

## Research Article

# Isolation and Characterization of Wilt-Causing Pathogens of Local Growing Pepper (*Capsicum annuum* L.) in Gurage Zone, Ethiopia

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The yield of pepper (*Capsicum annuum* L.) is extremely threatened by different diseases in Ethiopia. The objective of the study was isolation of wilt-causing pathogens and susceptibility test of local growing pepper. Eighteen pepper farming fields were selected for disease assessment study. The samples of Mareko Fana, Dubi, and Mitmita local cultivar pepper's pods, seeds, leaves, stems, and roots were collected, surface sterilized, and cultured on potato dextrose agar (PDA). Selective peptone pentachloronitrobenzene (PCNB) agar medium was used for fungus. Similarly, for bacteria isolation, nutrient agar (NA) was used. Morphological and biochemical tests revealed eleven fungal isolates of *Fusarium oxysporum* f. sp. that were isolated. The pathogenicity test confirmed nine of them were virulent to Mareko Fana, Dubi, and Mitmita local pepper. It is confirmed that *Fusarium oxysporum* f. sp. is the pathogen *Fusarium oxysporum* f. sp. *capsici*. Besides, *Ralstonia solanacearum* was identified as a bacterium pathogen causing complex pepper wilt disease. The highest mean PDI was registered in Remuga Keble (93.0%) and the lowest in Buyi Keble (58.3%). Similarly, the highest mean PSI was recorded in Buyi Keble (87.0%) and the lowest PSI (54.5%) was registered in Tawlla Keble. Among 60 seeds, Mareko local pepper inoculated by *F. oxysporum* f. sp. and *R. solanacearum* shows the highest susceptibility of 55 (91.0%) and 30 (50.0%), respectively. However, Mitmita local pepper was registered as the lowest susceptibility to both *F. oxysporum* f. sp. and *R. solanacearum* of 28.3% and 30.0%, respectively. Based on the finding, it can be concluded that pepper wilt was caused by a complex of fungus *Fusarium oxysporum* f. sp. *capsici* and bacteria *Ralstonia solanacearum* sp. in the study area. So, it is recommended that an integrated disease management approach should be implemented to manage the complex diseases of the site.

## 1. Introduction

Hot pepper (*Capsicum annuum* L.) is the most important vegetable crop belonging to the family *Solanaceae* and grown as a spice crop in different parts of the world [1]. It is the main part in the daily consumable diet of most Ethiopian societies. Pepper production accounts for 34.0% of the total spice cultivation in Southern Nations, Nationalities, and People's Region [2]. In Ethiopia, currently, an income generating by pepper cultivation scaled up to 509.44 million Ethiopian birr for small holder farmers per year as the study conducted by Tameru et al. [3]. This indicates that hot pepper is the important source of income to farmers as an exchange earning commodity in the country.

In Southern Nations, Nationalities, and People's Region (SNNPR), Mareko, Meskan, Abeshge, Lanfro, Dallocha, Silte, Ginbo, Gibe, Gojeb, Shashego, Halaba, Meirab-Abaya, and Hawassa Zuria are high pepper-producing districts. However, the yield of the crop is low in the region. This might be attributed to the use of low-yielding varieties, drought, insect pests, and disease susceptibility [4]. Unfortunately, the total crop failure due to diseases has been common in the region and farmers are sometimes forced to abandon their production due to excessive infection pressure in the field [5].

Fungal diseases are a major risk to food security [6]. *Fusarium* wilt is a serious disease attacking pepper plants in Central Java which causes loss of chili yield. *Fusarium* wilt is

caused by fungal pathogens. The symptoms of *Fusarium* wilt are wilting, vein clearing in younger leaflets, stunting, and yellowing of older leaves [7]. Furthermore, according to the report by Joshi et al. [8], wilt diseases caused by different fungal and bacterial pathogens are the major constraints of *solanaceous* crops.

As mentioned in the study by Ali [9], wilt is the main disease in pepper crops and *F. oxysporum* is one of the causal agents of wilting in pepper-producing countries. *Fusarium* isolates are the causative agent of wilt disease in a wide range of economically important crops. It is an anamorphic species circumscribed by different morphological criteria: principally, the size and shape of the macroconidia, the presence or absence of microconidia and chlamydospores, colony color, and conidiophore structure [10]. *Fusarium oxysporum* produces microconidia in false heads on short conidiogenous cells of monophialidic type and also produces chlamydospores, which makes it distinct. It is difficult to distinguish *F. solani* and *F. subglutinans*. However, *F. solani* forms microconidia in false heads on very long monophialidic conidiogenous cells. *F. subglutinans* is distinguished from *F. oxysporum* by the formation of microconidia from mono- and polyphialidic conidiogenous cells and lack of chlamydospores [11].

In this study, Abeshige, Mareko, Meskan, and Kebena districts are potential areas for pepper crops [4]. However, yield is highly affected by pepper wilt-causing pathogens, which leads to total loss of productivity. Type and relative importance of each disease across locations have not been well characterized and profiled in the study area. So, it is important to isolate and identify types of pathogens for disease control and management in the area. The objective of the study was isolation and characterization of wilt-causing pathogens and analyzing susceptibility of local growing pepper (*Capsicum annum* L.).

## 2. Materials and Methods

**2.1. Description of the Study Areas.** The study was conducted in three districts of Ethiopia: Gurage Zone of Southern Nations, Nationalities, and People's Region (SNNPR). Abeshige district (Lache-omancho and Dalga Keble), Kebena district (Remuga and Qola-kabada Keble), and Mareko district (Enseno and Buyi Keble) were selected to represent the major pepper-growing areas of the zone. Experimental activity was conducted in the Biotechnology Department laboratory of Wolkite University. Wolkite town is located 171 km away from Addis Ababa, the capital city of Ethiopia. The districts have average latitude and longitude of 8° 17'N and 37°4'E and an elevation between 1537 and 2010 m above sea level.

**2.2. Field Assessment and Sampling Techniques.** Three potentially pepper-growing districts, namely, Abeshige, Kebena, and Mareko, of Gurage Zone were selected. From each district, six pepper farming fields were selected. Farm fields at each selected area were systematically sampled to

collect disease specimens. Totally, 100 pepper plant samples were collected for disease measurements from 3 m × 2 m quadrant in each farm field. During this study, 100 peppers from each field, that is, 1800 from eighteen fields, were assessed for percent disease incidence (PDI) and percent severity index (PSI) determination in the 2019 cropping season. Pepper wilt was observed in all surveyed localities, with varied disease intensity.

**2.3. Isolation and Characterization of Wilt-Causing Pathogens.** Pepper leaves, stems, and roots were collected from wilt disease symptom-showing peppers, kept separately into polythene bags, placed inside the ice box, and brought to the Biotechnology Laboratory of Wolkite University. Pepper plant leaves, stems, and roots were cut into smaller pieces, and seeds were surface sterilized in 70% alcohol and 0.5% sodium hypochlorite for 30 and 60 s, respectively. Then, they were rinsed in sterile distilled water and transferred to Petri plates. For isolation of different fungal and *Fusarium* fungi pathogens, potato dextrose agar (PDA) and selective peptone pentachloronitrobenzene (PCNB) agar medium were used. The peptone PCNB agar medium ingredients were 15 g Difco peptone, 1 g KH<sub>2</sub>PO<sub>4</sub>, 0.5 g MgSO<sub>4</sub>·7H<sub>2</sub>O, 20 g agar, 1 g pentachloronitrobenzene (PCNB, 75% WP), 1 mL lactic acid, 0.5 g chloramphenicol, and 1 L distilled water. The ingredients were mixed and dissolved, the pH was adjusted to 4.5, and then the medium was autoclaved [12]. The Petri plates were incubated at 28°C for 4 days followed by colony morphology identification. Four-day grown fungal hyphae were further subcultured on the potato dextrose agar (PDA) medium amended with 300 mg/L chloramphenicol. A colony of fungus with different colors on PDA near the pepper sample parts was selected and transferred into new PDA and incubated for 7 days to get pure culture of the isolate. For bacterial isolation, nutrient agar was used.

**2.3.1. Morphological Examination of Isolated Fungus.** After pure colony development, identification of fungi was performed based on the cultural characteristics and microscopic examination using the standard manuals [13]. Colony color, number of septation, and the shapes of spore (macroconidia and microconidia) were recorded and used for morphological classification. The spores of fungi were taken aseptically, mounted on a slide, stained with methyl blue, and covered with a cover slip to examine under a microscope (100x). Identification was made on the basis of their colony morphology (shape and color), spore shape, shape and size of microconidia, size and septation of macroconidia, presence of isolate, or chain chlamydospores. Morphology results were compared using text books for identification of fungi of their taxonomic keys, as stated by Ronhede et al. and Ulhan et al. [10, 14]. The identity of the culture was further confirmed only with the presence of macroconidia and microconidia. Pure cultures of all the isolates were stored on PDA [15].

**2.3.2. Morphological and Biochemical Characterization of Isolated Bacteria.** The pure colonies were subjected to various morphological and biochemical characterization to determine the isolated bacteria according to Sonkar et al. [16]. The performed morphological tests were Gram staining and tests for color and shape of colony. The performed biochemical tests were the catalase test, oxidase test, citrate utilization test, starch hydrolysis test, KOH test, and urease tests. The bacterial streaming test was applied as a method to distinguish bacterial wilt. The infected plant stem was cut and dipped into a water-containing test tube. The bacterial exude from the cut end was analyzed [6].

**2.4. Disease Incidence Assessment.** This was made by observing wilting and leaf-yellowing expression symptoms on representative plants to determine the general presence or absence of diseases [17]. Pepper-farming fields were visited diagonally, and the disease incidence was estimated by using the 3 m × 2 m quadrant. The percent disease incidence was calculated by using the following formula:

$$\begin{aligned} &\text{percent disease incidence (PDI)} \\ &= \frac{\text{number of plants affected}}{\text{total number of plants observed}} \times 100. \end{aligned} \quad (1)$$

To know disease severity, symptoms such as wilting, vascular discoloration, and root rotting were considered as indices for sample collection. Root rot and wilt severity were estimated from 10 days to 35 days by 5 days of interval after transplanting by following the method outlined by Ismail et al. [11]. The disease rating scale of 0–5 was based on leaf yellowing and wilting grading: 0 = healthy, 1 = one leaf yellowing, 2 = more than one leaf yellowing, 3 = one wilted leaf, 4 = more than one leaf wilted, and 5 = completely dead/wilted plants.

Disease severity scores were converted into percentage severity index (PSI) as follows:

$$\begin{aligned} &\text{percent severity index (PSI)} \\ &= \frac{\text{sum of numerical rate}}{\text{number of plants scored} \times \text{maximum score on scale}} \times 100. \end{aligned} \quad (2)$$

**2.5. Pathogenicity and Susceptibility Test.** Three different genotypes of local peppers, Mareko Fana, Dubi, and Mitmita, were used for both *R. solanacearum* and *Fusarium oxysporum* pathogenicity tests. A total of 60 seeds were selected for each cultivar and then surface sterilized and planted on a plastic pot with 25 cm diameter containing autoclaved top soil, composite, and sand at 3:2:1 ratio, respectively, according to Fekadu and Dandena [4]. Each pot was made to have 12 seeds with three replications for each local pepper cultivar. The experiment was carried out under greenhouse condition at 25°C. A completely randomized design was used with three replicates (pots). A healthy plant

was maintained as the control. Percent disease incidence and disease severity were recorded.

The pathogenicity test for *R. solanacearum* was carried out according to the method outlined by Mimura and Yoshikawa [18]. The bacterium was cultured in nutrient broth media overnight on an incubator shaker, and the concentration was adjusted using a UV spectrophotometer. Soil on plastic pots was infested with 5 mL of ( $4 \times 10^8$  cfu/mL) bacterial suspension into the center. After thirty days, three pepper seedlings were transplanted into a *R. solanacearum* inoculated pot with 25 cm diameter under greenhouse condition. The inoculated pepper plants were examined for wilting symptoms starting from 10 DAI (days after inoculation). Disease symptom and proportion of wilted plants (PW) were recorded by 5 days of intervals till 35 DAI.

The pathogenicity test for isolated *Fusarium* sp. was performed using the method designed by Khalifa [19]. Five milliliters of conidial suspensions containing  $6.4 \times 10^6$  conidia/mL were used to infest the soil on pots. Thirty-day-old three local peppers used in this study were transplanted onto the infected soil in pots with three replications. Sets of control pepper from all genotypes were subjected to sterile soil-containing pots. Pepper wilting symptom examination and proportion of wilted plants were registered starting from 10 DAI every 5-day interval till 35 days as stated for *R. solanacearum*. For further confirmation test, isolates identified using cultural or morphological characteristics and yellowing lesions on the vascular bundle were examined. The pathogen isolated in the laboratory was compared with that isolated from disease symptom-developing pepper. Disease susceptibility was calculated, and grading was performed on the basis of wilt characteristics which is expressed by percent as follows: highly resistant (HR), 0% wilting; resistant (R), 1–10% wilting; moderately resistant (MR), 11–20% wilting; moderately susceptible (MS), 21–30% wilting; susceptible (S), 31–50% wilting; and highly susceptible, >50% wilting, according to Bayoumi and El-Bramawy [20].

### 3. Results and Discussion

A total of eleven fungal colonies (PF1-PF11) and two bacterial colonies (BC1 and BC 2) were isolated from pepper leaves, seeds, stems, roots, and pods. From the isolated fungi, nine isolates were found to be pathogenic to Mareko Fana, Dubi local, and Mitmita local pepper plant showing typical wilt symptoms on the foliage growth, stems, and xylem vesicles. Similarly, bacteria isolates were found to be virulent to these three selected genotypes of local pepper plants.

**3.1. Isolated and Identified Types of Pepper Wilt-Causing Fungi Pathogens.** Table 1 shows that all colonies grown on the plate showed light brown pigmentation in PDA except PF-10 and PF-11 isolates. This result was similar to that reported by Ismail et al. and Sonkar et al. [11, 16]. The fungal isolates coded as PF-3, PF-4, PF-6, and PF-9 registered the largest colony with averages between 5.3  $\mu\text{m}$  and 5.5  $\mu\text{m}$  diameter.

TABLE 1: Morphological characterization of isolated fungi.

S/N	Isolate code	Colony color	Colony cover on the plate	Reverse colony color/pigmentation
1	PF-1	Dirty white	Plate fully covered with colony	Very small light brown pigmentation
2	PF-2	Dirty white	Plate fully covered with colony	Very small light brown pigmentation
3	PF-3	Dirty white	Plate fully covered with colony	Light brown pigmentation covered all parts of the plate
4	PF-4	Dirty white	Plate fully covered with colony	Large concentrated light brown pigmentation
5	PF-5	Dirty white	Plate fully covered with colony	Light brown pigmentation on one side only
6	PF-6	Dirty white	Plate fully covered with colony	Large part covering light brown pigmentation
7	PF-7	Dirty white	Colony concentrated at the middle only	Very small light brown pigmentation at the center
8	PF-8	Dirty white	Colony concentrated at the middle only	Very small light brown pigmentation at the center
9	PF-9	Dirty white	Fully covered with small aerial mycelium	Large part covering light brown pigmentation
10	PF-10	Cottony white	Colony at the center	White (no pigmentation)
11	PF-11	Dirty white	Colony at the center	White (no pigmentation)

Isolates such as PF-1, PF-2, PF-5, PF-7, and PF-8 formed colonies that cover a small part of the plate on PDA media with white mycelia and red-brown pigmentation, which was the same as observed in the study reported by Balali and Iranpoor [21]. On the basis of mycelium growth up pattern, all isolates showed adherent smooth surface except PF-10 and PF-11 with fluffy growth on PDA.

All isolates' microconidia were devoid of septation and macroconidia were with septation (Figure 1). Microconidia were found singly or in mass with cylindrical shape. The isolates in this study registered nonseptate microconidia formed in false heads on short monophialides. Septation of macroconidia were found to be 2, 3, and 4 on PF-1, PF-3, and PF-9; PF-4, PF-5, and PF-6; and PF-2 and PF-7, respectively. Macroconidia found in this study were the same related to the shape falciform, number of septation (2–4), and abundance as the finding by Ciampi et al. [22]. For *Fusarium oxysporum* sp., *F. solani* forms cylindrical macroconidia, with no convex curvature, but *F. oxysporum* f. sp form falciform/ellipsoid shape according to Ulhan et al. [10]. Oval-shaped microconidia without septation and falciform-shaped macroconidia possessing 2–4 septation were registered. All fungal isolates were identified to have short phialides. Cultural and biochemical analysis of the bacterial study showed that *R. solanacearum* was found as a wilt-causing pathogen on peppers.

All fungal isolates except PF-9 and PF-2 were identified to contain short phialides of less than 18  $\mu\text{m}$ . This characteristic made our isolate, *F. oxysporum*, different from *F. solani* that contains long phialides. It is the best characteristic for differentiating these two species according to Balali and Iranpoor [21]. So, in concert with colony characteristics and microscopic structures, all the nine fungal isolates showed the characteristics of *F. oxysporum* f. sp. In this regard, the result was in line with the work of Pawasker et al. [23]. The average macroconidia length and width ranged from 5.5  $\times$  3  $\mu\text{m}$  to 10  $\times$  3  $\mu\text{m}$ . As indicated in this study, chlamydospores existed in single and intercalary in PDA cultures except on PF-2 and PF-9 isolates.

**3.2. Morphological and Biochemical Characterization of Isolated Bacteria.** One of the causative agents of pepper wilt disease identified in this study was a bacterial pathogen. As morphological and biochemical tests revealed, two bacterial

colonies (BC1 and BC 2) were identified as *Ralstonia solanacearum* (Table 2). The biochemical test result was similar to the work of Assefa et al. and Pawasker et al. [6, 23]. This bacterial isolate was found infecting the pepper alone and with the association of *F. oxysporum* f. sp.

**3.3. Disease Measurement.** Mean percent disease severity (PSI) and percent disease incidence rate (PDI) were found to be 65.9% and 73.2%, respectively (Table 3).

Percent severity index and percent disease incidence of pepper wilt disease within the selected kebles are shown in Figure 2. This study showed that, in three pepper-farming districts, the highest mean disease incidence (PDI) was registered in Remuga Keble (93.0%) and the lowest was observed in Buyi Keble (58.3%). The highest mean PSI was also recorded in Remuga Keble (87.0%), and the lowest PSI (54.5%) was registered in Tawlla Keble.

The mean PSI (65.9) showed high severity of disease in the area according to the disease severity rating set by Bayoumi TY [20]. During the study period, the mean PDI was found to be 73.2% which is higher than the result reported by Shiferaw and Alemayehu [24] in Abeshige district. The PSI of the study area was higher than the result reported by Mekonnen et al. [6], a research conducted in Bako Tibe district, Oromia Regional State. The occurrence of high PSI of pepper wilt disease in the area may be owing to the high soil-water holding capacity, cultural practices, type of the cultivars used plant spacing, and level of inoculums present in the soil [25].

Both the fungal isolate *F. oxysporum* and the bacteria isolate *R. solanacearum* were found from stems, roots, leaves, pods, and seeds of infected pepper plants. Both the isolates were found together forming a complex pepper wilt disease-causing pathogen [24]. This study shows the reason for limited productivity and total loss of pepper yield through identification of the main causative agent of pepper wilt disease. Also, Temam [26] reported *Fusarium wilt* (*Fusarium oxysporum*) as being the most widespread fungal disease of hot pepper in Ethiopia.

The field observation symptoms of wilt-causing diseases were wilting, vein clearing in younger leaflets, stunting, and yellowing of older leaves (Figure 3).

The pathogenicity test of *F. oxysporum* isolates revealed that the nine tested isolates were pathogenic to Mareko Fana, Dubi local, and Mitmita local pepper plants showing typical



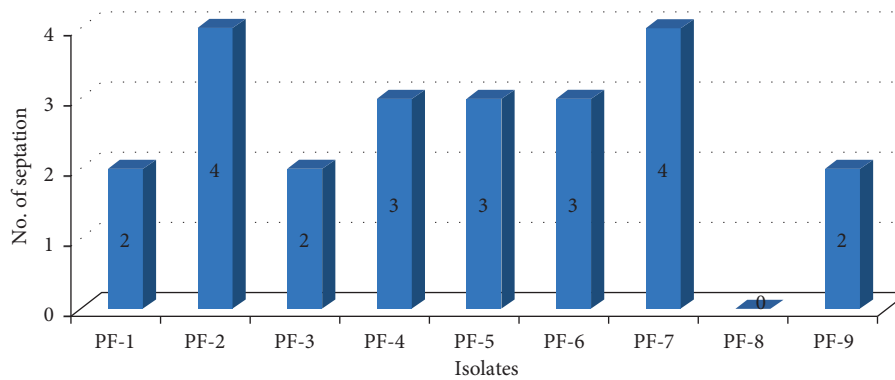


FIGURE 1: Fungal isolates with macroconidia septation number.

TABLE 2: Morphological and biochemical characterization of isolated bacteria.

	S/N	Types of tests	Test results
Morphological tests	1	Gram staining test result	Negative
	2	Colony shape	Short rod
	3	Elevation	Raised
	4	Margin	Entire
	5	Surface	Smooth
	6	Color	White
Biochemical tests	1	KOH solubility test	Positive
	2	Catalase test	Positive
	3	Starch hydrolysis test	Negative
	4	Oxidase test	Positive
	5	Citrate utilization test	Positive
	6	Cellulose decomposition	Negative
	7	H <sub>2</sub> S	Negative

TABLE 3: Average percent severity index and percent disease incidence of pepper wilt disease.

Disease condition	Minimum	Maximum	Mean	Std. deviation	Std. error
PSI %	54.5	87.0	65.9	12.90	3.04
PDI %	58.3	93.0	73.2	12.78	2.91

PSI, percent severity index; PDI, percent disease incidence.

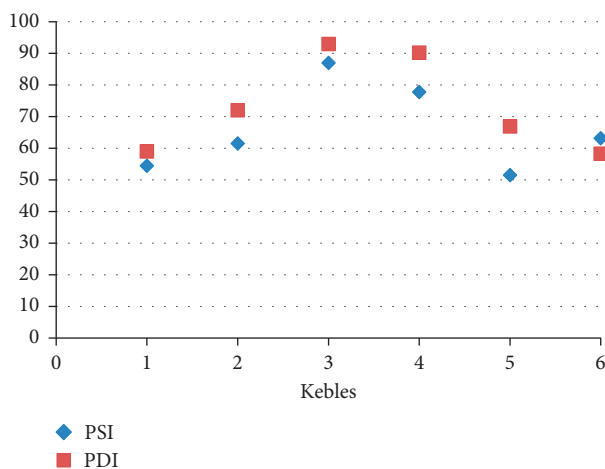


FIGURE 2: Percent severity index and percent disease incidence of pepper wilt disease within selected kebles. 1, Tawlla; 2, Dalga; 3, Remuga; 4, Qola-kabada; 5, Enseno; and 6, Buyi.

wilt symptoms on the foliage growth, stems, and xylem vesicles. Moreover, *R. solanacearum* bacteria inoculated onto local growing pepper showed symptom spreading from the youngest leaves to the oldest leaves which made it different from the fungus *F. oxysporum* which is in line with the description given by Lowell et al. [17]. The wilting characteristics due to *F. oxysporum* started from the older leaves and spread to the younger ones. A transverse cut of stem also showed vascular browning that confirms most likely *F. oxysporum* and bacterial ooze for *R. solanacearum*. Roots and stems of the diseased pepper plant that were cut and placed in a small test tube containing water showed yellowish ooze coming from the cut end. This ooze was a key feature in confirming the disease caused by bacterial pathogens. This finding was similar to the work of Chaudhuri et al. [27].

3.4. Pathogenicity and Disease Susceptibility Evaluation of *F. oxysporum* sp. and *R. solanacearum*. The pathogenicity test conducted on Mareko Fana, Dubi local, and Mitmita



FIGURE 3: Field work sample pictures showing disease severity and symptom. (a) Fusarium wilt symptom on leaves. (b) Fusarium wilt symptom on the external stem. (c) Fusarium wilt systemic symptom. (d) Symptom on a root.

TABLE 4: Disease susceptibility evaluation after inoculation of *F. oxysporum* and *R. solanacearum* on local pepper (no. of seedlings used per cultivar was 60).

Pathogens	Local pepper genotype	Wilted plants (%)					
		Days after inoculation					
		10 DAI	15 DAI	20 DAI	25 DAI	30 DAI	35 DAI
<i>F. oxysporum</i> f. sp.	Mareko Fana	0	0	15 (25.0%)	29 (48.3%)	47 (78.3%)	55 (91.0%)
	Dubi local	0	0	5 (8.33%)	30 (50.0%)	40 (66.7%)	40 (66.7%)
	Mitmita local	0	0	0	13 (21.7%)	15 (25.0%)	17 (28.3%)
<i>R. solanacearum</i>	Mareko Fana	0	0	0	10 (16.7%)	25 (41.7%)	30 (50%)
	Dubi local	0	0	0	13 (21.7%)	20 (33.3%)	20 (41.7%)
	Mitmita local	0	0	0	5 (8.3%)	15 (25.0%)	18 (30.0%)
Control	Control	0	0	0	0	0	0

local pepper confirmed that the nine *F. oxysporum* f. sp. isolates and two bacteria were pathogenic (Table 4). Despite the fact that PF-2 and PF-9 isolates were identified as *F. oxysporum*, they were confirmed as no pathogenic in this study. Among 60 seeds inoculated by *F. oxysporum* f. sp. and *R. solanacearum*, Mareko local pepper shows the highest susceptibility of 91.0% and 50.0%, respectively. Also, Dubi local pepper is 66.7% susceptible to *F. oxysporum* f. sp.

Similarly, Mareko Fana (50.0%) and Dubi local pepper (41.7%) were susceptible to *R. solanacearum*. Among these three genotypes, Mitmita local pepper was

registered as the one with lowest susceptibility to *F. oxysporum* f. sp. and *R. solanacearum* of 28.3% and 30.0%, respectively. Disease severity indices varied significantly ( $p < 0.05$ ) for the three local genotypes. That means, Mareko Fana > Dubi local > Mitmita local pepper for disease susceptibility. Control treatment did not show wilting which was significantly different ( $p < 0.01$ ) from all local inoculated genotypes. Similar to the study conducted by Joshi et al. [8], Melka Awaze and Melka Zala local genotype pepper were recognized as moderately resistant against *F. oxysporum* f. sp. Therefore, all the nine *F. oxysporum* f. sp. and two *R. solanacearum*

isolated from wilted pepper parts, stems, roots, leaves, pods, and seeds, were recognized pathogenic in this study.

#### 4. Conclusion

Cultural, microscopic analyses and biochemical tests supported by the pathogenicity test revealed that fungi *Fusarium oxysporum capsici* and bacteria *R. solanacearum* were the causative agents for pepper wilt disease in the study area. From the findings, local growing pepper, Mareko Fana, and Dubi local pepper were identified highly susceptible to *Fusarium oxysporum capsici* and *R. solanacearum*, but Mitmita local was identified moderately susceptible to both pathogens. The current study indicated that a complex of diseases exists at each growth stage of pepper and the occurrence across districts is highly variable. Therefore, an integrated approach is required to manage the complex diseases in the area. Also, pepper cultivars should be selected, tested, and genetically improved for the wilt-causing pathogen control.

#### Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

#### Conflicts of Interest

The authors declare that they have no conflicts of interest.

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