Effect of Neem Kernel Cake Powder (NKCP) on *Fusarium* Wilt of Tomato when Used as Soil Amendment

S.K. Kimaru¹, S.W. Waudo¹, E. Monda¹, A.A. Seif¹ and J.K. Birgen^{1*}

Abstract

A study was conducted to investigate effect of Neem Kernel Cake Powder (NKCP) at 1.75, 3.5 and 7g rates on development of tomato *Fusarium* wilt in 1997 at the National Horticultural Research Center, Thika, Kenya. Inoculum density of *Fusarium oxysporum f.sp. lycopersici* (Sacc.) was two 14mm - diameter disks per planting hole taken from 10 day old cultures growing on PDA. Plant performance was based on shoot height and weight; stem diameter; number and weight of tomato fruits. Disease assessment was based on wilt index of shoots and length of discolouration of vascular tissues. Performance of plants grown in NKCP amended and non-amended soils was significantly (p=0.05) different (33.3 - 93.3%). Disease severity based on the wilt index (0.53-2.87) and length of discoloured vascular tissues (7.4cm - 25.62cm) differed significantly (p=0.05) among treatments.

Keywords: Neem kernel cake powder, *Fusarium oxysporum f. sp lycopersici*, tomato, wilt, soil amendment, plant performance, disease severity

1 Introduction

Tomato (*Lycopersicon esculentum* Mill) is an important source of vitamin C, calories, phosphorus and calcium (DAVIS and HOBSON, 1981; HOBSON and KILBY, 1985; LANGER and HILL, 1991).

In 1990, the estimated area under tomato cultivation in Kenya was about 19,000 hectares with total annual production of 494,000 tons (KIBATA, 1980). By 1995 the production had declined from the 494,000 tons to 225,310 (HCDA, 1995) due to constraints caused by environmental stress, diseases and poor agronomic practices.

Important tomato diseases include blights and wilts (DIXON, 1985; ALABOUVETTE *et al.*, 1993; KEDERA, 1996; GOTH and KEANE, 1997; LARKIN and FRAVEL, 1998; AVRDC, 2001). Annual losses of up to 30,000 tons of canning tomatoes or 10-15% of the crop damage in the USA are associated with *Fusarium* tomato wilt (WESTCOTT, 1971; BENHAMOU *et al.*, 1989). In Kenya, *Fusarium* wilt is known to cause significant tomato losses, (RRC-KISII, 1994; ONYANGO and MAKWORO, 1997). In spite of the high tomato losses associated with *Fusarium* wilt its control is limited to use of fungicides which are unaffordable by the many poor resource Kenyan farmers. There is, therefore,

¹ Department of Botany, Kenyatta University, P.O. BOX 43844, Nairobi, Kenya

 $^{^{\}ast}$ corresponding author

need to seek alternative control measures that can be attractive to a poor resource farmer.

This study was undertaken to evaluate the effect of NKCP against *Fusarium* wilt when used as a soil amendment in the green house as a possible available alternative in the management of the *Fusarium* wilt disease in both poor and rich resource agricultural systems.

2 Materials and Methods

2.1 Isolation of Fusarium oxysporum f.sp. lycopersici

Fusarium oxysporum f.sp. lycopersici (Fol) was isolated from infested tomato plants collected from a farm at Kigumo, Kiambu District, Kenya in September 1996. Roots were thoroughly washed in tap water, rinsed with sterile distilled water and then aseptically cut into 1- cm-long pieces/segments. The root pieces were surface sterilized in 0.5% sodium hypochlorite solution for 30 seconds. The sterilized root segments were placed on Potato Dextrose Agar (PDA), five segments per petri dish (WAUDO *et al.*, 1995) and incubated at 23°C for 10 days. Fol was identified using morphological characteristics (NELSON *et al.*, 1983). A pathogenity test was done to validate the identification. Pure cultures of Fol were maintained on PDA at 4° C.

2.2 Soil infestation

Soil infestation involved aseptically transferring of a 14mm - diameter disc of PDA taken from 10-day old cultures of Fol using a cork-borer into sterile soil, two disks per planting hole. The infested soil was then thoroughly mixed.

2.3 Greenhouse test

A test was conducted in a greenhouse at the National Horticultural Research Centre, Thika, Kenya to assess the effects of Neem Kernel Cake Powder (NKCP) on the development of *Fusarium* wilt of tomato, in soil amended with NKCP at the rate of 1.75, 3.5 and 7g. Inoculum density per planting hole was two PDA disks of culture transferred to sterile soil contained in 18cm- diameter pots as descried previously. Pots without NKCP served as controls.

Tomato seeds were germinated in sterilized sand contained in a $15\times20\times10cm$ wooden box. Twenty-eight day old tomato seedlings were transplanted (one plant per pot) and treated with Di-amminium phosphate (DAP) fertilizer at the rate of 2g per pot. A completely radomized block design (CBD) with six replicates was used. Plants were watered appropriately and staked four weeks after transplanting.

Plant performance based on shoot height and weight, stem diameter, and number and weight of fruits was carried out. Shoot length was taken from the first basal node to the tip of the youngest apical bud. Stem diameter was taken just below the first basal node. Tomato shoots were cut at the first node and dried at 80° C for 72 hours before weighing. Shoot length and stem diameters were measured after every two weeks for eight weeks. Shoot weight, number and fruit weight were obtained at harvesting time.

Similarly, disease assessment based on length of discoloured vascular tissue and wilt index was done on harvesting day.

3 Results

Tomato plants grown in soil amended with different rates of NKCP differed (p=0.01) significantly in their performance. Tomato plants grown in soil amended with 7g of NKCP had the highest mean shoot height and stem diameter 42, 56, and 98 days after transplanting (Table 1). Mean shoot heights and stem diameter of the plants were lowest and highest in soils amended with 1.75 and 7.0g of NKCP. Plant performance decreased with reduction in the rate of NKCP 98 days after NKCP and Fol application (Table 1 and 2).

		Days after NKCP application and Fol inoculation †							
Treatment *	14	2	8	4	2	5	6	9	8
(NKCP in g)	SH	SH	SD	SH	SD	SH	SD	SH	SD
7	10.9 ^b	17.5 ^{ab}	0.67 ^a	38.3 ^a	0.86 ^a	51.4 ^a	1.06 a	80.8 ^a	1.14 a
3.5	13.2 a	19.3 a	0.72 ^{<i>a</i>}	35.5 ^a	0.90 ^a	50.7 ^a	0.99 ^a	76.0 ^a	1.03^{a}
1.75	9.75 ^c	16.4 ^{<i>b</i>}	0.53 ^b	27.9 ^b	0.81 ^b	36.4 ^b	0.81 ^b	55.7 ^b	0.82 ^b
Non-amended	9.58 ^c	13.9 c	0.32 ^c	21.3 c	0.54 ^c	25.9 ^c	0.54 c	41.8 c	0.59 ^c

 Table 1: Mean shoot height (SH) in cm and stem diameter (SD) in cm of tomato cv

 money maker after NKCP and Fol application.

* Each treatment was replicated 6 times

[†] Figures followed by the same letter within a column do not differ significantly (p=0.01) according to Duncan's Multiple Range Test (DMRT).

Tomato plants grown in soil amended with different rates of NKCP differed (p=0.01) significantly in their dry shoot weight (Table 3). Tomato plants grown in soil amended with 7g of NKCP had the highest dry shoot weight while those grown in non-amended soil had the lowest shoot weight 98 days after NKCP and Fol application (Table 3). Tomato plants grown in soil amended with 3.5g of NKCP had the second highest mean dry shoot weight while those grown in soil amended with 1.75g of NKCP had the second lowest mean dry shoot weight 98 days after NKCP and Fol application (Table 3). Similar trend was observed for tomato fruit weight (Table 3).

Tomato plants grown in soil, amended with different rates of NKCP differed (p=0.01) significantly in the number of mature red fruits 98 days after NKCP and Fol application (Table 3). However tomato plants grown in soil amended with 7g and 3.5g of NKCP did not differ significantly in the number of fruits they produced 98 days after NKCP and Fol application (Table 3). Tomato plants grown in soil amended with 1.75g of NKCP did not also differ significantly from those grown in non-amended (control) in the number

Treatment * (NKCP in g)	SH (cm)	% change	SD (cm)	% change
7	80.8	93.3	1.14	93.2
3.5	76.0	81.8	1.03	74.6
1.75	55.7	33.3	0.82	39.0
Non-amended (control)	41.8	0	0.59	0
* Each treatment was replicate	d 6 times.			

Table 2: Percentage (%) change in mean shoot height (SH) and stem diameter (SD)of tomato cv money maker 98 days after NKCP and Fol application.

of mature red fruits 98 days after NKCP and Fol application (Table 3). Tomato plants grown in soil amended with 3.5g of NKCP had the highest mean fruit number while those grown in non-amended soil had the lowest mean fruit number (Table 3). Tomato plants grown in soil amended with 7g and 1.75g of NKCP had the second highest and second lowest mean fruit number, respectively (Table 3).

Table 3: Mean dry shoot weight (DSW) in grams, fruit weight (FW) in grams and fruitnumber (FN) of tomato cv moneymaker 98 days after NKCP application andFol inoculation.

Treatment *	Plant growth parameters			
(NKCP in g)	DSW	FW	FN	
7	38.59 ^{<i>a</i> †}	50.77 ^{<i>a</i>}	16 a	
3.5	29.48 ^b	36.27 ^b	19 ^{<i>a</i>}	
1.75	25.38 ^c	26.95 ^c	11 b	
0 (Control)	$17.8^{\ d}$	11.93 d	8 ^{<i>b</i>}	

* Each treatment was replicated 6 times.

 † Figures followed by the same letter within a column do not differ significantly (p=0.01) according to DMRT.

Tomato plants grown in soil amended with different rates of NKCP differed (p=0.01) significantly in disease severity based on length of discoloured vascular tissue (LDV) and wilt index (WI), Table 4. Tomato plants from non-amended soil had the highest mean LDV and WI while those from soil amended with 7g of NKCP had the lowest LDV and WI means (Table 4). Tomato plants grown in soil amended with 3.5g and 1.75g of

Treatment *	Disease Parameters		
(NKCP in g)	LDV	WI	
7	7.42 ^d	0.53 ^d	
3.5	13.97 ^{<i>c</i>}	0.95 ^c	
1.75	22.77 ^b	2.53 ^{<i>b</i>}	
0 (Control)	25.62 ^{<i>a</i>}	2.87 ^a	

 Table 4: Mean length (cm) of discoloured vascular tissue (LDV) and wilt index (WI) of tomato cv money maker 98 days after NKCP application and Fol inoculation.

* Each treatment was replicated 6 times.

[†] Figures followed by the same letter within a column do not differ significantly

(p=0.01) according to DMRT.

NKCP had the second lowest and second highest mean LDV and WI respectively 98 days after NKCP and Fol application (Table 4).

Disease severity (based on LDV and WI) on tomato plants decreased with increase in NKCP rates used as soil amendment (Table 4).

4 Discussion

The significantly (p=0.01) higher performance for tomato plants grown in soil amended with NKCP compared to those grown in non-amended soil (control) indicate that NKCP suppressed pathogenic effects of Fol (Table 1, 2, 3). The suppressive effect might have been due to production of fungistatic substances such as azadirachtin and improved host resistance perhaps as a result of improved host nutritional status (SCHAFER, 1971; KHAN *et al.*, 1973; MAUKAU, 1980; AGRIOS, 1988). These possibilities, however, need to be investigated. Neem seed cake contains higher levels of nitrogen, phosphorus, potassium, calcium and magnesium than those in farmyard manure or sewage sludge (RADWANSKI and WICKENS, 1981).

The significantly (p=0.01) short discoloured vascular tissues of plants grown in soil amended with 7g of NKCP (Table 4) indicates that high amount of NKCP suppressed the pathogenic effect of Fol. The poor disease development could be associated with enhanced performance of the tomato plants due to the high nitrogen nutrition from the neem seed cake (HUBER and WATSON, 1974; TISDALE *et al.*, 1985; PACUMBABA *et al.*, 1997).

References

AGRIOS, G. N.; *Plant Pathology, 3rd edition*; Academic Press Inc., New York; 1988.
 ALABOUVETTE, C., LEMANCEAU, P. and STEINBERG, C.; Recent advances in the biological control of *Fusarium* wilts; *Pesticide Science*; 37:365–373; 1993.

- AVRDC; Asian Vegetable Research and Development Center Report for 2000; Shanshua Taiwan, pp. 11 12; 2001.
- BENHAMOU, N., CHAREST, P. M. and JARVIS, W. R.; Biology and host parasite relationships of *Fusarium oxysporum*; in: *Vascular Wilt Disease of Plants. Basic Studies and Control*, edited by TJAMOS, E. C. and BECKMANN, C. H.; NATO ASI H28; Springer Verlag; 1989.
- DAVIS, K. M. and HOBSON, G. E.; The constituents of tomato fruit the influence of environment, nutrition and genotype; *Critical Reviews of Food Science and Nutrition*; 15:205–280; 1981.
- DIXON, G. R.; Vegetable Crop diseases; pages 337-377; MacMillan; 1985.
- GOTH, R. W. and KEANE, J.; A detached leaf method to evaluate late blight resistance in potato and tomato; *American Potato Journal*; 74:347 352; 1997.
- HCDA; The horticultural news HCDA magazine of the horticultural industry; Horticultural Crops Development Authority, Kenya; 1995.
- HOBSON, G. E. and KILBY, P.; Methods for tomato fruit analysis and indicators of consumer acceptability; Annual Report of the Glasshouse Crops Research Institute for 1984, pp. 129-136; 1985.
- HUBER, D. W. and WATSON, R. D.; Nitrogen form and plant disease; Ann. Rev. Phytopathol.; 12:139–164; 1974.
- KEDERA, J.; Plant Diseases; vol. 30 of Review of Kenyan Agricultural Research; pages 28 –30; KARI/Bangor; 1996.
- KHAN, M. W., KHAN, A. M. and SAXENA, S. K.; Influence of oil cake amendment on nematodes and fungi in tomato field; *Acta Bot. indica*; 1:49–54; 1973.
- KIBATA, G. N.; I.P.M. studies in Kenya; Special Report of the National Agri. Res. Inst., 41 pp.; 1980.
- LANGER, R. H. M. and HILL, G. D.; *Agricultural plants. Second Edition*; Cambridge University Press; 1991; 387 pp.
- LARKIN, R. P. and FRAVEL, D. R.; Efficacy of various fungal and bacterial biocontrol organisms for control of *Fusarium* wilt of tomato; *Plant Disease*; 82:1022–1029; 1998.
- MAUKAU, R.; Biological control of nematode pests by natural enemies; Ann. Rev. Phytopathol; 18:415–440; 1980.
- NELSON, P. E., TOUSSOUN, T. A. and MARASAS, W. F. O.; Fusarium species; An illustrated manual for identification; University Park, USA: Penasylvania State University Press; 1983.
- ONYANGO, M. and MAKWORO, S.; Fruit and vegetable production practices and constraints in South West Kenya as a Basis for selecting Research Priorities; in: A Review of Agriculture Practices and constraints in South West Kenya; pages 47 – 61; KARI/DFID; 1997.
- PACUMBABA, R. P., BROWN, G. F. and PACUMBABA, R. O. JR.; Effect of fertilizer and rates of application on incidence of soybean disease in northern Alabama; *Plant Dis.*; 81:1459–1460; 1997.
- RADWANSKI, S. A. and WICKENS, S.; Vegetative value and potential value of Neem tree in tropics; *Econ. Bot*; 35:398–414; 1981.
- RRC-KISII; KARI Regional Research Center Annual report for 1994; Kisii; 1994.

- SCHAFER, J. F.; Tolerance to plant diseases; Ann. Rev. Phytopathol.; 9:235-252; 1971.
- TISDALE, S. L., NELSON, W. L. and BEATON, J. D.; *Soil fertility and fertilizers*; Macmillan publishing company, New York; 1985.
- WAUDO, S. W., OWINO, P. O. and KURIA, M.; Control of *Fusarium* wilt of tomatoes using soil amendments; *E. Afric Agri. For J*; 60(4):207–217; 1995.
- WESTCOTT, C.; *Westcott's Plant disease Handbook, 3rd edn.*; Van Nostrand Reinhold Co., New York, London; 1971.