BIODIVERSITY STUDY IN RHIZOSPHERE SOILS OF FRUIT TREES AND BIO-EFFICACY TEST OF *CATENARIA ANGUILLULAE* IN SIX LOCAL GOVERNMENT AREAS OF NIGER STATE, NIGERIA

NIRANJAN KUMAR*, DANJUMA, S., GUDUGI, I. A. S., ISAH, M. K. AND HARUNA, U. F.

Department of Crop Production, Faculty of Agriculture, Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria.

***Corresponding author: niranjanibbu@gmail.com**

Article received: 14th May, 2015; Article accepted: 5th July, 2015.

ABSTRACT

Studies on biodiversity of Catenaria anguillulae in different rhizosphere of fruit plants six different Local Government Areas of Niger State, Nigeria in years 2014 and bio-efficacy test of C. anguillulae against free living nematodes were carried out in the Laboratory, Department of Crop Production, Faculty of Agriculture, Ibrahim Badamasi Babangida University, Lapai. Among 48 soil samples collected, 45 samples yielded infected nematodes by C. anguillulae. However, the percentage infection of nematodes by C. anguillulae varied significantly from different Local Government Areas and also from different rhizosphere of fruit plant. The degree of infection of nematodes by C. anguillulae varied from 12.47 to 67.57% irrespective of the types of soil samples. Soil sample from citrus rhizosphere yielded the highest (67.57%) biodiversity of this fungus, whereas, in least biodiversity (12.47) was in banana soil samples. In bio-efficacy test of isolates of C. anguillulae, isolate mango (I_M) has the highest percentage (65.33%) of killing nematodes, whereas, the minimum (19.33%) bio-efficacy was observed in banana isolate (I_B).

Keywords: *Catenaria anguillulae*, fruit trees, rhizospheric soil, free-living nematode

INTRODUCTION

Catenaria anguillulae Sorokin is a zoosporic fungus (Sorokin, 1876) and facultative endo-parasite of free living and plant parasitic nematodes (Jaffee, 1986; Singh and Gupta, 1986; Singh *et al.*, 1996; Decan and Saxena, 1997; Singh *et al*, 2007; Singh *et al*., 2012). Moreover, zoospores of the fungus also parasitised eggs (Martin 1978; Singh and Gupta, 1986; Singh *et al*., 2007) and 2nd stage juveniles of root-gall and cyst nematodes (Singh and Gupta, 1986; Singh *et al*., 2007). The fungus was classified as member of the order Chytridales, until Couch (1945) transferred it to the order Blastocladiales. This change was made based on similarities between the genus *Catenaria* and members of the order Blastocladiales with respect to zoospore structure and behaviour, nature of hyphen reputations, origin and germination of the resistant sporangium and life cycle.*C. anguillulae*is one of the commonest parasites of tiny invertebrates in soil and fresh water ecosystem (Whisler, 1985; Dick, 2003; Barron, 2004; Gleason *et al*., 2010). Zoospores are attracted to and encysted near the natural openings such as mouth, excretory pore and anus of nematode body (Singh *et al.*, 1993; Decan and Saxena, 1997). Tunlid *et al*. (1991) reported that zoospores adhere to the cuticle by a layer of extra cellular protein which functions as an adhesive substance. Eventually, zoospores encyst on the surface of the host. The cyst germinates forming a narrow germ tube. After growing a short distance the germ tube produces in intercalary vesicle from which the rhizoidal system emerges and penetration usually occurs directly through the cuticle but can occur rarely through natural openings (Jaffee, 1986; Decan and Saxena, 1997).*C. anguillulae* found in different types of soil (Barron, 1977; Parmark*et al.*, 1995; Vaish and Singh, 2002; Singh *et al.*, 2007) and also recorded from different type of leaf litter substrates (Kumar *et al*., 2013).It has been reported that the fungus attacks nematodes with varying degrees of severity, (Esser and Ridings, 1973; Barooti *et al*., 1985; Jaffee, 1986; Singh and Gupta, 1986; Singh *et al*., 1996; Singh *et al.*, 2007; Singh *et al*., 2012). Singh and Gupta (1986) reported that *C. anguillulae* caused epidemic in a sorghum field severely infested by cyst nematode (*Heterodera sorghi*) disease, which resulted in decline in the population of nematode. Barooti*etal.* (1985) also reported natural parasitism of plant parasitic nematodes by *C. anguillulae* to the extent of 3.5%, further, Gupta *et al*.(2003) studied natural parasitism of nematodes by *C. anguillulae* in the rhizosphere soil of different fruit plants and observed that it varied between 0.60% and 36%.

Plant parasitic nematodes destructive pathogens of many agricultural crops and cause over 100 million losses per year worldwide (Chitwood, 2003). These nematodes are ubiquitous, particularly in areas with warm or hot climates and in green houses (Agrios, 2005; Dropkin, 1980; Kolev*aet al*., 2006; Das *et al.*, 2011). These nematodes can be managed by cultural practices and synthetic chemicals especially nematicides. The use of synthetic nematicides has limitations because of environmental and human health concerns in addition nematicides are expensive. Therefore, there is urgent need to develop alternative to manage the nematode biodiversity in the agricultural soil. Since *C. anguillulae* is a polyphagous type of fungus, can attack several kinds of nematodes in nature and *in vitro*. The fungus has been studied and appears to be the most important bio-control agents for regulating population dynamic in the soil especially those soils which are severely infested by plant parasitic nematode (Singh and Gupta, 1986; Singh *et al*., 2007; Singh *et al*., 2012).In view of the above facts, the aims of this research paper were to find out the biodiversity of *C. anguillulae* from rhizosphere soil of different fruit plants viz., mango, guava, citrus, banana, African shea butter tree, papaya, cashew**,** African locust bean tree,and to assess the bio-efficacy of isolated isolates of the fungus against free living nematodes *In vitro*.

MATERIALS AND METHODS

Experimental Site

The experiment was conducted in the Laboratory, Department of Crop Production, Faculty of Agriculture, Ibrahim Badamasi Babangida University, Lapai, Niger State in 2014. However, collection of samples was done from different Local Government Areas of Niger State, Nigeria. This lies between longitude 9º-02'N and latitude 6º-34'E of the equator. The area is located in the vegetative zone of Guinea Savannah middle belt of Nigeria. It has an average temperature of 23ºC-34.4ºC, minimum rainfall of 1073mm. The soil is laterite or ferruginous. The areas visited in the State the main occupation of people was farming, only few people were involved in trading (Geographical reformist, 2010). The treatments were laid out in a Completely Randomized Design (CRD). The collected data were converted into % mortality of nematodes or % infection caused by *C. anguillulae*.

Evaluation of Biodiversity *Catenaria anguillulae* from rhizosheric Soil of different fruit crops

Approximately 500 g of soil samples were collected in separate polyethylene bags from the rhizosphere of different fruit crops viz: mango (*Mangifera indica*), guava (*Psidium guajava*), citrus (*Citrus limon*), banana (*Musa* spp.), African *shea* butter tree (*Bulyrospermum parkii*), papaya (*Carica papaya*), cashew (**(***Anacardium occidentale***),** African locust bean tree **(***Parkia biglobosa***)**, from different Local Government Areas of Niger State, Nigeria. From each location, soil samples were collected from July, 2014 to September, 2014and mixed thoroughly.

The mixed soil samples were used for extraction of nematodes following the decanting and sieving techniques given by Cobb (1918). Natural parasitism of nematodes by *C. anguillulae* was recorded after 48 hours and calculated in terms of percentage as given below:

Percentage Natural Parasitism of = No. of nematodes parasitized by *C. anguillulae* X 100
Total number of nematodes extracted

All the freshly isolated isolates of *C. anguillulae* were maintained in the laboratory for further experiments.

Bio-efficacy test of eight isolates of *C. anguillulae* against free living nematodes

The bio-efficacy test of eight isolates of *C. anguillulae* against free living nematodes was tested by the method described by Singh *et al.*(1998). The free-living nematodes were extracted from rhizosphere soil. The population of the extracted nematodes was maintained in water in the Petri dishes.

Approximately 50 free-living nematodes were poured into sterilized cavity blocks, 10 infected nematodes by *Catenaria anguillulae* were inoculated in each of the cavity blocks with the help of sterilized glass pipette, and observation was taken after 48 hours. Virulence test of all the isolates of *C. anguillulae* against nematodes was carried out at room temperature (25±1ºC) by transferring five infested nematodes with developed sporangia before releasing zoospores into cavity having 50 nematodes. The cavity blocks were incubated at room temperature. Three (3) cavity blocks were used as replicates. Observations on infected nematodes were recorded and calculated in terms of mortality percentage as mentioned above.

RESULTS

Biodiversity of *Catenaria anguillulae* in rhizospheric soil of fruit plants

The observation on biodiversity on *Catenaria anguillulae* from different Local Government Areas in years 2014 and bio-efficacy test of *C. anguillulae* against free living nematodes are presented in (Table 1) and (Table 2) respectively. It was clearly shown from the data that most of the soil sample collected showed the presence of *C. anguillulae*. Among 48 samples, 45 samples yielded infected nematodes by

C. anguillulae (Table 1). However, the percentage infection of nematodes by *C. anguillulae* varied significantly from different Local Government Areas and also from different rhizospheric fruit plant soils. The degree of infection of nematodes by *C. anguillulae* varied from 12.47 to 67.57% irrespective of the types of soil sample. However, the percentage infection of the eight (8) samples viz; mango, citrus, guava, banana, African shea butter tree, papaya, cashew, African locust bean tree which yielded the following percentage of *C. anguillulae*. From mango rhizospheric of *C. anguillulae* was observed in soil maximum percentage Paikoro (64.28) followed by Lapai (61.53) and Minna(57.89), whereas the minimum (42.85) was in Wushishi. From Guava rhizospheric soil the maximum population of *C. anguillulae* was (48.70) in Minna followed by Lapai (42.00) and Paikoro (36.33) whereas the minimum (21.50) was in Wushishi.

From citrus the maximum (67.57) was in Lavun followed by Lapai (53.66) and Paikoro (48.00) whereas minimum (31.36) was in Wushishi. From Banana maximum (25.00) was in Kontagora followed by Lavun (19.33) and Lapai (17.75) whereas the minimum (12.47) was in Paikoro. However, Wushishi did not yield any population of *C. anguillulae*. From African shea butter tree, the maximum (29.75) was in Wushishi followed by Lapai (24.37) and Paikoro (20.00) whereas the minimum (13.56) was in Lavun. However, Minna Local Governement Area did not yield any significant information. Papaya maximum (28.81) was in Lapai followed by Kontagoro (23.76) and Paikoro (18.52) whereas minimum (15.24) was in Minna, Lavun Local Government did not yield any number of the fungus. African locust bean tree maximum (33.33) was in Lavun followed by Lapai (28.67) and Kontagora (22.00) whereas minimum (12.50) was in Wushishi (Table 1). Irrespective of Local Government Area, it is clear from data that citrus rhizosphere so yielded the highest % (67.57) biodiversity of this fungus, whereas, in least biodiversity (12.47) was in banana soil samples.

Bio-efficacy test of eight isolates of *Catenaria anguillulae* against free living nematodes.

Bio-efficacy test of eight (8) isolates of *Catenaria anguillulae* against free-living nematodes. The observations on bio-efficacy test of eight isolates of *C. anguillulae* against free-living nematodes are presented in (Table 2). It is clear from the observation that percentage infection of free-living nematodes by *C. anguillulae* differed significantly among eight (8) isolates of *C. anguillulae* which evidently indicated that all the isolates tested for the present bio-efficacy test differed significantly. It was clear from observation that I_M (65.33%) has the highest percentage of killing nematodes. The data also showed that isolate citrus showed the second highest (60.67%). The minimum bio-control potential was observed in isolate banana (19.33%).

Table 1: Biodiversity of *Catenaria anguillulae* in rhizospheric soil of fruit plants from six Local Government Areas of Niger State, Nigeria.

FRUIT PLANTS	LOCAL GOVERNMENT AREAS					
	Lapai	Lavun	Minna	Paikoro	kontogora	Wushishi
Mango (*Mangifera indica*)	61.53	50.00	57.89	64.28	55.55	42.85
Citrus (*Citrus limon*)	53.66	67.57	42.28	48.00	38.50	31.36
Banana (*Musa* spp.)	17.75	19.33	14.65	12.47	25.00	0.00
African shea butter tree *(Butyrospermumparkii)*	24.37	13.56	0.00	20.08	18.85	29.75
Papaya (*Carica papaya*)	28.81	0.00	15.24	18.52	23.76	16.23
Cashew (*Anacardium occidentale*)	52.00	27.27	29.43	34.21	45.38	41.29
African locust bean tree (*Parkia biglobosa*)	28.67	33.33	16.66	18.89	22.00	12.50

Table 2: Bio-efficacy test of eight isolates of *Catenaria anguillulae* against free living nematodes.

Isolates	Days				
	4th	5th	6th	7th	8th
I_M	6.67	25.00	39.67	58.00	65.33
I_G	3.33	7.67	16.33	38.67	53.33
I_{CI}	4.00	13.33	28.00	47.33	60.67
I_B	-	-	5.00	13.67	19.33
I_{SB}	-	1.33	10.33	17.00	26.00
I_P	-	-	9.67	14.00	23.67
I_{CA}	3.67	9.00	21.33	41.33	56.33
I_{PAR}	1.33	4.33	13.67	29.67	47.00

I_M = Isolate of mango, I_G= Isolate of guava, I_{CI}= Isolate of citrus, I_B= Isolate of Banana, I_{SB}= Isolate of African shea-butter tree, I_P= Isolate of papaya, I_{CA}= Isolate of cashew, I_{PAR}= Isolate of African locust bean tree

DISCUSSION

The study on biodiversity of *C. anguillulae* from rhizosphere soil of different fruit plants viz., mango (*Mangifera indica*), guava (*Psidium guajava*), citrus (*Citrus limon*), banana (*Musa* spp.), African *shea* butter tree (*Bulyrospermum parkii*), papaya (*Carica papaya*), cashew (**(***Anacardium occidentale***),** African locust bean tree **(***Parkia biglobosa***)** from different Local Government Areas of Niger State, Nigeria in year 2014 clearly shows varying degree of infection of nematodes by the fungus. The varying degree of infection of nematodes shows that *C. anguillulae*

parasitizes and kills nematodes in these rhizospheric soil samples. Similar data on varying degree of natural and *In Vitro* parasitism of nematodes in soil sample have been reported by (Singh *et al.,* 2012;Barooti *et al.,* 1985; Jaffee, 1986; Gleason *et al.,*2010, Singh *et al.,* 2007; Singh *et al*., 2012; Kumar *et al*., 2013). Singh *et al*. (1993) reported that, zoospores of the fungus generally attracted to and encyst near the mouth, excretory pore and anus of infected nematodes where they aggregated. Similar phenomenon was also observed in the present work that zoospores were attracted at natural openings of the host body. Since 1876 when Sorokin reported that *C. anguillulae can* parasitize and kill nematodes, its potential to parasitize the nematodes has been a controversial issue, while some workers have reported *C. anguillulae* as a virulent pathogen of nematodes (Stirling and Platzer, 1978; Singh and Gupta, 1986; Singh *et al.,* 2007; Singh *et al*., 2012), and others have reported that this fungus is a weak parasite (Sayre and Keeley, 1969; Boosalis and Maukau, 1965). Bio-efficacy test of *Catenaria anguillulae* against free-living nematodes differs significantly. It was clear from the observation that isolate mango has the highest percentage of killing free-living nematodes. It attributes that the isolate of mango has the highest virulence against nematodes. It might be due to the impact of high population of nematodes present in the rhizosphere of mango root system. The minimum bio-control potential was observed in isolate banana I_B, it might be due to least extensive root system of the fruit plant and least population present in rhizosphere of banana plant. Sayre and Keeley (1969), Singh *et al.* (1996), Singh *et al*. (2007) and Singh *et al*. (2012) reported that some saprophytic and plant parasitic nematodes are also highly susceptible to *C. anguillulae* which clearly support the present work.

CONCLUSION

The present research shows clearly the presence of *C. anguillulae* from different Local Government Areas of Niger State of Nigeria in rhizosphere soil of different fruit plants.Some of this isolates of *C. anguillulae* were highly virulent.Since it is a preliminary research work in the State, further study is needed on these aspects so that this valuable fungus can be utilized for the integrated management of plant parasitic nematodes.

REFERENCES

Agrios, G.N. (2005). *Plant Disease Caused by Nematodes.* In: Plant Pathology, Agrios, G.N. (Ed.). Elsevier Academic Press Ltd., London pp:608.

Barooti, S.B., Daneshpazuk, B., Torabi, M., 1985.Two predaceous and parasitic fungi to nematodes in Iran. *Iranian Journal of Plant Pathology* 21, 13–16.

Barron, G.L., 1977. *The nematode-destroying fungi*, In Topics in Mycobiology-1.Canadian Biological Publications Ltd., Guelph, Canada.140 p.

Barron, G.L., 2004. *Fungal parasites and predators of rotifers, nematodes, and other invertebrates*. In: Mueller, G.M., Bills, G.F., Foster, M.S. (Eds), Biodiversity of Fungi, Inventory and Monitoring Methods. Elsevier Academic Press, Amsterdam, pp. 435–450.

Boosalis, M.G., Mankau, R., 1965. *Parasitism and predation of soil microorganisms*. In: Barker, K.F., Snyder, W.C. (Eds.), Ecology of Soil-Borne Plant Pathogens. California University Press, Berkeley, USA, pp. 374–391.

Chitwood, J. 2003. Research on plant parasitic nematodes biology conducted by the United State Department of Agriculture. Agriculture Research Service. *Pest Management Science*. 59, 748-759.

Cobb, N.A. (1918). Estimating the nematode population of the soil. *Agric. Cric. Bur. Pl. Ind. U.S. Department Agri*c. No. 1 p48.

Couch, J.N., 1945. Observations on the genus *Catenaria*. *Mycologia* 37, 163–193.

Das, S., Wesemael, W.L.M., Perry, R.N. (2011). Effect of temperature and time on the survival and energy reserves of juveniles of *Meloidogyne* spp. *Agricultural Science Research Journal* 1:102-112.

Deacon, J.W., Saxena, G., 1997.Oriented zoospore attachment and cyst germination in *Catenaria anguillulae*, a facultative endoparasite of nematodes. *Mycological Research* 101, 513–522.

Dick, M.W., 2003. *The Peronosporales and other flagellate fungi*. In: Howard, D.H. (Ed), Pathogenic Fungi in Humans and Animals, second ed. Marcel Dekker, Inc, New York, pp. 17–66.

Dropkin, V.H. (1980). *Introduction to Plant Nematology.* John wily and Sons, New York.

Esser, R. P. and Ridings, W. H. 1973.Pathogenicity of selected nematodes by *Catenaria aguillulae*. *Proceedings of the soil and Crop Science Society of Florida*. 33: 60-64.

Gleason, F.H., Marano, A.V., Johnson, P., Martin, W.W., 2010. Blastocladian parasites of invertebrates. *Fungal Biology Reviews* 24, 56–67.

Gupta, R.C., Singh, R.K., Singh, N.K., Singh, K.P., 2003. Natural parasitism of nematodes by *Catenaria anguillulae* Sorokin in the rhizosphere of different fruit plants. *Indian Journal of Plant Pathology* 21, 33–40.

Jaffee, B.A., 1986. Parasitism of *Xiphinema rivesi* and *X. americanum* by zoosporic fungi. *Journal of Nematology* 18, 87–95.

Koleva, L., Neshev G., Masheva S., Loginova E., Samaliev H., Naidenov M., Simidchiev H., Kanazirska V., Yankova V., Kokoranova K. (2006). Handbook for introduction with the most important pests in greenhouse vegetable crops and control against them with alternatives of methyl bromide. NSPP, FAO, GEF, UNDP, UNEP, 136.

Kumar, Niranjan; H.M. Majiya, M.K. Isah; S. Mustapha.(2013). Natural isolation of *Catenaria anguillulae* from different substrates and its virulence test. *Development Journal of Science and Technology Research*, 2(1): 68-74.

Martin, W.W., 1978. Two additional species of *Catenaria* (Chytridiomycetes, Blastocladiales) parasitic in midge eggs. *Mycologia* 70, 461–467.

Sayre, R.M., Keeley, L.S., 1969. Factors influencing *Catenaria anguillulae* infections in a free living and a plant parasitic nematode. *Nematologica* 15, 492–502.

Singh, K. P.; Vaish S.S., Niranjan Kumar, Singh K.D., Minakshi Kumari (2012).*Catenaria anguillulae* as an effective biological control agent of *Anguinatritici* in vitro. *Biological Control*, 61:185-193.

Singh, K.P., Bandyopadhyay, P., Stephen, R.A., Vaish, S.S., Makesh Kumar, T., 1998.Techniques for selective isolation, semiquantification and rapid virulence testing of *Catenaria anguillulae*. *Mycological Research* 102, 658–660.

Singh, K.P., Gupta, P., 1986. Observations on *Catenaria anguillulae* parasitic on *Heterodera sorghi*. *Advances in Biological Research* 4, 240–250.

Singh, K.P., Jaiswal, R.K., Niranjan, Kumar., 2007. *Catenaria anguillulae* Sorokin: a natural biocontrol agent of *Meloidogyne graminicola* causing root knot disease of rice (*Oryzasativa* L.). *World Journal of Microbiology and Biotechnology* 23, 291–294.

Singh, K.P., Stephen, R.A., Makesh Kumar, T., 1993. Development of *Catenaria* in *Heterodera cajani*. *Mycological Research* 97, 957–960.

Singh, K.P., Stephen, R.A., Vaish, S.S., 1996. Pathogenicity and development of *Catenaria anguillulae* on some nematodes. *Mycological Research* 100, 1204–1206.

Sorokin, N., 1876. Note Sur les vegetaux parasites des Anguillulae. *Annels des Sciences Naturalles, Botanique* 64, 62–71.

Southey, N.W., 1970. *Laboratory Methods for work with Plant and Soil Nematodes*. Ministry of Agriculture, Fisheries and Food, Technical Bulletin No. 2. HMSO: London.

Stephen, R.A., 1992. Studies on *Catenaria anguillulae* Sorokin.Ph. D. Thesis, Banaras Hindu University, Varanasi, India.

Sterling, A.M., Platzer, E.G., 1978. *Catenaria anguillulae* in the mermithid nematode *Romanomermis culicivorax*. *Journal of Invertebrate Pathology* 32, 348–354.

Tunlid, A., Nivens, D.E., Jansson, H.-B., White, D.C., 1991.Infrared monitoring of the adhesion of *Catenaria anguillulae* zoospores to solid surfaces. *Experimental Mycology* 15, 206–214.

Vaish, S.S., 1999. Studies on *Catenaria anguillulae* Sorokin as biocontrol agent against some plant parasitic nematodes.Ph. D. Thesis, Banaras Hindu University, Varanasi, India.

Vaish, S.S., Gupta, R.C., Singh, K.P., 1997. Pathogenicity and performance test of *Catenaria anguillulae* on *Heterodera avenae*. *Current Nematology* 18, 1–6.

Vaish, S.S., Mir, A.A., 2002. *Catenaria anguillulae*Sorokine a facultative endoparasite of nematodes from soils of cold region of Leh (Ladakh). *Indian Journal of Plant Pathology* 20, 87–89.

Vaish, S.S., Singh, K.P., 2002. Distribution of *Catenaria anguillulae*, a facultative endoparasite of nematodes in soils from different locations of India. *World Journal of Microbiology and Biotechnology* 18, 65–67.

VoB, B., Wyss, U., 1990. Variation between strains of the nematophagous endoparasitic fungus *Catenaria anguillulae* Sorokin. Factors affecting parasitism In vitro. *Journal of Plant Diseases and Protection* 97, 416–430

Whisler, H.C., 1985. *Life history of species of Coelomomyces*. In: Couch, J.N., Bland, C.E. (Eds), The Genus Coelomomyces. Academic Press Inc, New York, pp. 9–22.