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Spatial Distribution of *Enset* Bacterial Wilt (*Xanthomonas campestris* Pv. *musacearum*) and its Association with Biophysical Factors in Southwestern Ethiopia

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የእንሰት አጠውልግ በሽታ በዛንቶሞናስ ካምቴስትሪስ ፓቶቫር ሙሳሴረም በሚባል የባክቴሪያ ተህዋስያን አማካኝነት የሚከሰትና በኢትዮጵያ ውስጥ እንሰትን ከሚያጠቁ በሽታዎች ውስጥ ቀዳሚውን ስፍራ የያዘ አደገኛ በሽታ ነው። የዚህ ጥናት አላማ በደቡብ ምዕራብ እንሰት አብቃይ አካባቢዎች ላይ የዚህን በሽታ ስርጭትንና በሽታው በአካባቢው በሚለዋወጡ ከሥነ-ሕይዎታዊና ሥነ-አካላዊ ነገሮች ጋር ያለውን ዝምድና ለማጥናት ነው። ለዚህም በ10 ወረዳዎች ውስጥ በሚገኙ የ120 የእንሰት ማሳዎች ውስጥ የዳሰሳ ጥናት ተካሂዷል። በበሽታው የተጠቁ የእንሰት መጠን (Incidence) በአማካይ በወረዳዎች መካከል 23.67- 31.92 በመቶ ነው። እንደዚሁም የበሽታው የጥቃት መጠኑን በተመለከተ (Severity) ከፍተኛ የሆነ ጥቃት (62.50 በመቶ) በሰሜን ቤንች ወረዳ የተመዘገበ ሲሆን በአንድራቻ ወረዳ ዝቅተኛ የጥቃት መጠን (49.58 በመቶ) ተመዝግቧል። ሎጅስቲክ ሪግሬሽን ሞዴል እንደሚያስረዳው የእንሰት አጠውልግ በሽታ ስርጭቱ ከ25 በመቶ በላይ ሆኖ የመገኘት አጋጣሚው የእንሰት ማሳ የአፈሩ pH ከ5.5 - 7 መሆን፣ የእንሰት ሰብል ብቻ በአንድ ማሳ ውስጥ መትከል፣ በሽታ ተቋቋሚ ያልሆነ የእንሰት ዝርያ፣ ዝርያውን ከሌላ አርሶ አደር ማሳ መጠቀም እና ምንም ዓይነት አረም ማረምና የበሽታ መከላከያ ያልተካሄደበት ማሳ ናቸው። በሌላ በኩል ደግሞ የበሽታ ጥቃት መጠን ከ55በመቶ በላይ የመሆኑ አጋጣሚ የአየር ፀባይ የሰሜን-ቤንችንና የየኪ ወረዳዎችን የሚመስሉ አካባቢዎች፣ አረም በሚታረምበት ወቅት እንሰትን በገጀራ መቁረጥ፣ ተቋቋሚ ዝርያ ያልሆነ የእንሰት ተክል፣ የእንሰቱ ዕድሜ ከመካከለኛው እስከ ምርት ለመስጠት ያለው ጊዜ፣ የአርሶ አደሩ ለበሽታው ያለው ዝቅተኛ ግንዛቤ ናቸው። የዚህ ዳሰሳ ጥናት ግኝቶች እንደሚያመለክቱት የእንሰት አጠውልግ በሽታ በከፍተኛ ሁኔታ በደቡብ ምዕራብ ኢትዮጵያ አካባቢዎች ላይ የተሰራጨ ሲሆን የስርጭቱን መጠን ለመቀነስ እንሰትን እንደ አንድ አማራጭ የማሳው አፈሩ pH ከ5.5 እስከ 7 መካከል ውጪ መትከል፣ ከሌላ ሰብል ጋር አቀላቅሎ መትከል፣ በእንሰት ማሳ ውስጥ የምንጠቀምባቸውን የመገልገያ መሳሪያዎችን ማፅዳትና መጠቀም፣ በበሽታ የተጠቃውን ከማሳው ነቅሎ ማውጣትና ማቃጠል፣ የባለሙያ ምክር መጠቀም መቻል እና በአርሶ አደሮች መካከል የእንሰት ዝርያ መለዋወጥ እንዳይኖር ማድረግ ናቸው።

Abstract

Enset (**Ensete ventricosum**) *bacterial wilt (EBW), caused by* **Xanthomonas campestris** ***pv.*** **musacearum**, *is one of the highly destructive diseases of enset in Ethiopia. Field survey was conducted to determine the distribution of EBW and its association with biophysical variables in Southwestern Ethiopia. In the survey, 120 enset fields in 10 major enset growing districts were assessed. The mean disease* *incidence across districts ranged from 23.67 to 31.92%, and significantly different levels of disease severity were recorded among districts. Thus, among districts, the highest mean disease severity of 62.50% was recorded from Semen-bench, whereas Andiracha district showed the lowest (49.58%) mean severity. Logistic regression analysis indicated that EBW incidence of >25% had high probability of association with enset grown on soils with pH of 5.5-7, sole cropped, susceptible clones, using planting materials obtained from other farmers and enset fields with no weeding and EBW management practices. EBW severity of >55% had high probability of association with growing enset in Semen-bench and Yeki districts, weed management through machete slashing, growing local susceptible enset clones, vegetative to maturity growth stages, and low to medium levels of farmer's awareness about EBW. Findings of this survey indicate that EBW is widely distributed and could be minimized through growing enset preferably on soils out of pH 5.5-7 ranges, intercropping system, proper weeding, access to disease-free planting material, disinfecting farm tools before using, rouging out and burning of infected plants, accessing of advisory services, and limiting free exchange of planting material among enset growers.*

Keywords: Biophysical factors, EBW, *enset*, Incidence, Logistic regression analysis, Severity

Introduction

Enset (*Ensete ventricosum* (Welw.) Cheesman) is a monocarpic herbaceous plant belonging to the genus *Ensete* and Musaceae family. It is domesticated in Ethiopia and cultivated for food, feed and fiber (Yemane and Fassil, 2006; Ajebu *et al.*, 2008). The Ethiopian highlands had long been considered to be the primary centers of origin for *enset* culture. Currently *enset* distribution is restricted to the south, southwest and the central parts of Ethiopia and it is not known as food crop in the northern part of Ethiopia (Bezuneh *et al.*, 1967). *Enset* is well known for its tolerance to drought and for its high productivity that makes it as one of the priority crops for food security in Ethiopia. The *enset* plantations restrict soil erosion and preserve soil, thereby adding more nourishment to the soil (Woldetensaye, 2001). It also attracts farmers because of its ability to produce more food on a small piece of land with minimum inputs than other cultivated staple crops such as wheat, barley and maize (Melesse *et al.*, 2014).

Enset serves as a staple or co-staple food crop for more than 20 million populations in Ethiopia, which accounts for 20% of the total population in the country (Zerihun *et al.*, 2013). The edible parts of the plant are the underground stem (corm) and pseudo stem, which are pulverized and fermented into a starch-rich product traditionally known as *kocho* that would be stored for long being intact. The corm can be harvested at almost any stage of the crop growth, and is cooked and consumed in the same way with other root and tuber crops, relieving hunger during periods of critical food shortages (Brandt *et al.*, 1997). Apart from its medicinal values (Melesse *et al.,* 2014), a chemical substance phenylphenalenone, from *enset* clones, could serve as antitumor, antibacterial, nematicidic and antifungal substance to both human and animal diseases (Holscher and Schneider, 1998).

Diseases, insect pests and wild animals are among the most important production and productivity constraints of *enset*. Various diseases including leaf damaging fungal diseases (*Phyllosticta* spp., *Pyricularia* spp. and *Cladosporium* spp), corm rot (*Sclerotium rolifsii* and *Fusarium oxysporum)*, bacterial sheath and dead heart-leaf rot, nematodes such as root-knot *(Meloidogyne* spp.), root lesion (*Pratylenchus* spp.) and black leaf streak (*Aphelenchoides* spp.) , and mosaic and chlorotic leaf streak viral diseases had been reported (Quimio and Mesfin, 1996). However, based on the occurrence and the magnitude of damage incurred on *enset* production, *enset* bacterial wilt (EBW), caused by *Xanthomonas campestris* PV *musacearum* (Yirgou and Bradbury) Dye, is known to be a major constraint to *enset* production in Ethiopia. The disease is widely distributed and affects the crop at all growth stages (Fikre and Alemar, 2016; Mekuria *et al*., 2016).

Xanthomonas campestris pv. *musacearum* (Xcm) was first reported on *enset* in Ethiopia in 1968 (Yirgou and Bradbury, 1968). It was later described on banana in the country (Yirgou and Bradbury, 1974), Uganda in 2001(Tushemereirwe *et al*., 2001) and eastern part of Democratic Repubilic of Congo in 2003 (Ndungo *et al.*, 2005). It has also been reported on banana in Tanzania (Mgenzi *et al.*, 2006), Rwanda (Biruma *et al.*, 2007) and Kenya (Aritua *et al.*, 2008). The typical symptoms of EBW are recognized by wilting of the heart-leaf, followed by wilting of the neighboring overlapping leaves. When petioles and leaf sheaths are dissected, pockets of yellow or cream colored bacterial masses (oozes) are clearly observed in the air pockets, and bacterial slime oozes out from cut vascular tissues (Gizachew *et al.,* 2008). Eventually, infected plants wither and the plant rots. Symptom development is rapid during wet season and typically evident within three to four weeks under field conditions (Tripathi *et al.*, 2009).

A serious outbreak of EBW was reported by Dereje (1985) with losses of up to 70%. The results obtained from recent EBW assessment made in some *enset* fields of southern Ethiopia showed losses of up to 100% (Tariku *et al.*, 2015). Many researchers (Desalegn and Addis, 2015; Mekuria *et al*., 2016) have reported that both the area and productivity of *enset* is declining continuously due to EBW. The disease has also forced farmers to abandon *enset* production, resulting in critical food shortage in the densely populated areas of southern Ethiopia. Moreover, EBW could cause social impacts on the farmers. For example farmer whose *enset* fields infected by the disease are not able to participate in social works during *enset* management due to tool contamination with the pathogen (Mekuria *et al*., 2016).

Efforts have been made to reduce the damage caused by EBW through evaluation and identification of tolerant *enset* clones (Gizachew *et al.,* 2008; Mengistu *et al*., 2014; Fikre and Alemar, 2016), identification of promising cultural management practices such as field sanitation (Dereje, 1985); surveys of infected areas (Desalegn and Addis, 2015) and determination of alternate hosts (Alemayehu *et al*., 2016). However, EBW remained a constraint due to lack of clones having stable tolerance across locations and over years. Although enormous advisory services on field sanitation (disinfection of farming and processing tools, rouging of infected *enset* plants) have been under taken to curb the diseases problem, the management strategy was reported difficult to implement by small holder farmers.

Therefore, it is important to have the detailed information of EBW with regard to factors that influence the disease occurrence, distribution and the importance of EBW in the production systems of southwestern Ethiopia. Disease occurrence, development and damage to crops is influenced by cropping systems and production practices, crop genotypes, altitudinal ranges, cropping areas and field management practices under a given environment (Zhu *et al*., 2000). Assessing different factors associated with disease development is important to obtain relevant data for gaining understandings into the occurrence, distribution and relative importance of different crop diseases (Rusuka *et al*., 1997). Moreover, disease management requires a thorough understanding of all interacting factors which contribute to disease epidemics (Kijana *et al.,* 2017). However, detail information on the distribution, relative importance and how the different cropping practices and environmental factors affect EBW epidemics is lacking in the southwestern Ethiopia. Lack of such data constrains the development of robust, efficient and sustainable management interventions to EBW in these areas, where *enset* is widely grown. Conducting EBW survey and understanding the factors that influence its occurrence and spread will accelerate development of effective EBW management options. Thus, the objectives of this study were to determine (1) the distribution of EBW, and (2) its association with agro-ecological variables, cropping systems and farmers cultural practices in southwestern Ethiopia.

Materials and Methods

Descriptions of the survey areas

Disease survey was conducted in ten major *enset* growing districts in three administrative zones namely Bench-maji, Keffa and Sheka of southwestern Ethiopia during June to August 2017 (Figure 1, Table 1). Southwestern Ethiopia is characterized by relatively high total annual rainfall (9578 mm); humid and tropical rainforest climate type and forest coffee systems. Districts were selected on the basis of accessibility and potentials of enset production in the region. The altitude in the surveyed areas ranged from 1470 to 2393 meters above sea level (m.a.s.l.) and the survey areas are located within 4.43°-8.58°N latitude and 34.88°-39.14°E longitude. Monthly average temperatures and total rainfall of the districts were obtained from the Ethiopian National Meteorological Agency. During the survey period, months from April to September were considered as the most rainy months with 193 to 220 mm of average rainfall, and mean annual temperature ranging from 11.1 to 29.8°C was recorded in the surveyed areas (Appendix Figure 1).

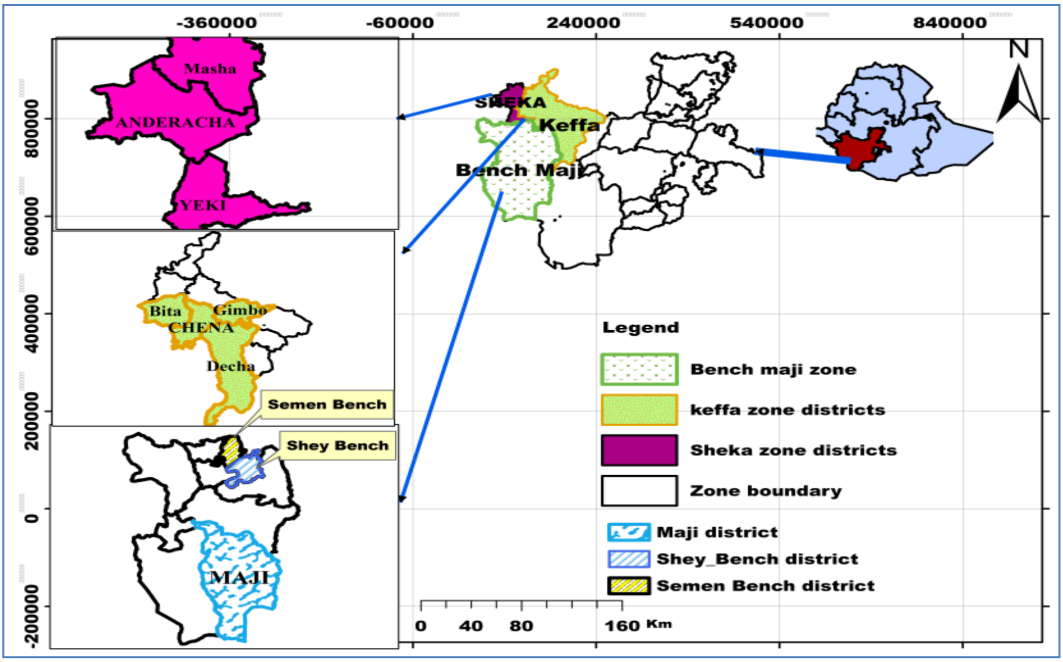

Figure 1. Map showing surveyed districts for *enset* bacterial wilt (*Xanthomonas campestris* pv. *musacearum*) in southwestern Ethiopia, during the 2017 cropping season

Table 1. Description of enset bacterial wilt survey districts in Southwestern Ethiopia, 2017

Zone	District	Altitude range (m.a.s.l.)	Dominant crops in the cropping system
Bench-Maji	Maji	2344-2393	*Enset* and maize
	Semen-bench	2118-2239	*Enset*, maize, coffee and taro
	She-bench	2023-2097	*Enset*, maize and taro
Keffa	Bita	1868-1922	*Enset*, maize and coffee
	Chena	1969-2207	*Enset*, maize and coffee
	Decha	1935-2023	*Enset*, maize and cardamom
	Gimbo	1637-2077	*Enset*, maize and coffee
Sheka	Andiracha	1932-2423	*Enset*, maize, coffee and potato
	Masha	2121-2309	*Enset*, maize, faba bean and potato
	Yeki	1470-1886	*Enset*, coffee, avocado, mango and taro

Sampling procedures and sample unit

Survey of EBW was conducted at two consecutive stages. The first stage was the administration of designed questionnaires through oral interview and/or focus group discussions with *enset* growing farmers to clearly understand *enset* growing culture and areas. In the second stage, on spot visiting of sample *enset* fields were made to assess EBW intensity across districts. Accordingly, a total of 120 *enset* fields (12 fields per district) were assessed, based on the importance of the crop and EBW, three representative Farmer Associations (FAs) were purposively selected from each district. Four *enset* fields were inspected in each FA. The selection of districts was done in consultation with the extension advisory service staff of the respective districts' Agriculture and Natural Resource Bureau.

Disease assessment

Within each selected field, by moving diagonally in an 'X' pattern, 30 *enset* plants were randomly tagged and assessed for disease incidence. Disease incidence was rated as mean percentage of *enset* plants showing typical wilt disease symptoms (yellowing of leaves and oozing out of bacteria during opening of the suspected leaf petioles) of each tagged *enset*. Therefore, disease incidence (DI) was calculated using the formula:

$$\mathrm{DI} = \frac{\text{Number of } enset \text{ plants with visible wilt symptom}}{\text{Total number of } enset \text{ plants assessed}} \times 100$$

As some *enset* clones in the surveyed areas were recovering after bacterial wilt infection, disease severity was also assessed from six randomly taken *enset* plants per field using a 0-5 disease scoring scale (Winstead and Kelman, 1952); where 0 = no visible disease symptom, 1 = 1 leaf wilted, 2 = 2 - 3 leaves wilted, 3 = 4 leaves wilted, 4 = all leaves wilted, and 5 = plant dead. Disease severity values were converted into percent severity index (PSI) for analysis for each field as:

$$\mathrm{PSI} = \frac{\text{Sum of numerical ratings}}{\text{Total number of } enset \text{ plants assessed} \times \text{Maximum score on the scale}} \times 100$$

The disease prevalence (DP) was determined as the presence and absence of EBW in *enset* fields assessed, and calculated using the formula:

$$\mathrm{DP} = \frac{\text{Number of fields with } enset \text{ bacterial wilt}}{\text{Total number of fields assessed}} \times 100$$

In addition, biophysical factors like, cropping system, *enset* clones grown, source of planting material, growth stage of *enset*, weeding practices, disease management practices, presence/absence of EBW in the neighboring fields and farmers' awareness about EBW were collected using formal and informal discussions with growers and extension staff. The altitude ranges and geographic positions of fields were recorded by using GPS device. Common weed species in infested *enset* fields were recorded and identified based on weed identification guides (Stroud and Parker, 1989). Disease specimens were also collected to identify and confirm the identity of Xcm. As *enset* is not common at lowlands, *enset* fields were grouped into 1470-2000 m.a.s.l (midland) and >2000 m.a.s.l (highland). The soil pH of surveyed areas was determined and grouped into 5.5-7.0 (slightly acidic) and ≤ 5.5 (acidic). Farmers' awareness levels about EBW were recorded and grouped as low (when farmers had no knowledge about the disease), medium (farmers received advisory services on how to manage the disease, but not managed the disease appropriately) and high (farmers received advisory services on how to manage the disease and he/she were trying to manage the disease).

Data Analyses

Descriptive statistics was used to summarize disease incidence and severity data obtained from field assessment using SPSS version 20.0 for windows to explain the distribution and relative importance of EBW. Where significant difference for DI and PSI existed, means were separated using the T- test at $P < 0.05$.

DI and PSI were classified into distinct groups of binomial qualitative data as described by Fininsa and Yuen (2001) and Yuen (2006). Class boundaries were selected so that binary variable classes were set for DI and PSI. Thus, ≤ 25 and >25% were chosen for incidence, and ≤ 55 and >55% for PSI yielding a binary variable for EBW in the survey. Contingency tables of DI, PSI and independent variables were built to represent the bivariate distribution of the fields according to data classifications (Table 2). The association of EBW incidence and severity with independent variables was analyzed using logistic regression model (Yuen, 2006) with the SAS Procedure of GENMOD (SAS, 2008). The importance of the independent variables was evaluated twice in terms of their effect on DI and PSI. First, the association of all the independent variables with the DI and PSI was tested in a single-variable model. Second, the association of an independent variable with the DI and PSI was tested when entered first and last with all the other variables in the model. Lastly, those independent variables with significant association with the DI or PSI were added to a reduced multiple-variable model. The parameter estimates and their standard errors were analyzed using the GENMOD procedure both for the single and multiple models. The odds ratio was obtained by exponentiation of the parameter estimates for comparing the effect based on a reference point, which is interpreted here as the relative risks (Yuen, 2006). The deviance, the logarithm of the ratio of two likelihoods, was used to compare the single-and multiple-variable models. The difference between the likelihood ratio tests (LRTs) was used to examine the importance of the variable and was tested against the χ^2 value (McCullagha and Nelder, 1989).

Table 2. Independent variable by disease contingency table for logistic regression analysis of the distribution and relative importance of *enset* bacterial wilt epidemics in ten enset growing districts (n = 120) of southwestern Ethiopia, during 2017

Variable	Variable class	Number of fields	Diseases incidence (%)		Percent severity index (%)		Variable class	Variable class	Number of fields	Diseases incidence (%)		Percent severity index (%)	
			≤ 25	>25	≤55	>55				≤ 25	>25	≤55	>55
District	Decha	12	6	6	6	6	Growth stage [c]	Sucker	8	5	3	4	4
	Gimbo	12	5	7	6	6		Seedling	11	3	8	5	6
	Chena	12	5	7	6	6		Vegetation	13	5	8	5	8
	Bita	12	7	5	6	6		Maturity	18	7	11	5	13
	Maji	12	5	7	3	9		All growth stages	70	37	33	29	41
	She-Bench	12	5	7	3	9	Disease management practices [g]	Rouging out and throwing	47	31	16	29	18
	Semen-bench	12	4	8	4	8		Cut into pieces and left in the field	6	2	4	0	6
	Masha	12	7	5	5	7		Use of clean farm tool	17	17	0	13	4
	Andiracha	12	7	5	8	4		Cattle grazing of infected fields	9	3	6	2	7
	Yeki	12	7	5	5	7							
Altitude [a] (m.a.s.l)	1470 - 2000	44	25	19	24	20		Planting *Yeero* around infected *enset*	7	2	5	3	4
	> 2000	76	33	43	32	44							
Soil pH	5.5 – 7.0	56	12	44	15	40		None	34	3	31	7	27
	≤ 5.5	64	46	18	39	25	Presence/absence of EBW in the neighboring field	Present	57	9	48	16	41
Source of planting material	Owen field	73	48	25	43	30		Absent	63	49	14	30	23
	Other farmer	47	9	38	11	36	Farmer's awareness to EBW [d]	Low level	69	19	50	21	48
Enset clones	Local susceptible clone	55	5	50	6	48		Medium level	23	13	9	11	12
								High level	28	25	3	23	5
	Mixed	65	53	12	48	17	Weed management practice	Hand weeding	48	35	13	30	18
Cropping system [b]	Sole	55	8	47	14	41		Machete slashing	35	12	23	12	23
	Inter- cropping	65	50	15	41	24		None	37	11	26	13	24

[a] Altitude ranges > 2000 m.a.s.l = highland and 1470 ≤ 2000 m.a.s.l. = midland. [b] Cropping system = sole (fields covered only with *enset*) and intercropping (fields with *enset* and maize and/or faba bean and/or mango and/or avocado).[c] Growth stage = sucker (≤1 year old), seedling (1-2 years old), vegetative/young (2-4 years old), maturity stage (≥ 4 years old) and any growth stage. [d] Farmer's awareness level = Low (no knowledge about the disease), medium (farmers received advisory services on how to manage the disease but not managed the disease appropriately) and high (farmers received advisory services on how to manage the disease and he/she was managing the disease appropriately).

Result and Discussion

Distribution, incidence and severity of EBW

The enset fields

In southwestern Ethiopia, *enset* plant is diverse consisting of various clones. More than 25 *enset* clones, known by their vernacular names, were commonly grown in the region. Of these, only two *enset* clones, namely *Nobo* and *Gudiro* were found to be tolerant to EBW during the survey and from farmers' response feedback. All the surveyed *enset* fields were infected with EBW and consequently the prevalence was 100%. The EBW causing bacteria, *Xcm*, was frequently isolated from infected *enset* plants. The mean DI across districts ranged from 23.7 to 32%. Different levels of PSI were recorded among districts. Significantly (P<0.05) the highest (62.5%) mean PSI was recorded from Semen-bench district, whereas Andiracha district showed the lowest (49.6%) mean PSI (Table 3). The highest EBW epidemics recorded in Semen-bench district could have been resulted from high annual rainfall and extended rainy days for about seven months (from April to October) and moderate temperature (Appendix Figure 2), and growing of susceptible *enset* clones in such district. However, Andiracha district records the lowest disease severity (as the farmers commonly practice growing of locally known tolerant clones and susceptible ones) though the weather conditions were conducive for the disease.

Enset fields at an altitude of > 2000 m.a.s.l. had higher (28.6%) mean DI than fields at 1470-2000 m.a.s.l., which had mean DI of 23.1%. The *t*-test also showed significant (P<0.05) difference between altitudinal ranges for incidence. These results agree with Brandt *et al*. (1997); Maina *et al*. (2006) and Mekuria *et al.* (2016), who noted higher EBW levels in the highlands than in lowland areas. This might be due to agro-ecological requirements of the pathogen for higher moisture and lower temperature levels. In agreement with this finding, Dereje (1985) reported that the *Xcm* requires humid condition for survival. As a general principle, weather conditions along with other factors such as susceptibility of plants and field cultural practices affect the spread of the inoculum in an area (Spring *et al.*, 1996; Maina *et al*., 2006). Obviously, higher rainfall and prolonged leaf wetness were suspected to contribute to increased disease caused by Xcm (Maina *et al*., 2006); and water availability on the leaf surface is an important factor for the pathogen to gain entry into plants and for its establishment (Agrios, 2005). Extended precipitation and cloudy weather in high altitudes increase the relative humidity and reduces evaporative demand on the plant and keeps the leaves wet for longer periods (Maina *et al*., 2006).

The highest EBW mean incidence (33.29%) and PSI (59.59%) were recorded in fields within soil pH range of 5.5-7.0 compared with fields with soil pH ≤ 5.5, which had mean DI of 23.36% and PSI of 52.31%. In this regard the *t*-test results also showed significant (P<0.0001) variation between pH ranges for both DI and PSI (Table 4, Figure 3). Present findings demonstrated that slightly acidic soils are more conducive to EBW development than acidic soils. Similarly, Fikre (2014) indicated that the *Xcm* pathogen was frequently isolated from processed *kocho* obtained from bacterial wilt infected *enset* plants and found a positive correlation between *Xcm* colonies and lower *kocho* pH levels.

Enset cropping systems, management practices and farmers' awareness of EBW

Farmers grow *enset* in sole and intercropping systems. Crops commonly associated with *enset* in intercropping systems were maize, faba bean, common bean, barley, taro, cardamom, avocado and mango. Significantly (P<0.0001) higher EBW mean incidence (34.15%) and PSI (60.60%) were recorded in sole cropped *enset* fields than in intercropped fields that had mean DI of 22.78% and PSI of 50.57% (Table 4, Figure 3). In the intercropping system component crops might have increased spatial distance between *enset* plants and act as a physical barrier between infected and healthy *enset*. Intercropping might also reduce *enset* population and modify microclimate that might disfavour the wilt causing pathogen. In this regard, component crops reduced host population, increased spatial distance between hosts to impede the transfer of diseases carrying propagules from infected plants to healthy plant (Mekuria *et al.*, 2016; Getachew *et al*., 2018).

Enset fields covered with planting materials obtained from other farmers showed a higher DI (32.9%) than from own source, with mean DI of 24.59% (T = -0.912, P = 0.000). Similarly, higher mean PSI (61.2%) was recorded from fields grown with planting materials collected from other farmers than fields planted with own source with mean PSI of 52% (T = -4.555, P = 0.000) (Table 4, Figure 3). This might be due to the latent nature of *Xcm* especially in the early stages. With this regard, previously Getachew *et al*. (2018) reported that the presence of the strong association of source of planting material and the wilt epidemics. However, suckers from own field and from other farmers were the only means of obtaining the planting materials in the inspected fields. This may mislead farmers to plant the already infected suckers that could serve to spread diseases across fields. Suckers are an important means of spread for systemic bacterial diseases (Getachew *et al*., 2018).

Mean EBW incidence of 34.4% and PSI of 64.3% were recorded for *enset* fields planted with only locally known susceptible *enset* clones. Conversely, mean DI of 22.7% and PSI of 48.7% were recorded in fields planted with a mixture of tolerant and susceptible clones (T = 9.477, P = 0.000 and T = 9.942, P = 0.000), respectively. This could have resulted from the differential response of local *enset* clone to EBW as reported by Mengistu *et al.* (2014) and Tushemereirwe *et al.*

(2003), as heterogeneity of plantations could possess different levels of resistance to a disease. For instance, Girma (2004) reported that heterogeneous coffee plantations reduced wilt disease due to varying levels of resistance conferred by different coffee populations compared with homogeneous coffee cultures. *Enset* fields closer to EBW infected neighboring enset fields showed higher mean DI of 33.7% (T = 9.027, P = 0.000) and PSI of 61% (T = 1.263, P = 0.000) than their counter parts (Table 4, Figure 3).

Based on field observations and farmers experiences, EBW found to infect *enset* plants at all growth stages. However, *enset* plants in vegetative growth stage had higher mean DI of 32.2% than the sucker, seedling, all growth stage in combination and maturity growth stage with mean DI of 25, 27.6, 26.5% and 27.8%, respectively. The highest (63.39%) disease severity was recorded at maturity growth stage of the crop (Table 3). This might be due to the less vigorous nature of *enset* plants during the early growth stages. On the other hand, matured *enset* plants have a long period of exposure to the disease that would result in the accumulation of pathogen propagules through time. And, as a result of new infections that might have resulted from frequent inoculations, when farmers use contaminated farm tools for different purposes. In agreement with this finding, Mekuria *et al.* (2016) concluded that suckers had no or little significant role in the transmission of the disease but they might cause latent infection. But, high wilt incidence at middle age due to long exposure time of the host to the pathogen and crop management practices. It is also reported that EBW is mostly observed on old plantations of more than four years (Desalegn and Addis, 2015).

Weed species commonly found in *enset* fields included goat weed (*Ageratum conyzoides*), mech (*Guizotia scabra*), bermuda grass (*Cynodon dactylon*), black lack (*Bidens pilosa*), black nightshade (*Solanum nigrum*), water maker (*Commelina latifolia*), aluma (*Amaranthus hybridus*), thorn apple (*Digitaria scularum*) and mexican marigold (*Tagetes minuta*). Hand weeding had significantly ($P<0.001$) lower mean disease incidence (22.15%) and PSI (51.75%) than fields weeded using machete for slashing (DI of 31% and PSI of 59.63%) (Figure 2C) and unweeded (DI of 34% and PSI of 57%) (Figure 2A) *enset* fields (Table 3). Similar scenarios have been reported for different host-pathogen systems as high weed infestation could reduce crop vigor and promote disease development through competition for available resources that render crops susceptible to diseases (Eshetu *et al*., 2013; Getachew *et al*., 2018). Effective weed management and removal of asymptomatic alternate hosts, along with others, from infected fields can prevent and check the spread of the pathogen (Hennessy *et al*., 2005; Getachew *et al*., 2018).

On the contrary, slashing by machete for weed control might result in cross inoculation thereby increasing the incidence and severity of EBW in *enset* fields (Figure 2C). Similar phenomena were reported by Girma (2004) that most of the coffee trees are found with wounds, where slashing is employed to control coffee weeds that predisposes coffee plants to wilt disease. Mekuria *et al.* (2016) also reported that transmission from *enset* to *enset* within a field is mechanically accomplished by cutting *enset* with infected farm tools. Moreover, Dereje (1985) reported that bacterial inocula were found on surfaces of contaminated tools and survived for up to four days under humid conditions and up to three days under dry conditions; thus, contaminated tools are potential means of pathogen spread.

In the surveyed districts, *enset* growers were found to use different kinds of EBW management practices. Significantly (P<0.001) the lowest disease incidence was recorded from fields managed by cleaned use of farm tools (19.7%) and rouging out and throwing of infected *enset* plants from the field (23.8%) as compared to other management practices employed by the growers, which recorded 31.43-32.11% of DI. None managed *enset* fields registered a DI of 35.35% (Table 3). Most farmers in the surveyed area did not apply EBW management practices. In these fields, the infected *enset* plants were found standing in the field even when they had long died, which could serve as sources of inocula. On the other hand, some *enset* growers were found to carry out different kinds of EBW management practices that may or may not result in disease spread. Among EBW management practices, a small number of farmers were managing their fields by a careful utilization of the farm tools and rouging and throwing out infected plants from the *enset* field that resulted in low mean disease incidence. In other studies also wilt management through cutting of wilted plants and on spot burning or burying, careful utilization of farm tools and restricted movement of infected plant parts lowered wilt epidemics close to 100%. Such practices are supposed to reduce inoculums build up, disease development and farm tool transmission of plant disease (Mengistu *et al.*, 2014; Getachew *et al.*, 2018).

Conversely, even though there were no significant disease reduction was observed, farmers in the survey area believe and practice that, planting *Yeero (Pychnostachis abyssinica*) around the infected *enset*, prevents transmission of the EBW from infected to healthy *enset* plants (Figure 2D). This might be due to the bio-fumigant effects of the plant at the time when volatile chemicals released from the plant that adversely affects the pathogen, thereby reducing disease. Previously, Kidist (2003) reported that, the crude extracts from bract and leafs of *Pychnostachis abyssinica* were evaluated against EBW pathogen and showed a promising growth inhibition of the pathogen.

Because of the giant nature of the *enset*, some farmers in the surveyed region cut infected *enset* in to pieces and leave them in the field (Figure 2E&F). However, by so doing they increase the EBW incidence by 25% and PSI by 17% when the practice was compared with lower disease intensity recorded from fields managed by rouging out the infected *enset* (Table 3). This practice might disseminate the causal pathogen within and across the field while wind and rain splash moves through it. Yemataw *et al*. (2017) reported that the overflow of water from infected to uninfected field spreads the EBW thereby increasing DI. Other studies also indicated that pathogen spread is thought to happen through soil, planting materials, wind, rain, surface run-off and contaminated irrigation water (Ghag *et al*., 2015).

Low and medium levels of farmer's awareness to EBW contributed significantly ($P<0.001$) to the highest mean DI of 31.9% and 29.9%, respectively as compared to high level of farmer's awareness to the disease. A similar trend was obtained for severity (Table 3). This might imply that many farmers in the surveyed areas have either little knowledge or no knowledge at all regarding the management of EBW. Supporting this finding, Muchuruza and Melchior (2013) reported that low level of farmers' awareness to banana xanthomonas wilt resulted in the development of banana wilt to epidemic level in a region. Moreover, Getachew *et al*., 2018 also stated that moderate to high level of farmers' awareness towards wilt diseases could help growers to access planting materials from disease free fields; otherwise, lack of awareness could restrict an attempt to effectively manage the disease.

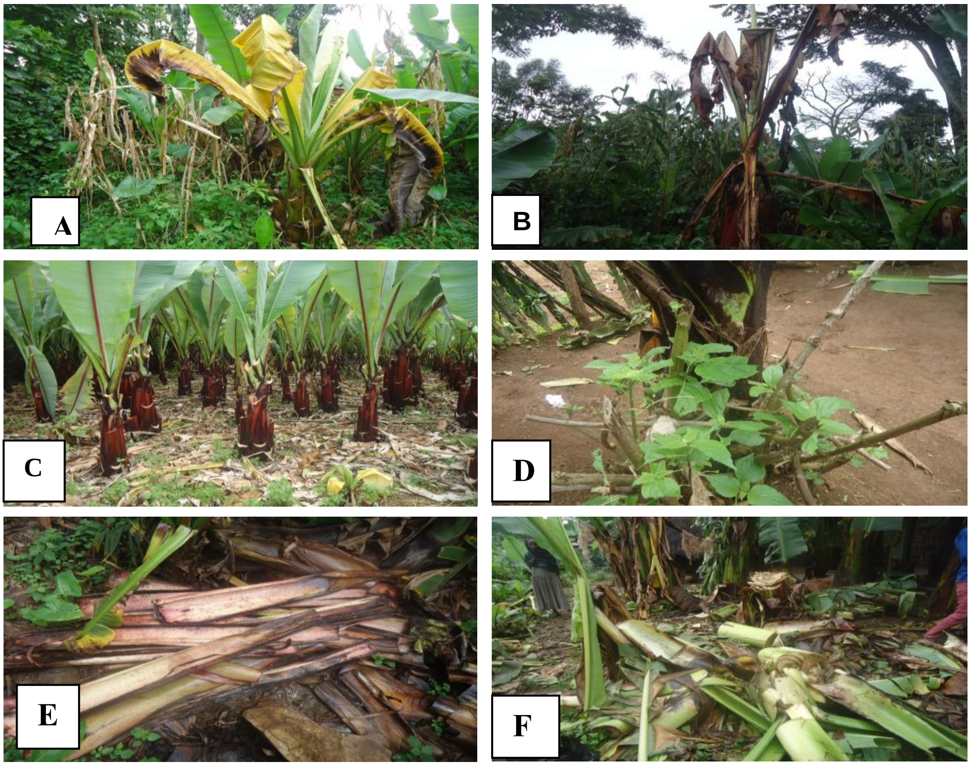

Figure 2. Unweeded enset fields with typical bacterial wilt symptoms (A), completely wilted enset plant (B), infected enset field weeded by slashing with machete (C), EBW management practice using growing yeero (Pychnostachis abyssinica) around diseased enset (D), and EBW management practice through cutting diseased enset into pieces and left in the field that result into disease spread (E and F)

Table 3. Disease incidence and percent severity index (mean ± SE) of *enset* bacterial wilt for different independent variables in Southwestern Ethiopia, during the 2017 cropping season

Variable	Variable class	Disease incidence (%)	*P-value*	Percent severity index (%)	*P-value*
District	Decha	25.83±1.66	0.911	54.58±4.32	0.050
	Gimbo	28.92±2.32		53.56±3.09	
	Chena	27.75±2.61		55.58±3.64	
	Bita	26.58±2.37		54.08±3.53	
	Maji	29.42±2.75		56.58±3.37	
	She-Bench	30.67±3.66		57.75±2.74	
	Semen Bench	31.92±3.04		62.50±2.81	
	Masha	26.33±1.97		55.33±3.67	
	Andiracha	23.67±2.31		49.58±3.15	
	Yeki	26.83±2.74		56.50±3.37	
Growth stage [a]	Sucker	25.00±1.96	0.010	51.88±4.39	0.032
	Seedling	27.64±2.89		53.27±3.46	
	Vegetation	32.15±2.83		56.00±3.12	
	Maturity	27.83±2.06		63.39±3.03	
	All growth stage	26.51±1.02		54.90±1.32	
Weed management practice	Hand weeding	22.15±0.57	0.000	51.75±1.56	0.004
	Machete slashing	30.71±1.18		59.63±2.02	
	None	33.95±1.72		57.14±1.76	
Disease management practices	Rouging out and throwing	23.86±0.59	0.000	51.53±1.83	0.000
	Cut into pieces and left in the field	31.67±4.24		62.00±2.66	
	Cleaned use of the farm tools	19.71±0.72		48.53±1.96	
	Grazing cattle in the infected fields	32.11±2.98		59.44±2.47	
	Planting *Yeero* around infected *enset*	31.43±2.67		60.29±2.98	
	None	35.35±1.61		62.03±1.62	
Farmers' awareness [b]	Low level	31.91±1.06	0.000	60.37±1.26	0.003
	Medium level	29.87±1.34		54.36±2.17	
	High level	20.07±0.62		46.68±1.60	

[a] Growth stage = sucker (≤ 1year old), seedling (1-2 year old), vegetation/young (2-4 years old), matured (≥ 4 year old) and all growth stage. [b] Farmers' awareness level = low (farmer had no knowledge about the disease), medium (farmers received advisory services on how to manage and control the disease, but not managed/controlled the disease appropriately) and high (farmers received advisory services on how to manage and control the disease and he/she was trying to manage the disease).

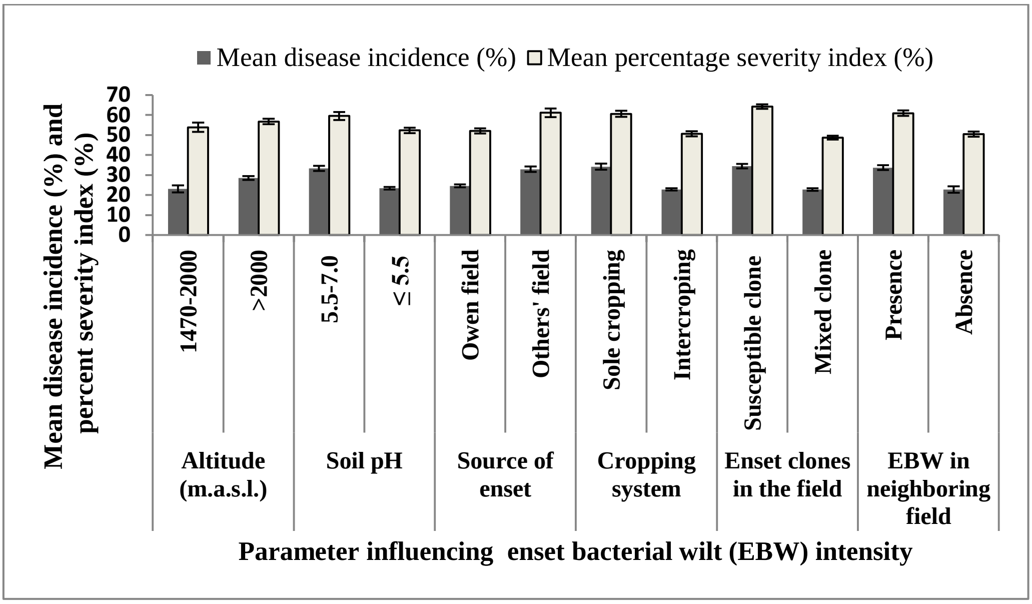

Figure 3. Mean disease incidence and percent severity index of enset bacterial wilt for different independent variables in Southwestern Ethiopia, during the 2017 cropping season

Table 4. Contingency t-test analysis of mean disease incidence and severity difference of enset bacterial wilt as influenced by independent variables

Variable	Variable class	Disease incidence (%)			Percent severity index (%)		
		SE a	t-value	P-value	SE a	t-value	P-value
Altitude (m.a.s.l)	1470-2000	1.342	-0.886*	0.037	1.734	-1.338ns	0.183
	> 2000	1.020			1.317		
Soil pH 2	5.5-7.0	1.258	7.331***	0.000	1.496	3.621***	0.000
	≤ 5.5	0.627			1.349		
Cropping system 3	Sole cropping	1.208	9.034***	0.000	1.483	4.628***	0.000
	Intercropping	0.543			1.284		
Source of planting material	Own field	0.734	-0.912***	0.000	1.264	-4.555***	0.000
	From other	1.321			1.531		
Enset Clones	Local susceptible	1.142	9.477***	0.000	1.146	9.942***	0.000
	Mixed	0.612			1.063		
EBW in the neighboring fields	Present	1.146	9.027***	0.000	1.393	1.263***	0.000
	Absent	0.509					

a SE = standard error of the mean; *and *** = mean differences detected at P ≤ 0.05 and P ≤ 0.0001, respectively. ns = non-significant difference at 5% probability level.

Association of enset bacterial wilt intensity with biophysical factors

Among the EBW influencing factors tested in the model, soil pH, cropping system and weed management practice showed very highly significant (P<0.0001) associations with mean DI. Also, *enset* clones and source of planting material found highly significant (P<0.001) associations with DI. While, district and disease management practice showed significant (P<0.01) associations with mean DI when entered into the logistic regression model as a single variable. Similarilly, when all variables entered last into the regression model, only pH, cropping system, weed management practice, *enset* clones, source of planting material, and disease management practices remained significant (P<0.001 and P<0.05) in their association with EBW incidence. However, district lost its' importance when entered last into the model (Table 5).

Of all the independent variables, soil pH (χ^2 = 141.60 and 4.11, 1df), cropping system (χ^2 = 102.81 and 17.27, 1df), weed management practice (χ^2 = 35.16 and 11.80, 2df), disease management practices (χ^2 = 16.76 and 14.09, 5df), *enset* clones (χ^2 = 12.61 and 5.75, 1df) and source of planting material (χ^2 = 11.69 and 4.45, 1df) were the most important variables in their association with mean DI when entered first and last into the model, respectively (Table 5). The deviation analysis of these variables in a reduced multiple variable model showed different levels of importance of their association with wilt incidence (Table 6). The probability of mean disease incidence of >25% was highly associated with sole cropping, *enset* fields without any management practice, growing only local susceptible *enset* clones, planting material sourced from other farmers, unweeded *enset* field and growing on soils with pH of 5.5-7.0. In contrast, low disease incidence (≤ 25%) had high probability of association with intercropping, rouging out and throwing of infected *enset* or cleaned use of farm tools, growing locally known susceptible and tolerant clones in mixture, using planting material from own field, hand weeding of *enset* fields and growing *enset* on soils with pH ≤ 5.5 (Table 6).

On the other hand, altitude, soil pH, cropping system, *enset* clones and weed management practice showed very highly significant (P<0.0001) associations with mean PSI. District, growth stage and farmers awareness towards the disease also found highly significant (P<0.001) relations with PSI. While, disease management practices showed significant (P<0.05) associations with mean PSI when entered into the logistic regression model as a single variable. Similarilly, when all variables entered last into the regression model, only district, *enset* clones, weed management practices, growth stage and farmers awareness level remained significant (P<0.001and P<0.05) in their association with mean PSI. However altitude, soil pH, cropping system and disease management practices were lost their association with mean PSI when entered last into the model (Table 5).

The deviation analysis of these variables in a reduced multiple variable model showed different levels of importance of their association with wilt PSI (Table 7). The probability of EBW PSI of >55% was highly associated with growing *enset* at growing conditions of Semen-bench and Yeki districts, slashing by machete for weed management practice, growing local susceptible *enset* clone alone in the field, vegetative to maturity growth stages of *enset* and low to medium level of farmer's awareness to the disease. But, growing mixed *enset* clones at Andiracha district in the presence of high level of farmer's awareness towards the disease had low probability of association with mean PSI (Table 7).

Table 5. Logistic regression model for *enset* bacterial wilt incidence and perecent severity index and likelihood ratio test on independent variables in southwestern Ethiopia, during 2017

Independent Variable	df	*Enset* bacterial wilt incidence, LRT[a]				*Enset* bacterial wilt PSI, LRT[a]			
		VEF		VEL		VEF		VEL	
		DR	Pr > χ^2	DR	Pr > χ^2	DR	Pr > χ^2	DR	Pr > χ^2
District	9	23.67	0.0049	13.79	0.1301	21.91	0.0092	25.55	0.0024
Altitude (m.a.s.l.)	1	0.69	0.4064	0.50	0.4797	16.39	<0.0001	1.30	0.2549
Soil pH	1	141.60	<0.0001	4.11	0.0428	63.59	<0.0001	1.95	0.1629
Cropping system	1	102.81	<0.0001	17.27	<0.0001	58.61	<0.0001	0.62	0.4302
Weed management practice	2	35.16	<0.0001	11.80	0.0027	17.74	0.0001	9.14	0.0103
Enset clones	1	12.61	0.0004	5.75	0.0165	161.81	<0.0001	103.56	0.0001
Source of planting material	1	11.69	0.0006	4.45	0.0350	1.59	0.2077	0.32	0.5702
Growth stage	4	6.06	0.1944	4.49	0.3440	13.49	0.0091	12.68	0.0129
Farmer’s awareness to EBW	2	0.95	0.6210	1.51	0.4702	12.47	0.0020	7.59	0.0225
Disease management practices	5	16.76	0.0050	14.09	0.0150	11.79	0.0378	9.73	0.0832
Presence/absence of EBW in the neighboring field	1	2.30	0.1290	2.30	0.1290	1.15	0.2830	1.15	0.2830

[a] LRT = likelihood ratio test; VEF = variable entered first in the model; VEL = variable entered last model; DR = deviance reduction; Pr = probability of an χ^2 value exceeding the deviance reduction; χ^2 = chi square; df = degrees of freedom; EBW = *enset* bacterial wilt

Table 6. Analysis of deviance, natural logarithms of odds ratio and standard error of enset bacterial wilt incidence (%) and likelihood ratio test on independent (added) variables in reduced model in the 2017, southwestern Ethiopia

Added variable	Residual deviance [a]	df	*Enset* bacterial wilt, LRT[b]		Variable class	Estimate Log_e (odds ratio)[c]	SE	Odds ratio
			DR	Pr > χ^2				
Intercept	446.51	0	40.08	<.0001		-1.0859	0.1715	0.338
Soil pH	280.55	1	4.12	0.0425	5.5-7.0	0.1139	0.0561	1.121
					≤ 5.5	0*		.
Cropping system	177.74	1	17.24	<.0001	Sole cropping	0.2596	0.0625	1.296
					Intercropping	0*		.
Weed management practice	142.58	2	11.62	0.0007	Hand weeding	-0.2204	0.0647	0.802
			3.96	0.0465	Slashing by machete	-0.1088	0.0547	0.896
					None	0*		.
Enset clones	129.97	1	5.75	0.0165	Local susceptible clone	0.1491	0.0622	1.161
					Mixed	0*		.
Source of planting material	118.29	1	4.45	0.0349	Owen field	-0.1141	0.0541	0.892
					Other farmer	0*		.
Disease management practices	94.51	5	11.78	0.0006	Rouging out and throwing	-0.2270	0.0661	0.797
			0.94	0.3317	Cut into pieces and left in the field	-0.1019	0.1050	0.903
			4.54	0.0332	Clean the equipment after cutting the infected *enset*	-0.2156	0.1012	0.806
			4.72	0.0299	Grazing cattle in the infected field	-0.2092	0.0963	0.811
			0.26	0.6072	Planting *Yeero* around the infected enset	-0.0523	0.1016	0.949
					None	0*		.

[a] Unexplained variations after fitting the model; [b] LRT = likelihood ratio test; DR = deviance reduction; Pr = probability of an χ^2 value exceeding the deviance reduction; [c] * Reference group; df = degrees of freedom; χ^2 = chi square; SE = standard error.

Table 7. Analysis of deviance, natural logarithms of odds ratio and standard error of *enset* bacterial wilt percent severity index and likelihood ratio test on independent (added) variables in reduced model in the 2017, southwestern Ethiopia

Added variable	Residual deviance [a]	df	*Enset* bacterial wilt, LRT[b]		Variable class	Estimate Log_e (odds ratio)[c]	SE	Odds ratio
			DR	Pr > χ^2				
Intercept	661.6124	0	0.79	0.3729		-0.1391	0.1561	0.870
District	639.7025	9	0.01	0.9419	Decha	-0.0065	0.0887	0.994
			2.84	0.0918	Gimbo	-0.1761	0.1045	0.839
			0.83	0.3630	Chena	-0.0952	0.1047	0.909
			0.01	0.9384	Bita	-0.0069	0.0896	0.993
			8.30	0.0040	Maji	-0.1959	0.1249	0.822
			0.07	0.7934	She-Bench	-0.0551	0.1241	0.946
			0.20	0.6571	Semen Bench	0.0321	0.1226	1.033
			0.20	0.6521	Masha	-0.0549	0.1219	0.947
			2.46	0.1166	Andiracha	-0.3481	0.1208	0.706
			-	-	Yeki	0*		.
Weed management practice	483.3737	2	5.63	0.0177	Hand weeding	0.1401	0.0591	1.150
			7.71	<.0001	Machete slashing	0.1439	0.0518	1.155
			-	-	None	0*		.
Enset clones	321.5663	1	102.59	0.5703	Local susceptible clone	0.6164	0.0609	1.852
			-	-	Mixed	0*		.
Growth stage	306.4856	4	0.49	0.4820	Sucker	-0.0617	0.0877	0.940
			8.27	0.0040	Seedling	-0.2177	0.0757	0.804
			0.24	0.6257	Vegetative	0.0345	0.0706	1.035
			2.13	0.1447	Maturity	0.0866	0.0594	1.090
			-	-	All growth stage	0*		.
Farmer's awareness to EBW	294.0139	2	4.55	0.0330	Low level	0.1468	0.0688	1.158
			6.89	0.0086	Medium level	0.1780	0.0678	1.195
			-	-	High level	0*		.

[a] Unexplained variations after fitting the model; [b] LRT = likelihood ratio test; DR = deviance reduction; Pr = probability of an χ^2 value exceeding the deviance reduction;
[c] * Reference group; df = degrees of freedom; χ^2 = chi square; SE = standard error.

Conclusion

The survey data analysis using the regression model indicated that growing of *enset* plants at an altitude of >2000m.a.s.l (highlands), growing on soils with pH of 5.5-7.0 (slightly acidic) soils, sole cropping, slashing by machete during weed management and un-weeded *enset* field, growing only susceptible local *enset* clones, using planting material from other farmer where the disease were common, vegetative/young growth stage of *enset*, growing *enset* nearby infested fields, *enset* fields that was no management action taken and cutting infected *enset* in to pieces and leaving it in the field, and low/medium level of farmers awareness to disease were associated with EBW intensity and made significant contribution to the epidemics of the disease in the surveyed region. Our results from this study suggest growing *enset* at midlands preferably rather than highlands, using disease-free planting material, intercropping with unrelated plants, mixed use of local tolerant and susceptible *enset* clone, avoiding frequent harvesting/cutting of leaves mostly during vegetative growth stage of *enset*, hand weeding practices, cleaned use of farm tool and rouging out the infected *enset* and burning should be carried out to reduce EBW impact on *enset*. Breeding for resistance to EBW should be given high priority and should be supported with good agronomic management practices that do not favor EBW epidemics. As enset suckers are used for planting material and infected suckers are source of inoculum in addition to infected soil, sucker and soil treatment method(s) have to be developed in addition to clone resistant to control the disease.

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