Science Park Journals

Vol. 2(1), pp. 012-017, April 2018
ISSN ISSN 2315-6279
DOI: 10.14412/JARNR2018.019
Copyright© 2018
Author(s) retain the copyright of this article
Available online at http://www.scienceparkjournals.org/jarnr

Journal of Agricultural Research and Natural Resources

Full Length Research Paper

Isolation of Nematodes and Determination of Best Habitats of Nematode Species

S.D Randive, S.M. Pore

Asst. Professor, Dept. of Biotechnology, Walchand College of Arts and Science Solapur (MS)
Correspondence Email: sonali_dream1@rediffmail.com

Abstract: Nematodes are used to cure the diseases. Recent testing suggests that more than 65% of human disease genes have equivalents in the genome of *Caenorhibditis elegans* nematode. They are also applicable in agriculture and horticulture field. In this study nematodes were isolated from varity of soil samples like compost soil, play ground soil, sandy soil and garden soil to determine which type of soil is the best habitats for nematode by performing assay of nematodes in laboratory. The result shows that compost soil is the best habitats for nematodes. According to assay of nematodes high numbers of nematodes were obtained from raw material from commercial vegetables and field crop, Landscape Plants and trees and vegetable gardens.

Keywords: Caenorhabditis elegans, compost soil, soil & plant nematodes, agriculture

Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution http://creativecommons.org/licenses/by/4.0/

Introduction:

Caenorhabditis elegans is belongs to genus *Caneorhabditis* and family *Rhabditida. C.elegens* is free-living transparent nematode, about 1mm in length, which lives in temperate soil environment. Research on molecular and developmental biology of *C.elegans* was started in 1974. It has been extensively used as model organism. Nematodes have successfully adapted to nearly every ecosystem from marine to fresh water, from polar region to the tropics as well as highest to lowest elevations. Nematodes are identified and counted using standard council of Canada accredited Barman pan method for nematode extraction. *C.elegans* was first multi cellular organism to have its genome completely sequence. It has 100 million base pairs long sequence containing approximately 20,100 protein coding genes. Nematodes are useful in agriculture and horticulture field. Also nematodes are useful to cure the disease. Nematodes are used to test biological effect of space light such as genetic damage caused by exposure to cosmic radiation, some nematodes kill crop pests, it is also useful in studying meiosis. Because of lots of economic importance it is necessary to increase the number of nematodes. This study is carried out to determine the best soil and plant habitat for nematodes.

Material -Live *E.coli* Culture, Loria broth (LB) or Nutrient Broth (NB), Nutrient agar media, Macconkey's agar media, bleach

Soil Samples:- Sandy samples, Compost samples, Garden Soil & Playground dirt.

Methodology:-

Macconkey's agar media was prepared. This media was autoclaved for 1 hrs. *E.coli* plates were prepared by spreading soil sample suspension. These plates were incubated for 12-14 hrs. Isolated colonies were collected from the plates and *E.coli* suspension was prepared. Spread this suspension on nutrient agar media and kept it for incubation for 12-14 hrs or 3 day's at room temperature. Lawn formation was observed on each plate. The lawn of *E.coli* was transferred to the nutrient agar plates and kept it for incubation for 12-14 hrs. 2 gm of each soil sample was mixed with 2 ml of distilled water. These dampen soil samples were spread on each plate and replicate it. Number of nematode on each agar plate was counted by using magnifying glass after 30 minutes.

Nematode Assay in Laboratory:-

1.Collection of the sample:-

a) Before planting:- Most nematode management practices must be performed before planting. Therefore nematodes samples need to be taken well before planting. Usually it is best to collect samples at the end of previous crop. Soil was collected from 10-20 field locations.

b) After Planting:- Some times it is necessary to determine what causing plant to get sick after they are planted. For these samples both roots and soil are required. Roots and soil should be collected 8-10 in. deep from around 10-20 sick plants. Avoid dead or dying plant, since decomposing roots will often harbour fewer nematodes. Whole plants with adhering soil may be taken for analysis. A minimum of one cup of soil and one to two cups of roots are required. The roots and soil need to be placed in to the same plastic bag for submission of nematodes assay.

Collect nematodes samples from edges of declining areas.

2.Landscape Plants and trees:-

a) Before planting:- soil from 8-12 locations was collected in a planting bed. Samples should be taken 8-12 in. deep. About a handful of soil from each location is adequate. Combine all the soil in to single plastic bag. The total volume of soil from the sample should de between 1 pint and ½ gallon. Sample may be taken with shovel. If using a shovel you can put a part of soil from 8-12 shovels full into a bucket. Thoroughly mix the soil in the bucket and taken out a pint for analysis.

b) After planting:-for this type of soil and roots was taken.dig soil and roots from around the drip line of the plant. Sample depth depends on the size of plant. For the bedding plants 6 in. deep is adequate. For most woody plants 8-10 in. deep. Place the soil and roots together in same plastic bag. A minimum of 1 pint of soil and 1-2 cups of roots are required and observe for nematode formation.

3.Vegetable gardens:-

a) Before planting:-Soil was collected from 8-10 locations in garden .samples should be taken 10 in. deep. Total volume of soil sample should be between 1 pint and half gallon. Thoroughly mix the soil and taken out a pint for analysis.

b) After planting:- For collection of roots and soil of vegetable sample should be taken 8-10 inch. deep. Place the soil and roots together in a plastic bag and allow it for observation.

4.Nurseries: -

a) Root feeding nematodes:- For smaller plotted plants 1 gallon size , submit several entire plant in their pots. The plant should be laced into plastic bags. For large plotted plants a cup of soil and roots was taken in same plastic bag.

b) Foliar nematodes: - For smaller potted plants, submit several entire plants in their pots. The plants should be placed in to plastic bags. For larger plant cut off twig with affected leaves. A damp paper towel may be wrapped around the base of the twig to help keep them from drying out. Place the twigs into a plastic bag and seal.

5.Citrus:-

Take several samples from the drip line of the trees. Samples should be 12 in deep for most nematodes, but need to be deeper for detection for burrowing nematodes. Both soil and feeder roots are taken together in a plastic bag. A minimum of 1 pint of soil and 1-2 cup of roots are required. Sampling for detection of burrowing nematodes is more involved than for other nematodes of citrus.

Fig i. *E.coli* growth

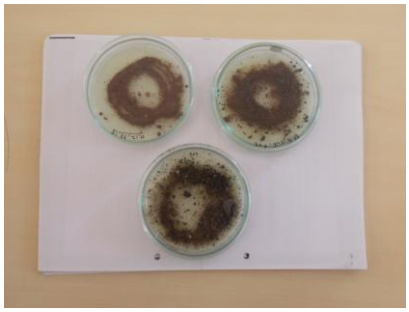

Fig ii. Lawn of Soil

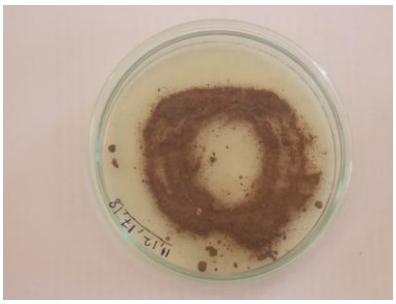

Fig iii. Isolated Nematode

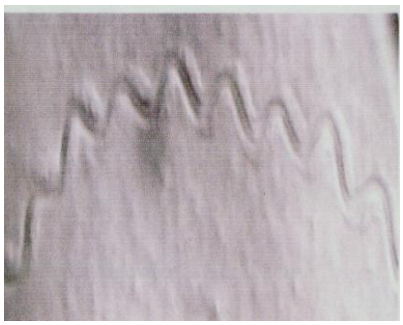

Fig vi. Move of Nematode on Agar

Observation:-

Sr. no	Soil Sample	Replicates	No. of Nematodes
01	Playground Soil	3	2
02	Compost Soil	3	10
03	Sandy Soil	3	1
04	Garden Soil	3	4

Table i. Observations with soil sample

Sr no	Sample	Replicates	No. of Nematodes
01	Commercial vegetables & field crop	3	20
02	Landscape Plants& trees	3	18
03	Vegetable gardens	3	12
04	Nurseries	3	9
05	Citrius	3	10

Table ii. Observations with plant samples

Results- Isolated *caenorhibditis* elegans nematodes were observed.

From above observation table it was concluded that the playground soil sample contain 2 nematodes, Compost soil contain 5 nematodes, sandy soil contains 1 nematodes and garden soil contains 4 nematodes. Recent testing suggests that more than 65% of human disease genes have equivalent in the genome of *caenorhibditis.* According to assay of nematodes high numbers of nematodes were obtained from raw material from commercial vegetables and field crop, Landscape Plants and trees and vegetable gardens.

Discussion:- In this project isolation of nematodes by using different types of media, in various types of soils were tested. It was concluded that compost soil is the best habitat for nematode species as compared to other soil samples. Determination of different soil the nematode Assay Laboratory determines the types and number of plant parasitic nematodes in soil and plant samples.

References:-

1. Barriere, A. & Felix,M Isolation of C. elegens & related nematodes, wormbook, ed. the C.elegens research community2006.
2. Caenorhabditis isolation guide, Wormbase.
3. Dolgin, Elie , Slime for dime Science 317(5842) , 2007.
4. Felix, M., Braendle, C., the natural history of c. elegens, Current biology 20(22), 2010.
5. Greg Walker, Sardi, A Guide to soil and crop sampling for nematodes.
6. http://www.cell.com/content/article/absract?uid=PIIS0092867406012955
7. http://www.sciencemag.org/cgi/content/abstract/282/5396/2012.
8. Hugot J. Baujard P., and Morand S. biodiversity in helminthus and nematodes as a field of study an overview, Nematology 3 2001 199-208.
9. Jagdale , G**.,** Sampling for Plant-Parasitic Nematodes Identification and Diagnosis.
10. Kiontke,K., and Sudhaus,W., ecology of *Caenorhibditis* species 2006.
11. Kosinski, R., and Zaremba,M., Dynamics of the model of the C. elegens acta physica polonicaB 38(6), 2007.
12. Lortkipanidze, M., Chkhubianishvili, T., and Burjanadze, M., Bullitin of the Georgian National Acadamy of science, vol. 4, no. 2, 2010
13. Tree of life web project Nematode version of 2002, 2008.SSS
14. Watts, D., and Strogatz, S., Collective dynamics of small world networks Nature 393(6684), 1998, 440-442.
15. Wood, Willium B. Introduction to C. elegenes biology 2009. 12-13.
16. Wormbasewiki ws 169 release notes, Wormbase.
17. Feng, z., Ward, w., *et.al* C.elegans model of nicotine dependant behaviour regulation by TRP family channels. Cell 127 (3), 2006, 621-633.
18. http://www.wikihow.com/Isolate-Entomopathogenic-Nematodes