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Response of Arabica Coffee collections to Coffee Berry Disease (Colletotrichum kahawae), In Oromia Regional State, Ethiopia

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Research Article

Keywords: attached berry test, detached berry test, hypocotyl test, infection percentages, inoculation

Posted Date: December 19th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3748115/v1

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Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Phytoparasitica on May 21st, 2024. See the published version at https://doi.org/10.1007/s12600-024-01162-6.

Abstract

Ethiopia mainly uses resistant varieties and cultural disease management strategies to tackle this disease. However, still there is a lack of improved and resistant varieties in coffee growing areas of the country including Arsi Zone. The objectives of this study were to investigate the resistance of Arsi coffee collections to Coffee Berry Disease through artificial inoculation of green berries on mother trees, and younger coffee seedlings under controlled conditions. The field experiment was conducted at Gololcha (Arbagugu state farm) and Mechara Agricultural research center on station. A hypocotyl test was conducted at Jimma Agricultural research center. Field test was carried out on 13 Arsi coffee collections and the Coffee Berry Disease -susceptible Arusa coffee variety. Standard checks like susceptible variety (370) and resistant varieties 741 and 754 were used for hypocotyl tests. Seventeen treatments were used for hypocotyl tests. Result showed the presence of a significant difference in Coffee Berry Disease reaction levels among genotypes ($P < 0.001$) under field conditions. The infection percentage of berries varied from 2.89 to 25.93% at Gololcha (Arbagugu state farm) and 3.25 to 34.92% at Mechara three weeks after treatment applications. According to the results, the resistant variety 741 showed the lowest infection percentage (15.04%) in comparison to all other collections and/or varieties at the seedling stage. Therefore, coffee collections that showed the lowest level of infection percentage under both experimental conditions (Ar15/11, Ar05/11, and Ar70/11) were recommended for further breeding strategies to develop resistant coffee varieties against the disease.

1. INTRODUCTION

The coffee genus (*Coffea*) comprises 124 species and is indigenous to the old world tropics (Razafinarivo *et al.*, 2012). Coffee has a significant economic, social and spiritual impact on many communities with diverse cultural and/or psychological backgrounds (Chauhan *et al.*, 2015). Coffee is the world's second most traded commodity next to petroleum and serves as a direct source of income for growers in different parts of the world (FAO, 2015). In the second half of the nineteenth-century coffee was transformed into an industrial product as a consequence of the accelerated expansion of coffee production in Brazil, which in turn, nurtured the growth of a mass consumer market in the United States (Morris *et al.*, 2018).

It is now grown in over 80 countries on more than 10.6 million hectares of land in the sub-tropical regions, especially in Africa, Asia and Latin America (FAOSTAT, 2020). On a social basis, it plays a relevant role notably in the subsistence of nearly 20 million coffee-farming families in under developed countries of Asia, Africa, and Latin America (Zhou *et al.*, 2016). Coffee in Ethiopia accounts for 40% of total export and 10% of total government revenue (ICO, 2020).

The country has wider ecologies containing indigenous germplasm which offers the greatest opportunity to produce superior *C. arabica* that allowed Ethiopia to be competitive in the world market (Zenebe and Dawit, 2020). About 700,447 ha of land have been cropped with coffee (CSA, 2017). The Oromia Regional State is the homeland of *C. arabica* evidenced by the existence of wild coffee trees in the region with

464,426 ha (66%) of total area coverage and 317,316 tons of production per annum (CSA, 2017). Kaffa, Illubabor, Jimma, Wollega, Sidamo, Gedeo, Yirgachefe, and Hararghe are the topmost coffee-growing and producing areas (Hinsene *et al*., 2015).

Despite these, world coffee production has grown steadily since the 1960s, although it will be difficult to maintain this trend due to the continued rise in production costs, problems related to climate change, and the higher incidence of pests and diseases (Krishnaan, 2017). Arabica coffee in Ethiopia is threatened by several fungal diseases, including coffee leaf rust, coffee berry disease (causal agent *Colletotrichum kahawae* Waller & Bridge), coffee wilt disease (causal agent *Gibberella xylarioides* Heim & Saccas), and Armillaria root rot (causal agent *Armillaria mellea* Vahl ex and Fries) (Beyene *et al*., 2020). Coffee berry disease is an anthracnose of green and ripe berries caused by *Colletotrichum kahawae* (Waller and Bridge, 1993). In Ethiopia, the major factors contributing to low yield are the lack of improved cultivars for each ecological zone of the country, diseases, mainly coffee berry disease (CBD), and traditional management practices(Beyetta,2001). *Colletotrichum* species that cause anthracnose symptoms have been reported in several African countries (Cristobel *et al*., 2017).

Coffee berry disease, was first reported in Kenya in 1922 (McDonald 1926) and is caused by the fungal pathogen *Colletotrichum kahawae*, which is a specialized hemibiotrophic pathogen of *C. arabica L*. (Vieira *et al*., 2019). Unlike other Arabica coffee diseases, CBD is still restricted to the African continent despite favorable climatic conditions in certain high-altitude Arabica coffee-growing areas of Latin America and Asia (Van der Vossen *et al*., 2015). *Colletotrichum kahawae* infects green berries at the rapid expansion stage (4–16 weeks after flowering) and may also attack mature berries, 28 weeks after flowering (Gichimu *et al*., 2014). Epidemics of this disease can quickly destroy 50–80% of the developing berries on susceptible Arabica coffee cultivars during prolonged wet and cool weather conditions (Hindorf and Omondi, 2011).

In Ethiopia, CBD alone caused about a 24–30% loss of the national average yield on local landraces (Derso *et al*., 2000). Bayetta (2001) reported that the national average yield loss due to the disease in Ethiopia is estimated to be 20–25%, and also he reported that the loss may reach 100% during favorable seasons in some areas where altitude and rainfall are high.

Preventive control by fungicide sprays accounts for 30–40% of total production costs and leads to environmental pollution (Gichuru *et al*., 2008). Crop loss and cost of CBD control in Africa are estimated to be 300–500 million USD (Van der Vosen and Walyaro, 2008). Coffee Berry Disease, caused by *Colletotrichum kahawae* Waller and Bridge (Waller *et al*., 1993) is, to a large extent, responsible for reduced productivity and increased cost of production, thereby reducing the competitiveness of the region's coffee.

Symptoms on green berries appear as small dark sunken lesions typical of anthracnose which may spread to cover the whole berry. Under wet and cold conditions, the fungus readily sporulates forming a mass of pink conidia and penetrating the interior of the berries destroying the beans. The resulting dry, black mummified berries have no commercial value and may be shed off the tree or remain intact. The

disease severity is higher on coffee trees exposed to sunlight than on those located under the shade (Mouen *et al.*, 2008).

To overcome these biotic constraints management strategies have been used worldwide. Among these resistant varieties, cultural and chemical methods are widely used. Ethiopia mainly uses resistant varieties, mechanical and cultural disease management strategies to save the coffee crop and contribute to increased foreign exchange earnings from the coffee industry. Host resistance to CBD has been the subject of intense studies in the East African region (Van der Vossen and Walyaro, 1981).

It is widely accepted that resistant varieties provide an opportunity for farmers to produce coffee in a manner that is sustainable, competitive, and environmentally benign. The most reliable screening test for CBD resistance of mature trees to detect true resistance with little chance of escape will be artificial inoculation of berries on the trees at their most susceptible stage, i.e. 12–15 weeks after flowering when the berries are fully expanded but still soft (Mulinge, 1970). The discovery of hypocotyl infection on a four to six-week-old seedling using artificial inoculation with *Colletotrichum kahawae* spores contributes significantly to *Arabica coffee* breeding by shortening the time required to identify resistant progenies from crosses involving resistant and susceptible donors (Gichuru *et al.*, 2008). A detached green berry inoculation test was used for resistance screening of *C. Arabica* accessions against CBD under artificial conditions. This offers the advantage of direct assessment of *C. Arabica* and *Colletotrichum kahawae* interaction at the fruit level and allowed an easy way of resistant genotypic characterization under field conditions (Pinard *et al.*, 2012).

Coffee Berry Disease affected the production and productivity of *Arabica* coffee in all assessed areas in the Arsi zone, particularly the local cultivars which are preferred by the growers for their bean weight and typical quality (Bersisa *at al.*, 2018). In most coffee-growing areas of Ethiopia, disease-resistant varieties have been developed and utilized by coffee growers. However, still, there is a lack of improved and resistant variety development in a few coffee growing areas including Arsi Zone. From Arsi zone, the "*Gololcha* "district is 14th among the top 25 coffee-producing districts in Ethiopia and 7th among the top 18 coffee-producing districts in Oromia (James *et al.*, 2015). However, the "*Gololcha"* district still doesn't have improved and resistant varieties developed for the respective area. Therefore, the objectives of this study were to investigate the resistance of Arsi coffee collections to Coffee Berry Disease through artificial inoculation of green berries on mother trees, and younger coffee seedlings under controlled conditions.

2. MATERIALS AND METHODS

2.1. Description of Study Area

The field experiment was conducted at the *Gololcha*, Arbagugu state farm and Mechara Agricultural Research Center (McARC) station during the main cropping season. "*Gololcha*" district is located in the Eastern part of Oromia about 218 km away from Addis Ababa capital city of Ethiopia, and 206 km

southeast of Asela town the capital of the Arsi zone. The altitude of the area is about 1400 to 2500 m.a.s.l. The average temperature of the district is 35^{o}c and the average rainfall is 900 mm/year. The major soil of the area is sandy to silt type.

Mechara has located 434 km to the east of Addis Ababa in the Daro Labu district of the west Hararghe Zone of Oromia Regional State. It has an altitude of 1760 m.a.s.l. with an annual average temperature and rainfall of 16^{o} C and 963mm, respectively. The major soil type of the center is sandy loam clay, which is reddish (Amin *et al*, 2018).

A hypocotyl test was conducted at Jimma Agricultural Research Center (JARC).

2.2. Experimental Materials, Treatments, and Design

Plant materials

Thirteen Arsi coffee collections were promoted to a variety verification trial (Table 1) and the Arusa coffee variety which is susceptible to CBD was used as a standard check for field and detached berry inoculation tests. Standard checks like susceptible variety (370) and resistant varieties 741 and 754 (Teferi 2014), were used for hypocotyl tests. Fourteen treatments were used for the attached berry tests. Seventeen treatments were used for hypocotyl tests. Treatments were arranged in Randomized Complete Block Design (RCBD) in three replications for field inoculation test and CRD in three replications for hypocotyl inoculation tests

Table 1
Arabica coffee collations/or varieties and their description used for the study

No.	Collection code	Location /Woreda	Altitude	No.	Collection code	Location /Woreda	Altitude
1	Ar03/11	Chole	1757	9	Ar64/11	Gololcha	1801
2	Ar05/11	Chole	1743	10	Ar66/11	Gololcha	1784
3	Ar15/11	Chole	1646	11	Ar70/11	Gololcha	1742
4	Ar34/11	Chole	1765	12	Ar72/11	Gololcha	1714
5	Ar46/11	Chole	1859	13	Ar78/11	Gololcha	1529
6	Ar54/11	Chole	1774	14	Arusa	Chiro	1200–1900
7	Ar62/11	Gololcha	1734	15	370	Jimma	1750
8	Ar63/11	Gololcha	1764	16	741	Gera / Jimma	1990
17	754	Kaffa	1650				

2.3. Experimental Procedure and Methods of Application

2.3.1. Inoculum preparation and inoculation

To obtain the inoculum for further infections, green CBD-infected berries with active black lesions were collected from Arsi "*Gololcha"* district. The diseased berries were surface-sterilized with 5% sodium hypochlorite (NaOCl) solution for 3 minutes and then rinsed with sterilized distilled water twice for one minute. Sterilized berries were placed on sterilized tissue paper for drying. The sample was plated on PDA and incubated at 24°C for 7 days.

The advanced mycelium was taken from the margin of the ten-day-old culture by using a sterile scalpel and multiplied on potato dextrose agar (PDA). After 10 days of incubation, the mycelia were grown out and the conidia were harvested. The conidial density was determined using a hemocytometer. Suspension 2×10^6 conidia mL^{-1} was used to inoculate coffee seedlings and berries under controlled and field conditions(Bayetta 2001; Zeru 2006).

2.3.2. Hypocotyl test

There were 120 seeds collected from each progeny of the test plants. Then, the seeds of each cultivar were prepared by removing the parchment and soaking them in sterilized water and kept for 48 hours. Thereafter, 30 seeds per box were sown on sterilized moist sand in plastic boxes which were arranged on benches, in three replications. Finally, the seedlings become ready for inoculation between 4–6 weeks period. Two days before inoculation the hypocotyls of the seedlings were preconditioned at 100% Relative Humidity (RH) for forty-eight hours and then tightly covered with a misted plastic sheet. Coffee seedlings were inoculated with suspension 2×10^6 conidia mL^{-l}. Inoculations were conducted by dipping small and soft camel hairbrushes in the spore suspension in a beaker and gently brushing individual seedlings. Then inoculated seedlings were immediately covered for another 48 hours with a plastic sheet misted on the inner sides. Forty-eight hours after the first inoculation the same process was repeated to double the inoculation (Bayetta 2001; James *et al*. 2019). The RH and temperature during the first and second inoculation were maintained at 100% and 20 ^{o} c, respectively. Finally, three weeks after the first inoculation, the seedlings were recorded using a 0–4 score scale based on lesion size and color (Fig. 1). where: 0 = no symptom; 1 = from very tiny to 1 or 2 narrow brown lesions up to 0.5 mm wide; 2 = more than 2 brown lesions or brown coalescing lesions exceeding 0.5 mm, black dots if present are rare; 3 = wide brown lesions with numerous black dots and/or black lesion may surround the stem but the top remains alive and 4 = black lesion girdling the stem and top killed.

2.3.3. Attached Berry Test (ABT)

Evaluations of 14 coffee genotypes were carried out under field conditions at two locations. The attached berry test was conducted following methods and procedures used by (Zeru 2006). One mature-bearing coffee tree of 8 years old per genotype was artificially inoculated using the virulent strain of *C. kahawae*. From each selected tree, three branches of the selected coffee trees (1 from the top, 1 from

the middle, and 1 from the bottom) were marked bearing healthy berries of the same age. Individual branches of a tree were used as replications. The inoculation was done in the evening and the inoculated branches were subsequently covered with plastic sleeves and paper bags overnight for 12 hours. A concentration of 2 x 10^6 spores/ml of spore suspension was used. Eventually, numbers of healthy and diseased berries were recorded three weeks after inoculation(Zeru 2006). The assessment of CBD development was done by counting the number of CBD coffee-infected berries against that of uninfected berries after 7, 14, and 21 days after inoculation.

2.4. Data Analysis

The data were subjected to analysis of variance (ANOVA) using R Studio software. A significant difference between treatments means was separated using the least significant difference (LSD) at a 5% probability level. Before the analysis of variance, all data sets were tested for normal distribution using the normality test.

3. RESULTS AND DISCUSSIONS

3.1. Response of Arsi coffee collections to CBD (*C. kahawae*), using the attached berry test

Under artificially inoculated field conditions at both sites, there was a significant difference in CBD reaction levels among collections (P < *0.001*) (Table 2). The highest infection percentage was recorded from Ar34/11 (25.93) and the lowest infection percentage was recorded from Ar15/11 (2.89) three weeks after inoculation at Gololcha (Arbagugu state farm). At Mechara on the station, the highest infection percentage was recorded from Ar78/11 (27.38) next to the check. The lowest was recorded from Ar15/11 (3.25) three weeks after treatment application (inoculated).

According to the results obtained at both locations and time intervals of 14 and 21 days after the application of the treatment, there was significance at both locations and time intervals (Table 2). At both locations, data were collected three times in week intervals. However, the initial recording time indicates that no black lesion occurred on the berry one week after treatment application. Berries showed produced CBD symptoms during the second recording period or 14 days after inoculation. The infection percentage of attached berry disease increased from 14 to 21 days after inoculation. Three weeks after inoculation, the infection percentage of berries varied from 2.89 to 25.93% at Gololcha (Arbagugu state farm) and from 3.25 to 34.92% at Mechara. Similarly, previous studies showed that Arabica coffee resistance to *C. kahawae* also results from constitutive and induced mechanisms operating at different stages of pathogenesis(Loureiro *et al*. 2012). The genotypic variation in pathogen infection under field conditions could be examined by ABT via artificial inoculation but better knowledge of both the pathogens and the coffee plant diversity allowed to identify of durable resistant varieties, which are novel and economical approaches against CBD(Wubshet *et al*, 2022). The nature of resistance in coffee was considered horizontal in which the resistance is controlled by 3–5 recessive genes (Van

der Graff, 1981). Generally, resistant varieties have the potential to reduce the cost of production and are the safe ways of disease management approach, and need great focus on sustainable use(Newton 2016).

According to an analysis of variance, there was a significant difference between treatments, and CBD development was also influenced by geographical locations (as a combined analysis) ($P < 0.001$). As the result obtained, the highest infection percentage was recorded from Ar34/11 (24.64) next to check three weeks after inoculation. The lowest infection percentage was recorded from Ar15/11 (3.07) three weeks after inoculation and Ar70/11 (1.05) infection percentage was recorded two weeks after inoculation as the result of the combined analysis. In the present experiment, it was found that the variations in CBD development might have been mostly caused by genetic differences and environmental influences on the selected lines. In other words, as a result of geographical locations, the severity of disease reactions might also be influenced. The result indicated that there existed a different level of infection in the Arsi coffee collection.

Previous studies have shown that the susceptibility of berries to CBD is affected by altitude, temperature, and humidity (Mouen Bedimo *et al*. 2008, 2012). Similarly, CBD development depends on climatic factors such as rainfall, temperature, and relative humidity (Mouen Bedimo *et al*. 2010).

To increase resistance against diseases in cultivated coffee, wild genetic variation is believed to be of utmost importance for coffee breeding(Davis *et al*. 2019; Scalabrin *et al*. 2020). From previous studies, the different diseases have different niches for various environmental and management variables (Zewdie *et al*. 2020, 2021). Generally, there is a close relationship between the host, environment, and pathogen for the development of coffee berry disease.

Table 2
Percent infection of attached berries at Gololcha, Mechara, and their combined analysis of Arsi coffee collections inoculated with *C. kahawae*

	At Gololcha		At Mechara		Combined analysis	
Genotypes	**14 DAI**	**21 DAI**	**14 DAI**	**21 DAI**	**14 DAI**	**21 DAI**
Ar34/11	20.41 ^{a}	25.93 ^{a}	20.57 ^{b}	23.34 ^{cd}	20.49 b	24.64 b
Ar46/11	14.63 ^{bc}	22.69 ^{a}	16.57 ^{c}	21.32 ^{de}	15.60 c	22.01 c
Ar64/11	18.51 ^{ab}	21.63 ^{ab}	12.04 ^{ef}	24.07 ^{c}	15.27 c	22.85 c
Ar03/11	7.43 ^{de}	17.98 ^{bc}	16.96 ^{c}	19.84 ^{ef}	12.20 d	18.91 d
Ar78/11	10.49 ^{cd}	17.02 ^{c}	13.69 ^{de}	27.38 ^{b}	12.09 d	22.20 c
Ar63/11	9.60 ^{d}	15.54 ^{c}	5.68 ^{g}	8.71 ^{h}	7.64 e	12.13 f
Ar72/11	4.38 ^{ef}	10.93 ^{d}	15.45 ^{cd}	19.19 ^{f}	9.92 de	15.06 e
Ar62/11	7.20 ^{de}	9.71 ^{de}	9.90 ^{f}	15.46 ^{g}	8.55 e	12.59 f
Ar54/11	3.84 ^{ef}	7.01 ^{def}	2.19 ^{hi}	6.04 ^{i}	3.02 f	6.52 g
Ar66/11	2.40 ^{f}	6.09 ^{ef}	0.00 ^{i}	6.25 ^{i}	1.20 f	6.17 gh
Ar05/11	2.84 ^{f}	4.91 ^{f}	2.71 ^{h}	4.34 ^{ij}	2.78 f	4.62 hi
Ar70/11	2.09 ^{f}	3.67 ^{f}	0.00 ^{i}	5.98 ^{i}	1.05 f	4.83 h
Ar15/11	1.88 ^{f}	2.89 ^{f}	0.54 ^{hi}	3.25 ^{j}	1.21 f	3.07 i
Arusa	17.83 ^{ab}	22.41 ^{a}	32.76 ^{a}	34.92 ^{a}	25.30 a	28.66 a
Significance level	***	***	***	***	***	***
Mean	8.82	13.46	10.65	15.72	9.74	14.59
CV	29.27	11.92	13.83	7.89	20.85	10.01

*Mean values followed with the same letter(s) in column are not significantly ($P < 0.05$) different from each other. ******* Highly significant. 14 DAT = 14 Days after Inoculation, 21 DAT = 21 Days after Inoculation

3.2. Response of Arsi coffee collections to CBD (*C. kahawae*) at seedling inoculation test

The data revealed a high level of significance ($P < 0.001$) among coffee collections and/or varieties (Table 3). According to the results, the resistant variety 741 showed the lowest infection percentage

(15.04%) in comparison to all other collections and/or varieties (Fig. 2). There was a range of 15.04–100.00% infection percentages in reality among tested collections and/or varieties. According to the results, the relatively resistant variety 741 showed the lowest infection percentages (15.04%) followed by Ar15/11(22.55) in comparison to all other collections and/or varieties under seedling inoculation tests. As compared to the well-known susceptible variety 370, these two Arsi coffee collections (Ar34/11, and Ar64/11) did not show significant differences in infection percentages at the seedling stage.

In controlled conditions in the laboratory, collections Ar34/11 and Ar64/11 which were highly susceptible under field conditions became susceptible. Similarly, result genotypes which are susceptible under field conditions could be susceptible under controlled conditions if no change in pathogen strains (Bayetta 2001; Wubshet *et al*, 2019).

So, seedling inoculation tests can be used directly to examine their interaction with Coffee arabica and *Colletotrichum kahawae* within a short period for an immediate solution. The discovery of hypocotyl infection on a four to six-week-old seedling using artificial inoculation with *Colletotrichum kahawae* spores contributes significantly to *Arabica coffee* breeding by shortening the time required to identify resistant progenies from crosses involving resistant and susceptible donors(Gichuru *et al*. 2008).

Table 3
Percent infection of seedlings of Arsi coffee collection inoculated with *C. kahawae*

Collections/Varieties	Infection Percentage
Ar34/11	100.00 [a]
Ar46/11	95.47 [b]
Ar64/11	100.00 [a]
Ar03/11	98.33 [a]
Ar78/11	91.39 [c]
Ar63/11	94.66 [b]
Ar72/11	93.40 [bc]
Ar62/11	93.61 [bc]
Ar54/11	86.70 [d]
Ar66/11	79.21 [e]
Ar05/11	31.25 [g]
Ar70/11	45.16 [f]
Ar15/11	22.55 [h]
Arusa	98.34 [a]
370	100.00 [a]
754	29.35 [g]
741	15.04 [i]
Significance level	****
Mean	**74.97**
CV	**2.38**

*** Highly significant. *Mean values followed with the same letter(s) in column are not significantly ($P < 0.05$) different from each other.

4. CONCLUSION AND RECOMMENDATION

Genetic differences and environmental influences on the selected coffee collections were mainly responsible for the variation in CBD development in this study. The infection percentage of attached berry disease increased from 14 to 21 days after inoculation. According to the results, the resistant variety 741 showed the lowest infection percentages (15.04%) followed by Ar15/11(22.55) in comparison to all other coffee collections and/or varieties under seedling inoculation tests. Under controlled conditions, collections Ar34/11 and Ar64/11 which were highly susceptible under field conditions became susceptible. The green berries might have some physiological barrier that prevented pathogens from germination on the surface and/or killed them after they had germinated. Generally, further breeding work should use genotypes Ar15/11, Ar05/11, and Ar70/11. This is because they showed a low infection percentage under both experimental conditions and might offer the most effective alternatives to CBD management, specifically in the study area. Further study is necessary to research the wax content and the biochemical composition of these Arsi coffee collections to determine what contributed to their CBD resistance.

Declarations

Acknowledgment

The authors are grateful to Mechara Agricultural Research Center and Jimma Agricultural Research Centre for financial and material supports.

Data availability statement

Data will be made available on request.

Declaration of interest statement

The authors declare no conflict of interest.

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Figures

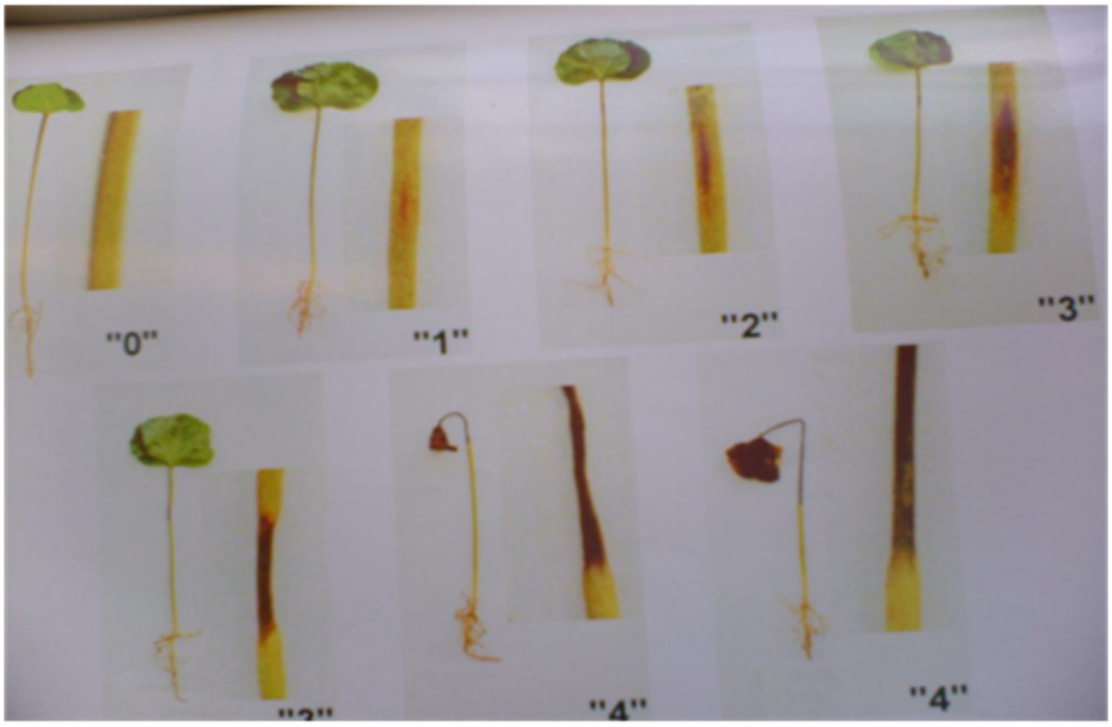


Figure 1

CBD score scale (0-4) under controlled conditions Source:(Bayetta 2001)

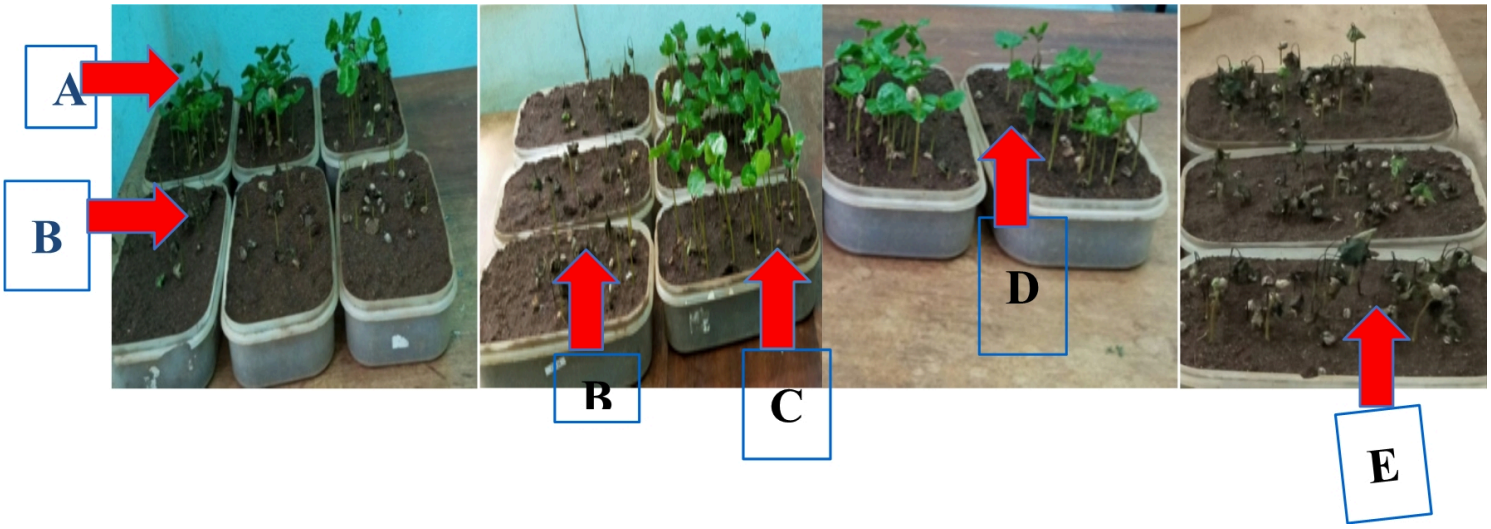


Figure 2

Comparison between standard resistant check, 741 (A), 370 (B) susceptible check, and Arsi coffee collections Ar15/11 (C), Ar05/11 (D), and Ar34/11 (E) and.