

**SCREENHOUSE EVALUATION OF THE EFFICACY OF BIO-FUMIGANT CROPS IN THE  
MANAGEMENT OF FUSARIUM WILT OF TOMATO  
(*FUSARIUM OXYSPORUM* F. SP. *LYCOPERSICI*)**

<sup>1</sup>Haruna\* S. G., <sup>2</sup>Adebitan, S. A., <sup>3</sup>Gurama, A. U. and <sup>4</sup>Ahmed B. I

<sup>1</sup>Department of Crop Protection, Faculty of Agriculture, Bayero University Kano

<sup>2</sup>Department of Crop Science and Horticulture, Federal University Oye, Ekiti State

<sup>3</sup>Department of Agronomy, Faculty of Agriculture, Federal University Kashere, Gombe State

<sup>4</sup>Department of Crop Production, Faculty of Agriculture and Agricultural Technology,  
AbubakarTafawaBalewa University Bauchi

\*Corresponding Author: [sgharuna.cpp@buk.edu.ng](mailto:sgharuna.cpp@buk.edu.ng)

#### ABSTRACT

Fusarium wilt of tomato caused by *Fusariumoxysporum* f. sp. *lycopersici* is one of the most important diseases of tomato especially in the Savannah ecological zone of Nigeria. Management of the disease through conventional strategies are not completely effective due to the development of new pathogenic races and resistance to fungicides. Screen house experiment was conducted at Federal College of Horticulture DadinKowa to determine the efficacy of bio-fumigation in managing Fusarium wilt of tomato. The experiment was a 4 x 4 factorial experiment fitted into randomized complete block design, replicated three times. Bio-fumigation was done two weeks before transplanting tomato seedlings by amending 3.5 kg of sterile soil with 850g of poultry manure or cow dung and 200g of cabbage/ onion or 100 g of garlic, respectively. Soils were inoculated with conidial suspension of *Fusariumoxysporum* f. sp. *lycopersici* 24 hours before application of the organic amendments. Poultry manure and cabbage- amended soil significantly (P<0.01) showed lower disease incidence, severity and vascular discolouration on tomatoes grown on soil amended with poultry manure and cabbage in comparison with the other treatments. Higher tomato yield was obtained on tomatoes grown on poultry manure and cabbage - amended soil. This is attributable to low incidence/severity of Fusarium wilt and the released of essential nutrients by the decomposed organic amendments. Volatile toxic compounds emitted by the decaying cabbage residues also suppressed the growth of *Fusariumoxysporum* f. sp. *lycopersici*. The use of poultry manure and cabbage residues is recommended to be tested in the field to ascertain their efficacies against Fusarium wilt of tomato.

Key words: Bio-fumigant crops, cabbage, tomato, Fusarium wilt, poultry manure

#### INTRODUCTION

Tomato (*Solanumlycopersicum* L.) is one of the major vegetable crops widely cultivated in Nigeria where it has been an important component of the daily diets. The crop is rich in vitamins, minerals and antioxidant compound lycopene which play a significant role in human health due to its anti-cancer effect (Miller *et al.*, 2002). Nigeria is ranked as the 14<sup>th</sup> world producer and second leading producer in

Africa with average production of 1.7tonnes (FAO 2010). The production of the crop is hampered by so many pests and diseases. Fusarium wilt is one of the major fungal diseases of tomato caused by *Fusariumoxysporum* f. sp. *lycopersici*(Sacc.) Snyder & Hansen. The pathogen is a soil-inhabiting fungus highly destructive and difficult to control once established as reported by several researchers (Hadianet *al.*, 2011. The difficulty experienced in the management of the disease is attributed to the emergence of new pathogenic races (2 and 3), injudicious use of pesticides leading to the attenuation and death of antagonistic bio-control agents (Dewaardet *al.*, 1993).

Management of tomato wilt incited by *Fusariumoxysporum* f. sp. *lycopersici* under large scale production depends on the use of the fumigant methyl bromide which has been implicated in the cause of environmental hazards and destruction of beneficial microorganisms (Santos *et al.*, 2006). Apart from human and environmental concerns continuous use of chemicals may lead to the development of multi-resistant strains of the fungal phytopathogens as reported by Njueet *al.* (2012). These necessitate the search for eco-friendly ways that are effective and affordable in managing the disease. The objectives of this study is to evaluate the efficacy of applying animal manure and bio-fumigant crops as organic amendments in managing Fusarium wilt of tomato under screenhouse conditions.

#### MATERIALS AND METHODS

##### Experimental Site

Screen house experiment was conducted at Federal College of Horticulture, DadinKowa in Gombe State between the months of February and May in 2015. The site of the experiment is located on latitude 10° 18' N and longitude 11° 31' E in the Sudan Savannah ecological zone of Nigeria (Kowal and Knabe 1972).

##### Isolation of *Fusariumoxysporum* f sp. *lycopersici*

The pathogen was isolated from diseased tomato plants that had shown symptoms of wilting and brown discolouration of vascular vessels. The stems of the infected plant were washed in distilled water to remove any foreign materials. Five-millimetre pieces were cut, dipped in 70% alcohol and then sterilized in 0.5 % sodium hypochlorite solution for 3 minutes. Chloramphenicol at 0.5 mg per litre of molten PDA

was added to suppress bacterial growth. The dried sterile infected plant samples were placed on the centre of Petri dishes containing amended PDA media and incubated at 38 °C for seven days. Colonies showing morphology of *Fusarium* was sub-cultured for another 7 days on potato dextrose agar (PDA) to obtain pure cultures as described by Booth (1977). Spore suspension of *FOL* was prepared and adjusted to 10<sup>7</sup> spores/ml using haemocytometer. Pathogenicity test was done on tomato and pepper plants. *Fusarium* wilt symptoms was only recorded on tomato seedlings which was compared with those observed in the field and was found to be similar.

**Experimental set up and procedures**

Eight hundreds grams of poultry manure and cow dung were separately mixed with 4 kg of sterile soil while 200 g of cabbage and 100 g of garlic or onion were sliced into 2 – 3 cm and incorporated into the respective containers. The containers were covered with polythene sheet after moistening to field capacity and pots were left for 14 days in the screen house for decomposition. Before amending soil with animal manure and bio fumigant crops, pots containing soil were inoculated with 30 ml each with conidial suspension of the pathogen (10<sup>7</sup> conidia ml<sup>-1</sup>) based on the methods used by Misraket *al.* (2004). The experiment was laid out in a randomised complete block design which consisted of 16 treatments. Two bio-disinfectants; poultry manure and cow dung; CAMAZEB® as check and un-amended soil as control and three bio-fumigant crops (cabbage, onion and garlic) and un-treated soil. The treatments were replicated three times. The experiment was terminated at 13 weeks after transplanting. Data on number of leaves, branches and plant height was recorded bi-weekly. Disease incidence and severity were recorded at weekly intervals. Yield was measured in kg<sup>-1</sup> plant, shoot and root weights (g<sup>-1</sup> plant) were assessed at final harvest.

*Fusarium* wilt severity was determined using the following scale: 1 = no symptoms, 2 = plant showing yellow leaves and wilting (1 – 20%), 3 = plant showing yellow leaves and wilting (21 – 40%), 4 = plant showing yellow leaves and wilting (41 – 60%), 5 = plant showing yellow leaves and wilting (61 – 80%), 6 = plant showing yellow leaves and wilting (81 – 100%) or death (Sibounnavong *et al.*, 2012). Vascular discolouration of the stem was measured using a Scale of 1 – 9 (Marley and Hillocks, 1996). Where 1 = no browning, 2 = browning only around stem base, 3 = patchy browning but limited below the first stem node, 4 = strong browning but limited below the first stem node, 5 = browning visible and extending above the first stem node, 6 = browning visible above first stem node and in up to half the total number of nodes, 7 = browning visible in more than half the stem node, 8 = strong vascular browning in all but uppermost internode, 9 = strong browning throughout the vascular tissue. Data on disease incidence and severity were transformed using arcsine and square root transformations, respectively. Analysis of variance was used to analyse the data using GenStat 17<sup>th</sup> Edition, Release 17.1 (2014). Treatment means were separated using LSD at 5% level of significance.

**RESULTS**

**Effect of bio-fumigation on incidence and severity of *Fusarium* wilt of tomato**

At 7 weeks after transplanting tomatoes grown on soil treated with poultry manure exhibited same effect with those tomatoes on CAMAZEB® - amended soil which significantly (P≤0.001) had lower disease incidence (15.6%) than tomatoes on cow dung treated soil (16.1%) and those in the control treatments (35.4%) as shown on Table 1. However, the efficacies of PM and CD outstandingly

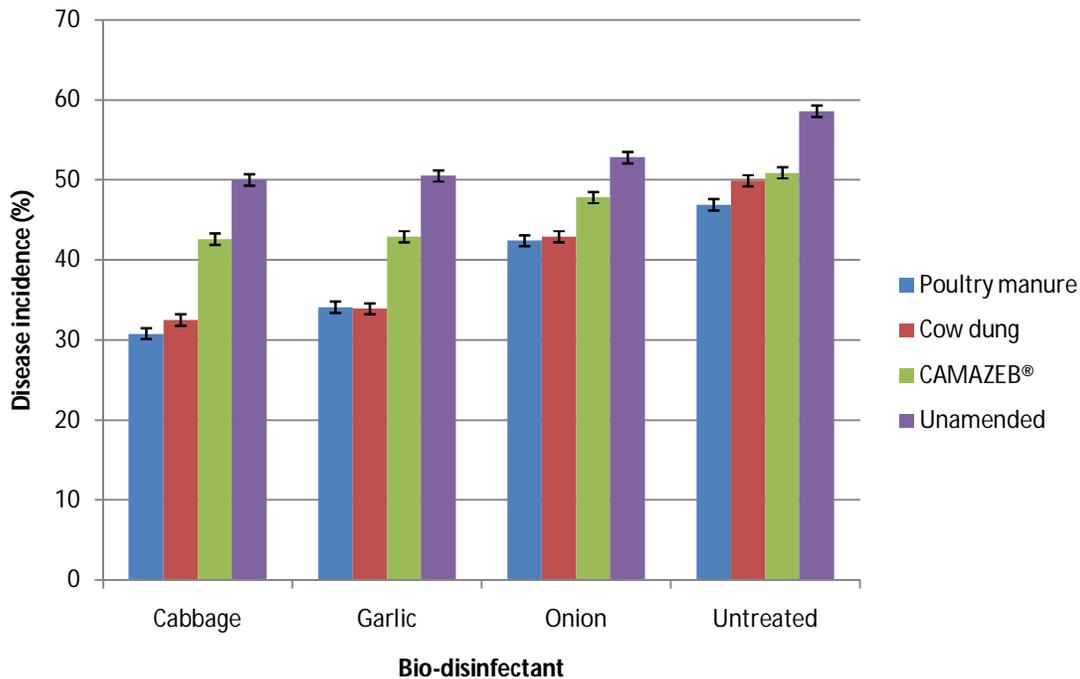
Table 1: Effect of bio-fumigation on incidence of tomato wilt caused by *Fusarium oxysporum* f. sp. *lycopersici*

Treatment	Weeks after transplanting						
	7	8	9	10	11	12	13
<b>Bio-disinfectant (Bio-D)</b>							
Poultry manure	15.3	33.9	40.8	39.3	35.4	35.1	33.4
Cow dung	16.1	35.8	41.2	39.9	37.8	36.7	35.0
CAMAZEB®	15.6	40.1	46.8	45.4	45.0	44.9	44.8
Un-amended soil	35.4	44.8	51.0	51.1	51.7	51.9	51.3
LSD (P≤0.01)	0.48	0.55	0.34	0.57	0.83	0.29	0.54
<b>Bio-fumigant crop (Bio-F)</b>							
Cabbage	7.8	36.9	43.0	41.8	40.2	39.9	39.7
Garlic	8.4	37.5	44.4	43.3	41.6	41.0	40.0
Onion	32.6	39.3	45.7	43.9	41.8	41.8	40.8
Un-treated soil	33.6	41.7	45.0	45.4	45.7	46.8	47.6
LSD (P≤0.01)	0.33	0.52	0.31	0.20	0.67	0.21	0.29
<b>Interaction</b>							
Bio-D X Bio-F	*	**	**	**	**	**	**

CAMAZEB® = (60% Mancozeb + 40% Carbendazim WP) as check, Un-amended soil = control for bio-disinfectant, Un-treated soil = control for bio-fumigant crops; \* significant at 5%, \*\* significant at 1%.

increased at the later stages of tomato growth until harvested at 13 WAT. Amendment with bio-fumigant crops significantly ( $P \leq 0.01$ ) led to low disease incidence. Tomatoes grown on soil amended with cabbage showed lower disease incidence than garlic and onion –amended soils, respectively as indicated at week 8 – 10 after transplanting. At 11 – 13 garlic and onion – amended soils significantly ( $P \leq 0.01$ ) had same effect on Fusarium wilt incidence. There was significant interaction between amendments with animal manure and bio-fumigant crops on the disease incidence@  $P \leq 0.01$ . Figure 1 shows interaction between the use of animal manure (bio-disinfectants) and bio-fumigant crops on incidence of Fusarium wilt of tomato at harvest (13

WAT). Tomatoes transplanted on soil amended with poultry manure and cabbage (33.8%) or garlic (34.1%) exhibited statistically same effect on the disease incidence. They recorded lower disease incidence than tomatoes grown on soil amended with PM and onion (44.3) and those grown on soil treated with only PM (45.1%). Similar observation was made on tomatoes grown on soil amended with cow dung and bio-fumigant crops in the following order: CD + cabbage (33.9%) and CD + garlic (34.5%) < CD + onion (44.7%) < CD + untreated (45.4%). Tomatoes on soil not amended with either animal manure or bio-fumigant crop recorded highest Fusarium wilt incidence (50.6%) compared to the other treatments



**Figure 1: Interaction between of bio-disinfectants and bio-fumigant crops on incidence (%) of Fusarium wilt of tomato at harvest**

Analysed results presented on Table 2 shows the effect of amendment with animal manure and bio-fumigant crops on Fusarium wilt of tomato. At the early stages of Fusarium wilt development (7 – 9 WAT), the effect of CAMAZEB® on the disease severity was at par with poultry manure and cow dung. However, at 10 – 13 WAT, tomatoes transplanted on soil amended with PM and CD showed lower severity than those recorded on tomatoes from soil amended with CAMAZEB® which also differed significantly with those in the

control treatment. The three bio-fumigant crops (cabbage, garlic and onion) exhibited same effect on Fusarium severity but, differed significantly ( $P \leq 0.01$ ) with tomatoes grown on un-amended soil (7 – 9 WAT). However, at 10 and 11 WAT, amendment with garlic (2.2) and onion (2.3) exerted same on Fusarium wilt severity. While as tomatoes reached 12 and 13 weeks after transplanting, lower severity was obtained on those tomatoes grown on soil amended with cabbage, followed by those tomatoes from soil amended with garlic and onion,

Table 2: Effect of bio-fumigation on severity of tomato wilt caused by *Fusarium oxysporum* f. sp. *lycopersici*

Treatment	Weeks after transplanting						
	7	8	9	10	11	12	13
<b>Bio-disinfectant (Bio-D)</b>							
Poultry manure	1.2	1.4	1.6	2.1	2.2	2.6	2.8
Cow dung	1.2	1.5	1.8	2.1	2.2	2.7	2.9
CAMAZEB®	1.2	1.4	1.6	2.5	3.0	3.2	3.5
Un-amended soil	1.5	2.2	2.6	2.9	3.5	3.8	4.1
LSD (P≤0.05)	0.21	0.55	0.43	0.38	0.43	0.46	0.57
<b>Bio-fumigant crop (Bio-F)</b>							
Cabbage	1.1	1.2	1.4	1.7	1.8	2.6	2.7
Garlic	1.2	1.5	1.9	2.2	2.2	2.9	3.0
Onion	1.3	1.7	1.9	2.3	2.3	3.2	3.4
Un-amended soil	1.5	2.2	2.3	2.7	2.7	3.6	4.1
LSD (P≤0.01)	0.14	0.36	0.35	0.31	0.36	0.20	0.28
<b>Interaction</b>							
Bio-D x Bio-F	ns	ns	ns	ns	ns	*	*

CAMAZEB® = (60% Mancozeb + 40% Carbendazim WP) as check, Un-amended soil = control for bio-disinfectant, Un-treated soil = control for bio-fumigant crops; \* significant at 5%, \*\* significant at 1%.

respectively. Amendment with CAMAZEB® resulted in lower severity compared to those in the control treatments. Significant interaction (P≤0.05) was also recorded between the use of animal manure and bio-fumigant crops on disease severity.

Figure 2 depicted interactions between animal manure and bio-fumigant crops on Fusarium wilt severity at 13 WAT. Growing tomato on soil amended with poultry manure and cabbage led to

significantly (P≤0.01) lower severity (1.28) than tomatoes grown on soil amended with poultry manure + garlic (1.52) which showed same effect with amendment with cow dung + cabbage (1.52) and cow dung + garlic (1.59) on the disease severity.. Tomato on the control treatments showed higher disease severity compared to the remaining treatments.

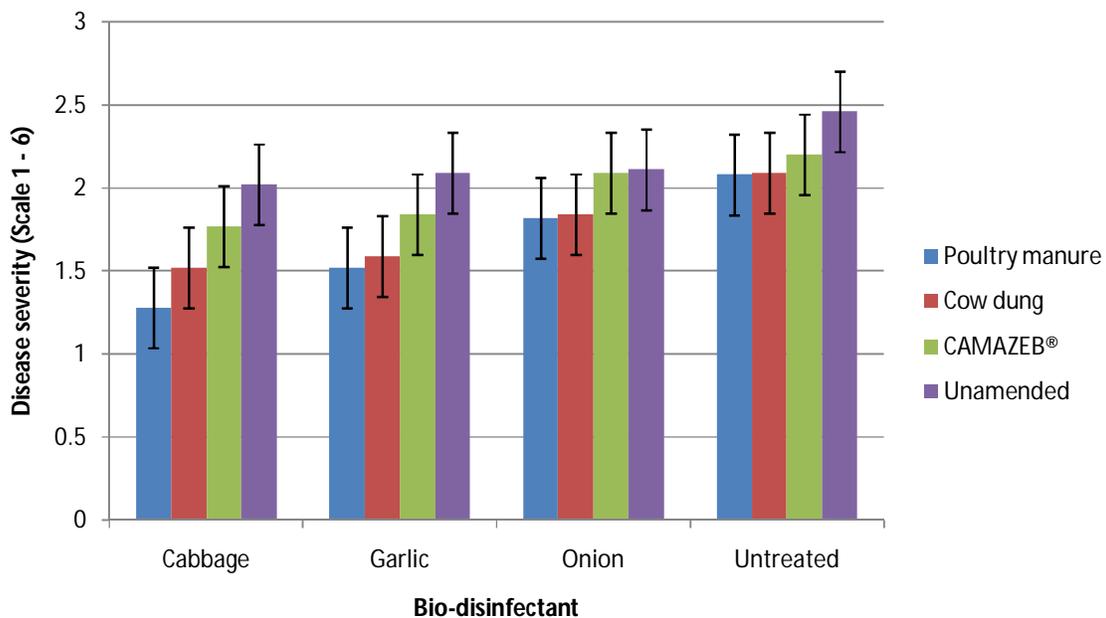


Figure 2: Interaction between application of bio-disinfectants and bio-fumigant crops on severity of Fusarium wilt of tomato at harvest

**Effect of bio-fumigation on vegetative growth of tomato infected with *Fusarium wilt***

Soil amendment with bio-disinfectants and bio-fumigant crops significantly ( $P \leq 0.01$ ) improved the height of the tomato plant as shown on Table 3. Application of poultry manure and cow dung at 2 and 4 weeks after transplanting were at par in producing taller tomatoes than CAMAZEB<sup>®</sup> and those in the control treatment, respectively. However, at 4 - 10 WAT, poultry manure and cow dung were at par in

producing taller tomatoes than those grown on soil amended with CAMAZEB<sup>®</sup>.

Table 4 shows increased in Leaf production with bio-fumigation. Application of poultry manure as soil amendment led to significant ( $P \leq 0.01$ ) production higher leaves than the use cow dung and CAMAZEB<sup>®</sup> throughout the growth stages of tomato. Least numbers of leaves were produced by tomatoes grown on un-amended soil. Similarly, soil amended with cabbage

Table 3: Effect of bio-fumigation on plant height (cm) of tomato infected with tomato wilt caused by *Fusarium oxysporum* f. sp. *lycopersici*

Treatments	Weeks after transplanting				
	2	4	6	8	10
<b>Bio-disinfectants (Bio-D)</b>					
Poultry manure	19.3	27.6	44.2	62.3	80.2
Cow dung	18.7	27.4	43.9	62.4	78.8
<sup>a</sup> CAMAZEB <sup>®</sup>	18.1	24.4	41.0	49.3	62.2
Un-treated soil	16.8	23.2	39.8	40.6	52.6
LSD ( $P \leq 0.01$ )	0.32	0.41	0.50	0.33	0.55
<b>Bio-fumigant crops (Bio-F)</b>					
Cabbage	19.5	27.2	43.8	55.2	71.0
Garlic	18.7	26.3	42.9	51.4	69.0
Onion	18.0	25.1	41.7	48.9	67.2
Un-amended soil	16.7	24.0	40.5	46.7	64.6
LSD ( $P \leq 0.01$ )	0.26	0.27	0.33	0.50	0.44
Interaction					
Bio-D x Bio-F	**	**	**	**	**

CAMAZEB<sup>®</sup> = (60% Mancozeb + 40% Carbendazim WP),<sup>b</sup>Other interactions are not significantly different at  $P \leq 0.005$ .

Table 4: Effect of bio-fumigation on leaf production of tomato infected with tomato wilt caused by *Fusarium oxysporum* f. sp. *lycopersici*

Treatment	Weeks after transplanting				
	2	4	6	8	10
<b>Bio-disinfectants (Bio-D)</b>					
Poultry manure	33.2	52.5	72.3	113.7	46.0
Cow dung	26.9	50.2	63.9	104.8	36.8
CAMAZEB <sup>®</sup>	19.8	32.8	54.8	96.8	28.9
Un-treated soil	15.6	28.0	35.3	77.8	09.3
LSD ( $P \leq 0.01$ )	2.45	0.80	3.38	2.54	2.63
<b>Bio-fumigant crops (Bio-F)</b>					
Cabbage	27.4	45.2	63.9	105.8	137.9
Garlic	24.9	43.0	60.2	101.9	134.2
Onion	22.3	38.9	54.8	96.5	128.9
Un-amended soil	20.8	36.3	47.2	88.8	120.2
LSD ( $P \leq 0.01$ )	1.41	1.23	1.43	0.99	1.24
Interaction					
Bio-D x Bio-F	ns	ns	**	**	**

CAMAZEB<sup>®</sup> = (60% Mancozeb + 40% Carbendazim WP),<sup>b</sup>Other interactions are not significantly different at  $P \leq 0.005$ , \* significant at 5%, \*\* significant at 1%, ns = not significant.

significantly ( $P \leq 0.01$ ) enhanced leaf production, followed by amendments of soil with garlic and onion bulbs, respectively.

Tomato profusely produced branches when grown on soil amended with animal manure and bio-fumigant crops as presented on Table 5. At 2 WAT, poultry manure and cow dung showed same effect on branching of tomato. Poultry manure and cow dung significantly produced more branches than those grown on soil amended with CAMAZEB®. However, at 4 – 8 WAT, poultry manure led to the production

of more number of branches than those grown on cow dung and CAMAZEB®, respectively. The least number of branches were produced by tomatoes grown on un-amended soils. Amending soil with cabbage led to higher number of branches than the use of garlic and onion, respectively (2 WAT). At 4 – 8 WAT, same results was recorded with more numbers of branches on tomatoes grown on soil amended with cabbage, followed by garlic and onion, respectively.

Table 5: Effect of bio-fumigation on branching of tomato infected with Fusarium wilt

Treatment	Weeks after transplanting				
	2	4	6	8	10
<b>Bio-disinfectant (Bio-D)</b>					
Poultry manure	9.7	11.6	14.4	17.3	19.4
Cow dung	9.7	11.3	14.2	16.6	18.4
CAMAZEB®	7.3	9.2	12.0	13.5	15.5
Un-treated soil	5.2	7.2	9.5	11.1	13.1
LSD ( $P \leq 0.01$ )	0.78	1.00	0.46	0.59	0.73
<b>Bio-fumigant crops (Bio-F)</b>					
Cabbage	8.9	10.8	13.6	15.5	17.6
Garlic	8.3	10.1	12.2	14.4	16.7
Onion	7.5	9.4	11.5	13.1	15.4
Un-amended soil	6.1	8.9	10.6	12.7	14.5
LSD ( $P \leq 0.01$ )	0.53	0.50	0.56	0.58	0.74
Bio-D x Bio-F	ns	ns	ns	ns	ns

CAMAZEB® = (60% Mancozeb + 40% Carbendazim WP),<sup>b</sup> Other interactions are not significantly different at  $P \leq 0.05$ , \* significant at 5%, \*\* significant at 1%, ns = not significant.

**Effect of bio-fumigation on yield of tomato infected with Fusarium wilt**

Greater yield was recorded on tomatoes grown on soil amended with poultry manure ( $1.63 \text{ kg plant}^{-1}$ ), cow dung ( $1.61 \text{ kg plant}^{-1}$ ) and CAMAZEB® ( $1.57 \text{ kg plant}^{-1}$ ), respectively as shown on Table 6. Low yield was recorded on the control treatment ( $1.43 \text{ kg plant}^{-1}$ )

<sup>1</sup>). Soil amended with cabbage significantly ( $P < 0.01$ ) produced tomatoes with the highest yield ( $1.65 \text{ kg}^{-1} \text{ plant}$ ) followed by tomatoes grown on soil amended with garlic ( $1.62 \text{ kg}^{-1} \text{ plant}$ ) and onion bulbs ( $1.60 \text{ kg}^{-1} \text{ plant}$ ). Least yield was obtained on soil not treated with bio-fumigant crop ( $1.37 \text{ kg}^{-1} \text{ plant}$ ).

Table 6: Effect of bio-fumigation on yield of tomato and vascular discolouration of tomato stem

	Yield ( $\text{kg}^{-1} \text{ plant}$ )	vascular discolouration (Scale 1 – 9) <sup>b</sup>
	<b>Bio-disinfectant</b>	
Poultry manure	1.63	3.9
Cow dung	1.61	4.3
CAMAZEB®	1.57	5.7
Un-treated soil	1.43	6.5
LSD ( $P \leq 0.01$ )	0.030	0.74
<b>Bio-fumigant crops</b>		
Cabbage	1.65	4.5
Garlic	1.62	4.6
Onion	1.60	5.3
Un-amended soil	1.37	6.0
LSD ( $P \leq 0.01$ )	0.010	0.49
<b>Interaction</b>		
Bio-D x Bio-F	**	*

CAMAZEB® = (60% Mancozeb + 40% Carbendazim WP), \* significant at 5%, \*\* significant at 1%.

## DISCUSSION

Incidence and severity of Fusarium wilt of tomato was significantly influenced by combine application of poultry manure and cabbage. This could be attributed to the release of biocidal volatiles by the bio-fumigant crop as it decomposed as reported by Matthiessen and Kirkegaard (2006). In a study conducted by Ramirez-Villapudua and Munnecke (1988), incorporation of cabbage decreased the severity of *Fusariumoxysporum*f.sp. *conglutinans* due to the released some fungitoxic gases during the decomposition. Garlic also contained biologically active compounds that inhibited the growth of a wide range of soil borne fungal pathogens (Avato *et al.*, 2000). This finding conformed to the work of Ramsey *et al.* (2007) who effectively used garlic extract and suppressed the growth of *Pythium* spp., *Phytophthora* spp., *Rhizoctonia solani*, *Fusariumoxysporum*, and *Thielaviopsis basicoli*.

Application of poultry manure and cabbage amended soil inhibited the growth of Fusarium wilt pathogen and enhanced vegetative growth and yield of tomato. This could be due to presence of essential nutrients on the decayed poultry manure and cabbage which stimulated the growth and proliferation of bio-control agents and increased the soil organic carbon content used as energy source for the microbial processes (Misra *et al.*, 2008). Several researchers have confirmed the antimicrobial activity of cabbage against wide range of fungal and bacterial pathogens (Matthiessen and Kirkegaard 2006). Decomposition of plant materials and animal manure enhanced the activities of soil micro-organisms which had adverse effect on Fusarium wilt pathogen and improved tomato growth and yield. This conforms with the findings of Smolinska, (2000) and Pakeerathan *et al.* (2009) who reported dual effect of combined use of animal manure and plant materials for improvement of diseases control and plant growth. Combined application of poultry manure or cow dung with cabbage or garlic could therefore be use as integrated management alternative to the application of pesticide in managing *Fusarium* wilt in screen or green houses.

## CONCLUSION

From the studies conducted it was evident that combined application of poultry manure and cabbage residues effectively managed Fusarium wilt and also increased tomato growth and yield. This technology could therefore be use as an integrated management package against tomato wilt incited by *Fusarium oxysporum* f. sp. *lycopersici* and the package could also be use as bio-fertilizer under screen or green house conditions.

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