
Prevalence of Bacterial Wilt of Ginger (*Z. Officinale*) Caused by *Ralstonia Solanacearum* (Smith) in Ethiopia

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Abstract: *Ginger is one of the most important spices, largely for small scale farmer in Ethiopia. In many production areas of Ethiopia up to 85% of the farmers and 35% of the total arable land were allotted for ginger production. However, this potential spices crop wiped out by a sudden outbreak of bacterial wilt epidemics in 2011 and 2012. A survey was conducted on the status of ginger bacterial wilt incidence in major growing areas of Ethiopia and laboratory work was done to identify the causal agent of ginger wilt. The diseases were found distributed in all ginger growing areas and the loss were estimated up to 100%. During 2012 survey season the wilt incidence percentage was recorded maximum (93.5) in Sheka zone followed by Benchmaji zone (91.6) and Majang zone (65.7) while the lowest wilt incidence was recorded in gamogofa zone (10.7). During 2014 survey season wilt incidence percentage was recorded maximum (98.9) in Benchmaji zone followed by Majang zone (98.8) and Sheka zone (97.4) while the lowest wilt incidence was recorded in Keffa zone (78.4). The syptomology and pathogenicity test confirmed the bacteria were *R. solanacearum* causing bacterial wilt of ginger. All groups of *R. solanacearum* isolates were found virulent producing pink or light red color or characteristic red center and whitish margin on TZC medium after 24 hours of incubation. Since no resistance genotype was found in the country and the nature of the bacteria is difficult to control by chemical means, an integrated management program needs to be started.*

Keyword: *Ginger, Ethiopia, R. solanacearum, bacterial wilt.*

1. BACK GROUND INFORMATION

Ginger is one of the most important spices, largely for small scale farmer in Ethiopia. At south part of the country in eight woredas 2.9 million quintal of fresh ginger was produced from an area of 18,240ha during 2006/2007 and average rhizome yield of 160 quintal per hectare (BOARD, 2008). From two woredas (Hadaro tunto and Boloso Bombe) during 2008 alone, more than 1.2 million quintal of fresh ginger was produced from area of 8986 hectare. In the production area up to 85% of the farmers and 35% of the total arable land were allotted for ginger production (Endrias and Asfaw, 2011).

The first Ginger bacterial wilt diseases were reported from India in 1941 by Thomas, then after a lots of reports came from Australia (Hayward et al., 1967), China (Li et al., 1994), Hawaii (Rosenberg, 1962), Indonesia (Sitepu et al., 1977), South Korea (Choi and Han, 1990), Malaysia (Lum, 1973), Mauritius (Orian, 1953), Nigeria (Nnodu and Emehute, 1988), Philippines (Zehr, 1969) and Japan (Morita et al. 1996). In Ethiopia, the bacterial disease has been reported on Potato, Tomato, Pepper, Enset, Banana and ornamentals but ginger bacterial wilt is not yet reported and new to Ethiopia.

In many tropical and subtropical regions the pathogen has been widely distributed and associated with a wide range of hosts (Agrios, 2005). *Ralstonia solanacearum*(RS) grouped in to five races based on the different in host ranges and geographic distributions; those races principally attack Bananas (race 1), ornamental planes (race 2) , potato (race 3), Ginger (race 4) and mulberries (race 5) (Kelman, 1997). Race 4, of the pathogen limits the production of ginger in the tropics (Paret, et al, 2010) and has a narrow host range, and restricted to ginger (Kelman, 1997). Lemessa and Zeller (2007) identified sixty two RS strains from Ethiopia out of this 19 were grouped as biovar I and rest of the strains as biovar II. It was further observed that biovar II strains had limited host range (affecting mainly potato) as compared to biovar I strains (affecting eggplant, tomato and potato) but so far race 4 is not yet reported in association with ginger or other crops in Ethiopia

Despite the increasing importance of the disease in the country, there was no information generated on the distributions and identification of the pathogen. The objectives of the study were to survey and map ginger bacterial wilt distribution and identify the causal agent of wilt diseases of ginger in Ethiopia.

2. MATERIALS AND METHODS

2.1. Survey and Mapping

Survey of Ginger Bacterial Wilt (GBW) was done in 2012 and 2014 to study the disease distribution in major ginger producing area of Ethiopia. A stratified sampling technique was employed to sample zones and woredas and apply simple random sampling technique to sample ginger field. Data on disease prevalence, incidence, and severity were collected. A total of 165 ginger producing farmer's field which found in twenty seven woredas, nine zone and two special woreda in south Nation and Nationality of People Regional State (SNNPRS) and one zone and one woreda in Gambela regions were assessed. In order to determine the incidence of the disease, field diagnosis was done based on symptoms of the disease. In conditions where distinct symptom to the disease is confusing, a rhizome segment (about 5-6 cm) immersed in the glass of water and watched for milky continuous threads streaming downward from the cut; and the internal tissue was examined for vascular brown ring and creamy bacterial exudates. All surveyed fields were geo referenced to integrate data into GIS for analysis and map the distributions of GBW incidence. Information based on the check list that includes the socio economic aspect and the cropping system was gathered to see how this affects the incidence of the disease. The incidence of bacterial wilt was recorded and calculated based on the description by Horita and Tsuchiya (2001).

$$\% \text{ Wilt incidence} = \frac{\text{Number of wilted plants in each field}}{\text{Total number of plants in each field}} \times 100$$

Finally average incidence was calculated for each zone.

2.2. Isolation and identification of *Ralstonia solanacearum*

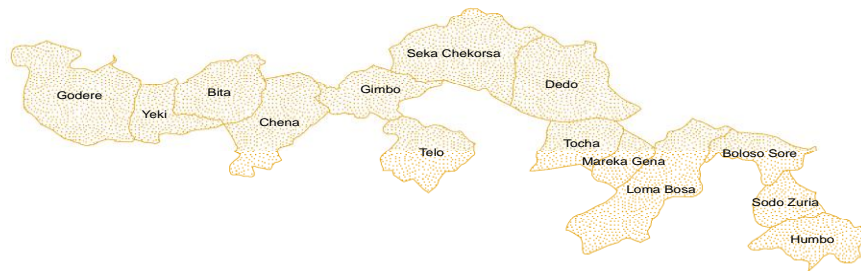
Diseased ginger plant parts (leaf, pseudo-stem, and rhizome) and soil samples were collected from the survey areas of Ethiopia. Field diagnosis of diseased plant samples were done by critically observing the bacterial wilt symptoms. *R. solanacearum* was isolated in Nutrient Agar (NA) plate by streaking the bacterial ooze streamed out into the water from the Rhizome and Pseudo-stem. The plates were then incubated at 28°C for at 24 hrs. After isolation, *R. solanacearum* isolates were purified by streaking a single colony of each isolate on Triphenyl Tetrazolium Chloride (TTC) medium as described by Kelman (1954). The pathogenicity test was performed in three month old healthy ginger seedlings by steam inoculation method. A single colony of *R. solanacearum* showing virulent, fluidal, irregular and creamy white with pink at the center was selected and adjusted to 3.2×10^8 cfu ml⁻¹ for inoculation. Pure culture of *R. solanacearum* were transferred to Nutrient agar media slants and maintained at 4°C for further studies.

3. RESULT AND DISCUSSIONS

3.1. Map of Ginger Bacterial Wilt (GBW) Distribution in Ethiopia

Bacterial wilt of ginger was found widespread in all area surveyed. Among the different places surveyed, wilt incidence were found ranged from 93.5% sheka zone to 10.7 Gamogofa zone in 2012 survey season. The first bacterial wilt syndrome were reported from Benchmaji zone Bebek coffee estate farm, then after it progress to the neighboring zone Sheka within a short period of time and cause up to 67% yield loss. During 2012 the diseases were less prevalent around Wolayta zone even if the area have been known for producing ginger as a major crop; this may be due to the difference in geography, agro-ecology and the prevailing weather condition (Elphinstone, 2005) Direct yield losses and incidence by *R. solanacearum* vary widely according to the host, cultivar, climate, soil type, cropping pattern, geography and strain (Yuliar, Yanetri and Koki, 2015).

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Ginger bacterial wilt distribution map in Ethiopia
 yellow color on the map indicated that the survey woreda

Date: 8/26/2015

Fig1. Ginger bacterial wilt distribution map in Ethiopia

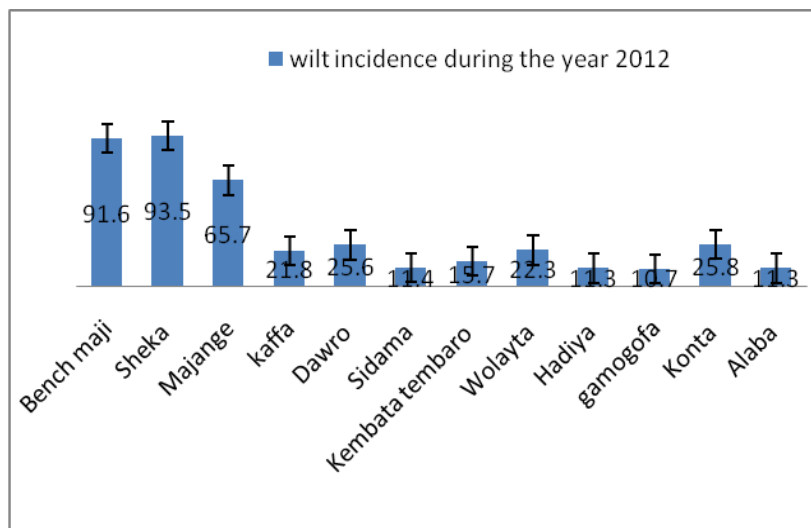


Fig2. Ginger bacterial wilt incidence during 2012 survey season

At the same year even if the diseases is less prevalent at Wolayta area which is different in geography and agro-ecology to that of Benchmajji and Sheka zones, we collect and check the seed rhizomes from wolayta and surrounding for latent infection, from the laboratory result the seed rhizomes were found 100% latently infected (table 1).

Table1. Ginger bacterial wilt incidence on seed rhizomes

No.	Zone	Woredas covered	Kebele	Rhizome tested	Rhizome infected	Latent infection (%)
1	Hadiya	7	10	12	12	100
2	Welaita	2	9	35	35	100
3	KambataTambaro	3	18	61	61	100
4	Dauro	7	18	70	70	100
5	Teppi research center	-	-	56	3	5.4
Total		19	55	234	181	405.4
	Mean					81.08

In subsequent year 2014 survey result, SNNPRS (Dawro, Wolayta, Kenbata tenbaro, Hadiya, Gomogofa, Konta, Alaba, Sheka, and Bench maji) and Gambella region (Majang) zone ginger were found devastated by the bacterial wilt and cause yield loss up to 98%. This is due to the prevailing ideal weather condition for the bacteria epidemics (average rain fall, 287.9mm, Tmax, 27.8°C & T min. 17.1°C) and using of latently infected seed rhizome. The pathogen transmitted through rhizome seed caring latent infection (Tall, 1997), Bowman, 1980 reported that *R. solanacearum* is seed and soil born and easily disseminated from one area to the other through planting material.

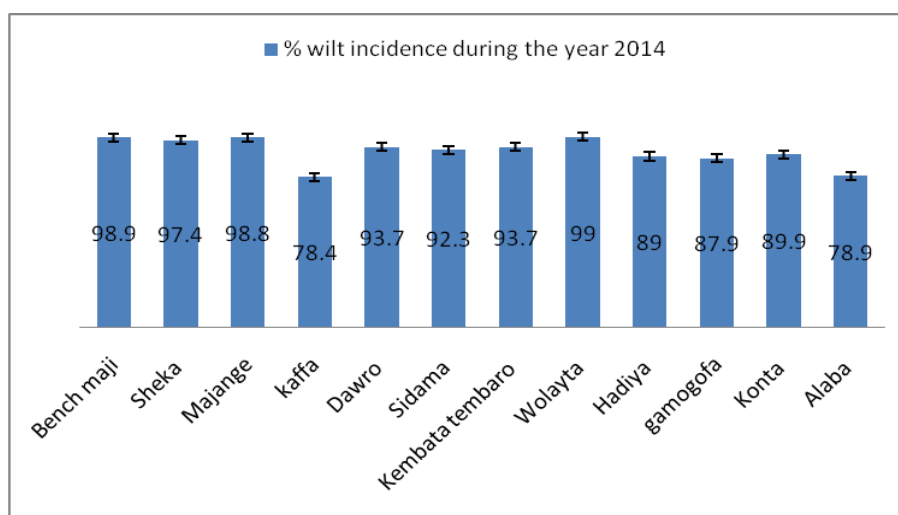


Fig3. Incidence of ginger bacterial wilt during 2014 survey season

A sharp relationship was observed between ginger bacterial wilt incidence and the prevailing weather condition during the survey period (Table 2). The disease on set was found at the end of June to mid of July which is the main rainy season of the country as well as warm humid weather dominates. Diseases progress and wilt of ginger continues till September and October. After October the moisture and temperature as well as wilt incidence dramatically reduced. In most cases wilting process of ginger started from end of Jun to July (av. T.max. 29°C, T.min. 16.5°C and RF. 234.5mm) when moisture gradually rises. The maximum wilt intensity 98.9% was recorded during August-September (Av. T.max. 29.5°C and T.min. 17°C and RF. 207.5mm) and death of such plants ceased at the end of October (Av. Tmax.29.3°C, T.min. 16.7°C and RF. 131.4mm). Due to the increase in rainfall during July-September the active inoculums in the field could easily be transported to nearby fields by rain run-off, and farm activities, these condition increases the incidence, severity and disease distribution (Mondal, Bhattacharya and Khatua, 2014). According to EPPO (2004) the optimum growth temperature of *R. solanacearum in the tropics* was (35°C) whereas strains occurring at higher altitudes in the tropics and in subtropical and temperate areas is lower (27°C); no growth has been observed at 40°C or 4°C. According to Kelman (1953) Approximate minimal and maximal growth temperature values would be 8-10°C and 37-39°C respectively.

the correlation result of bacterial wilt incidence to weather parameter (Rain fall, Temperature maximum and Temperature minimum) shows that high Rain fall and high temperature were found a positive significant correlation whereas, low daily temperature and low rainfall were found negatively correlated. It also noted that PH, Soil and air temperature and moisture, have great influence and relationship on the survival and incidence of the pathogen (EPPO, 2004 and Kelman, 1953).

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Table2. Correlation result of GBW incidence, Temperature (Maximum and minimum) and Rain fall

Correlations					
		RF	T.min.	T.max.	GBW incidence
RF	Pearson Correlation	1	-.057	.227	.991**
	Sig. (2-tailed)		.785	.275	.000
	N	25	25	25	25
T. min.	Pearson Correlation	-.057	1	-.024	-.106
	Sig. (2-tailed)	.785		.909	.614
	N	25	25	25	25
T max	Pearson Correlation	.227	-.024	1	.191
	Sig. (2-tailed)	.275	.909		.359
	N	25	25	25	25
GBW incidence	Pearson Correlation	.991**	-.106	.191	1
	Sig. (2-tailed)	.000	.614	.359	
	N	25	25	25	25

** . Correlation is significant at the 0.01 level (2-tailed).

3.2. Range of Host Infected by the Bacteria During Survey

During the survey major crops which have been cultivated in the area such as tomato (*Lycopersicon esculentum.*), banana (*Musa accuminata*), turmeric (*Curcuma domestica*) and capsicum were found not attack by the bacteria. However, ginger (*Z. officinale*) was attacked and wilted within 5 to 12 days after observing the first symptom. *R. solanacearum* have different host ranges and geographic distributions; race 1, 2, 3, 4 and 5 principally attacks bananas, ornamental planes, potato, Ginger and mulberries respectively (Kelman, 1997). *R. solanacearum* race 4 was found restricted to ginger (Paret, et al, 2010). According to Kumar and Sarma, (2004) race 4 didn't attack banana, potato, tomato, pepper and curcuma and cause wilt on ginger within 5 to 7 days, The causal pathogens were resembling as race 4 based on their specific pathogenicity to *Zingiberaceae* crops (Morita *et al.* 1996, Tsuchiya *et al.* 1999 &2005, Yano *et al.* 2005 & 2011) (Fig 8 a & b).



(a) Wilted ginger and healthy turmeric

(B) wilted ginger and healthy banana

Fig8. Host specificity of ginger bacterial wilt pathogen during survey

3.3. Symptomology of Ginger Bacterial Wilt

3.3.1. Symptom on above Ground Plant Part



(a)Initial symptom

(b) wilted tiller

(c) diseased shoots
Break off easily

(d) Water soaked spots
on leaves

Fig4. Areal symptom of ginger bacterial wilt diseases

The wilt symptom on the above ground ginger plant part were found the first wilt symptoms were a slight yellowing and wilting of the lower leaves. The wilt progresses upward, affecting the younger

leaves, followed by a complete yellowing and browning of the entire pseudo-stem. Under conditions favorable for disease development, the entire shoot becomes flaccid and wilts with little or no visible yellowing. However, the plant dries very rapidly and the foliage becomes yellow-brown in 5 to 10 days. Wilt of the pseudo-stem advances make young succulent shoots frequently become soft and completely rotted and the diseased shoots break off easily from the underground rhizome at the soil line (fig 4, a , b , c , and d). The same ginger bacterial wilt symptom was described by Kumar and Sarma (2004)

3.3.2. Symptom on Underground Plant Part of the Plant

From the analysis of underground plant part symptom, grayish-brown discoloration of the rhizomes were seen at early stage of infection, in advanced stage of disease development major part of the rhizome became discolored, soft, water-soaked and rotted (Fig 5, f). From the cut surface of diseased rhizome extensive bacterial ooze that shows slimy, creamy exudates when pressed or immerse in a glass of water (Fig 5, g) the same result have been reported by Belén Álvarez, Elena G. Biosca, and María M. López. (2010) and Kumar and Sarma (2004)

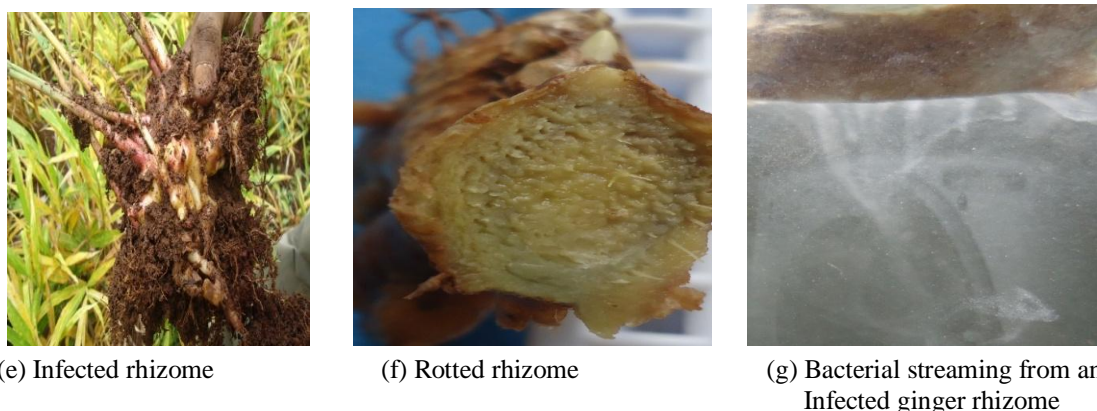


Fig5. Below ground symptom of ginger bacterial wilt disease on rhizome

The symptoms observed on initial developmental stage on leaves, on pseudo-stems and rhizomes of infected ginger plants were identical to the symptom descriptions by (Trujillo, 1964). In vascular tissue, fine milky white strands, compound of mass of bacteria in extra cellular slime, stream down from the cut ends of xylem vessels. This bacterial exudates combined with symptoms observed on leaves distinguishes this wilt from fungus wilt (Hayward, 1964).

3.4. Isolation and Identification

Since the diseases is new to Ethiopia a team of different discipline mobilized to check and identify the true causal agent of ginger wilt diseases. As a result field diagnosis for characteristic symptom and isolation frequency as well as existence of minimal number of parasitic nematode in 100gram of soil confirms that nematodes were not causal agents. Isolation of fungal pathogen from infected ginger samples shows that none appeared. Which confirm that fungi were hardly suspected as causal agent of the diseased happened, The samples were also diagnosed for presence and isolation of bacteria, which reviled that similar bacterial colonies were observed per all samples of all plant parts (Leaf, Rhizome and Pseudo stem) and the biochemical characterization of the bacteria reviled that it *R. solanascearum* biovar III (Ambo plant protection research Center, result of diagnosis report unpublished).

3.5. Culture based Identification

The culture based identification were found the bacteria is fluidal, presents irregular shape and white with pink centered colonies on tetrazolium chloride (TZC) media, which is similar with the description of *R. solanascearum* by Kelman (1954) and Hayward, (1964) (Fig 6).



Fig6. Virulent colonies of *R. solanacearum* in TZC agar medium

3.6. Pathogenicity Test

Result of pathogenicity test of *R. solanacearum* isolate under artificial stem inoculation method revealed that wilt of ginger occur within 5 to 15 days after inoculation (Fig 7, a & b). The result were found in range of pathogenicity study in India which is wilt of ginger occur within 5-7 days after inoculation (Kumar and Sarma, 2004)



(a) symptom on tiller and leaf

(b) healthy ginger

Fig7. Result of pathogenicity test of *R. solanacearum* on ginger

4. CONCLUSION

Hence, the study revealed that the disease that threat Ginger in Ethiopia is caused by *Ralstonia solanacearum* biovar III race 4 and the diseases was found distributed in major ginger growing areas of Ethiopia. Since no resistance genotype was found in the country and the nature of the bacteria is difficult to control by chemical means, an integrated management program needs to be started. Detail work also needed on the biology, ecology, and epidemiology of the bacteria. The pathogen is soil and/or seed born, diseases management option focused on soil and seed is required. An urgent need also required on establishing diseases free ginger seed rhizome production scheme both tissue culture and greenhouse culture.

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