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Anaerobic soil disinfestation using locally available carbon sources as a potential management strategy for bacterial wilt in tomatoes

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Abstract

Tomato production in Tanzania is far lower than the 27.5 t/ha global average. Important factors such as deteriorating soil fertility, using vulnerable and low-yielding varieties, unreliable rainfall, diseases, pests, and poor farming practices contribute to reduced tomato production. Bacterial wilt is the most devastating tomato disease in terms of yield losses and complicated management considering pathogen diversity and the soil-borne nature of the disease. On-farm trials and bioassays were conducted to determine the efficacy of anaerobic soil disinfestation (ASD) with wheat bran, rice bran, molasses, and cow manure as carbon sources in suppressing bacterial wilt disease of tomato. We established randomized complete block design (RCBD) experiments in nine fields in Misufini, Mlali villages in Morogoro and Image of Iringa region in mainland Tanzania. The bioassay experiment was also laid in a RCBD and conducted at Sokoine University of Agriculture (SUA) greenhouses using soil naturally infested with *Ralstonia pseudosolanacearum* collected from the same nine fields. The treatments reduced bacterial wilt incidence in tomatoes grown in ASD-treated soils compared to non-treated control soils in field trials at Misufini 1 (P=0.0205), Misufini 2 (0.0061), and Mlali 2 (P =0.019). There were no significant differences among ASD treatments with different carbon sources in disease incidence. This trend was also observed in the bioassays in which bacterial wilt incidence and area under disease progress curves in tomato seedlings grown in field-treated soils from Image, Mlali, and Misufini were significantly lower than in non-treated controls (P <0.0001). This study confirms that ASD can be used as an important suppressor of pathogens through a soil rejuvenation process that involves the creation of an anaerobic environment in water-saturated soil amended with high carbon-based organic materials.

Key words: *Anaerobic soil disinfection; bioassay; Ralstonia pseudosolanacearum; latent infection*

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Introduction

Tomato is among the widely cultivated horticultural crops in Tanzania for local consumption and export to neighboring countries (Maerere *et al*., 2006; De Putter *et al*., 2011). Tomato farmers consider qualities such as shape, size, yield and market in choosing varieties (Minja *et al*., 2011; Testen *et al*., 2016). However, the most preferable cultivars are highly susceptible to diseases and pests that cause damage and reduced yield. Pest and diseases account for 56% of 88% of tomato yield losses (UMADEP, 2003; CABI, 2004) where up to 100% yield losses were recorded in areas with high disease and pest pressure (UMADEP, 2003). Bacterial wilt is among the damaging soilborne diseases of tomatoes that lead to total wilting of plants especially at the flowering and fruiting stage; hence causing huge yield losses (Elphinstone, 2005). The disease is instigated by members of the RSSC (*R. solanacearum* species complex), the second most recognized bacterial pathogen causing devastating crop and yield loss globally, especially in Solanaceous crops (Champoseu and Momol, 2009; Mansfield *et al*., 2012; Meng, 2013). *R. solanacearum,* a pathogenic soil proteobacterium, is widely spread in sub-tropical, tropical, and temperate areas (Hayward, 1991; Yabuuchi *et al*., 1995; Elphistone, 2005). In Tanzanian tomato-producing regions, *Ralstonia* phylotype I and III strains have predominated to date, which are designated *R. pseudosolanacerum* (Remenant *et al*., 2011; Safni *et al*., 2014; Prior *et al*., 2016; Aloyce, 2020; Kanyagha, 2021). The fact that *R. solanacearum* is highly diverse with variable hosts complicates management approaches to wilt disease, especially in important solanaceous crops like tomatoes. Previous studies indicated that bacterial wilt management approaches ranged from phytosanitary measures, cultural practices, biological control, and chemical treatments to host resistance (Elphinstone, 2005; Saddler, 2005; Champoseau and Momol, 2009, Shutt *et al*., 2018). Phytosanitation approaches may be effective in areas where the pathogen has not yet been introduced to minimize disease introduction or spread. Cultural approaches including intercropping, crop rotation, delayed planting periods, and solarization reduce bacterial wilt (Kinyua *et al*., 2001; Saddler, 2005). However, in the African environment, insufficient land availability and the short period between crops limit these approaches. In typical African tomato farming systems, tomato is planted after the main crops (maize or rice) that are cultivated twice annually (Luzi-Kihupi *et al*., 2015), thus a very short time separates the rotation programs. Besides, intercropping is rarely practiced especially in areas with land scarcity where main crops are given priority with limited spacing. Biocontrol is not commonly used in African farming systems as research on their local adaptability, efficacy, dosage, and species recommendations is currently insufficient. The use of disease-resistant tomato varieties is constrained in lower-income countries due to their lack of availability, high cost, and/or failure to match local market preferences. Therefore, soil treatment can be the most preferable management option.

There are several soil treatments like solarization, fumigation and anaerobic soil disinfestation that have been proven to be efficient in the management of soil-borne diseases. Among soil treatments that suppress bacterial wilt pathogen effects, solarization has been effective only for *Ralstonia* biovar 2 strains that inhabit cool areas, as strains from other biovar groups easily adapt to higher temperatures (French, 1994). Anaerobic soil disinfestation (ASD) has been shown in numerous studies to reduce *R. solanacearum* populations to undetectable levels or symptoms have been delayed or reduced in susceptible plants (Blok *et al*., 2000; Momma *et al*., 2006; Messiha *et al*., 2007; Van Overbeek *et al*., 2013). No ASD studies have been reported in Eastern Africa or Tanzania in particular. This study therefore was aimed at studying the efficacy of ASD treatment in reducing populations of *R pseudosolanacearum* or delaying and reducing disease symptoms in farmers preferred tomato varieties that are highly susceptible to bacterial wilt. Different locally available carbon sources were tested for their efficacy in both field and greenhouse conditions. Obtained results lead to the development of recommendations for soilborne disease approaches that will be added to IPM packages and extended to tomato farmers.

Materials and methods

Study site

Three villages in the Morogoro and Iringa regions

of Tanzania mainland were selected for conducting the ASD trial (Figure 1). These are Mlali and Misufini (in Morogoro region) and Image village (in Iringa region). The villages are characterized by different soil types and rainfall patterns, but again they are known as tomato production hotspots in which the tomatoes are available all year long.

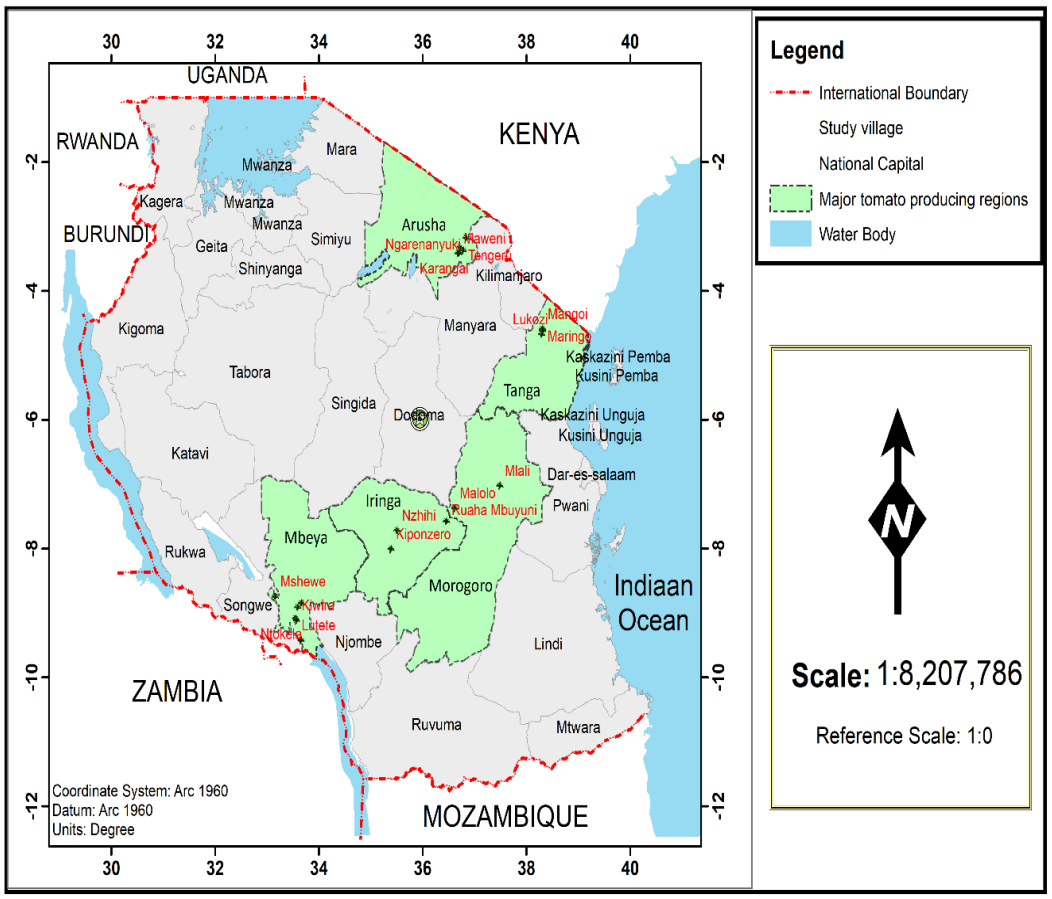
Misufini is located in Mvomero district Morogoro region and lies between (6°17' 29.16°S, 37°28 19° 92'E) approximately 400m above sea level (absl). The area lies in a flat Wami river plain with characteristic fertile clay loamy soil. The area receives heavy rainfall (March -June) and shorter rains (October-December). Tomato is normally planted after the main crop (rice or maize) season together with other vegetables that include eggplant, melons, onions, and cucumber**.**

Mlali village lies between 6°57′ 39.60′ S, 37°3′ 11°64′ E6° 57' 0" S, 37° 32' 0" E. The area is a flat river plain that is depended on for irrigation and used all year round for vegetable production. Tomato is always rotated with sweet pepper, eggplants, and maize. The area is characterized by sandy loamy and moderately high temperatures all year round. It receives rain with a long season (March to June) and a short season (October to December)

The image is in Kilolo district Iringa region. Image has an altitude of 1500m absl and is characterized by sandy loam and cool temperatures of up to 10°C in cooler months. The main rainy season is November to April and the short season is February to April. Many farms are irrigated, and tomato is rotated with main crops (maize and sunflower) throughout the year. Tillage in vegetable and tomato fields is by tractors and ox-drawn ploughs.

Figure 1

Map of Tanzania indicating major tomato producing regions highlighted with light green coloration



Field experiment

Anaerobic soil disinfestation field experiments were conducted at the nine fields located in Morogoro (6) and Iringa (3), in October 2019 and February 2020 to evaluate the efficacy of four selected carbon sources in the reducing *R. pseudosolanacearum* load in naturally infested soil. We also assessed the performance of selected susceptible tomato varieties on bacteria wilt disease on ASD-treated soils located in disease hotspots. The soils were amended with rice bran, wheat bran, molasses, and cow manure at the rate of 20.2 Megatonnes per hectare (Mg/ha) equivalent to 2.5 kg/m^2. Rice and wheat bran were collected from nearby mills while molasses was collected from sugar-producing industries and ripe cow manure from nearby livestock keepers. In the experiment controls were saturated soils that were non-amended and not covered (aerobic control) and saturated not amended and covered soil (anaerobic control). The experiment was set in a completely randomized block design with four blocks each having all six treatments in plots of 1 m × 2.5 m with 0.5 m spacing within rows and 1 m between blocks. The six treatments were aerobic control, anaerobic control, rice bran, wheat bran, molasses and cow manure-treated plots. A hand hoe was used to mix amended organic sources with the soil at the 15 cm top layer. Polythene tubes (IRIS) painted with iron iii oxide (30cm long) was inserted into each treatment to assess the soil reductive conditions. The treatment plots were saturated with water using a water can or pump then tarped with a plastic sheet (black), the edges were sealed with soil to limit the exchange of air.

The ASD treatments lasted 3 weeks. Soil temperatures were measured using a probe field digital thermometer (Fisherbrand™, Thermo Fisher Scientific Inc., UK). Immediately after the removal of the plastic sheet, the thermometer probe was inserted into the soil in the four corners and center of each plot to a depth of 10cm and the average temperature was computed for each treatment in a field. IRIS tubes were removed from the soil immediately after the removal of the plastic sheet, rinsed, and allowed to dry. Percentage paint removal (PR) was estimated by the grid method, in which 1mm squares were drawn all over the tubes. The total number of squares was counted, followed by counting the number of squares from which the paint was removed. The percentage of paint removal was calculated using the formula (Sanabria *et al*., 2020):

1. Percentage IRIS tube paint removal = 100% - ((PA - PR)/PA×100)

Where (PA) = painted area and (PR) = area with paint removed. From each plot, 10g of soil was sampled in separate 10cm Falcon tubes for pH analysis at SUA soil science laboratories. Soil PH was determined by mixing the sampled soils (1:1) with distilled water, agitating vigorously on a shaker for 30 minutes, and centrifuging at 30,000 revolutions per minute (rpm) for 10 minutes. The pH meter electrode Cyberscan pH 510 (Eutech Instruments, Singapore) was inserted into the supernatant and the displayed pH value was recorded. Measurements of pH, temperature, and PR are reported as the average of measurements of four reps per treatment.

On-farm evaluation of ASD efficacy

The efficacy of ASD with four different carbon sources was assessed in on-farm plots located in bacterial wilt disease hotspots. The ASD-treated plots were left for 7 days to aerate after the removal of plastic sheets before tilling them with a hand hoe at approx. 15 cm depth. Ten holes approx. 10X10 cm were dug in each plot, in two rows (five holes each) separated by 100cm spacing and 50cm between holes. A 20-day-old tomato seedling (bacterial wilt-susceptible variety Assila (Seminis, Holland)) rose by the farm owner was planted in each hole. The seedlings were raised in 0.5kg polythene bags filled with soil heated for 5-6 hrs in firewood-fuelled local stoves and amended with cow manure before seed sowing. The bags were kept in nurseries near fields and watered once daily. Planted seedlings were fertilized with NPK (15-9-20) fertilizer (Yaramila Winner, Yara TZ) 2 and 6 weeks after planting at the rate of 5g/seedling, followed by UREA and sulfur (40N, 5.6S) (Yaravera AMIDAS, Yara TZ) at 8 weeks after planting and Nitrabor with nitrogen, calcium, and boron (YaraLiva Nitrabor, Yara) at fruit set. The plots were watered twice weekly on dry days, otherwise, they were rainfed. The plants also received applications of fungicides (Linkmill WP; metalaxyl-M 40g/Kg, mancozeb 640g/Kg) and insecticide (Abamectin 5% EC) every two weeks. Weeding was done manually as needed to maintain the plots weed-free. Disease scores for bacterial wilt incidence were taken every two weeks by counting dead and wilting plants. The plants were raised for three months.

Greenhouse Experiment

Naturally bacterial wilt-infested soil was collected from the nines fields that were identified to be used for solanaceous plant production and have a history of being infected by *R. pseudosolanacearum*. Experiments were conducted at SUA greenhouses from November to February 2019 and repeated from March to June 2020. Four different carbon sources namely wheat bran, molasses, rice bran and cow manure at the rate of 20.2 Mg/ha. Carbon sources were integrated into the 300g of infested soil by hand and mixed thoroughly. Soils were saturated with water and each treatment pot was covered by a piece of nylon sheet safeguarded with a rubber band Controls were not amended with carbon sources but saturated with water were uncovered saved as aerobic control and covered as anaerobic control. The experiment was set in a complete randomized block design with four blocks each containing all six treatments. The ASD treatment lasted 3 weeks. Greenhouse temperature was recorded during the ASD experiment using a thermometer and averaged to save as ASD treatment temperature.

Assessing the efficacy of ASD treatments

The bags were opened to aerate the soil for seven days, and then the soil was pulverized by hand and placed in 500ml disposable cups. A 20-day-old tomato variety Assila seedling was transplanted into each cup. The tomato seedlings were previously raised in plastic trays with 50x50 mm holes filled with autoclaved soil amended with cow manure. The cups were arranged in

RCBD within each field collection. The tomato seedlings received the same fertilization and pesticide applications as field plants except for the amounts of fertilizer applied adjusted to small-sized pots. The seedlings were raised for 8 weeks and disease assessments were done weekly.

Plants were assessed weekly for the incidence of wilting symptoms. Disease incidence was assessed by counting the total number of plants (N) and the number of plants with bacterial wilt symptoms (n) for each replicate. The incidence of bacterial wilt was calculated using the equation

2.

$$Incidence\ of\ bacterial\ wilt = \frac{n}{N} * 100$$

The area under the disease progress curve (AUDPC) was calculated according to the Excel formula (Madden *et al*., 2007).

3.

$$\text{AUDPC} = \sum_{i=1}^{n} \left(\left(\frac{y_i + y_{i+1}}{2} \right) + (t_i + 1 - t_i) \right)$$

Where y_i = measures of disease level at ith observation and t_i time of disease measure at ith observation.

To assess latent infection, plants remaining asymptomatic eight weeks after inoculation were sampled by cutting a 2 cm stem section from the base of the plant and placing it in a tube containing 2.5 ml of sterile distilled water to allow for bacterial streaming for one hour. Suspensions (100µl) were pipetted into wells of a 96-well microtiter plate and an enzyme-linked immunosorbent assay (*R. solanacearum*) ELISA kit; Agdia Inc. Elkhart, IN, USA) was conducted according to manufacturer instructions. Negative and positive controls provided with the kit were used as color change guides to score positive or negative results. Wells with a blue color visibly darker than the negative control were scored positive whereas wells with no color or lighter blue than the negative control were scored as negative for latent infection. Number of positives and negative samples were recorded. The percentage of plants with latent infection was calculated by dividing the number of plants that tested positive for *R. pseudosolanacearum* by the total number of plants sampled for latent infection x 100.

Statistical analyses

Statistical analyses were carried out in SAS 9.4.4 (2017) using the statistical package Proc GLM. All data were tested for normality using the Shapiro test and equal variance using Bartlett's test; when needed data were transformed using Arcsin (n) or $\log_{10}$ (n). Treatment means were analyzed and compared using the Tukey Kramer test at P =0.05. The area under the disease progress curve means was compared in SAS using Proc GLM.

Results

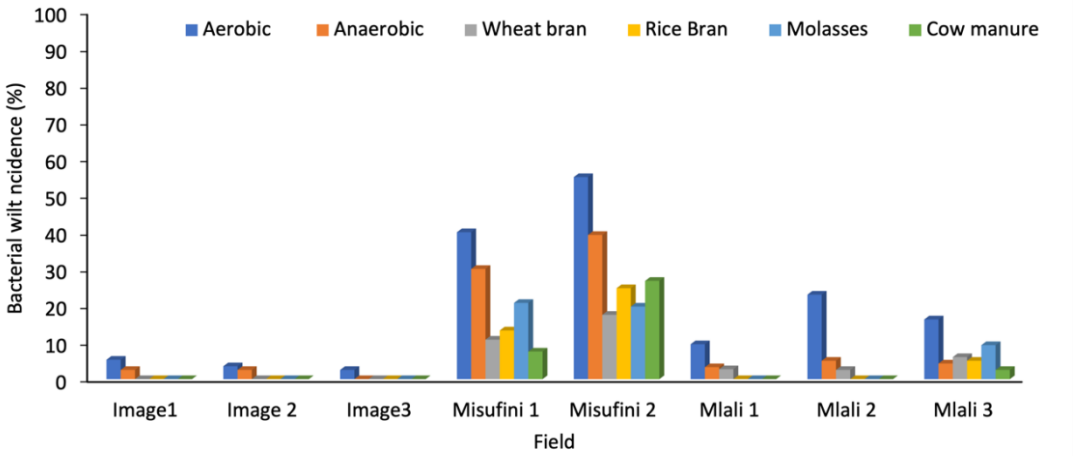
Field Experiment

Efficacy of different carbon sources on reducing the effect of bacterial wilt disease incidence, the area under the disease progress curve and latent infection

Tomato bacterial wilt incidences were measured on tomato seedlings that were planted on ASD-treated plots to assess the efficacy of ASD treatment. Mean bacterial wilt incidence was significantly higher in the aerobic and anaerobic control as compared to carbon source-amended ASD treatments across all sites (Figure. 2). Carbon source-amended treatments revealed relatively low incidence as compared to non-amended treatments (T1 and T2). Response of ASD varied among carbon sources for each site. A significant variation in bacterial wilt incidence was observed among treatments at Misufini (P= 0.026), Image (P= 0.003) and Mlali fields (P=0.019). Manure (T6) wheat bran (T3) and rice bran (T4) respectively had relatively low disease incidence and molasses (T5) did show a difference in incidence as compared to controls (T1 and T2) (Figure 2) for Mlali site. Wheat bran (T3), rice bran (T4) and molasses (T5) had extremely low disease incidences while manure (T6) performed the same as anaerobic control in terms of disease incidences and relatively low to aerobic control (Figure 2). For Misufini site all amended (T3, T4, T5, T6) treatments revealed relatively low disease incidences as compared to aerobic controls (T1) and relatively similar to anaerobic control (T2) (Figure 2).

Figure 2

Bacterial wilt incidence in tomato seedlings twelve weeks after planting in anaerobic soil disinfestation (ASD)-treated soils in fields located at Image (Iringa) and Mlali and Misufini (Morogoro) villages, Tanzania



Note. Values are the mean disease incidence for four blocks of each of six ASD treatments, including aerobic and anaerobic controls and ASD-treated plots amended with wheat bran, rice bran, molasses, or cow manure carbon sources. Values for bars with the same letters in a field are not significantly different from each other at $P \leq 0.05$

A slight difference in latent infection was observed across all treatments and all sites. No significant difference was observed between latent infection between carbon source amended treatments (T3, T4, T5 and T6) and controls (T1 and T2) at Mlali (P= 0.011), Misufini (P=0.049) and Image (Table 1). Aerobic controls revealed significantly higher latent infections as compared to carbon source-amended soils and anaerobic control (Table 1).

Table 1

Tomato seedlings mean latent infection (%) recorded at the termination of the Field experiment; the seedlings were transplanted on pretreated ASD soil

Field ID	Treatment	Latent infection (%)
Image	T1[a]	24.83
	T2[b]	20.83
	T3[c]	0.00
	T4[d]	9.67
	T5[e]	0.00
	T6[f]	8.33
		P=0.28
Misufini	T1[a]	41.50 A
	T2[b]	16.67 B
	T3[c]	13.83 B
	T4[d]	8.83 B
	T5[e]	9.67 B
	T6[f]	9.67 B
		P=0.049
Mlali	[a]T1	55.50 AB
	[b]T2	69.50 A
	[c]T3	48.67 AB
	[d]T4	52.83 AB
	[e]T5	16.67 B
	[f]T6	40.33 AB
		P=0.0115

Note. [a] Aerobic control with no carbon source amendment and cover

[b] Anaerobic controls without carbon source amendment but covered

[c] Wheat bran amended and covered

[d] Rice bran amended and covered

[e]Molasses amended covered

[f] Cow manure covered

P= Probability at 5% alpha value, treatments with the same letters are not significantly different from each other

There was no significant difference in bacterial wilt AUDPC among soils (P= 0.1843). Misufini revealed significantly higher AUDPC as compared to the rest of the sites (Table 2). All treatments revealed a significant difference in AUDPC among each other (P<0.0001). Aerobic and anaerobic controls (T1 and T2) revealed significantly higher AUDPC as compared to all amended carbon source treatments. T4 revealed the least AUDPC as compared to other treatments (Table 2). Within fields/location revealed a significant difference in AUDPC among ASD treatments at Misufini (P=0.0001) and Mlali (P=0.001). Relatively low AUDPC were observed in carbon source amended ASD treatments across all fields (Table 2) as compared to non-amended fields

Table 2
Percentage latent infection by Ralstonia pseudosolanacearum in bacterial wilt-susceptible tomato variety Assila seedlings after eight weeks of growth in soil from nine infested tomato fields in Tanzania treated in bioassays with anaerobic soil disinfestation (ASD) using different carbon sources

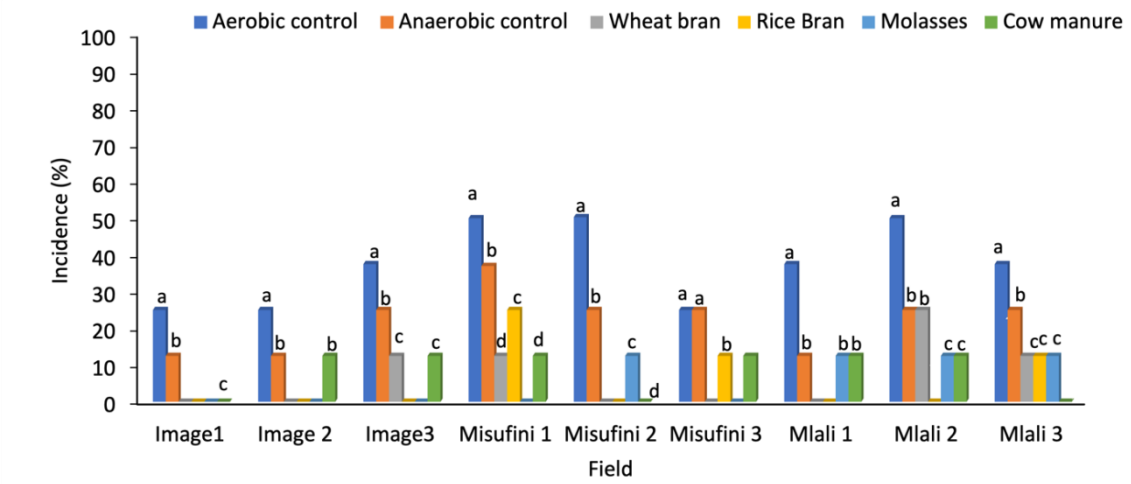
Carbon source	Mean percentage seedlings with latent infection [x, y, z]								
	IMG1	**IMG2**	**IMG3**	**MS1**	**MS2**	**MS3**	**ML1**	**ML2**	**ML3**
Aerobic control	25.0 a	16.5 b	33.0 a	25.0 ab	66.5 a	33.8 a	100.0 a	16.5 ab	50.0 a
Anaerobic control	0.0 b	50.0 a	12.5 b	0.0 c	45.9 b	0.0 c	100.0 a	33.5 a	75.0 a
Wheat bran	0.0 b	0.0 b	0.0 c	29.0 a	0.0 c	12.5 b	58.5 b	12.5 b	75.0 a
Carbon course	**IMS 1**	**IMG 2**	**IMG3**	**MS1**	**MS2**	**MS3**	**ML1**	**ML2**	**ML3**
Rice Bran	16.5 a	12.5 b	0.0 c	0.0 c	12.5 c	9.4 b	100.0 a	0.0 b	58.5 ab
Molasses	0.0 b	0.0 b	0.0 c	12.5 bc	16.5 c	0.0 c	25.0 c	0.0 b	12.5 c
Cow manure	12.5 ab	0.0 b	12.5 b	0.0 c	0.0 c	29.0 a	58.5 b	0.0 b	50.0 b
P value	0.0029	0.0012	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0016	<0.0001

Note. [x] Means from two combined experiments conducted under similar screenhouse conditions.
[y] Means with the same letters within a row are not significantly different from each other at $P \leq 0.05$.[z] *IMG=Image MS=Misufini ML=Mlali*

Greenhouse Experiment
Efficacy of selected carbon sources on reducing the effect of bacterial wilt disease
Tomato bacterial incidence, latent infection and bacterial wilt area under disease progress curve on tomato seedlings planted on ASD-treated soils were analyzed. Mean bacterial wilt incidence was significantly higher in the aerobic and anaerobic control as compared to carbon source-amended ASD treatments across all soils (Figure 3). Relatively low incidence was observed on carbon source-amended soils as compared to non-amended treatments (T1 and T2). The response of ASD varied among carbon sources for each site. A significant variation in bacterial wilt incidence was observed among treatments in Misufini and Mlali soils (P= 0.025 and P= 0.031) respectively, and a non-significant difference in treatments at image soil (P=0.086). Image rice bran treatment (T4) revealed no plant wilt, other treatments wheat bran (T3) and molasses (T5) and manure (T6) respectively revealed relatively low disease incidence as compared to aerobic control (T1) and non-significant difference with anaerobic control (T2) (Figure 3). Non-significant variation was observed for Misufini where wheat bran (T3) and molasses (T5) had extremely low disease incidences followed by rice bran (T3) and manure (T6) as compared to controls (T1 and T2) (Figure 3). For Mlali, all amended (T3, T4, T5, T6) treatments revealed relatively low disease incidences as compared to controls (T1 & T2) (Figure 3)

Figure 3

Bioassay of the effects of anaerobic soil disinfestation (ASD) with different carbon sources on bacterial wilt incidence in tomato variety Assila seedlings planted in soils collected from nine Ralstonia pseudosolanacearum-infested fields in Image (Iringa) and Mlali and Misufini (Morogoro), Tanzania



Note. Values for bars with the same letters within a field are not significantly different from each other at a 5% alpha value.

A slightly significant difference in latent infection was observed across all treatments and all sites. No significant difference was observed in latent infection between carbon source amended treatments (T3, T4, T5 and T6) and controls (T1 and T2) at Mlali (P= 0.011), Misufini (P=0.049) and Image (Table 3). Aerobic controls revealed significantly higher latent infections as compared to carbon source-amended soils and anaerobic control (Table 3).

There was a non-significant difference in bacterial wilt AUDPC among soils (P= 0.1843). Misufini revealed significantly higher AUDPC as compared to the rest of the sites (Table 4 and Figure 4). All treatments revealed a significant difference in AUDPC among each other (P<0.0001). Aerobic and anaerobic controls (T1 and T2) revealed significantly higher AUDPC as compared to all amended carbon source treatments. T4 revealed the least AUDPC as compared to other treatments (Table 4). Within fields/location revealed a significant difference in AUDPC among ASD treatments at Misufini (P=0.0001) and Mlali (P=0.001). Relatively low AUDPC were observed in carbon source amended ASD treatments across all fields (Table 4, Figure 4) as compared to non-amended controls.

Table 3

Mean comparison of bacterial wilt area under disease progress curve across all fields and all ASD treatments, Means with the same letter are not significantly different from each other at 5% alpha value

Farm ID	AUPDC mean	Treatment	AUPDC mean
MS1	88.54 a	T1	126.39 a
MS2	67.71 ab	T2	93.06 a
MS3	43.75 ab	T6	40.97 b

ML1	53.13 ab	T3	34.03 b
ML2	64.58 ab	T5	25.69 b
ML3	67.71 ab	T4	18.75 b
IMG1	21.88 b		
IMG2	35.42 b		
IMG3	65.63 ab		
LSD	**46.87**		**38.27**
P VALUE	**0.1843**		**<0.0001**

Table 4

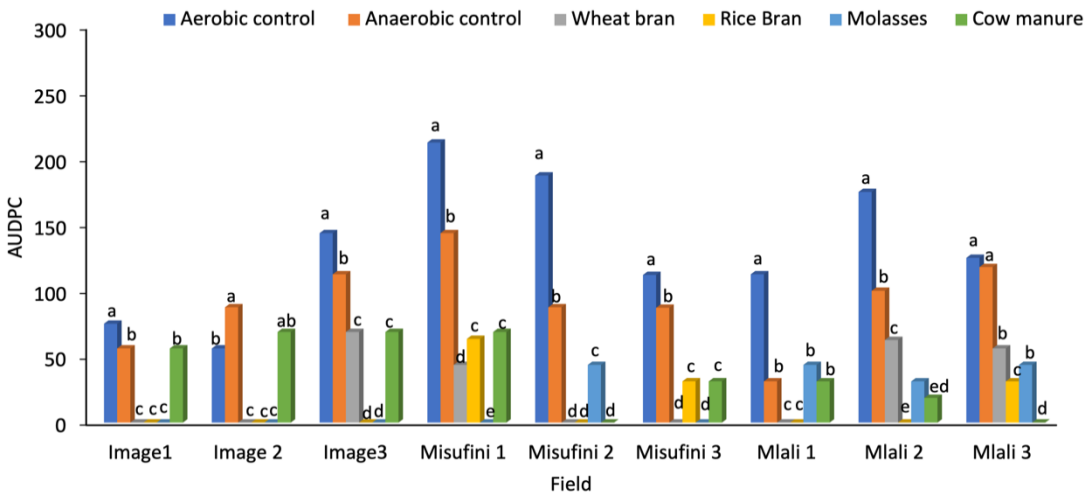
Mean comparison of bacterial wilt area under disease progress curve within each fields and ASD treatments, Means with the same letter are not significantly different from each other at 5% alpha value

Farm ID	**AUPDC mean**	**Treatment**	**AUPDC mean**
IMG1	21.88	T1	95.83 A
IMG2	35.42	T2	37.50 AB
IMG3	65.63	T3	41.67 AB
		T4	0.00 B
		T5	18.75 B
		T6	52.08 AB
P value	**0.2145**		**0.162**
LSD	**50.896**		**71.978**
MS1	88.54 *A*	T1	170.83 *A*
MS2	67.71 *AB*	T2	106.25 *B*
MS3	43.75 *B*	T3	14.58 *C*
		T4	45.83 *CB*
		T5	18.75 *C*
		T6	43.75 *C*
P value	**0.1352**		**0.0001**

LSD	**44.289**		**62.634**
ML1	53.13 *a*	T1	112.5 a

Figure 4

Bioassay of the effects of anaerobic soil disinfestation (ASD) with different carbon sources on bacterial wilt disease progress (area under the disease progress curve; AUDPC) in tomato variety Asila seedlings planted in soils collected from nine Ralstonia pseudosolanacearum-infested fields in Image (Iringa) and Mlali and Misufini (Morogoro), Tanzania



Note. Values for bars with the same letters within a field are not significantly different from each other at 5% alpha value

Table 5

Mean soil pH, Indicator of Reduction in Soils (IRIS) tube paint removal and soil temperature after 3 weeks of anaerobic soil disinfestation (ASD) treatment with different carbon sources in nine on-farm trial sites in Mlali (ML), Image (IMG) and Misufini (MS) villages

		Mean [x]								
	Carbon source	**IMG1**	**IMG2**	**IMG3**	**MS1**	**MS2**	**MS3**	**ML1**	**ML2**	**ML3**
pH	Aerobic control	5.6	6.8 a	6.6 a	7.0 a	6.7	7.1 a	6.5 a	6.8 a	7.1 a
	Anaerobic control	5.5	6.8 a	6.2 c	6.7 ab	6.3	6.0 c	6.1 d	6.3 c	7.1 a
	Wheat bran	5.5	6.6 b	5.8 e	6.4 bc	6.3	6.5 b	6.5 a	6.5 b	7.1 a
	Rice Bran	5.5	6.8 a	6.5 b	6.5 b	6.2	5.1 d	6.2 c	6.5 b	7.1 a
	Molasses	5.5	6.2 c	6.5 b	6.1 c	6.1	6.4 b	6.4 ab	6.2 c	7.1 b
	Cow manure	5.5	6.1 d	6.1 d	6.6 ab	6.3	6.2 c	6.4 ab	5.3 d	5.7
		Mean [x]								
	Carbon source	**IMG1**	**IMG2**	**IMG3**	**MS1**	**MS2**	**MS3**	**ML1**	**ML2**	**ML3**
	P value	0.3071	<0.0001	<0.0001	0.0021	0.1557	<0.0001	<0.0001	<0.0001	<0.0001
Paint removal (%)	Aerobic control	28.5 c	2.3 f	19.0 f	5.9 c	17	6.7 c	8.5 d	17.8 c	9.1 c
	Anaerobic control	36.6 c	13.8 e	24.2 e	18.0 ab	18.3	9.7 c	9.5 d	16.4 c	12.3 c
	Wheat bran	44.6 ab	38.0 a	44.9 c	26.5 a	18.7	20.0 b	32.4 a	29.2 ab	14.8 c
	Rice Bran	53.8 a	17.3 d	75.2 a	18.4 ab	21.6	39.1 a	25.1 b	25.7 bc	40.5 a
	Molasses	41.2 abc	36.7 b	36.1 d	15.4 bc	25	28.1 b	15.3 c	25.0 bc	40.2 a
	Cow manure	47.0 ab	34.3 c	52.8 b	19.3 ab	29.7	26.8 b	19.2 c	37.1 ab	29.1 b

	P value	0.0478	<0.0001	<0.0001	0.0167	0.4565	<0.0001	<0.0001	0.0066	<0.0001
Temperature (°C)	Aerobic control	27	24.7 c	24	26.5 b	34.7	31.0 c	27.9 b	33	34.1 c
					Mean [x]					
	Carbon source	**IMG1**	**IMG2**	**IMG3**	**MS1**	**MS2**	**MS3**	**ML1**	**ML2**	**ML3**
	Anaerobic control	27.8	25.7 ab	24.3	30.1 a	38.1	34.9 ab	32.5 a	35.6	37.6 ab
	Wheat bran	27.8	26.2 a	24.8	29.9 a	37.5	35.4 a	33.2 a	35.9	38.4 ab
	Rice Bran	27.6	26.1 a	24.2	30.8 a	38.2	35.5 a	32.4 a	36.4	39.1 a
	Molasses	27	25.7 ab	24.1	30.3 a	35.8	34.3 b	33.1 a	34.9	36.7 b
	Cow manure	27.9	25.3 bc	24.9	30.7 a	37	34.7 b	32.3 a	35.9	36.5 b
	P value	0.5749	0.0025	0.4319	<0.0001	0.1106	<0.0001	0.0078	0.1855	0.0018

Table 6

Mean soil pH and Indicator of Reduction in Soils (IRIS) tube paint removal after 3 weeks of anaerobic soil disinfestation (ASD) treatment with different carbon sources in bioassays of Ralstonia pseudosolanacearum-infested soils collected from tomato fields in Mlali (ML), Image (IMG) and Misufini (MS) villages in Tanzania

ASD indicator factor	Carbon source	Mean[x y]								
		IMG1	**IMG2**	**IMG3**	**MS1**	**MS2**	**MS3**	**ML1**	**ML2**	**ML3**
pH	Aerobic control	7.0 a	6.9 a	6.6	7.0 a	6.7 a	6.8 a	6.7 a	6.8	6.8 a
	Anaerobic control	6.6 b	6.5 b	6.2	6.6 bc	6.3 c	6.5 cb	6.5 c	6.6	6.7 ab
	Wheat bran	6.4 cb	6.4 bc	6.4	6.8 abc	6.5 b	6.5 cb	6.6 ab	6.6	6.5 c
	Carbon source	**IMG1**	**IMG2**	**IMG3**	**MS1**	**MS2**	**MS3**	**ML1**	**ML2**	**ML3**
	Rice Bran	6.2 cd	6.4 bc	6.4	6.7 bc	6.5 cb	6.6 b	6.5 c	6.7	6.5 c
	Molasses	6.2 cd	6.3 c	6.4	6.9 a	6.4 c	6.3 d	6.5 c	6.6	6.6 bc
	Cow manure	6.0 d	6.6 b	6.5	6.6 c	6.5 b	6.5 cb	6.5 c	6.6	6.6 bc
	P value	<0.0001	<0.0001	0.105	0.0341	<0.0001	<0.0001	0.0005	0.0977	0.0023
Paint removal(%)	Aerobic control	6.8 f	0.9 f	6.5 f	2.2 c	2.8 d	2.9 d	5.4 bc	4.7 d	12.1 dc
	Anaerobic control	12.1 e	4.5 e	8.1 e	3.0 cb	3.3 cd	3.6 cd	4.8 c	5.5 d	9.2 d
	Wheat bran	17.5 b	12.8 a	14.9 c	6.2 a	4.7 cbd	4.6 cb	4.7 c	9.8 b	15.0 c
	Carbon source	**IMG1**	**IMG2**	**IMG3**	**MS1**	**MS2**	**MS3**	**ML1**	**ML2**	**ML3**
	Rice Bran	17.8 a	5.8 d	25.1 a	4.3 b	4.9 cb	5.1 b	8.2 a	10.3 b	40.5 a
	Molasses	13.7 d	12.1 b	12.1 d	3.8 cb	5.2 b	5.2 b	4.7 c	7.8 c	40.2 a
	Cow manure	15.7 c	11.3 c	17.5 d	4.6 b	7.2 a	7.2 a	8.2 a	12.2 a	28.9 b
	P value	<0.0001	<0.0001	<0.0001	0.0003	0.0006	<0.0001	0.0165	<0.0001	<0.0001

Note. [x] Means from two combined experiments conducted under similar screenhouse conditions.
[y] Means with the same letters within a row are not significantly different from each other at $P \leq 0.05$.

Discussion

Experiments were carried out to evaluate the efficacy of ASD with different carbon sources in reducing the incidence of tomato bacterial wilt in disease hotspots in farm fields-controlled bioassays in Tanzania. Reduced Bacterial wilt disease incidence was achieved within all ASD treatments except for unamended treatments in both field and greenhouse experiments conducted. However, our results indicated variation in the efficacy of carbon sources in each field or soil collected from those locations. In the field environment, all amendments revealed more, less, or equal efficiency in reducing bacterial wilt incidence. Manure, wheat bran and rice bran were most efficient At Mlali and Misufini while molasses treatment was less effective and inconsistent in reducing the incidence of bacterial wilt at Mlali. On the other hand, greenhouse experiments revealed a similar trend with minor differences. Rice bran was the most efficient for Image soil while manure, rice bran and molasses were effective but not much different in performance as anaerobic control. Similar results were observed with Misufini and Mlali soils. Testen and Miller, (2018) and Sanabria *et al*. (2020) emphasized the variation of the efficacy of different carbon sources during ASD treatments. Sanabria *et al.* (2020) reported variation in the sensitivity of different c sources in reducing the viability of sclerotia. Wheat and rice bran and fresh grass were reported by Momma *et al*. (2006); Messiha *et al.* (2007) and Van Overbeek. (2013) in reducing symptoms and effects of bacterial wilt incidences. Findings of Messiha *et al*. (2007) and Van Overbeek *et al*. (2013) indicated the efficacy of <100% reduction of *R. solanacearum* reduction, we also observed the same trend in our greenhouse and field ASD.

Many factors may have contributed to the failure to achieve a 100% reduction. ASD efficacy involves many factors that work together. Soil types and conditions, amount and type of carbon source used, time used for ASD treatment as well as surrounding environmental conditions i.e. rainfall and temperature regimes (Momma *et al*., 2006; Mazola and Hewavitharana, 2014; Runia *et al*., 2014). Though our choice of carbon sources and dosage were guided by successful results from previous ASD experiments and future availability and practicality of the process especially in subsistence farming communities (Momma, 2008; Testen and Miller, 2018; Sanabria *et al.* 2020), we hypothesize that our results were affected by the selection of similar carbon sources and use of similar amounts regardless of the location. Selected locations differ in soil types, altitude, temperature rainfall regimes and probably types of microbial flora composition. Mlali is a warm area with sandy loam soil and receives less rainfall as compared to Image and Misufini which are characterized by cool temperatures clay loamy soils and relatively heavy rains. Soil types and temperature regimes affect inhabiting microbes, *R. solanacearum* population as well as their distribution (Elphinstone, 2005). Thus, the choice of variable carbon sources or doses could have helped improve the efficacy of our ASD experiments in the sense that different carbon sources have variable efficacy regarding soil type, the amount used and environmental factors Research insists carbon sources vary in efficacy and sensitivity to pathogens considering kind and amounts of metabolites produced during ASD (Hewavithrana *et al*., 2014; Testen and Miller, 2018). In addition to this, we used a similar time regime for our experiments. Soil temperature plays an important role in the efficacy of carbon sources as warm soil conditions enhance the metabolism of microbial population and degradation of carbon sources (Huang *et al*., 2016). Thus, devotion to more ASD time especially in cooler areas could have improved the efficacy of our ASD in reducing *R pseudosolanacearum* populations.

We also assume that the use of hand hoes in mixing carbon sources may have contributed to variable efficacy. In similar previous experiments by Momma *et al*. (2006), Runia *et al*. (2014) and Testen and Miller (2018) mixing of soil and farm preparations involved the use of tractors or power tillers; (Application of carbon source requires vigorous mixing and constant distribution, thus with a hand hoe is very hard to achieve the conditions probably may have led to variations in concentration even in an individual treatment plot. Inconsistent concentration may have formed pockets that did not or received less carbon source amendments hence reduced efficiency in wiping out bacterial wilt pathogen. We also encountered unusually heavy and frequent rains throughout our experiment; we had almost no single day without rain. The rains

led to flooding and landslides that could be a source of contamination to our treated fields, especially at bordering treatment plots as well as making affecting soil temperature and concentration of metabolic products produced from the breakdown of carbon sources. In addition to this hardship in availability, application and variability in concentration may render their practicality as ASD carbon sources, especially in subsistence farming communities.

Concerning ASD efficacy, various mechanisms are involved to make ASD efficiently reduce the soilborne pathogen population. As we observed the ASD treatment effects ranged from reduced soil pH, increased temperature, and paint loss from IRIS tubes (Tables 5 and 6). Change in pH to acidic indicates the production of organic acids (Momma *et al*., 2006; Sanabria *et al*., 2020), and the removal of paint indicates anaerobicity of soil caused by anaerobic microorganisms' reduction of iron oxide during their metabolism (Runia *et al*., 2014). Changes in temperature for anaerobic treatments indicate increased metabolism of microorganisms resulting in heat production (Runia *et al*., 2014). ASD enhances the activity of anaerobic microbial populations such as *Klebsiella*, (Huang *et al*., 2016; Testen and Miller, 2018). Degradation of carbon sources helps to perpetuate population density through the rapid multiplication of anaerobes as well as release metabolites with antimicrobial effects and formation of anaerobic conditions thus making the soil condition unfit for growth of aerobic microorganisms (Katase *et al*., 2009; Runia *et al*., 2014). An increased population of anaerobes plays a direct role in outcompeting soilborne pathogens (Huang *et al*., 2016). The metabolites released include increased production of toxic gases and organic acid that act as natural biocontrol hence helping in reducing harmful soil pathogens. While many ASD studies focused on optimization and determining the key mechanism of ASD efficacy, our research concentrated on determining the efficacy of selected carbon sources in reducing the incidences of bacterial wilt disease of susceptible tomato varieties in under controlled and natural environment concerning ASD efficacy predictor factors soil temperature, pH and soil reducing condition (Eh)

The type and amount of organic acids released during ASD determine how acidic and toxic microbes will be (Hewavitharana *et al*., 2014). Momma *et al*. (2006) declared the effectivity of low soil pH (5.5) from wheat bran to effectively reduce populations of soil pathogens. Organic acid production lowers soil PH which is intolerable to soilborne pathogens such as *R. solanacearum* (Momma *et al*., 2006). Momma, (2008) reported on the effectivity of organic acids (acetic and butyric) in reducing *R. solanacearum* populations at a concentration of 2000mg/kg and 1500 mg/kg respectively. In the same context Sanabria *et al*. (2020) declared reduced soil pH to correlate with reduced viability of sclerotia with varying sensitivity of different carbon sources. In our experiments, we did not see a strong relationship between pH reduction on reduced incidence of susceptible tomato seedlings and later latent infection. As explained in previous sessions several factors may have contributed to the invariable pH observed during our experiments. However, we observed a reduction of incidence indicating that more than one mechanism can be involved in suppressing soilborne pathogen populations during ASD.

Increased soil reductive conditions during ASD play a big role in the efficacy of the process (Runia *et al*., 2014). In ASD experiments, reductive soil conditions are correlated with IRIS tube iron oxide paint removal (Testen and Miller, 2018; Sanabria *et al*., 2020). Paint removal signifies low soil oxygen concentration because of high metabolic activities and by-products from anaerobes (Momma *et al*., 2006; Runia *et al*., 2014). In their experiments, Sanabria *et al*. (2020) correlated reduced viability of sclerotia and root-knot nematodes eggs with paint removal. We did not observe a strong correlation between paint removal and reduced incidence and latent infections. However, we observed reduced incidences in our experiments.

Bacterial wilt pathogens have been reported to survive in deep layers of soil, especially in environments with favourable growth conditions (approx. 75cm) (Van Elsas *et al.,* 2001). In this case, any soil treatment approach should target a reasonable depth to achieve a targeted efficient reduction of the pathogen. Ineffective ASD could result in massive disease occurrence especially when favorable conditions favor multiplication of a few bacterial cells that survived the treatments (Momma, 2008). We observed disease occurrence in the later stages of tomato production in our field experiments. We hypothesize that our

treatments were effective however; our method of soil mixing was not consistent or deep enough to cause disinfection to the soil's deeper layer that could be reached by roots of mature tomato plants. Thus, surviving cells multiplied and infected the plants at later growth stages. Thus, we suggest the use of integrated pest management approaches to take care of unexpected disease occurrences. These approaches may include cultural practices such as delayed planting time (cool months), rotation with non-host crops and use of resistant tomato varieties or seedlings that were grafted onto resistant rootstocks.

Conclusion and recommendations

Anaerobic soil disinfestation (ASD) treatment can efficiently reduce bacterial wilt disease incidence and severe effects. Locally available carbon sources can be efficiently used and produce effects that suppress bacterial wilt disease. Therefore, ASD can be an important tool for bacterial wilt management on smallholder farms in Tanzania. Findings from this study paved the way for using anaerobic soil disinfestation as a method of treating bacterial wilt-infested soils. ASD can efficiently reduce bacterial load in bacterial wilt-contaminated soils and reduce its devastating effect, thus can be used as a tool in integrated pest management packages. However, more experiments should be done for dosage recommendations and timing of ASD treatments to increase the efficacy of ASD

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