their nutritive value, have been reported [27-28]. Infected seeds play a key role in the dissemination of plant pathogens and disease establishment [29]. Toxins of the seed-borne fungi were responsible for; inhibition of normal growth of seedlings in different crops, germination failure, mycotoxin production and permanent contamination [30-31].

Table 2 Effects of seed-borne fungi on the germination of lentil seeds

Locations	Seed borne fungi isolated					Total infection	Total infection (%)	Germination (%)
	Fusarium spp.	*Cladosporium spp.*,	*Aspergilus spp.*	*Penicillium spp.*	*Rhizopus spp.*			
Golja	504	477	230	239	371	1821	45.53	55.63
Jijigu Weransa	518	358	357	350	384	1967	49.17	61.85
Ya'i Cabo	536	538	504	348	166	2092	52.3	64.53
Kure Gatira	524	576	522	388	237	2247	56.17	67.35
DaseAkililo	629	552	492	429	136	2238	55.95	73.03
Total fungi	2711	2501	2105	1754	1294	10365	51.83	

Effect of aqueous extracts on mycelial growth of test fungi

The result of *in vitro* antifungal assay of botanical extracts indicated that all treatments showed significant effect on the radial growth of the test fungi over untreated control plate. The botanical extracts significantly inhibit the radial growth of the test fungi at all concentrations. Plant extracts have played a significant role in reducing the incidence of seed borne pathogens and in the improvement of seed quality and the emergence of plant seeds in the field [26]. *Allium sativum* (garlic) cloves extracts at 15% concentration showed the

highest inhibition (79.26%) of the radial growth of the tested fungi as shown (Table 4, Fig 3). However, the least inhibition was obtained by *Rumex minima* root extracts at the same concentration (70.24%) when compared to the other treated treatments (Table 4, Fig 3).

Table 3 Determination of effect of seed mycoflora on seed germination and seed to seedling disease transmission

Locations	Sample code	Germination %	Seed infection					Seedling infection	TES
			FOL	*CS*	*AS*	*PS*	*RS*	*FOL*	*FOL*
Golja	GF001	45.0	66.0	19.0	21.0	16.0	20.0	13.0	19.69
	GF002	56.0	57.0	19.0	4.0	62.0	30.0	11.0	19.29
	GF003	55.3	60.0	60.0	2.0	29.0	30.0	12.0	20.00
	GF004	62.5	70.0	23.0	2.0	46.0	68.0	15.0	21.43
	GF005	64.0	45.0	53.0	62.0	11.0	40.0	4.5	10.00
	GF006	46.3	55.0	21.0	0.0	45.0	0.0	7.0	12.73
	GF007	63.8	49.0	55.0	3.0	53.0	55.0	6.0	12.24
	GF008	54.5	17.0	65.0	77.0	6.0	10.0	0.0	0.00
	GF009	38.5	49.0	20.0	2.0	10.0	10.0	6.5	13.26
	GF0010	70.5	64.0	56.0	4.0	59.0	59.0	12.0	18.75
Jijigu	JWF0011	56.0	50.0	63.0	4.0	0.0	48.0	8.5	17.00
Weransa	JWF0012	47.3	19.0	63.0	4.0	0.0	49.0	0.0	0.00
	JWF0013	58.0	41.0	19.0	78.0	0.0	59.0	3.0	7.32
	JWF0014	62.0	65.0	64.0	53.0	9.0	30.0	15.0	23.07
	JWF0015	57.3	47.0	23.0	68.0	41.0	2.0	5.0	10.64
	JWF0016	65.3	65.0	21.0	10.0	39.0	67.0	12.5	19.23
	JWF0017	52.0	70.0	33.0	10.0	4.0	66.0	17.0	24.28
	JWF0018	74.0	41.0	35.0	90.0	81.0	10.0	2.5	6.09
	JWF0019	69.3	35.0	67.0	73.0	8.0	28.0	1.0	2.86
	JWF0020	77.5	57.0	56.0	20.0	70.0	74.0	10.0	17.54
Ya'i Cabo	YCF0021	73.8	75.0	48.0	39.0	83.0	0.0	18.0	24.00
	YCF0022	56.5	57.0	58.0	45.0	2.0	6.0	9.0	15.79
	YCF0023	49.0	48.0	60.0	46.0	1.0	4.0	6.5	13.54
	YCF0024	56.0	65.0	63.0	11.0	23.0	3.0	12.0	18.46
	YCF0025	64.3	16.0	55.0	86.0	1.0	45.0	0.0	0.00
	YCF0026	78.0	54.0	48.0	62.0	75.0	28.0	8.0	14.81
	YCF0027	76.0	74.0	59.0	23.0	75.0	30.0	17.5	23.65
	YCF0028	56.0	44.0	49.0	38.0	4.0	45.0	3.0	6.82
	YCF0029	65.8	52.0	55.0	68.0	36.0	5.0	7.5	14.43
	YCF0030	70.0	51.0	43.0	86.0	48.0	0.0	9.0	17.65
Kure	KGF0031	64.8	51.0	64.0	42.0	15.0	32.0	10.5	20.58
Gatira	KGF0032	56.7	33.0	54.0	57.0	20.0	0.0	0.0	0.00
	KGF0033	65.5	76.0	71.0	3.0	58.0	0.0	20.0	26.32
	KGF0034	76.5	75.0	43.0	70.0	65.0	3.0	19.5	26.00
	KGF0035	66.0	75.0	86.0	54.0	6.0	0.0	19.0	25.33
	KGF0036	64.3	57.0	0.0	72.0	78.0	12.0	10.0	17.54
	KGF0037	77.0	78.0	69.0	55.0	0.0	29.0	22.0	28.21
	KGF0038	80.3	58.0	71.0	39.0	68.0	43.0	10.8	18.54
	KGF0039	63.5	54.0	48.0	76.0	4.0	27.0	6.5	12.04
	KGF0040	59.0	49.0	59.0	4.0	70.0	12.0	7.0	14.28
Dase	DAF0041	82.0	47.0	74.0	62.0	68.0	25.0	5.5	11.71
Akilio	DAF0042	90.8	74.0	75.0	62.0	60.0	10.0	18.0	24.33
	DAF0043	74.5	42.0	49.0	49.0	87.0	10.0	3.0	7.14
	DAF0044	57.3	50.0	34.0	90.0	0.0	0.0	8.0	16.00
	DAF0045	62.5	67.0	41.0	10.0	57.0	25.0	13.0	19.41
	DAF0046	76.0	49.0	78.0	48.0	0.0	58.0	7.0	14.28
	DAF0047	69.5	66.0	78.0	55.0	0.0	0.0	12.0	18.18
	DAF0048	95.0	53.0	81.0	46.0	77.0	58.0	6.0	11.32
	DAF0049	56.3	55.0	53.0	23.0	45.0	6.0	7.5	13.64
	DAF0050	66.5	44.0	0.0	97.0	39.0	23.0	0.0	0.00

FOL (*Fusarium oxysporium f,sp.lentis*), CS (*Cladosporium spp*), AS (*Aspergillus spp*), PS (*Pencillium spp*), RS (*Rizopus spp*) and TES (Transmission efficiency)

Fusarium wilt

Fusarium wilts on lentil Healthy seedling of lentil

Where: (A-B). Lentil seedling infected by wilt disease
(C). disease free lentil seedlings

Fig 2 Occurrence of the pathogen on seedlings in seed to seedling transmission test

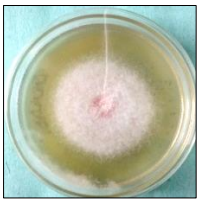
Table 4 Antagonistic effect of different botanicals on mycelial growth and inhibition zone percentage of the test pathogens isolated from lentil seed samples

Botanicals	Concentration %	*Fusarium oxysporium f. sp. lentis*	
		Mycelia growth (mm)	Inhibition zone %
Allium sativum cloves	0	43^{h}	0.00^{h}
	5	12.32^{bcde}	71.35^{bcde}
	10	10.5^{ab}	75.58^{ab}
	15	8.92^{a}	79.26^{a}
Moringa seeds	0	43^{h}	0.00^{h}
	5	13.73^{cde}	68.06^{cde}
	10	12.05^{bcd}	71.97^{bcd}
	15	10.35^{ab}	75.92^{ab}
Moringa leaf	0	43^{h}	0.00^{h}
	5	14.22^{cdef}	66.93^{def}
	10	12.25^{bcde}	71.51^{bcde}
	15	10.52^{ab}	75.54^{ab}
Aloe vera	0	43^{h}	0.00^{h}
	5	14.52^{defg}	66.23^{def}
	10	12.69^{bcde}	70.49^{bcde}
	15	10.99^{ab}	74.44^{ab}
Vernonia anygdalina	0	43^{h}	0.00^{h}
	5	14.77^{efg}	65.66^{efg}
	10	12.85^{bcde}	70.11^{bcde}
	15	11.54^{abc}	73.17^{abc}
Datura stramonium	0	43^{h}	0.00^{h}
	5	16.48^{fg}	61.66^{fg}
	10	13.8^{cdef}	67.9^{cde}
	15	12.39^{bcde}	71.18^{bcde}
Rumex minima L.	0	43^{h}	0.00^{h}
	5	17.2^{g}	59.99^{g}
	10	14.41^{def}	66.48^{def}
	15	12.8^{bcde}	70.24^{bcde}
LSD (0.05)		2.699	6.219
CV (%)		11.6	5.6
S.E.		1.64	3.779

*Mean values are mean of three replicates. Means followed by the same letter across the column are not significantly different. CV= Coefficient of variation and LSD= Least Significant Difference

The inhibitory action of the aqueous plant extracts on mycelial growth increased with increase in concentrations. Similar result was obtained by Hasan *et al*. [26] who reported that aqueous extracts of garlic cloves have been shown to be effective in inhibiting *Fusarium solani* of peanut; this is due to presence of sulfur content and other phenolic compounds in the garlic. The tested plant extracts at 5%, 10% and 15% concentrations significantly inhibited the mycelial growth of the tested fungi. From this output the antifungal activities of the extracts were enhanced by increasing the concentration from 5 to 15 % (w/v); hence the inhibition activities of the extracts were concentration dependent. This is in agreement with the report of Jasso *et al*. [55] who indicated that increase in the antifungal activities had corresponding increase in concentration of plant extracts.

Control- Fusarium oxysporium f.sp.lenyis *Allium sativum* (garlic) cloves



Moringa leaf *Aloe vera leaf extracts* *Rumex minima root extracts*

Fig 3 *In vitro* biological activity of plant extracts against *Fusarium oxysporium f. sp. Lentis*

Table 5 Bioassay against *Fusarium oxysporium f, sp. lentis*

Treatments	Mycelia growth (mm)*	Inhibition zone %
Control	42.11^{b}	0.00^{c}
T_1	8.91^{a}	78.83^{a}
T_5	10.7^{a}	74.62^{ab}
T_{15}	12.06^{a}	71.34^{b}
Cv %	15.3	5.8
LSD (0.05)	4.355	5.055
SE	2.827	3.281

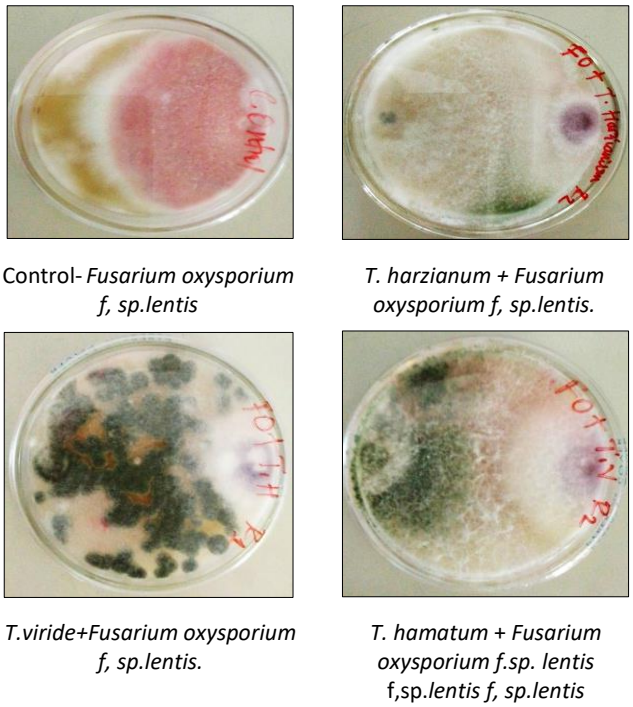
Where; C= Control, T_1: *Trichoderma harzianum*, T_5: *Trichoderma hamatum* T_{15}: *Trichoderma viride*
CV= Coefficient of variation and LSD= least significant difference

Note: *Mean values are of four replicates. Means followed by the different letter across the column are significantly different according to least significant difference test (P< 0.05)

In vitro evaluation of Trichoderma spp. against Fusarium oxysporium f. sp. lentis

All the isolates of *Trichoderma* spp. significantly inhibited the radial growth of the pathogen when compared to

the control (Table 5, Fig 4). *T. harzianum* showed the highest inhibition (78.83%) of the radial growth of the test pathogen in the dual culture followed by *T. hamatum* (74.62%) and *T. viride* (71.34%). These results indicated that all the three evaluated *Trichoderma* spp. isolates in the present study were potential antagonists against the tested fungi (Fig 4). Similarly previous research work indicates that *Trichoderma* species are more effective when integrated with moderately susceptible or resistant cultivars to control *Fusarium* wilt by 30-46% [32]. However, this method has been given only little attention in managing lentil wilt in Ethiopia.



Control- *Fusarium oxysporium f, sp.lentis*

T. harzianum + Fusarium oxysporium f, sp.lentis.

T.viride+Fusarium oxysporium f, sp.lentis.

T. hamatum + Fusarium oxysporium f.sp. lentis f,sp.*lentis f, sp.lentis*

Fig 4 Dual cultures of *Trichoderma* and *Fusarium oxysporium f. sp. lentis* on PDA medium

Similarly, the antagonistic effect of *Trichoderma spp*. isolates against tested seed borne fungi has been reported [32-33]. *Trichoderma* species are being extensively used as bio-control agents against soil and seed-borne diseases. Raique *et al*. [34] reported that two species of *Trichoderma* were employed against highly virulent isolate of *Fusarium* responsible for lentil wilt and the results revealed that *T. harzianum* was highly effective in controlling wilt disease in comparison to other isolate, when applied as a soil drench. Ngueko and Xu [35] also reported that *T. harzianum* reduced the mycelial growth of *F. oxysporum* with 52 - 87%.

CONCLUSION

In the current study a total of 5 fungal species belonging to 5 genera viz. *Fusarium spp., Cladosporium spp., Aspergillus spp. Penicillium spp. and Rhiizopus spp.* were found to be associated with lentil seeds. The seed germination and seed to seedling transmission results indicated that the seed borne pathogens reduce seed germination and cause seedling disease. Hence, seed health testing is a primary need to avoid crop failure and it is desirable that seeds of crop plants should invariably be tested for seed health before planting so as to prevent pathogen build up and introduction of pathogens in new areas. The results are also in conclusive idea that seed associated fungi are economically important in lentil seed system and there is a need for certified seed production and seed treatments to reduce the pathogen build up and disease distribution in potential lentil production areas of West Shewa. The inhibitory action of the aqueous plant extracts and *Trichoderma* species on mycelial growth of the test pathogen, *Fusarium oxysporium f. sp. lentis* confirm that the plant extracts and the *Trichoderma* spp. are potential candidate to manage seed borne pathogens. Therefore, further screening of active ingredients of botanicals and mass production techniques of bio-agents could be needed to formulate and industrialize the products for seed treatments against seed-borne pathogens.

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