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ANTIMICROBIAL EFFICACY OF THYMOL IN THE MANAGEMENT OF BACTERIAL WILT OF TOMATO

A. R. POPOOLA*, S.A. GANIYU AND S.O. DUROJAIYE

Department of Crop Protection, Federal University of Agriculture, Abeokuta, Nigeria.
***Corresponding author:** popoolaar@funaab.edu.ng

ABSTRACT

Bacterial wilt (BW), caused by *Ralstonia solanacearum,* is a devastating disease of tomato world-wide. The disease is responsible for more than 60% reduction of tomato yield in Nigeria. Volatile plant essential oil, thymol, was evaluated against BW in artificially-inoculated potted plants. Twenty milliliter of 10^8 cfu/ml suspension of *Ralstonia solanacearum* was introduced into 5 kg pot of sterilized soil and 20 ml each of three concentrations of thymol (0.2, 0.4, 0.6 g/L) applied as pre-plant soil drench 2 hours after bacterial inoculation. Eight tomato genotypes were transplanted into the pots five days after thymol application. The experiment was laid in a completely randomized design (CRD) with three replicates. At 0.2 g/L thymol application, disease severity index was 1.50-4.00 at 5 Weeks After Transplanting (WAT). Disease severity index was reduced significantly ($p<0.05$) by 77.78% in tomato variety Delila upon 0.4 g/L application of thymol. At 0.6 g/L thymol application, tomato varieties Delila, Perfect Pee and Kerewa each had 0 (zero) disease severity index, while tomato lines NG/AA/MAY/09/030, NG/TB/AUG/09/006 and NG/AA/SEP/09/037 had disease severity index of 1 (one) each. These results indicated that application of thymol (0.6 g/L concentration, 1:250, thymol:soil, v:w) in potted experiment was effective in reducing the severity index of bacteria wilt of tomato caused by *Ralstonia solanacearum*, and is therefore recommended for management of the disease.

Keywords: Bacterial wilt, Management, Thymol, Tomato

INTRODUCTION

Bacterial wilt pathogen, *Ralstonia solanacearum* (Smith, 1896) Yabuuchi *et al.,* 1996 (syn. *Pseudomonas solanacearum*) race 1 is the soil-borne causal agent of bacterial wilt of more than 50 families of host plants, especially solanaceous species - potato and tomato (Thwaites *et al.,* 2000). It is widely distributed in tropical and subtropical regions of the world (Fegan and Prior, 2004). The bacteria species has a very broad host range and infects hundreds of species in many plant families, including tomato – *Solanum lycopersicum* Mill (Tiwari *et al.,* 2012). It is an aerobic non-sporing, Gram-negative plant pathogenic bacterium. It is motile with a polar flagella tuft and colonises the xylem causing rapid wilting in host plants (Jacobs *et al*., 2012).

Soil treatment with traditional general purpose fumigants as methyl bromide did not provide satisfactory control of this disease (Chellemi *et al*., 1997). Control of infested soils is very difficult. Some resistant tomato cultivars are available, but they have not been generally accepted by growers and commercial tomato industry due to poor horticultural quality (Kurian and Peter, 2001). No conventional pesticides are

known to provide effective control of this soil borne pathogen (Deberdt *et al*., 2012).

The destructive nature of the disease and ineffective disease suppression has made development of effective disease control approaches desirable (Weissinger *et al.,* 2001). Thymol and palmarosa oil are antibacterial agents produced by Thyme (*Thymus* spp.) and Palmarosa (*Cymbopogon martinii*) and have been widely used as a general antiseptic or additive in cosmetics and food industry (Ji *et al.,* 2007). In agricultural studies, these compounds have broad-spectrum activities against fungi, bacteria, nematodes, and insects. Lee *et al.* (2012) reported the use of plant essential oils for managing bacterial wilt in tomato, which could serve as an alternative to copper compounds. Other natural resistance inducers have proved to be potentially useful in control of diseases in tomato (El-Mougy *et al.,* 2012). In this study, the efficacy of thymol as pre-plant soil treatment on the control of bacterial wilt in artificially-inoculated potted tomato plants was evaluated.

MATERIALS AND METHODS

***Experimental site*:** The study was carried out in the laboratory and screenhouse of the Department of Crop Protection, College of Plant Science and Crop Production, Federal University of Agriculture, Abeokuta, Nigeria.

Sources of tomato genotypes

Eight tomato genotypes (NG/AA/MAY/09/030, NG/TBAUG/09/006, NG/AA/SEP/09/037, Delila, Markis, Hypee 45, Perfect Pee and Kerewa) were sourced from National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan and the College of Plant Science and Crop Production (COLPLANT), Federal University of Agriculture, Abeokuta, Nigeria.

***Soil source and sterilization*:** Sandy-loam top soil containing 86% sand, 10.8% silt and 3.2% clay with pH 5.87 used for the pot experiment was sourced from Experimental Teaching and Research Farm of the Federal University of Agriculture, Abeokuta, Nigeria. The soil was mixed and sterilized at 102^{o}C for 60 minutes using an improvised heating system. The sterilized soil was stored in jute sacks and rested for six weeks to restore stability. It was then dispensed into pots at 5 kg per pot.

Preparation and test of pathogenicity of bacterial suspension: Pathogenic isolate of *Ralstonia solanacearum* was obtained by streaming bacterial ooze from an infected tomato plant, cultured and recovered on semi-selective tetrazolium chloride (TZC) plates and incubated at 28^{o}C for 48 hrs according to Pradhanang *et al.* (2000). *Ralstonia solanacearum* pathogenic isolate were distinctively bold, fluidal and creamy-white on the medium. Identity of these presumptive colonies was confirmed on the third day after inoculation by the observed characteristic red centre and whitish periphery on TTC Agar (Kelman, 1954; Hayward, 1991). The isolate was purified by subculturing onto Nutrient Agar medium and adjusted to 10^{8} cfu/ml. Pathogenicity of the isolate was confirmed by drenching the sterilized soil with 20 ml of 10^{8} cfu/ml pathogen. Susceptible Beske variety was transplanted onto inoculated soil to observe characteristic symptom of bacterial wilt.

Application of bacterial suspension: Twenty milliliter of 10^{8} cfu/ml bacterial suspension was used as drench in each pot containing 5 kg sterile soil. Drenching was con-

centrated on the middle part of the pot where tomato plants would be transplanted later.

***Preparation and application of thymol*:** Thymol (98%, Lot No. 10151883, product of Alfa Aesar®) was prepared into solution of 0.6 g/L (0.6 g thymol, 35 ml 70% ethanol, 964 ml water, 1 ml detergent). This was further diluted to obtain 0.2 and 0.4 g/L. Twenty milliliter each of three thymol concentrations (0.2, 0.4, and 0.6 g/L) was applied unto 5 kg soil 2 h after pathogen inoculation. For untreated control, 20 ml of the solution without the thymol (35 ml 70%, 964 ml water, 1 ml detergent) was used. The pots were then covered with polythene bags for 3 days after thymol application. There were fifteen plants per treatment per genotype.

***Tomato transplanting*:** Three-week old tomato seedlings were transplanted into the experimental pots 5 days after thymol application.

Disease incidence, severity score and severity index.

$$\text{Disease incidence} = \frac{\text{No of plants showing wilt symptom in a genotype}}{\text{Total number of plants in the genotype}} \times 100$$

Plants were monitored for disease severity at 3 and 5 weeks after transplanting. Disease severity score were rated on 0 – 5 modified scale of Roberts *et al.* (1988) :- 0, no wilting; 1, 1-20% plants showed wilt symptom; 2, 21-40% wilt symptom; 3, 41-60% wilt symptom; 4, 61-80% wilt symptom; 5, 81 – 100% wilt symptom or dead.

$$\text{Disease Severity Index} = \frac{\sum(\text{Severity Score x number of plants in a genotype})}{\text{Total number of plants in the genotypes}}$$

***Data collection and analysis*:** Data collected included plant height (cm), number of branches, number of leaves, number of days for development of visible symptoms, percentage plant survival, percentage plant mortality, disease incidence and disease severity index. Data collected were subjected to ANOVA. Significant differences between means were separated using LSD at $P \leq 0.05$.

RESULTS AND DISCUSSION

Thymol on indices of growth in tomato.

Table 1 showed the effect of thymol application on growth performance of tomato at 3-5 weeks after transplanting (WAT). Tomato genotypes differed significantly ($P \leq 0.05$) in their growth response to thymol application. At 3 WAT, plant height value ranges from 3.5 - 17.5 cm. Delila variety had the highest value of plant height (17.5 cm) on application of thymol at 0.2 g/L. At 5 WAT, mean value of height ranges from 6.5 cm to 32 cm. Tomato genotypes were not significantly different in number of branches on thymol application. At 3 WAT, Markis variety had the lowest number of branches (2.5) without thymol application while Kerewa variety produced the highest branches of 8 at 0.2 g/L of thymol. Number of leaves ranged from 7.5 - 103 at 3 and 5 WAT, respectively. Markis variety had the least mean value of leaves (7.5) at 0.2g/L of thymol application while NG/TB/AUG/09/006 tomato line had the highest mean

value of 103 at 0.2 g/L of thymol at 5 WAT. Thymol did not have any significant effect on the growth performance of tomato in this study. It could also indicate that the essential oil, thymol, did not have any negative influence on the growth and development of tomato plant. Lee *et al*. (2012) emphasized that thymol exhibited antimicrobial activity.

Table 1: Effect of thymol concentrations on growth of eight genotypes of tomatoes inoculated with *Ralstonia solanacearum*.

Genotypes	Thymol (g/L)	Plant Height (cm)		Number of Branches		Number of leaves	
		3WAT	5WAT	3WAT	5WAT	3WAT	5WAT
NG/AA/MAY/09/030	0.0	8.60	16.00	6.00	6.00	18.00	33.00
	0.2	10.50	17.60	7.50	18.50	29.50	54.00
	0.4	8.75	17.60	7.00	16.50	19.00	46.00
	0.6	9.85	22.50	5.00	9.50	19.00	46.50
NG/TB/AUG/09/006	0.0	13.60	15.50	5.50	5.50	49.00	62.00
	0.2	13.50	24.00	6.50	21.50	59.00	103.00
	0.4	10.80	18.00	6.00	18.00	48.00	65.00
	0.6	9.50	21.00	5.50	10.50	49.50	80.00
NG/AA/SEP/09/037	0.0	14.50	32.00	7.00	15.00	56.00	83.50
	0.2	12.75	30.30	6.50	13.50	58.50	84.50
	0.4	12.75	19.00	7.50	15.50	66.50	88.00
	0.6	13.50	36.50	7.00	25.00	54.00	75.00
DELILA	0.0	13.00	21.00	7.50	15.00	64.00	78.00
	0.2	17.50	30.00	7.50	14.00	70.00	86.00
	0.4	14.95	29.00	6.50	20.00	46.50	62.00
	0.6	17.15	24.00	6.00	18.50	41.50	67.00
MARKIS	0.0	3.50	7.00	2.50	8.50	9.00	15.00
	0.2	4.00	9.50	3.50	9.00	7.50	19.50
	0.4	3.60	13.50	3.50	10.00	11.50	20.00
	0.6	8.25	24.50	7.00	12.00	16.00	35.50
HYPEE 45	0.0	9.00	22.50	6.00	15.00	20.00	41.50
	0.2	8.90	18.50	5.50	13.50	19.50	49.50
	0.4	5.10	15.00	5.00	13.00	14.00	34.50
	0.6	7.00	11.50	7.00	18.00	20.50	50.50
PERFECT PEE	0.0	7.45	6.50	6.00	3.50	35.00	24.50
	0.2	10.25	20.00	6.50	8.00	22.50	35.00
	0.4	5.50	17.00	5.00	13.00	23.00	42.50
	0.6	7.00	15.50	6.00	12.50	29.00	39.50
KEREWA	0.0	11.20	10.50	6.00	10.00	40.50	42.00
	0.2	9.05	14.50	8.00	11.00	40.00	52.00
	0.4	11.85	19.50	6.50	20.50	27.50	50.50
	0.6	4.00	9.80	3.50	5.50	11.00	23.00
LSD 0.05		3.67	9.16	NS	NS	10.64	18.31

WAT: Weeks After Transplanting
NS: Not Significant.

Thymol application on incidence and severity of bacterial wilt

In Table 2, at 3 WAT, the mean value for incidence of bacterial wilt (BW) ranged from 0-35% with variety Perfect Pee having the highest disease incidence of 35% where no thymol was added. At 3 WAT, variety Hypee 45 did not record disease incidence with or without thymol application.

Table 2: Incidence and severity of tomato bacterial wilt in tomato genotypes at 3 and 5 weeks after transplanting

Genotypes	Thymol (g/L)	Disease incidence (%)		Disease severity index	
		3WAT	5WAT	3WAT	5WAT
NG/AA/MAY/09/030	0.0	13.00	33.00	5.00	5.00
	0.2	0.00	6.00	3.50	4.00
	0.4	0.00	9.50	2.00	2.00
	0.6	8.50	17.50	1.00	1.00
NG/TB/AUG/09/006	0.0	17.50	0.00	5.00	5.00
	0.2	0.00	0.00	2.00	3.00
	0.4	0.00	14.50	1.00	1.50
	0.6	0.00	0.00	0.50	1.00
NG/AA/SEP/09/037	0.0	0.00	16.50	4.00	4.00
	0.2	0.00	0.00	2.00	1.50
	0.4	20.00	42.00	0.50	1.00
	0.6	0.00	0.00	0.50	1.00
DELILA	0.0	14.50	37.00	3.00	4.50
	0.2	19.00	40.00	3.00	3.50
	0.4	0.00	0.00	1.00	1.00
	0.6	0.00	0.00	0.00	0.00
MARKIS	0.0	0.00	7.50	3.50	5.00
	0.2	0.00	9.50	3.50	3.50
	0.4	4.50	7.50	1.00	2.00
	0.6	12.50	0.00	0.00	0.00
HYPEE 45	0.0	0.00	11.00	2.50	3.50
	0.2	0.00	24.50	2.50	2.50
	0.4	0.00	7.50	0.50	1.50
	0.6	0.00	0.00	0.00	1.00
PERFECT PEE	0.0	35.00	24.50	5.00	5.00
	0.2	13.00	35.00	4.00	4.00
	0.4	7.00	17.50	1.50	2.00
	0.6	0.00	0.00	0.00	0.00
KEREWA	0.0	15.00	52.00	5.00	5.00
	0.2	23.50	20.00	2.00	2.50
	0.4	0.00	0.00	0.00	1.00
	0.6	0.00	0.00	0.00	0.00
LSD0.05		NS	NS	0.55	0.45

WAT: Weeks After Transplanting
NS: Not Significant.

At 5 WAT, disease incidence ranged between 0-52%. There was significant reduction of 100% in both Delila and Markis varieties in BW incidence on application of thymol at 0.6 g/l. This was supported by other studies that reported that thymol used for soil treatment before transplanting provided significant and season-long protection against bacterial wilt (Ji *et al*., 2007; Lee *et al.,* 2012). The present study was also consistent with previous findings of Pradhanang *et al*. (2003) and Abdel- Kader *et al.* (2012) stating that thymol provided effective control on bacterial wilt of tomato under greenhouse conditions. Varieties Delila and Markis had significant reductions of 77.78% and 60% respectively in disease severity indices at 5 WAT on application of 0.4 g/L thymol. As past workers had also shown that thymol possessed bactericidal, fungicidal and nematicidal activities (Eldoksch and Hassanein, 2007; Karam *et al.,* 2012), it then followed that thymol could be used in the integrated management of soil-borne disease in tomatoes.

Effect of thymol on plant survival, mortality and number of days to symptom appearance

At 0.6 g/L, thymol application enhanced tomato survival; in untreated control, percentage mortality was high, ranging from 50-100% (Table 3).

The number of days to the development of visible symptoms ranged from 0-29. On application of thymol at 0.4 and 0.6 g/L, variety Delila showed no symptom of bacterial wilt. Tomato line NG/AA/SEP/09/037 showed visible symptom 4 days after pathogen inoculation. Thus, application of thymol at 0.4 and 0.6 g/L concentration provided significant protection against bacterial wilt in tomato genotypes. Lee *et al*. (2012) reported effectiveness of cinnamon and clove oil at lower concentration of 0.01 – 0.02 % (0.1 – 0.2 g/L) against bacterial wilt of tomato. At higher concentration of 0.05% (0.5 g/L) the oils became toxic to tomato plant and foliage damage occurred. According to Lee *et al.* (2012), effect of thymol oil was milder compared to cinnamon and clove oil, effectively reducing severity index of bacterial wilt at concentrations of 0.4 and 0.6 g/L. Other workers found thymol effective in reducing yeast, mould and total microbial counts on lemon (Castillo *et al.,* 2013) and for control of *Botrytis cinerea* (Adebayo *et al.,* 2013). Thus, thymol seems to possess broad antimicrobial property against a wide range of bacterial and fungal pathogens.

Table 3: Number of days to symptom appearance, plant survival and mortality in tomato genotypes inoculated with *Ralstonia solanacearum.*

Genotypes	Thymol (g/L)	No of days to symptom appearance.	Plant survival (%)	Plant mortality (%)
NG/AA/MAY/09/030	0.0	11.00	0	100
	0.2	14.00	100	0
	0.4	21.50	5	95
	0.6	29.00	50	50
NG/TB/AUG/09/006	0.0	16.50	0	100
	0.2	21.00	20	80
	0.4	10.50	40	60
	0.6	NSA	100	0
NG/AA/SEP/09/037	0.0	4.00	50	50
	0.2	8.50	75	25
	0.4	19.00	60	40
	0.6	10.50	80	20
DELILA	0.0	18.00	15	85
	0.2	21.00	50	50
	0.4	NSA	100	0
	0.6	NSA	100	0
MARKIS	0.0	13.00	0	100
	0.2	13.00	18	82
	0.4	18.00	50	50
	0.6	21.00	50	50
HYPEE 45	0.0	7.00	18	82
	0.2	15.00	40	60
	0.4	19.50	75	25
	0.6	24.00	89	11
PERFECT PEE	0.0	26.50	0	100
	0.2	13.50	0	100
	0.4	17.00	50	50
	0.6	18.00	50	50
KEREWA	0.0	10.50	0	100
	0.2	22.00	50	50
	0.4	25.50	50	50
	0.6	23.00	95	5
LSD0.05		5.42	27	45

WAT: Weeks After Transplanting.
NSA: No Symptom Appearance

CONCLUSION

This study showed that *Ralstonia solanacearum* caused extensive wilting of tomato with symptom appearing visible as early as four days after inoculation for genotypes receiving no thymol treatment. At thymol concentrations of 0.4 and 0.6g/L, plant survival increased while incidence of BW was reduced by more than fifty percent. In farm areas endemic with bacterial wilt, variety Delila with 0.6 g/L thymol applied as soil treatment at 20 ml/5 kg soil in pot cultivation is

recommended.

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