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Nematicidal Efficacy of Soil Fungal Isolates on the Root-Knot Nematode (*Meloidogyne incognita*) of Tomato

U. S. Maishera,M. O. Adebola, J. M. Egubagi and A. Abubakar
Department of Plant Biology, Federal University of Technology, PMB. 65, Minna, Nigeria.

Abstract
The root-knot nematode (*Meloidogyne incognita*) is a polyphagous plant disease causing losses in a wide range of crops. Due to loss of effectiveness and environmental hazard of chemical nematicide, the search for new agents of control is becomes paramount. Therefore, nematicidal efficacy of soil fungal isolates on the root-knot nematode of tomato was carried out. Soil samples for the isolation of fungi and nematode were collected from the rhizosphere of botanical garden and nematode infested tomato farms. The fungi were isolated using serial dilution methods and extraction of nematodes was done using pie-pan method. The antagonistic effects fungi were assessed with potato dextrose broth as control. The results of fungi isolation revealed four fungi species; *Aspergillusniger, Aspergillusnidulans, Penicilliumchrysogenum* and *Rhizopusstolonifer*. Nematicidal activity of the fungal isolate on the pathogen after 24 and 48 hrs results in percentage mortality range of 39.28 to 78.47% (*A. niger*), 29.12 to 57.49% (*A. nidulans*),18.59 to 47.48% (*P. chrysogenum*) and 8.05 to 26.43% (*R. stolonifer*) respectively. Thefindingstudy suggests that the soil fungi could serve as biological agent and alternative source to chemical control of root-knot nematode.

Introduction
Tomato (*Lycopersiconesculentum* Mill) is an anuual crop belonging to the family solanaceae. It is one of the most important vegetable crops cultivated worldwide. The crop is a rich source of lycopene, used in the treatment of cancer especially prostate cancer (Singh *et al*., 2011). According to the National Cancer Institute, research had shown that people who consume large amount of tomato products have significantly low risk of prostate, lung, and stomach cancer. Nigeria is ranked has the second largest producer of tomato in Africa with an annual production of 17,500,000 metric tons (FAO, 2012). However, a large number of phyto-pathogens have been reported to be associated with reduction in tomato productivity. Among them, root-knot nematodes had been reported to the major pathogen affecting the crop (Keshari and Gupta, 2015; Singh *et al*., 2011).However, fumigant and non-fumigant nematicides have been developed for controlling this nematode over years,but there are increasing concerns over the risks o these chemicals on the environment and human health which led to the withdrawal or restriction of these chemicals (Nyezepir and Thomas, 2009). The use of some non-fumigant nematicides based on organophosphates and carbamates has been restricted depending on the region, crop, and production system, such as integrated production and organic farming. Consequently, the search for naturally occurring compounds with nematicidal activity has been stimulated as an alternative to using existing compounds (Huang *et al*., 2016). Soil fungi that inhabit the rhizosphere may serve as an alternative source since they share the same environment as the nematodes and produce metabolites as a strategy for protecting their habitat from plant-parasitic nematodes or other organisms (Moosavi and Zare, 2012).These fungi may release compounds that directly kill nematodes, suppress nematode motility, reduce egg hatching and interfere with metabolic processes, through such mechanisms, they could regulate nematode populations (Moosavi and Zare, 2012).Although, several research studies are available on biological nematode control, there is few or no research on fungal nematicides. Therefore, this study carried out to evaluate the*in-vitro*nematicidalefficacy of some soil fungi isolatein control of*M. incognita* of tomato crop.

Materials and Methods
Collection of materials and Experimental site: Soil samples free from hard particles were collected in a sterile polythene bags from…..selected tomato farms in Niger State that have been ravage with root-knot disease and transported to Laboratory, Department of Plant Biology, Federal University of Technology

Minna. Soil sample was also collected from the Biological Garden, Department of Plant Biology, Federal University of Technology Minnato serve as the control.

Preparation of potato dextrose agar (PDA): Two hundred grams (200g) of peeled Irish potato were washed and boiled for 20 minutes in a 1000 ml of sterile distilled water. The supernatant was drained into one litre flask and make up to 1000 ml. Twenty grams (20g) of agar powder and 20g of glucose powder were weighed and added to the supernatant. The conical flask was corked and sterilized at 121^0C for 15 minutes in an autoclave. The sterilisedmixture was allowed to cool at room temperatue for 10 minutes, and 0.5 ml of 100% concentration chloramphenicol was added to prevent bacterial growth (Adebola*et al*., 2014).

Isolation and identification of soil fungi: Serial dilution was performed for the isolation of fungi as follows: One gram (1g) of soil sample was aseptically suspended into 10 ml of sterile distilled water in test tubes and vortexes properly for suspension of the microorganism in the sample. One (ml) of the diluents 10^{-1} and 10^{-2} were aseptically taken from the suspension and transferred into sterile Petri plate. 0.1 ml of 100% concentration of chloramphenicol was added into the Petri plate, swirled gently and incubated at 27 ± 20^0C for 72 hours (Adebola*et al*., 2014), fungal isolate were identified using cultural and microscopic and characterization as outlined by Cannon and Kirk (2007).

Extraction and identification of nematodes from soil: Nematode was extracted from collected soil samples taken using pie-pan method described by Coyne *et al*. (2007). The isolated organism (*M. incognita* [J2]) was identified microscopically based on observable the characteristics features using standard identification keys (Panesar and Marshall, 2003).

***In vitro*efficacy of fungal isolates against *M. incognita* (J2).:** Agar discs 0.5cm were taken from 7 days, actively growingfungi isolates onPDA and inoculated into 300 ml flasks containing 200 ml Potato Dextrose Broth (PDB). The inoculated broths were incubated on a rotary shaker at 100r/min and 25^0C for 7 days. The mycelia growth and the filtrate were obtained from the broth using a filter paper and microspore filter. *In-vitro* toxicity of the culture filtrate against *M. incognita* (J2), 5 ml culture filtrates of each fungi isolate and 1 ml of *M. incognita* (J2) suspension were pipette into Petri dish with three replicate each. The control petri dishes contained 5 ml PDB and 1 ml of *M. incognita* (J2) suspension. The number of immotile (considered dead) nematodes was viewed and counted after 24 and 48 hours. The values were expressed in percentage (Meyer *et al*., 2004).

Data Analysis: The data obtained on fungi isolation were expressed in percentages. *In-vitro* efficacyresults was subjected to Analysis of Variance (ANOVA) using statistical package for social sciences (SPSS) version 20 determined level of significance and Duncan's multiple range tests (DMRTs) was used separate means were there was significance at 5% level of confidence.

Results and Discussions

Fungal isolation and identification: Based on the observable characteristics (Table 1, Plate 1) Four filamentous species of fungi, belonging to three different genera (*Aspergillus*, *Rhizopus* and*Penicillium*)was isolated from soil samples. *Aspergillusniger*had the highest percentage occurrence (47.00 %) followed by*Aspergillusnidulance*(28.00 %),*Rhizopusstolonifer*(16.00 %) and the least was *Penicilliumchrysogenum*with value of 9.00 % (Table 2.0). The isolated and identified soil fungi is in conformity with the work of Abou-Zaid *et al*. (2007) and Altalhi (2004); who reported the most common genera of soil fungi were *Aspergillus, Penicillium* and *Rhizopus.* Abou-Zaid*et al*., (2007) isolated 70 species of fungi from soil sample of Taif, Saudi Arabia. These results indicated that soil ecology is rich source of habitat for soil inhabiting microfauna

Nematode extraction and identification; The isolated *M. incognita* (J2) varied in length 200 to 300 µm, labial region not offset, elevated labial disc and absent of lateral lips. Stylet ranged from 11 to 25 µm in length, basal knobs offset, rounded to transversely elongated. Hemizonid anterior or adjacent to excretory pore. Tail ranged 15 to 60 µm in length, tip rounded, anal body width 8 to 19 µm. lateral lines four with incisures, phasmidssubterminal, dot-like near cloaca aperture. The isolation of *M. incognita* of the collected tomato farms soil indicated that, these soil fauna might be a sources of primary infection to the crop.

Nematicidal activity of the filtrates revealed that the highest mortality (39.28 %) was due to *Aspergillus niger,* followed by *Aspergillus nidulans* (29.12) and the lowest was due to *Rhizopus stolonifer*(8.06) after24 hours. but showed maximum mortality (78.94% and 57.89% respectively) after 48 hours. However, there was significant different anomg all the mortality for the different fungi. Similar, after 48 hours the highest mortality was obtained in *Aspergillus nidulans* (78.47 %) and the lowest (26.43 %) was exhibited by *Rhizopus stolonifer*. However, there was no mortality recorded in the control for both 24 and 48 hour. The trend of mortality in the fungi filtrates observed in this study agreed with the report of Radwan (2007). They reported that filtrate of *Aspergillus niger* exhibited highest (100%) mortality after 12 hrs followed by

Aspergillus nidulans. Similarly, Siddiqui *et al*. (2004) and Xalxo*et al*. (2013) recorded maximum mortality by filtrates of *Aspergillus nidulans* after 72 hrs. The inhibitory activity of the *Aspergillu*genera could the attributed to their ability of producing toxins which could be harmful to the survival of the nematodes. In concordance with increase in mortality of nematicide with increase in days record in this study, Xalxo*et al*. (2013) reported in nematicidal efficacy of each fungal filtrate increased with duration of exposure.

Conclusion

The finding this study it can concluded that the soil fungi could serve as biological agent and alternative source to chemical control of root-knot nematode.

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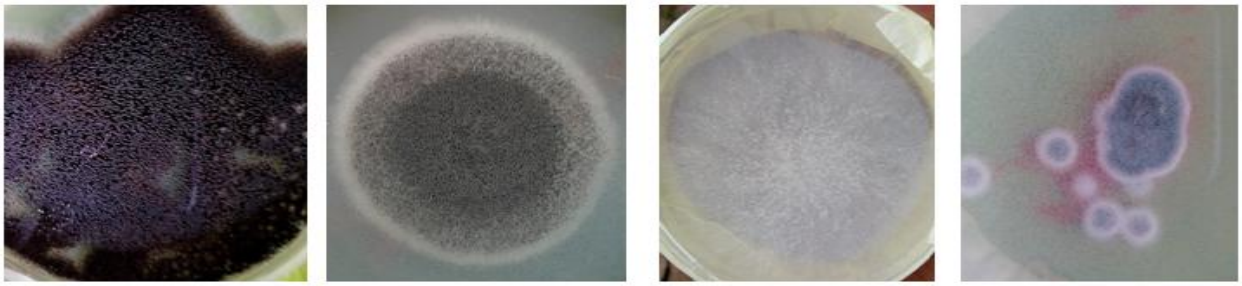
Table 1. Characteristic Features of Isolated fungi

Fungi	Characteristics Features
Aspergillus niger	Rapidly growth colonies on PDA with abundant submerged mycelium, Carbon black/ Deep brownish-black conidial heads. Colourless to pale yellow conidial on reverse Petri dish plate with at initial globose and then radiate well-defined columns.

Aspergillus nidulans	Colonies fast growing reaching 6-7 cm diameter in 7 days, smooth walled conidiophore, small echinulate conidia. Quickly ripening ascospores, purple black in colour, with equatorial binding and large thick walled, globose bodies termed "hulle cells" forming an irregular layer about the perithecia.
Penicillium chrysogenum	Colonies are usually fast growing, green-blue colour with white ring at the margin, sometimes white, mostly consisting of dense conidiophores. Conidiophores is hyaline, erect, branched and penicilately at the apexes with 2-3 metula, 3-4 verticilatephialides and catenulate conidia in each phialides, forming rather compact cylindrical.
Rhizopus stolonifer	Colonies very fast growing, 1-2 cm high, white at first, pale grey, dark brown grey, brownish black. Mycelium branched, non septate, stolons and rhizoids present, sporangiophores arise from these points, sporangia globose, hemispherical, zygospores round or oval.



Aspergillus niger *Aspergillus nidulans* *Rhizopus stolonifer* *Penicillium chrysogenum*

Plate 1: Morphological Features of Isolated Fungi

Table 2Percentage occurrence of fungi isolated from soil in botanical garden

Fungi	Percentage Occurrence (%)
Aspergillus niger	47f
Aspergillus nidulans	28c
Penicillium chrysogenum/notatum	16b
Rhizopus stolonifer	9a

Table 3: *In vitro* assessment of nematicidal activity in culture filtrates

Fungi	Mortality of *M. incognita* after	
	24 Hours	48 Hours
Aspergillus niger	39.28± 1.86^{e}	78.47± 0.56^{e}
Aspergillus nidulans	29.12± 1.52^{d}	57.49± 0.51^{d}
Penicillium chrysogenum	18.59± 1.53^{c}	47 48± 0.52^{c}
Rhizopus stolonifer	8.06± 1.52^{b}	26.43± 0.37^{b}
Control (PDB)	0.00± 0.00^{a}	5.00± 0.00^{a}