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Studies on the efficacy of different methods of nematode extraction with special reference to soil types

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Abstract
A study was conducted to know the efficiency of different nematode extraction methods from soil. Among different Pre-soaking times for extraction of nematodes from the soil by modified Baermann funnel method, Pre-soaking for 30 minutes showed increased recovery of nematodes from black soil (20.02% increase) and red loam soil (25.21% increase) respectively, when compared with the regular 5 minutes Pre-soaking with recovery of different nematode genera viz., *Longidorus elongatus* (58.33 percent) and *Pratylenchus coffeae* (41.00 percent) in both black and red loam soil respectively. Thirty minutes Pre-soaking was found to be the best for extraction of nematodes by centrifugal sugar floatation method from both black and red loam soil with 22.16 percent increase in nematode recovery. Among the extraction of nematodes by incubation technique with different mistifying intervals, mistifying with 5 minutes interval was found to recover the highest number of nematodes, which recorded 40.56 and 48.32 percent increased nematode recovery in both black and red loam soil respectively, when compared to 30, 60, and 120 minutes of spraying interval.

Keywords: Modified Baermann funnel method, Split roots, conical flask method, centrifugal sugar floatation method

Introduction
Plant parasitic nematodes play a major role in reducing the yield in a wide variety of crops throughout the world. The estimated yield loss due to nematodes is around US$ 125 billion annually in agriculture [2]. Nematodes are found in wide varieties of habitat namely terrestrial, fresh water and marine. The population assessment is essentially required for any nematological investigation. Appropriate extraction technique should be followed to obtain maximum recovery of nematodes. The extraction methods vary with type of nematodes, type of parasitism and the activeness of nematodes. To estimate the quality and quantity of the nematode population in soil and plant, appropriate extraction technique is essential. Over the years, the number of modification of basic methods has proliferated prodigiously. Usually the modifications were developed to satisfy the specific needs of individual investigator, motivation was usually due to particular characteristics of nematode infested soil, plant tissue substrate or expediency requirements of experiment [10]. Limited research had been done towards the optimization of the efficiency of the most extraction techniques. Hence fine tuning of the existing methods for extraction so as to get highest recovery of nematodes is required. As the nematodes are held in a thin film of water around the soil particles and most of the nematodes lie in quiescent or semi-quiescent state under anhydrobiotic condition, the recovery rate of these nematodes is less. In order to obtain highest recovery an attempt was made by fine tuning the existing methods of extraction in this experiment.

Materials and Methods
In the present investigation, attempts have been made to fine tune the extraction techniques already available in order to recover more number of nematodes. All the experiments in the present study were carried out at the Department of Nematology, Tamil Nadu Agricultural University, Coimbatore.

Nematode collection sites
For the present study, the nematode collection sites include Eastern Block region of TNAU campus, Coimbatore for black cotton soil, Theethipalayam of Coimbatore district for red loam soil.

Collection of soil samples from the fields

Soil samples were collected from the rhizospheres of the plants. About 500cc soil samples were collected individually at 20 to 30 cm depth and sampling was done from the four corners and centre of each selected crop field. From this, 200cc soil was taken for extraction of nematodes by quartering method. Samples were maintained at laboratory conditions until they were processed and analyzed further.

Extraction of vermiform nematodes from soil

Extraction efficiency of modified Baermann funnel method

In this experiment slight modification was done over the existing method to increase the recovery of nematodes from soil *viz*., Pre-soaking of the soil samples for 30, 60 and 120 minutes to enable the nematodes held in the thin film of water around soil particles to be released in the soil solution. A control was also maintained as per the normal practice with soaking for 5 minutes.

About 200cc of black cotton soil was taken in a basin with four different set and one litre of water was added to all the 4 basins containing 200cc soil individually and were allowed for presoaking with 4 different time duration *viz*., 30, 60, 120 minutes and

5 minutes as control. The individual samples were processed after the prescribed time by passing through various sieves like 20, 60, 100, 200 and 325 mesh sieves. Then the soil residues caught in the sieves were collected in a beaker and the collected residue was poured into the modified Baermann funnel arrangement which consists of wire mesh with tissue paper placed on it and this was in turn placed on the petridish containing distilled water where the residue collected is poured on the tissue paper [6]. The nematode penetrated the tissue paper and moved towards the clear distilled water present in the petridish. After 24 hours, the petridish containing the nematode was observed under a stereo zoom microscope, and the count of nematode is taken with a counting dish and for the genus identification, the nematodes were observed under the compound microscope by making water mounts.

The same method was followed for the red loam soil to know the comparative recovery of nematodes from red loam and black cotton soil.

Extraction efficiency of centrifugal sugar floatation method

Two soil types *viz*., black cotton soil and red loam soil and four soaking times *viz*., 30, 60, 120 and 5 minutes, were compared for extraction efficiency. The soil samples which are pre soaked with the different time duration were processed by the Cobb's decanting and sieving followed by modified Baermann funnel method and the residue containing the nematode obtained by modified Baermann funnel method was transferred to centrifuge tube, equalized for weight (with water) and centrifuged for 4 minutes at 3000 rpm.

After centrifugation the supernatant was discarded and to the residue pellet containing nematodes and soil particles, sugar solution of specific gravity of 1.18 was added, stirred well, weight equalized by using a top balance and centrifuged for 2 min at 3000 rpm. The supernatant was poured into a beaker containing water in order to dilute the concentration of sugar solution. The content were sieved through a 350 mesh sieve and the nematodes were collected into a vial by using a squeeze bottle. The nematodes collected from the mesh and were observed under stereo zoom microscope and counting was taken and the genera obtained were identified [1].

Extraction efficiency of nematodes by incubation techniques

The Baermann funnels set up were kept in a mist chamber designed to hold 4 Baermann funnels.

One hundred cc soil was taken and was placed on the tissue paper which was supported by the wire mesh over the funnel and rubber tube was fixed the below end of the funnel with a pinch cock so that the water flow was controlled which helped for collection of nematode. Spraying of water in mist chamber was done at different intervals like 30, 60 and 120 minutes with continuous mist with 5 minutes interval as a control and after 24 hours, the nematode which moved from soil to the funnel through which were collected in the tube was taken and observed under the microscope and the count was taken, various genera obtained were identified from both black cotton soil and red loam soil.

Results

Extraction of vermiform nematodes from soil

A. Extraction efficiency of modified Baermann funnel method with different Pre-soaking times (Black soil)

Different Pre-soaking times of 30, 60 and 120 minutes were tried to test the extraction efficiency along with a normal practice for 5 minutes of pre-soaking. All the treatments were found effective over control. Among the treatments 30 minutes Pre-soaking was found to be significantly superior, which yielded highest recovery of *Rotylenchulus reniformis* (22.53%), *Helicotylenchus incisus* (17.25%), *Xiphinema basiri* (20.00%), *Longidorus elongatus* (58.33%) and saprophytes (18.08%) over the control, followed by 120 minutes of pre-soaking. Among the different genera obtained, *Longidorus elongatus* showed the highest recovery of 58.33% over the control, followed by *Rotylenchulus reniformis* (22.53%). Pre-soaking of 60 minutes was least effective which recorded increased recovery of different nematode genera from 3.50 to 12.90%. The coefficient of variation in the recovery rate was highest with *Longidorus elongatus* (63.70%) and in rest of the species it ranged from 16.43 to 21.37% (Table. 1).

B. Extraction efficiency of modified Baermann funnel method with different Pre-soaking times (Red loam soil)

The extraction of nematodes was done from red loam soil with different Pre-soaking times viz., 30, 60, 120 and 5 minutes as control, prior to the extraction of nematodes by modified Baermann funnel method. Pre-soaking of soil for 30 minutes yielded the highest number of nematodes of different genera. In this experiment, all the other extraction methods with different Pre-soaking duration like 60 and 120 minutes were found to be superior over control. Among the Pre-soaking periods, 30 minutes Pre-soaking of black soil showed the highest number of recovery of nematodes with different genera to the tune of 30.37% (*Rotylenchulus reniformis*), 41.00% (*Pratylenchus coffeae*), 32.55% (*Helicotylenchus incisus*) and 16.95% (saprophytes). This was proved to be statistically significant followed by 120 minutes of pre-soaking. Among the different genera of nematodes, the recovery obtained was highest in *Pratylenchus coffeae* (41.00%) followed by *Helicotylenchus incisus* (32.55%). In different Pre-soaking times, 60 minutes Pre-soaking showed the lowest recovery of 5.12 to 27.43%. The coefficient of variation in the recovery rate was highest with *Pratylenchus coffeae* (33.80%) and in rest of the species it ranged from

14.71 to 30.32% (Table. 2).

Among the different types of soil *viz.*, black and red loam soils, which were compared for testing the extraction efficiency of different methods for nematode extraction, the recovery of nematodes obtained varied with different genera and highest recovery was in black soil in respect of *Longidorus elongatus* (58.33%) and that of *Pratylenchus coffeae* (41.00%) from red loam soil.

C. Extraction efficiency of centrifugal Sugar Floatation method with different Pre-soaking times (Black soil)

Pre-soaking of the soil samples for 30 minutes was significantly superior over other treatments *viz*., Pre-soaking times of 60 and 120 minutes and control with 5minutes of pre-soaking. Pre-soaking of 30 minutes followed by sugar floatation method yielded significantly higher proportion of *Rotylenchulus reniformis* (22.07%), *Helicotylenchus incisus* (28.49%), *Xiphinema basiri* (30.18%), *Longidorus elongatus* (60.00%) and saprophytes (15.16%) over the control, followed by 120 minutes of pre-soaking. Pre-soaking of 60 minutes revealed the lowest recovery of nematodes ranging from 3.40 to 27.27%. Among the different genera of nematodes obtained *Longidorus elongatus* showed the highest recovery of 60.00% than the other genera and lowest recovery is of *Rotylenchulus reniformis* (22.07%). A highest variation of 53.33% was noticed with *Longidorus elongatus* in respect of different soaking times. The variation with other nematodes ranges from 14.51 to 25.80%. Highest Pre-soaking of 60 and 120 minutes were found to be inferior to 30 minutes (Table. 3).

D. Extraction efficiency of centrifugal Sugar Floatation method with different Pre-soaking times (Red loam soil)

The extraction efficiency of sugar floatation with different Pre-soaking times in respective to red loam soil also reveals that the Pre-soaking of 30 minutes, resulted in highest recovery of *Rotylenchulus reniformis*, *Pratylenchus coffeae*, *Helicotylenchus incisus* and saprophytes (41.00, 37.00, 41.35 and 18.64% respectively) than the control. This was proved to be statistically significant followed by 60 minutes of pre-soaking. There is not much of differences in the variations among the species which ranged from 43.81, 37.81 and 37.20% in respect of *Rotylenchulus reniformis*, *Pratylenchus coffeae* and *Helicotylenchus incisus* (Table. 4).

In comparison with the black soil, red loam soil yielded more *Rotylenchulus reniformis*, *Pratylenchus coffeae* and *Helicotylenchus incisus* with recovery of 41.00, 37.00, and 41.35% respectively. Whereas that of black soil was 22.07, 28.49, 30.18 and 60.00% respectively for *Rotylenchulus reniformis, Helicotylenchus incisus, Xiphinema basiri* and *Longidorus elongatus*.

E. Incubation technique with different intervals of mistifying (Black soil)

Different mistifying intervals of 30, 60 and 120 minutes were tried to test the extraction efficiency along with a normal practice of 5 minutes interval of mistifying. All the treatments were found less effective over control. Among the treatments 5 minutes mistifying interval being a control was found to be significantly superior, which yielded highest recovery of nematodes. Mistifying interval of 120 minutes being statistically insignificant, showed lowest nematode population recovery of (-)31.42% for *Rotylenchulus reniformis*, (-) 35.88% for *Helicotylenchus incisus*, (-)35.26% for *Pratylenchus coffeae* and

(-)51.35% for saprophytes, when compared to control. Highest mean of nematode count was obtained with *Rotylenchulus reniformis*, *Helicotylenchus incisus*, *Pratylenchus coffeae* (184.03, 142.00 and 117.00 respectively), when subjected to 5 minutes mistifying interval. The coefficient of variation in the recovery rate was highest with *Rotylenchulus reniformis* (37.36%) and in rest of the species it ranged from (26.04 to 33.63%).

The lowest recovery of *Helicotylenchus incisus* was observed at 120 minutes mystifying (-35.88%) (Table. 5).

F. Incubation technique with different intervals of mistifying (Red loam soil)

Mistifying interval of 5 minutes, which was used as control was significantly superior over other treatments *viz.*, mistifying intervals of 30, 60 and 120 minutes. Among the treatments 5 minutes mistifying intervals being a control was found to be significantly superior, which yielded highest recovery of nematodes. Mistifying interval of 120 minutes being statistically insignificant, showed lowest nematode population recovery of *Rotylenchulus reniformis* (-38.07%), *Helicotylenchus incisus* (-41.96%) *Pratylenchus coffeae* (-45.64%) and saprophytes (-57.17). when compared to control. Highest mean of nematode count was obtained with *Rotylenchulus reniformis*, *Helicotylenchus incisus*, *Pratylenchus coffeae* (136.09, 142.01 and 137.10 respectively). There was not much of differences in the variations among the species which ranged from 35.51, 38.50 and 36.40% respectively. The lowest recovery of *Pratylenchus coffeae* (45.64%) was observed at 120 minutes mistifying (Table. 6).

Among the different types of soil *viz.*, black and red loam soils, which were compared for testing the extraction efficiency of different mistifying intervals. The recovery of nematodes obtained varied with different genera and lowest recovery was observed in red loam soil for *Rotylenchulus reniformis* (-38.07%), *Helicotylenchus incisus* (-41.96%) *Pratylenchus coffeae* (-45.64%) when compared with black soil.

Statistical procedures

CRD design with 5 replications was followed in all experiments. Statistical values (mean, standard deviation, coefficient of variation, variance, minimum, maximum values) were calculated [11]. On the basis of the coefficient of variation (CV %), the percentage recovery of different nematode genera in comparison with different extraction methods have been assessed.

Table 1: Extraction efficiency of vermiform nematodes by Modified Baermann funnel method with different Pre-soaking times (Black soil)

Treatments	Nematode population/200cc of soil (Mean of five replicates)				
	Rotylenchulus reniformis	***Helicotylenchus incises***	***Xiphinema basiri***	***Longidorus elongates***	**Saprophytes**
T_1-Pre-soaking for 30 min	174.31 (+23.53)	153.00 (+17.25)	147.11 (+20.00)	19.00 (+58.33)	493.13 (+18.08)
T_2-Pre-soaking for 60 min	118.10 (+3.50)	113.12 (+4.14)	112.10 (+6.66)	6.11 (+9.09)	437.00 +12.19)
T_3-Pre-soaking for 120 min	149.00 (+15.05)	131.33 (+11.48)	126.42 (+12.50)	10.11 (+33.33)	372.00 (+4.20)
T_4-Pre-soaking for 5 min (control)	110.26	104.01	98.00	5.21	342.09
Standard error (SE)	14.72	10.81	10.45	3.18	33.76
Standard deviation (SD)	29.44	21.63	20.9	6.37	67.53
CV (%)	21.37	17.26	17.30	63.70	16.43
SEd	1.3740	1.0333	1.1597	0.0765	4.5900
CD (p = 0.05)	2.9128	2.1905	2.4586	0.1622	9.7305

Figures in parentheses are percent increases (+) and decrease (-) of nematode recovery over control.

Table 2: Extraction efficiency of vermiform nematodes by Modified Baermann funnel method with different Pre-soaking times (Red loam soil)

Treatments	Nematode population/200cc of soil (Mean of five replicates)			
	Rotylenchulus reniformis	***Pratylenchus coffeae***	***Helicotylenchus incises***	**Saprophytes**
T_1-Pre-soaking for 30 min	103.11 (+30.37)	98.41 (+41.00)	114.10 (+32.55)	300.00 (+16.95)
T_2-Pre-soaking for 60 min	63.00 (+6.77)	72.32 (+27.43)	68.00 (+7.93)	236.15 (+5.12)
T_3-Pre-soaking for 120 min	74.19 (+14.72)	66.11 (+23.36)	82.00 (+17.14)	257.21 (+9.36)
T_4-Pre-soaking for 5 min (control)	55.00	41.16	58.12	213.00
Standard error (SE)	10.49	11.70	12.20	18.49
Standard deviation (SD)	20.99	23.41	24.40	37.00
CV (%)	28.31	33.80	30.32	14.71
SEd	0.6555	0.6594	0.8678	3.4413
CD (p = 0.05)	1.3897	1.3978	1.8398	7.2954

Figures in parentheses are percent increases (+) and decrease (-) of nematode recovery over control.

Table 3: Extraction efficiency of vermiform nematodes from Centrifugal Sugar Floatation method with different Pre-soaking times (Black soil)

Treatments	Nematode population/200cc of soil (Mean of five replicates)				
	Rotylenchulus reniformis	***Helicotylenchus incises***	***Xiphinema basiri***	***Longidorus elongates***	**Saprophytes**
T_1-Pre-soaking for 30 min	146.00 (+28.07)	124.31 (+28.49)	138.02 (+30.18)	16.06 (+60.00)	429.03 (+15.16)
T_2-Pre-soaking for 60 min	99.16 (+9.39)	87.11 (+11.53)	96.21 (+12.94)	7.12 (+27.27)	322.14 (+3.40)
T_3-Pre-soaking for 120 min	113.02 (+15.89)	107.10 (+21.59)	103.00 (+16.38)	10.11 (+42.85)	364.00 (+7.05)
T_4-Pre-soaking for 5 min (control)	82.00	69.01	74.10	4.00	316.00
Standard error (SE)	13.57	11.94	13.27	2.56	26.03
Standard deviation (SD)	27.14	23.89	26.55	5.12	52.07
CV (%)	24.71	24.60	25.80	55.32	14.51
SEd	1.3178	0.6837	0.8941	0.1001	4.0970
CD (p = 0.05)	2.7936	1.4495	1.8954	0.2123	8.6854

Figures in parentheses are percent increases (+) and decrease (-) of nematode recovery over control.

Table 4: Extraction efficiency of vermiform nematodes from Centrifugal Sugar Floatation method with different Pre-soaking times (Red loam soil)

Treatments	Nematode population/200cc of soil (Mean of five replicates)			
	Rotylenchulus reniformis	***Pratylenchus coffeae***	***Helicotylenchus incises***	**Saprophytes**
T_1-Pre-soaking for 30 min	98.10 (+41.00)	87.33 (+37.00)	94.00 (+41.35)	210.10 (+18.64)
T_2-Pre-soaking for 60 min	74.00 (+28.69)	67.00 (+25.23)	62.15 (+22.77)	187.01 (+12.99)
T_3-Pre-soaking for 120 min	54.21 (+13.68)	42.00 (+2.43)	40.08 (+1.26)	156.22 (+4.00)
T_4-Pre-soaking for 5 min (control)	41.00	40.01	39.20	144.00
Standard error (SE)	12.43	11.17	12.89	14.96
Standard deviation (SD)	24.86	22.34	25.78	29.93
CV (%)	37.20	37.81	43.81	17.10
SEd	0.5458	0.6857	0.9562	1.9131
CD (p = 0.05)	1.1570	1.4536	2.0271	4.0557

Figures in parentheses are percent increases (+) and decrease (-) of nematode recovery over control.

Table 5: Efficacy incubation technique with different intervals of mistifying (Black soil)

Treatments	Nematode population/10cc of soil (Mean of five replicates)			
	Rotylenchulus reniformis	*Helicotylenchus incises*	*Pratylenchus coffeae*	Saprophytes
T_1- Mistifying of water at 30 min interval	132.02 (-16.45)	79.00 (-28.50)	99.09 (-8.33)	265.00 (-11.81)
T_2- Mistifying of water at 60 min interval	114.00 (-23.48)	82.13 (-26.78)	81.00 (-18.18)	219.03 (-21.08)
T_3- Mistifying of water at 120 min interval	96.16 (-31.42)	67.11 (-35.88)	56.21 (-35.26)	108.20 (-51.35)
T_4- Mistiying of water at 5 min (control)	184.03	142.00	117.00	336.00
Standard error (SE)	18.98	16.81	13.02	47.82
Standard deviation (SD)	37.96	33.63	26.04	95.65
CV (%)	28.80	36.32	29.50	41.21
SEd	0.8503	1.2025	1.0574	3.1624
CD (p = 0.05)	1.8026	2.5492	2.2415	6.7041

Figures in parentheses are percent increases (+) and decrease (-) of nematode recovery over control.

Table 6: Efficacy incubation technique with different intervals of mistifying (Red loam soil)

Treatments	Nematode population/10cc of soil (Mean of five replicates)			
	Rotylenchulus reniformis	*Helicotylenchus incises*	*Pratylenchus coffeae*	Saprophytes
T_1- Mistifying of water at 30 min interval	92.11 (-19.29)	114.00 (-10.93)	109.07 (-11.38)	277.16 (-5.94)
T_2- Mistifying of water at 60 min interval	76.00 (-28.30)	89.03 (-22.94)	84.02 (22.94)	134.00 (-38.11)
T_3- Mistifying of water at 120 min interval	61.23 (-38.07)	53.02 (-45.64)	56.00 (-41.96)	85.05 (-57.17)
T_4- Mistifying of water at 5 min (control)	136.09	142.01	137.10	312.00
Standard error (SE)	16.20	18.90	17.69	54.80
Standard deviation (SD)	32.40	37.81	35.39	109.60
CV (%)	35.51	38.50	36.40	54.23
SEd	0.5213	1.1988	1.1854	2.9729
CD (p = 0.05)	1.1052	2.5414	2.5130	6.3024

Figures in parentheses are percent increases (+) and decrease (-) of nematode recovery over control.

Discussion

Extraction of vermiform nematodes from soil

Among the various methods, the modified Baermann funnel method is being used widely, as it is found to be superior over other methods in terms of highest nematode recovery and also overcoming the short falls in other methods. The literature shows that most of the soil dwelling nematodes are either in dormant or semi dormant condition under anhydrobiotic state [8, 3] and it takes considerable time for revival and come into soil solution. The implications of results of anhydrobiotic nematode extraction can be extended to agricultural situations and nematode control [5].

The present study was designed based on the above principles and time of soaking was increased to enable the nematodes to revive from quiescent or semi - quiescent stage and to bring them into soil solution before separation. Out of the Pre-soaking time of 30, 60, and 120 minutes tried, Pre-soaking for 30 minutes was found to be best which yielded highest recovery than the normal practice of soaking for 5 minutes. The recovery of plant parasites *viz*., *Rotylenchulus reniformis*, *Helicotylenchus incisus*, *Xiphinema basiri* and *Longidorus elongatus* were significantly higher along with saprophytes.

Cobb's wet sieving, decantation and sugar floatation methods

Sugar floatation method [4] is considered as the rapid method of nematode extraction and it is also useful to extract the sluggish and dead nematodes including the eggs. In the present investigation, Pre-soaking of soil for 30 minutes and Cobb's sieving and decantation followed by sugar floatation method yielded highest recovery of *R. reniformis*, *H. incisus*, *X. basiri* and *L. elongatus*. However no modification in sugar floatation was tried in this method. The black soil yielded more nematodes than the red soil.

Modified Baermann funnel method was compared with centrifugal floatation method in order to avoid loss of nematodes by sieving and also to save time and found that centrifugal method was best [1].

Mistifier technique

Spraying of water continuously as fine mist over the infested material will result in the emergence of nematodes from them and which can easily be extracted [7]. The principle behind the method is better oxygenation which removes the toxic decomposition products. Although this method is highly useful to extract the migratory endoparasitic nematodes from roots and plant materials and it is being employed for soil also.

In the present study, continuous mistifying with 5 minutes interval resulted in recovery of nematodes *viz*., *R. reniformis*, *H. incisus*, and *P. coffeae* when compared to mistifying at 30, 60 and 120 minutes interval, indicating that continuous mist increases the humidity in the chamber resulting in easy separation of active nematodes to go into the water media due to high oxygenation as postulated [1].

Highest recovery of *Pratylenchus vulnus* and *M. incognita*, when water is sprayed approximately for 1-5 minutes in a 10 minutes cycle with the residues of wet sieving. Substantial recovery was also noticed when the soil is directly placed on the funnel in the mist chamber assembly with all the soil type tested [9, 10]. The present finding is comparable with the results obtained earlier.

Summary

In order to achieve maximum recovery of nematodes and to know the efficiency of different nematode extraction methods from soil, slight modification has been done in existing methods for nematode extraction in the present study. Among different Pre-soaking times for extraction of nematodes from soil by modified Baermann funnel method, Pre-soaking for 30 minutes showed increased recovery of nematodes from black soil (20.02% increase) and red loam soil (25.21% increase) respectively, when compared with the regular 5 minutes Pre-soaking with recovery of different nematode genera viz., *Longidorus elongatus* (58.33 percent) and *Pratylenchus coffeae* (41.00 percent) in both black and red loam soil respectively. Thirty minutes Pre-soaking was found to be the

best for extraction of nematodes by centrifugal sugar floatation method from both black and red loam soil with 22.16 percent increase in nematode recovery. Among the extraction of nematodes by incubation technique with different mistifying intervals, mistifying with 5 minutes interval was found to recover the highest number of nematodes, which recorded 40.56 and 48.32 percent increased nematode recovery in both black and red loam soil respectively, when compared to 30, 60, and 120 minutes of spraying interval.

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