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Effect of compost extracts applied at different times of transplanting tomato seedlings on *Fusarium* wilt of tomato

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Abstract

Pot experiment was conducted at the screenhouse of Crop Production Programme, Abubakar Tafawa Balewa University, Bauchi, to study the efficacy of compost extracts applied at different times of transplanting tomato seedlings for suppression of *Fusarium* wilt on the crop. Three compost extracts: poultry manure based compost extracts (PMCE), cow dung based compost extracts (CDCE) and neem-leaf based compost extract (NLCE) were applied at one week before seedling transplanting (1WBT), at transplanting (ATRA), one week after transplanting (1WAT) and two weeks after transplanting (2WAT). The compost extracts were tested alongside a synthetic fungicide Team® for their efficacy on *Fusarium* wilt. Distilled water was used as control. The treatments were laid in a randomized complete block design (RCBD) which was replicated thrice. Each of the compost extracts was applied at 1: 5 (compost: water) at the rate of 100 litres ha^{-1}. The standard fungicide Team® was applied as soil drench at the rate of 100g / 100 litres of water. The result indicated that PMCE and CDCE applied at 1WBT and ATRA significantly reduced disease incidence and severitycompared with NLCE.Team was only effective in reducing incidence between the 4^{th} and 5^{th}week after transplanting. From this work, application of PMCE and CDCE at 1WBT is recommended as this will reduce the use of synthetic pesticide and additionally serve as an eco-friendly way of managing the disease. However, further research is necessary to identify the bio-control agents present in the compost extracts.

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Introduction

Fusarium wilt of tomato caused by *Fusarium oxysporum* (Schlecht.) f. sp. *lycopersici* (Sacc.), is known as one of the most devastating diseases of tomato worldwide (Mes *et al.,* 1999), and has caused important yield losses in Nigeria. The pathogen is a soil-inhabiting fungus which is resistant to chemical fungicides (Sibounnavong *et al.*, 2010).

The disease is responsible for significant economic yield loss of up to 10-50 % (Adebayo and Ekpo, 2004; Borrero *et al.*, 2004; Larkin and Fravel, 1988), and also up to 80% loss in severe cases (McGrath *et al.*, 1987; Malhotra and Vashistha 1993).The disease caused by this fungus is characterized by wilted plants, yellowed leaves and minimal or absent crop yield. The pathogen enters tomato through the roots and causes yellowing of the oldest leaves, often on only one side of the plant (Abdel-monaim 2012).

Currently, effective control measures for the disease are poorly documented. Hence, most efforts are directed towards the prevention of the disease. Fusarium wilt is not easily managed through crop rotation because the fungus produces resting spores (chlamydospores) that survives in soil even in the absent of suitable host. The use of resistant varieties is one of the most effective alternative approaches for controlling wilt disease (Hornouchi *et al.*, 2007; Silva and Bettiol, 2005). However, the breakdown of resistance in the face of high pathogenic variability in the pathogen population necessitates the need to develop alternative strategies to manage this disease. Similarly, the use of chemicals is not always effective because the pathogenic fungus may develop resistance to chemical fungicides (Silva and Bettiol, 2005). Hence, this study was conducted in potted soil inside the screen-house to evaluate the effectiveness of compost extracts applied at different times of transplanting tomato seedlings on *Fusarium oxysporum f. sp. lycopersici*, the causal agent of Fusarium wilt of tomato.

Materials and methods

Isolation of Fusarium Wilt Pathogen

The pathogen *Fusarium oxysporum* f. sp. *lycopersici* was isolated from the stem and roots of naturally infected tomatoes grown in DadinKowa, Gombe State in Nigeria. Small pieces of vascular tissues were surface-sterilized by soaking in 0.5 % NaOCl for 30 – 60 seconds, and then washed in three changes of sterile distilled water. The pieces were placed on acidified (with lactic acid) potato dextrose agar (APDA) sterilized and contained in Petri dishes which had been sterilized in an autoclave at 121°C for 15minutes. The inoculated plates were arranged on the bench at room temperature (27 ± 2 °C) under fluorescent light in the laboratory for seven days as described by Wong (2003). Using a No 3 cork-borer, agar plugs were taken from the actively growing region of the mycelial growth for sub-culturing in other sterilized Petri dishes containing APDA and left for 14 days under fluorescent light at the room temperature. Sub-culturing was done and repeated until the pure culture of the pathogen was obtained and kept as stock culture for subsequent use.

Inoculation of tomato plants

Spore suspension of the pathogen were prepared from 21-day-old culture by adding 10 ml of sterile distilled water to the Petri dish and the culture was scrapped with a rubber spatula. The inoculum concentration was adjusted with a haemocytometer to 10^7 conidiaml^{-1} suspension.

The seedlings of UC 82B (susceptible variety that is commonly grown by the farmers in the study area) were washed in distilled water to remove soils attached to the roots, and immersed in 30mls conidial suspension of the pathogen as earlier prepared. The experiment was laid out in a randomized complete block design in the screen house of Crop production programme, Abubakar Tafawa Balewa University, Bauchi. The treatments consisted of poultry manure based compost extract (PMCE), neem-leaf based compost extract (NLCE), cow dung based compost extract(CDCE), TEAM® (Carbendazim 12% + Mancozeb 63% W.P.) and distilled water (control). Compost extracts were applied at four different times: one week before transplanting tomato seedlings

(1WBT), at transplanting (ATRA), one week after transplanting (1WAT), and two weeks after transplanting (2WAT). The fungicide was applied once at transplanting as soil drenched at the rate of 100 grams/100 litres of water, while compost extracts were applied as soil drench at the rate of 0.4 L per plant. All the treatments were replicated three times.

Data collection and Statistical Analysis

Disease severity was scored according to Sibounnavong *et al.* (2010) as follows: 1= no symptom, 2= symptom on leaves covered 1- 20% with lower leaves yellow; 3= symptom on leaves covered 21- 40% with plants showing yellowing or wilting of two leaves; 4= symptom on leaves covered 41-60% with plants showing yellowing/wilting of two or more leaves, 5= symptom on leaves covered 61- 80% with plants showing vessel browning nearly to the leader shoot and most leaves wilting, except the leader shoot, 6= symptom on leaves covered 81- 100% with plants showing wilting of leaves up to the shoot or becoming dead.

Disease incidence was also recorded as (Number of diseased leaf / total number of leaves assessed) x 100. Data collected were subjected to statistical analysis using GenStat Release 10.3DE (PC/Windows 7), Copyright 2011, VSN International Ltd. (Rothamsted Experimental Station). Treatment means were separated using LSD at p = 0.05.

Results

Effect of compost extracts applied at different times of transplanting tomato seedlings on the incidence of Fusarium wilt

Table 1 shows *in vivo*effect of compost extracts and transplanting tomato seedlings at different timeson the incidence of tomato wilt. There was significant difference (*P*=0.05) among the treatments on the disease incidence at the different stages of the crop's growth. At 5 WAT, tomates treated with fungicide showed low disease incidence. These were followed by those tomatoes treated with PMCE and CDCE which significantly exhibited lower Fusarium incidence than tomatoes not treated.

Table 1. Effect of compost extracts and time of transplanting tomato seedlings on incidence (%) of Fusarium wilt pathogen.

Treatments	Weeks after transplanting				
	5	6	7	8	9
Compost extract [α]					
PMCE	25.0	36.7	40.6	37.5	32.9
NLCE	35.8	40.8	45.8	43.3	42.1
CDCE	25.0	35.8	38.3	37.9	33.8
TEAM®	30.8	36.3	48.3	60.0	67.1
CNTL	40.0	48.8	60.0	69.2	76.7
LSD (P=0.05)	1.32	2.41	2.35	2.26	2.40
Time of transplanting *					
1WBT	26.0	33.7	42.0	47.0	47.0
ATRA	28.0	38.3	44.7	47.7	48.7
1WAT	35.3	42.7	50.0	51.0	53.0
2WAT	36.0	44.0	51.7	51.7	54.3
LSD (P=0.05)	1.18	2.15	2.10	2.02	2.15

α PMCE: poultry manure based compost extract, NLCE: neem-leaf based compost extract, CDCE: cow dung based compost extract, TEAM®:Carbendazim 12% + Mancozeb 63% W.P., CNTL: control.

*1WBT: compost extracts applied at one weeks before transplanting, ATRA: compost extracts applied at transplanting, 1WAT: compost extracts applied at one week after transplanting, 2WAT: compost extracts applied at two weeks after transplanting.

Application of PMCE, CDCE and TEAM on tomatoes at 6 WAT, significantly showed same effect on the disease incidence which induced lower incidence than the use of NLCE. However, at 7th to 9th WAT, tomatoes treated with PMCE and CDCE induced significantly lower incidence than tomatoes treated with NLCE and TEAM respectively.

Soil drenching with compost extracts at one week prior to transplanting (1WBT) and at transplanting (ATRA) showed a progressive decrease in the incidence of the disease. Whereas, tomatoes treated at at one week after transplanting (1WAT) and at two weeks after transplanting (2WAT) exhibited same effect on the disease with high disease incidence.

Effect of compost extract application at different times of transplanting seedlings on the severity of Fusarium wilt of tomato

Application of PMCE and CDCE resulted in lower severity of *Fusarium* wilt at the various stages of tomato growth than NLCE and TEAM, respectively (Table 2). At 5 WAT, NLCE and TEAM have same effect on disease severity. However, from the 6th to the 9thWAT, PMCE and CDCE significantly induced lower disease severity than NLCE and TEAM, respectively. In similarity to what was earlier reported for disease incidence, application of compost extracts at 1WBT induced significantly the lower disease severity index. This was followed by the application of compost extracts at ATRA. Compost extracts applied at 1WAT and 2WAT did not differ significantly from each other in reducing wilt severity indices on tomato plants.

Table 2. Effect of compost extracts and time of transplanting tomato seedlings on severity [a] of Fusarium wilt pathogen.

Treatments	Weeks after transplanting				
	5	6	7	8	9
Compost extract [α]					
PMCE	2.17	2.54	24.6	2.42	2.26
NLCE	2.85	3.32	3.28	3.10	2.98
CDCE	2.24	2.77	2.57	2.54	2.52
TEAM®	3.25	3.37	3.62	3.78	3.95
CNTL	3.39	3.49	3.78	3.94	4.03
LSD (P=0.05)	0.056	0.181	0.182	0.051	0.050
Time of transplanting *					
1WBT	2.57	2.91	3.09	3.06	3.03
ATRA	2.67	3.07	3.19	3.10	3.07
1WAT	2.91	3.20	3.23	3.25	3.23
2WAT	2.97	3.20	3.27	3.24	3.25
LSD (P=0.05)	0.050	0.162	0.163	0.045	0.044

[b] PMCE: poultry manure based compost extract, NLCE: neem-leaf based compost extract, CDCE: cow dung based compost extract, TEAM®:Carbendazim 12% + Mancozeb 63% W.P., CNTL: control.
[c]1WBT: compost extracts applied at one weeks before transplanting, ATRA: compost extracts applied at transplanting, 1WAT: compost extracts applied at one week after transplanting, 2WAT: compost extracts applied at two weeks after transplanting.

Figure 1 shows the interaction effect between the different times of transplanting the seedlings and application of compost extracts on the severity of *Fusarium oxysporum* f. sp. *lycopersici*. Tomato plants whose seedling roots were treated with the compost extracts and fungicide at different times of seedling transplanting differed significantly at *P*=0.05. Infected tomato plants whose roots were treated with PMCE and CDCE at 1WBT showed lower disease severity indices compared to other stages of transplanted tomato seedlings whose roots were similarly treated with the extracts. These were followed by those tomatoes whose roots were drenched with NLCE at 1WBT and ATRA. Tomato plants whose roots were treated with fungicide were statistically similar to those in the control, and they both exhibited the highest severity indices at 1 and 2WAT.

Effect of compost extracts and time of transplanting seedlings on plant height of tomatoes inoculated with Fusarium oxysporum f. sp. lycopersici

As presented in Figure 2 (A), all the extracts were significantly similar in their effects on the pathogen resulting in production of tomatoes with similar height. However, they were different from the plants on which TEAM was applied and those that were maintained as control. As observed for the control plants, TEAM generally had no significant effect on plant height. Plant height was increased in response to application of compost extracts at the different times of tomato seedling transplanting, especially from the 3rd WAT (Figure 2A). However, no significant difference in plant height of the treated tomato plants was observed in the application of the extracts used in the study at the different times of transplanting the seedlings.

Effect of compost extracts and time of transplanting seedlings on branching of tomatoes inoculated with Fusarium wilt pathogen

Figure 2B shows the effect of compost extracts application at different times of transplanting tomato seedlings on the branching of tomatoes inoculated with Fusarium wilt pathogen. It was observed that all the compost extracts significantly (p =0.05) mitigated the effect of the pathogen on tomato plants which resultantly produced more branches than in plants whose roots were drenched with fungicide and those left untreated.

The result showed that the application of compost extracts at different transplanting periods differed significantly at *P*=0.05 (Figure 2B). At 2 and 3WAT, tomatoes on which compost extracts were applied at 1WBT and at ATRA exhibited higher number of branches than those treated with compost extracts at 1 and 2 WAT, whose plants separately had the same effect on branching. During sampling, from 3, 4 and 5WAT, increasingly higher number of branches were recorded on plants which received compost extracts at one 1WBT and ATRA, respectively.

Effect of compost extracts applied at different times of transplanting seedlings on leaf production of tomatoes inoculated with Fusarium wilt pathogen

The results in Figure 2C showed that application of PMCE and CDCE from 4 WAT significantly induced higher leaf production than applying NLCE and TEAM both of statistically the same effect on leaf production. Tomato plants whose seedling roots not treated had the least number of leaves.

Tomatoes seedlings whose were roots were drenched with the compost extracts at 1WBT, and at ATRA, produced significantly higher number of leaves than those treated at 1WAT and 2WAT, respectively.

Effect of compost extracts and time of seedling transplanting on the number of flower truss from tomatoes inoculated with Fusarium wilt pathogen

Results on Figures 3A and 3B indicated that application of PMCE and CDCE resulted in the significant (p=0.05) production of higher number flower trusses and flowers, compared to production from tomatoes that were treated with NLCE. The least production of flower trusses and flowers was obtained on tomatoes treated with TEAM, and on the untreated ones.

Compost extracts applied at 1WBT and at ATRA were statistically same and produced significantly (*P*=0.05) higher number of flower trusses and flowers that those treated at 1WAT and 2WAT, respectively.

Effect of compost extracts and times of transplanting seedlings on fresh shoot and root weight of tomatoes inoculated with Fusarium wilt pathogen

From Figure 3C tomatoes with the heaviest roots (52.5g) and shoots (343.6g) were obtained with the application of PMCE. These values compared favorably well with application of CDCE on plants which had their roots weights as 49.3g and shoot weight as 341.0g. Application of NLCE produced plants with lighter roots 43.5g and shoots (332.2g). Untreated tomatoes did not differ significantly (*P*=0.05) from those on which TEAM was applied with respect to their root and shoot weights.

Similarly, compost extracts applied at 1WBT and ATRA led to significantly (p=0.05) heavier development of fresh shoots and roots (46.1g, and 330g) and (43.9g, and 326g), respectively, compared to those that were treated at 1WAT and 2WAT which exhibited similar effects in reference to the yield parameters.

Discussion

Results indicated that compost extracts applied at one week before transplanting and at transplanting exhibited higher disease suppression. This conforms to the findings of Kerkeni *et al.* (2008) and Segarra *et al.* (2009) who reported successful control of *Fusarium oxysporum* f. sp. *radisis-lycopersici* and *Erysiphe polygoni* (powdery mildew) in tomato by foliar application of compost tea prior to transplanting. Application of compost extracts before transplanting was reported to induce systemic acquired resistance in cucumber and Arabidopsis (Zhang *et al.*, 1998). Similar results were also reported on the suppression of tomato wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* with poultry manure based compost extracts applied at transplanting (Adebitan *et al.*, 2011; Haruna *et al.*, 2011).

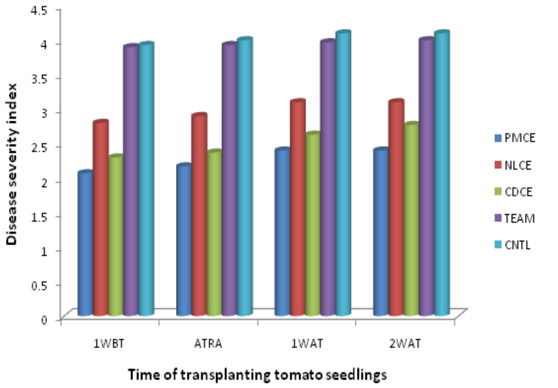

Fig. 1. Effect of interaction between compost extracts and time of transplanting Tomato seedlings on the severity of Fusarium wilt fungus.

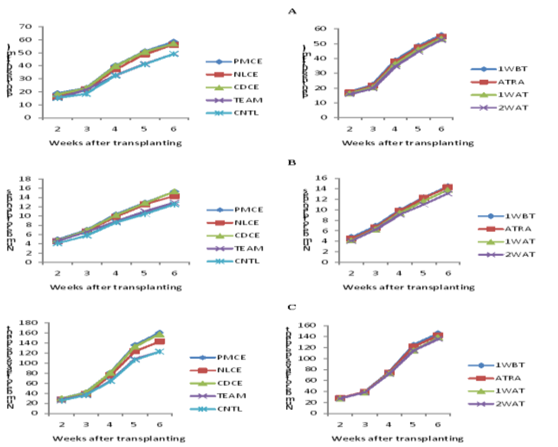

Fig. 2. Effect of compost extracts and time of application on plant height (A), branching (B) and leaf production (C) of tomato inoculated with Fusarium wilt pathogen.

The superiority of NLCE on fungicide against *Fusarium oxysporum* f. sp. *lycopersici* may be attributed to antimicrobial compounds such as nimolicinole, isolimolicinolide, azadirachtin, azadirachtol, nimilinone, nimibiocinol, nimbicinone and nimocine contained in neem plant as outlined by Tewari (1992) and Yar' adua (2007).

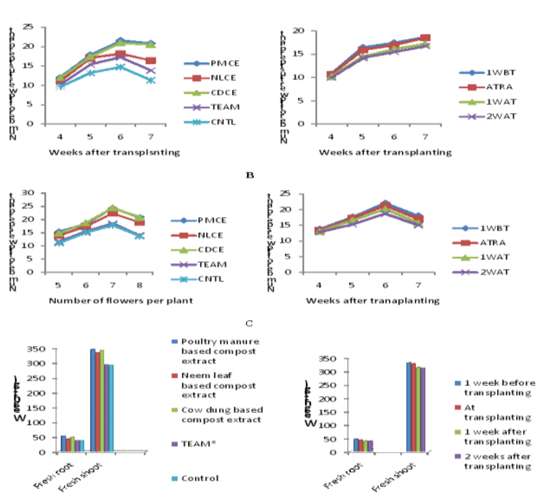

Fig. 3. Effect of compost extracts and time of seedling transplanting on number of flower truss per plant (A), flowering (B), fresh root and shoot weight (C).

The result on tomato yield obtained in this study is contrary to the findings of Ghorbani *et al.* (2006) and Koocheki *et al.* (2008) who reported that compost extracts did not cause significant increase in tomato yield. Application of compost extracts led to increase in crop yield due to addition of auxin, gibberellin, and cytokinenin (Grapelli *et al.*, 1998; Tomati and Galli, 1995). Therefore, soil drenching with compost extracts at one week before transplanting and at transplanting could be a potential eco-friendly approach for managing tomato wilts caused by *Fusarium oxysporum* f. sp. *lycxopersici*. However, further research is required to identify the antagonists in the compost extracts which are responsible for the suppression of this disease.

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