Taylor & Francis
Taylor & Francis Group

Acta Agriculturae Scandinavica, Section B — Soil & Plant Science

ISSN: 0906-4710 (Print) 1651-1913 (Online) Journal homepage: https://www.tandfonline.com/loi/sagb20

Effect of soil sterilisation on biological control of *Fusarium oxysporum* f. sp. *lycopersici* and *Meloidogyne javanica* by antagonistic fungi and organic amendment in tomato crop

M. W. Mwangi, W. M. Muiru, R. D. Narla, J. W. Kimenju & G. M. Kariuki

To cite this article: M. W. Mwangi, W. M. Muiru, R. D. Narla, J. W. Kimenju & G. M. Kariuki (2018) Effect of soil sterilisation on biological control of *Fusarium oxysporum* f. sp. *lycopersici* and *Meloidogyne javanica* by antagonistic fungi and organic amendment in tomato crop, Acta Agriculturae Scandinavica, Section B — Soil & Plant Science, 68:7, 656-661, DOI: 10.1080/09064710.2018.1450439

To link to this article: https://doi.org/10.1080/09064710.2018.1450439

ACTA AGRICULTURAE SCANDINAVICA, SECTION B — SOIL & PLANT SCIENCE, 2018
VOL. 68, NO. 7, 656–661
https://doi.org/10.1080/09064710.2018.1450439

Taylor & Francis
Taylor & Francis Group

SHORT COMMUNICATION

Effect of soil sterilisation on biological control of *Fusarium oxysporum* f. sp. *lycopersici* and *Meloidogyne javanica* by antagonistic fungi and organic amendment in tomato crop

M. W. Mwangi[a], W. M. Muiru[a], R. D. Narla[a], J. W. Kimenju[a] and G. M. Kariuki[b]

[a]Department of Plant Science and Crop Protection, University of Nairobi, Nairobi, Kenya; [b]Department of Agricultural Science and Technology, Kenyatta University, Nairobi, Kenya

ABSTRACT

One of the major challenges in the application of biological control agents into the soil is their inability to withstand competition from natural microflora. In this study a management programme involving fungal biological control agents *Trichoderma harzianum* (TH) and *Purpureocillium lilacinium* (PL), and the organic amendment neem was carried out for the control of *Meloidogyne javanica* and *Fusarium oxysporum* f. sp. *lycopersici* (FOL). The experiment was carried out in sterile and non-sterile soil in order to test the efficacy of biological control in natural soil conditions. This experiment was repeated twice. A Wilcoxon's Signed-Rank test indicated there were no significant ($P < 0.05$) differences in fusarium wilt control in sterile and non sterile soils in the treatments PL neem and TH neem, but there was significantly ($P < 0.05$) higher control of *M. javanica* in the same treatments in sterile soils compared to non sterile soils. The combinations of fungal antagonists and neem were effective in non sterile as in sterile soils in the control of fusarium wilt, while being less effective in the control of *M. javanica*. Thus the biological control agents can be very effective in the control of fusarium wilt in natural conditions where the soil is not sterilised, but be less effective in the control of *M. javanica* in the same conditions. There is therefore potential of integrated management of fusarium wilt and root-knot nematodes (RKN) by these biological control agents in natural soil field conditions as an alternative to using chemicals.

ARTICLE HISTORY
Received 4 November 2017
Accepted 5 March 2018

KEYWORDS
Antagonistic fungi; biological control; *Fusarium oxysporum* f. sp. *lycopersici*; *Meloidogyne javanica*; non sterile; sterile

Introduction

The resistance of pests and pathogens to chemical control, environmental pollution and hazardous effects on human beings has brought a shift in the control of pests and plant pathogens from reliance on chemicals to alternative methods that do not have a negative impact on the environment (Singh 2002). Biological control is the most researched alternative to use of chemicals. Generally, biological control agents have been found to be inconsistent and their effect do not persist for a long time (Agbenin 2011). It has also been reported that organic amendments can boost the effectiveness of biological control agents by providing nutrients (Nagesh et al. 2006). Neem is one of the most commonly used organic amendment that is applied to the soil in order to provide nutrients and also to control nematodes in greenhouse and field conditions (Lokanadhan et al. 2012).

Trichoderma harzianum is among the most commonly investigated fungi, in the control of fungal plant pathogens (El-mohamedy et al. 2014; Fatima et al. 2015), and nematodes (Dababat and Sikora 2007). *Purpureocillium lilacinium* syn. *Paecilomyces lilacinus* on the other hand is the most preferred fungal biological control agent in the control of root-knot nematodes (RKN) (Schenck 2004; Kiewnick and Sikora 2006).

The effectiveness of biological control agents is to a great extent determined by the soil conditions (Luambano et al. 2015). Therefore there is need for biological control agents to undergo vigorous tests on different types of soils. Heydari and Pessarakli (2010) in their review recommended the need of more research on effect of environmental factors on biological control agents in order to have more effective biological control. In order to come up with good recommendations on the efficacy of *P. lilacinium, T. harzianum* and neem in the biological control of FOL and *M. javanica* it is important to test the efficacy of biological control agents in natural soils. Most screening tests on biological control competences are carried out on

CONTACT M. W. Mwangi wanjirumargie00@gmail.com Department of Plant Science and Crop Protection, University of Nairobi, College of Agriculture and Veterinary Sciences, P. O. Box 29053, Kangemi 00625, Nairobi, Kenya

sterile soils. The insecticide ACT, 1968 (www.cibrc.nic.in/guidelines.htm) stated the importance of carrying out tests on non-sterile soils to rule out the possibility of interference of native microflora in the bio efficiency assay.

Therefore the experiment was set up to investigate effect of combined applications of fungal antagonists and neem (TH neem and PL neem) on FOL wilt pathogen and *M. javanica* in sterile and non sterile soils in tomato cultivar Prostar F1.

Materials and methods

The study was carried out in a greenhouse located at the University of Nairobi, College of Agriculture and Veterinary Sciences (CAVS), Upper Kabete Campus field station.

Soil sterilisation

The soil was collected from Kabete field station and had the following constituents; % nitrogen (0.04), % organic carbon (0.65), pH (6.31), Pottasium (0.65 centimoles/kg and Phosphorous (11.20 Phosphorous parts per million). The soil was mixed with sand in the ratio 3:1 and was sterilised at 121°C for 15 min and the procedure repeated after a period of three days.

Preparation of FOL inoculum

An isolate of FOL was grown on PDA for five days at room temperature (24°C ±2). Two PDA cores of fungal growth were obtained by use of a cork borer (4 mm) and were used to inoculate 100 ml czapek dox growth media put in a rotatory shaker (J. P. Selecta, S. B. Made in Spain 564222 S/W) at 50 rpm, and at room temperature for a period of one week. After one week, the mycelia was harvested by sieving using a sterilised tea strainer and was used to inoculate one kilogram of sterilised sand-maize meal medium (900 g sand, 100 g maize flour, 200 ml water) in a transparent three kilograms polyethylene bag. This was a modification of sand-maize meal medium preparation described by Nene and Haware (1980). The fungus colonised the surface within a period of two weeks. One hundred grams of the fungal growth on the surface was scraped using a sterilised spatula and was suspended in 900 ml of (0.001%) water agar. One hundred grams of the fungal growth on the surface was scraped using a sterilised spatula and was suspended in 900 ml of (0.001%) water agar. The suspension was filtered through four layers of muslin cloth to remove the mycelia and media. The spore concentration was determined by serial dilution and plating. The filtrate was centrifuged at 2250 rpm for 10 min, and the top layer siphoned out by means of a pipette and then adjusting to a concentration of 1×10^7spores per ml by adding sterile distilled water. Inoculation of FOL was performed by drenching each pot with the fungal suspension at the rate of 1×10^7 spores per gram of soil.

Source and production of P. lilacinium, T. harzianum *inoculum*

Purpureocillium lilacinium was obtained from a commercial product (Bio-Nematon 1.15WP by Osho chemical industries Ltd.). One gram of the product was dissolved in nine millilitre of 0.001% water agar. The 10^{-4} dilution was cultured on a PDA agar plate and was incubated for seven days at room temperature (24°C ±2). A single colony of the fungus was then cultured on a PDA agar plate for one week at room temperature. Two PDA cores of fungal growth were obtained by use of a cork borer (4 mm) and were used to inoculate 100 ml czapek dox growth media put in a rotatory shaker (J. P. Selecta, S. B. Made in Spain 564222 S/W) at 50 rpm and at room temperature for a period of one week. The rest of the procedure was carried as in the preparation of FOL inoculums. The filtrate concentration was adjusted to a concentration of 1×10^7spores/ml. *Purpureocillium lilacinium* was applied by drenching each pot with the fungal suspension at the rate of 1×10^7 spores per gram of soil. The *T. harzianum* (Trianum, strain T-22:1 × 10^9 by Koppert Biological Systems) was applied as a drench by dissolving 2.5 g in 250 ml water.

Preparation and inoculation of M. javanica *J2s*

Second stage juveniles (J2s) were extracted from infested tomato roots of *M. javanica* using a modification of method of extraction described by Coyne et al. (2007). One gram of one centimetre pieces roots in 20 ml water was blended in a domestic blender at high speed for 15 s. The J2s were extracted from the blended root-water mixture using the modified Baermann tray method (Whitehead and Hemming 1965). The mixture was placed on a plastic sieve lined with a two ply tissue paper placed in a plastic plate. Water was then poured into the plate between the edge of the mesh and the side of the tray and the set up left for 48 h for J2s to migrate to the water below. The J2s were then concentrated by sieving through a 250 and 38 µm sieves. An estimate of total J2s was made using a compound microscope and a nematode counting chamber.

The seedlings were each infested with *M. javanica* J2s one day after transplanting. A hole was made two centimetres near each plant using a plastic

spoon. Thirty millilitres of suspension contained 2000 juveniles was dispensed into the holes which were then covered.

Experimental design

Three week old tomato seedlings of the tomato cultivar Prostar F1 were transplanted separately into plastic pots (14 cm diameter and 8 cm height) filled with one kg sterilised soil. Other seedlings were planted in plastic pots containing same amount of non sterile soils. The experiment had the following treatments in both sterile and non sterile soil; co-infected plants but untreated control, co-infected + Carbendazim, co-infected + Neem, co-infected + PL + Neem and co-infected + TH + Neem. The neem imported from India but available in local agronomic shops was applied at the rate of 20 g per plant. The neem had a C/N ratio greater than 19 (≤ than 19), was to provide optimal growth conditions for biological control fungi. Carbon/nitrogen (C/N) ratio is very important for fungal growth (Lamour et al. 2000). In the positive control treatment 40 ml of 0.25% Carbendazim 500 WP (Bavistin $^{(TM)}$) manufacturd by BASF SE, Germany, was applied by drenching. Carbendazim 500 WP is a commercial systematic fungicide that was also found to control nematodes in this investigation. Biological and chemical applications to experimental plants were done four days after infesting with FOL inoculum and *M. javanica* juveniles as described by Kamali et al. (2015).

Each treatment was replicated four times and the experiment was laid out in a randomised complete block design and was repeated twice. Plants were sprayed with a fungicides and pesticides once per week to control fungal diseases and pests. The fungicide Ridomil Gold$^{(Tm)}$ (Mancozeb 640 g/g + Metalaxyl-M 40 g/g) by Syngenta Crop Protection AG, Switzerland was applied at the rate of 5 g per 2 litres of water. Different pesticides were used interchangeably and these included Evisect $^{(Tm)}$ (Thiocyclam SP 50%) by Nippon Kayaku Co. Ltd. Japan, at one gram per litre. Coragen $^{(Tm)}$ 20 SC (Chlorantranilip 200 g/litre) by E. I. Dupont Nemours and Company, U.S.A. was applied at 2 ml per litre of water.

Extraction of juveniles from the soil and the roots

From a homogeneous 500 g sample of soil used in each experiment, 250 g was used for the extraction of J2s. The juveniles were extracted from the soil of each individual plant and from the whole root system using modified Baermann tray method (Whitehead and Hemming 1965) after termination of the experiment.

Estimation of FOL fungal propagules in the soil and roots of experimental plants

Data on number of fungal propagules in terms of colony forming units (CFU) of FOL from the roots was taken after termination of the experiment. The FOL fungal propagules were obtained in terms of CFU per gram of soil and root by dilution plate technique. One gram of the soil was suspended in 9 ml dilution media (0.01% of water gar) and was diluted to 10^{-1} and 10^{-2}. From the dilutions one millilitre was placed on PDA agar and incubated at 25°c for 14 days. After incubation the colonies of the FOL fungus were counted and the counts were used to estimate the number of fungal propagules per gram of soil. Numbers of fungal propagules in the roots were estimated per gram of ground roots following the same procedure used for soil.

Data collection

The test plants were harvested eleven weeks after transplanting. The plants height and dry shoot weight were measured. Roots from treatments inoculated with nematodes were stained with cold eosin yellow (0.1 g L^{-1} of water) for 30 min and gall indices scored according to a scale by Taylor and Sasser (1978) using the following values: 0 = zero gall; 1 = 1 or 2 galls; 2 = 3 to 10 galls; 3 = 11 to 30 galls; 4 = 31 to 100 galls and 5 = > 100 galls per root system. Disease severity was assessed by modification of a wilting index described by Akram et al. (2014). The severity scale 1 represent no symptom, 2 = wilting and yellowing covered less than 25%, 3 = wilting and yellowing was less than 50%, 4 = wilting and yellowing was equal to or more than 50%.

Data analysis

Data on effect of treatments on plant height and dry shoot weight was analysed by one way ANOVA, using GenStat statistical package (Discovery Edition 14). Counts of J2s in the roots and soil was normalised before analysis by logarithmic transformation using the formula (Log base 10). Data on number of fungal propagules was normalised by square root transformation. Means were compared using Least Significant Difference (L.S.D) at 5% level of significance. The means were separated by Fisher's Protected Least Significant Difference test. Data on disease severity and galling indices was analysed by Friedman test a non parametric test using Statistical Programmes for Social Sciences SPSS [SPSS (IBM SPSS Statistics 20)]. Significant differences were tested by Wilcoxon's Signed-Ranks Test pair wise comparisons.

Results

The TH neem and PL neem significantly decreased FOL disease, FOL fungal propagules, *M. javanica* juveniles compared to the control in both sterile and non sterile soils (Tables 1 and 2). A Wilcoxon's Signed-Rank test indicated that there were no significant ($P < 0.05$) differences in the treatments PL neem and TH neem in fusarium wilt disease control in sterile and non sterile soil (Table 1). Similarly, there were no significant differences in the number of FOL propagules in sterile soils compared to non sterile soils in the treatment PL neem. However, the treatment TH neem had significantly higher number of FOL propagules in sterile than non sterile soil (Table 2). There were no significant differences in the galling indices in non sterile compared to sterile soils in the treatments PL neem and TH neem according to Wilcoxon's Signed-Rank test. There was also significantly higher control of J2s in sterile soils than in non sterile soils for the treatments, PL neem and TH neem (Table 2). There were no significant differences in the dry shoot weight and tomato plants height when grown in sterile soil than in non sterile in the treatments, PL neem and TH neem (Table 3).

Table 1. Median and Friedman test analysis ranking the disease severity and galling indices after application of fungal antagonists and neem in the control of FOL in fusarium wilt and RKN disease complex in sterile and non sterile soils.

Soil	Treatments	Mean rank FOL disease	Median	Gall index	Median
Non-sterile	Control	9.75	3.5	8.5	4
	Carbendazim	2.38	1	2.13	1.5
	Neem	8.13	2	7.75	3.5
	PL neem	3.25	1	4.75	2.5
	TH neem	3.25	1	6.38	3
Sterile	Control	9.75	4	9.25	4
	Carbendazim	2.38	2	2.13	1.5
	Neem	8.13	3	7.75	3.5
	PL neem	4.13	1.5	3.25	2
	TH neem	4.13	1.5	3.25	2
	Chi-test (χ^2)	26.47		29.02	
	Df	9		9	
	P -value	>.001		>0.01	

Analysis based on Friedman test while the median provides a descriptive summary of the central value in each treatment. FOL = *Fusarium oxysporum* f. sp. *lycopersici* PL = *Purpureocillium lilacinium,* TH = *Trichoderma harzianum,* control = inoculated with *M. javanica* and FOL but not treated.

Table 2. Effect of fungal antagonists and organic amendements on FOL propagules and *M. javanica* J2s in sterile and non sterile soils.

	Treatment	[1]Total J2s	FOL [2]g/root	FOL [2]g/soil
Non-sterile	Control	5021 (3.7)	157 (12.5)	40 (6.2)
	Carbendazim	1446 (3.2)	133 (11.5)	1.3(1.1)
	Neem	4010 (3.6)	296 (17.2)	51 (7.1)
	PL neem	2387 (3.4)	30.67(5.5)	24 (4.8)
	TH neem	3440 (3.6)	3.3 (1.1)	3(1.5)
Sterile	Control	4663 (3.7)	400 (20)	127 (11.1)
	Carbendazim	1167 (3.1)	150.3 (12.4)	1.3 (1.1)
	Neem	3619 (3.6)	453 (20.55)	64 (8.0)
	PL neem	1720 (3.2)	40.0 (6.3)	17.7 (4.2)
	TH neem	2253 (3.4)	10 (3.16)	20 (4.5)
	L.S.D ($P = 0.05$)	0.10	3.8	1.8

Data are mean of four replicates. Values in brackets are [1]$\log_{10}$ and [2]square root transformed means used in comparison with L.S.D. J2s = stage two juveniles, FOL = *Fusarium oxysporum* f. sp. *lycopersici* PL = *Purpureocillium lilacinium,* TH = *Trichoderma harzianum*, control = inoculated with *M. javanica* and FOL but not treated.

Table 3. Effect of TH, PL and neem on growth in tomato inoculated with *M. javanica* and FOL pathogen in sterile and non sterile soils.

	Treatment	Height (cm)	Dsw (g)
Non -Sterile	Control	74.7a	14.63a
	Carbendazim	86abc	21.97bc
	Neem	96cd	23.83bcd
	PL neem	89.7bcd	26.7de
	TH neem	97bcd	24.7bcde
Sterile	Control	81.33ab	15.4a
	Carbendazim	86.67abc	20.73b
	Neem	90bcd	25.07cde
	PL neem	91bcd	27.60e
	TH neem	101d	25.77de
	L.S.D ($P = 0.05$)	13.0	3.6

Data are mean of four replicates. Means followed by the same letter are on the same column are not significantly ($P < 0.05$) different according to Fisher's L.S.D test. DSW = Dry Shoot weight, PL = *Purpureocillium lilacinium,* TH = *Trichoderma harzianum,* control = inoculated with *M. javanica* and FOL but not treated.

Discussion

The treatments TH neem and PL neem controlled FOL propagules and *M. javanica* juveniles in sterile and non sterile soils. There are reports of inhibition of FOL by application of a combined treatment of *T. harzianum* and neem (TH neem) (Nagesh et al. 2006; Singh et al. 2014), though inhibition from application of PL neem have not been reported but was observed in this experiment. Nagesh et al. (2006) also reported inhibition of RKNs when the treatments TH neem and PL neem were applied separately. Generally, the treatments TH neem and PL neem, were as effective in non sterile soils as in sterile, in the control of FOL wilt disease. In the control of *M. javanica* juveniles the treatments TH neem and PL neem were more effective in sterile than in non sterile soils. The establishment of biological control agents in sterile soils may have been higher in the absence of competition and this made them more effective against the *M. javanica* J2s. In order for bio control agents to be effective, they must first establish in the soil (Thomashow et al. 2007; Heydari and Pessarakli 2010). Wang et al. (2004) reported that nematophagous fungi establish better in sterile than in non sterile soil.

The combinations of fungal antagonists and neem were as effective in sterile soils as in non sterile in the control of fusarium wilt. However they were less effective

in the control of *M. javanica* in non sterile compared to sterile soil conditions. Many experiments of neem and fungal antagonists have been carried out in sterile soils (Nagesh et al. 2006; Seenivasan and Poornima 2010; Singh et al. 2014). This experiment supports the use of TH neem and PL neem as fungal antagonists in the control of FOL and *M. javanica* in natural (non sterile soil) conditions. There are other reports on effectiveness TH neem and PL neem in natural soil field conditions in the control of RKNs (Khan et al. 2012). This indicates there is potential of using the biological control agents in natural soil conditions (non sterile soil) to control plant diseases and RKNs as an alternative to using chemicals.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by University of Nairobi [grant number UON/FO/500-661-364].

Notes on contributors

M. W. Mwangi is a Senior Lecturer at Nairobi Technical Training Institute, Nairobi, Kenya. Her teaching interests are in Microbiology and Plant Pathology. She received her MSc degree in Microbiology from Kenyatta University in Kenya.

W. M. Muiru is an a Agricultural Scientist, a Senior Lecturer, Department of Plant Science and Crop Protection in the University of Nairobi (UON), Kenya. He holds a PhD degree in Plant Pathology from the UON.

R. D. Narla is an Agricultural Scientist, a Professor in Plant Pathology in the Department of Plant Science and Crop Protection in the UON. She holds a PhD degree from Osmania University in India.

J. W. Kimenju is a Professor of Nematology in the Department of Plant Science and Crop Protection in UON. He holds a PhD degree in Crop Protection from the UON.

G. M. Kariuki is a Lecturer, Department of Agricultural Science and Technology in Kenyatta University, Kenya. He holds a PhD degree in Plant Nematology from the University of Florida, U.S.A.

References

Agbenin NO. 2011. Biological control of plant parasitic nematodes: prospects and challenges for the poor Africa farmer. Plant Protect Sci. 47:62–67.

Akram W, Anjum T, Ahmad A. 2014. Basal susceptibility of tomato varieties against different isolates of *Fusarium oxysporum* f. sp. *lycopersici*. Int J Agric Biol. 16:171–176.

Coyne DL, Nicol JM, Claudius-Cole B. 2007. Practical plant nematology: a field and laboratory guide. Ibadan (Nigeria): IITA.

Dababat AA, Sikora RA. 2007. Use of *Trichoderma harzianum* and *Trichoderma viride* for the biological control of *Meloidogyne incognita* on Tomato. JJAS. 3:297–309.

El-Mohamedy RSR, Abdel-Kareem F, Jabnoun-Khiareddine H, Daami-Remadi M. 2014. Chitosan and *Trichoderma harzianum* as fungicide alternatives for controlling Fusarium crown and root rot of tomato. Tunis J Plant Prot. 9:31–43.

Fatima K, Noureddine K, Jamal E, Henni JE, Mabrouk K. 2015. Antagonistic effect of *Trichoderma harzianum* against *Phytophthora infestans* in the North-West of Algeria. Int J Agron Agri R. 6:44–53.

Heydari A, Pessarakli M. 2010. A review on biological control of fungal plant pathogens using microbial antagonists. J Biol Sci. 10:273–290.

Kamali N, Pourjam E, Sahebani N. 2015. Elicitation of defense responses in tomato against *Meloidogyne javanica* and *Fusarium oxysporum* f. sp. *lycopersici* wilt complex. J Crop Prot. 4:29–38.

Khan MR, Mohiddin FA, Ejaz MN, Khan MM. 2012. Management of root-knot disease in eggplant through the application of biocontrol fungi and dry neem leaves. Turk J Biol. 36:161–169.

Kiewnick S, Sikora RA. 2006. Biological control of the root-knot nematode *Meloidogyne incognita* by *Paecilomyces lilacinus* strain 251. Biol Control. 38:179–187.

Lamour A, Van den Bosch F, Termorshuizen AJ, Jeger MJ. 2000. Modelling the growth of soil-borne fungi in response to carbon and nitrogen. IMA J Math Appl Med Biol. 17:329–346.

Lokanadhan S, Muthukrishnan P, Jeyaraman S. 2012. Neem products and their agricultural applications. J Biopest. 5:72–76.

Luambano ND, Manzanilla-López RH, Kimenju JW, Powers SJ, Narla RD, Wanjohi, JW, Kerry BR. 2015. Biol Control. 80:23–29.

Nagesh M, Hussaini SS, Ramanujam B, Chidanandaswamy BS. 2006. Management of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *lycopersici* wilt complex using antagonistic fungi in tomato [*Lycopersicon esculentum* Mill.]. Nematol Medit. 34:63–68.

Nene YL, Haware MP. 1980. Screening chickpea for resistance to wilt. Plant Dis. 64:379–380.

Schenck S. 2004. Control of nematodes in tomato with *Paecilomyces lilacinus* Strain 251. Vegetable Report. 5:1–5.

Seenivasan N, Poornima K. 2010. Bio-management of root-knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood in jasmine (*Jasminum sambac* L .) Pest Manag. Hort Ecosyst. 16:34–40.

Singh KP. 2002. Eco-friendly approaches for plant disease management-a report. J Sci Ind Res. 239–240.

Singh R, Biswas SK, Nagar D, Singh J, Singh M, Mishra YK. 2014. Sustainable integrated approach for management of fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Synder and Hansen. SAR. 4:138–147.

Taylor AL, Sasser JN. 1978. Biology, identification and control of root-knot nematodes (Meloidogyne spp). Washington: North Carolina State University Graphics, Cooperative Publication of Department of Plant Pathology, North Carolina State University and USAID.

The insecticide ACT, 1968. Guidelines and data requirements for registration of antagonistic fungi under section 9(3B) and 9(3). www.cibrc.nic.in/guidelines.htm.

Thomashow LS, Weller DM, Mavrodi OV, Mavrodi DV. 2007. Selecting monitoring, and enhancing the performance of bacterial biocontrol principles, pitfalls, and progress. In: Vurro M, Gressel J, editors. Novel biotechnologies for biocontrol agent enhancement and management. New York: Springer; p. 87–105.

Wang KH, McSorey R, Gallaher RN. 2004. Effect of *Crotalaria juncea* amendment on squash infected with *M. incognita*. J Nematol. 36:290–296.

Whitehead AG, Hemming JR. 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. Ann Appl Biol. 55:25–38.