

Studies on the Efficacy of Different Methods of Vermiform Nematode Extraction from Roots

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Abstract: A study was conducted to know the efficiency of different nematode extraction methods from root. Among the methods tested to extract nematodes from roots, highest increased nematode recovery of 25.32 per cent (*Pratylenchus coffeae*) and 11.00 per cent (*Helicotylenchus incisus*) was obtained from incubation of split roots for 24 hours and maceration of roots followed by removal of sap and placing on modified Baermann funnel, when compared with regular maceration along with modified Baermann funnel method.

Keywords: Modified Baermann funnel method, Split roots, maceration, *Pratylenchus coffeae*, *Helicotylenchus incisus*

1. 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^[1]. Hence 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. The easiest and most simple method is to submerge a plant sample in water in a petridish and directly select the nematode for further identification using microscopes. Although this procedure can provide results in a very short time, it is only suitable for small samples and cannot be standardized and its overall extraction efficacy is low. Over the years, the number of modification of basic methods have 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^[11]. Limited research had been done towards the optimization of the efficiency of the most extraction techniques. Not much work had been done on fine tuning the existing methods of extraction so as to get highest recovery of nematodes. In order to obtain highest recovery an attempt has been made by fine tuning the existing methods of extraction in present study.

2. Materials and Methods

2.1 Testing the efficacy of different methods to extract vermiform nematodes from roots

This experiment was carried out to find out the efficacy of different methods to extract the migratory endoparasitic nematodes from the root samples. Various methods of extraction were tried and the comparison was done with different methods to know which method was efficient.

Nematode infested young feeder roots of banana were collected (5gm) and macerated in a waring blender and the macerate was directly poured in a modified Baermann pan. ^[2]Slight modification was done wherein the sap of the

macerate was removed by filtering through the filter paper and the residue kept on modified Baermann pan. In another modification the roots were split longitudinally and oblique sections of the root were taken and kept on modified Baermann pan. Incubation of split roots, root sections and root bits were also formed the treatments. The oblique section was made in order to have a more exposed area of the sections. Centrifugation method was also followed with root macerate, and macerate after removing sap. The nematodes isolated by various methods or modification were counted under stereo zoom microscope.

2.2 Treatment details

T₁_Maceration- Modified Baermann funnel (control)
 T₂_Maceration-removal of sap-Modified Baermann funnel
 T₃_Maceration-centrifugation
 T₄_Maceration- removal of sap-centrifugation
 T₅_Split root- Modified Baermann funnel
 T₆_Root sections-Modified Baermann funnel
 T₇_Incubation of split roots for 24 hours
 T₈_Incubation of root section for 24 hours
 T₉_Incubation of root bits for 24 hours
 Design: CRD; Replications: 4

2.3 Statistical procedures

Statistical values (mean, standard deviation, coefficient of variation, variance, minimum, maximum values) were calculated by using the methods^[6]. 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.

3. Results

Efficiency of different methods of extraction of endoparasitic nematodes from roots

The extraction efficiency of different methods to extract endoparasitic nematodes from roots revealed that incubation of split roots for 24 hours yielded the maximum recovery of nematodes like of different genera from soil like *Helicotylenchus incisus* (36.42%), *Pratylenchus coffeae* (23.66%) and saprophytes (25.27%). This was proved to be statistically significant followed by maceration-removal of sap-modified Baermann funnel with the recovery of

Helicotylenchus incisus (25.61%), *Pratylenchus coffeae* (12.28%), and saprophytes (1.84%). Among the different genera *Helicotylenchus incisus* showed the highest recovery of 36.42% by incubation of split roots for 24 hours, whereas lowest in case of incubation of cut root bits for 24 hours which recorded (-)18.43%. Highest recovery of 23.66% was observed in *Pratylenchus coffeae* by incubation of split roots for 24 hours and lowest was (-)11.11% in incubation of cut root bits for 24 hours. Maceration with removal of sap and centrifugation showed the recovery of *Helicotylenchus*

incisus as (5.62%) which was on par with the recovery of 5.91% by split root with modified Baermann set up. For the extraction of *Pratylenchus coffeae*, maceration with centrifugation and incubations of root sections for 24 hours were found to be on par which recorded 3.84 and 5.66% respectively. The coefficient of variation in the recovery rate was highest with *Helicotylenchus incisus* (42.00%) and was lowest in *Pratylenchus coffeae* (22.01%) (Table. 1; Figure. 1).

Table 1: Efficiency of different methods of extraction of endoparasitic nematodes from roots

Treatments	Nematode population /5gm of root (Mean of four replicates)		
	<i>Helicotylenchus incisus</i>	<i>Pratylenchus coffeae</i>	Saprophytes
T ₁ -Maceration-modified Baermann set up (control)	151.16	50.00	221.00
T ₂ -Maceration-removal of sap-modified Baermann set up	255.00 (+25.61)	54.01 (+12.28)	213.31(+1.84)
T ₃ -Maceration-centrifugation	110.03 (-15.70)	54.08 (+3.84)	135.02 (-24.15)
T ₄ -Maceration-removal of sap-centrifugation	169.00 (+5.62)	45.00 (-5.26)	117.11 (-30.76)
T ₅ -Split root-modified Baermann set up	170.12 (+5.91)	48.61 (-2.04)	124.07 (-28.11)
T ₆ -Root sections- modified Baermann set up	137.08 (-4.86)	43.03 (-7.52)	115.00 (-31.54)
T ₇ -Incubation of split roots for 24 hours	324.11 (+36.42)	81.08 (+23.66)	307.63 (+25.28)
T ₈ -Incubation of root sections for 24 hours	114.16 (-13.96)	56.00 (+5.66)	124.09 (-28.11)
T ₉ -Incubation of cut root bits for 24 hours	104.10 (-18.43)	40.11 (-11.11)	144.21 (-21.09)
Standard error (SE)	24.50	4.00	22.10
Standard deviation (SD)	73.71	12.00	66.30
CV (%)	42.00	22.01	37.00
SEd	32.5661	5.5886	28.2083
CD (p = 0.05)	66.8217	11.4672	57.8801

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

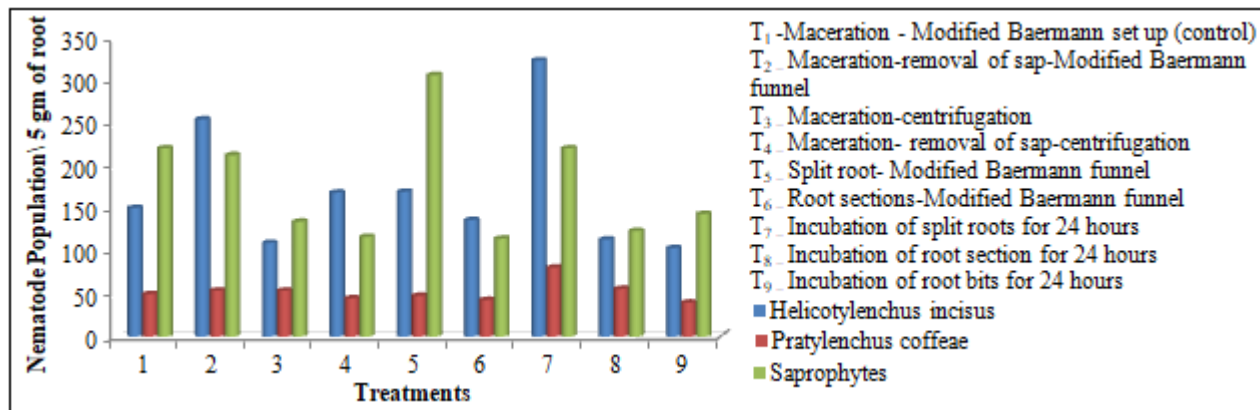


Figure 1: Efficiency of different methods of extraction of endoparasitic nematodes from roots

4. Discussion

Extraction of endoparasitic nematodes from roots

The earlier studies revealed that the migratory endoparasitic nematodes from the roots can be extracted either by keeping the infested materials on a modified Baermann pan, or macerate the roots and the residues placed on the modified Baermann pan. The major problem faced in this method is the release of toxic compounds in the root macerate which kills the nematodes instantly resulting in poor recovery rates of nematodes.

The results of the present study revealed that incubation of split roots yielded highest recovery of *H. multicintus* and *P. coffeae* from banana roots over other methods tried, viz., incubation of root bits, slantingly cut root sections, maceration-Baermann funnel, maceration- centrifugation

and maceration- removal of sap - modified Baermann funnel. This result is in accordance with findings^[7], who observed that splitting of banana roots and their incubation at 25°C (±2 °C) in a plain tap water yielded a significantly higher and physiologically active population of *Radopholus similis*. Conversely, split root incubation in hydrogen peroxide (20ml/lit) at 20°C yielded a higher population as compared to split root incubation at 20 °C in tap water. By and large root splitting had a positive influence on nematode emergence from roots compared to unsplit roots.

The possible reason for this could be that, the active nematodes emerge from the roots as they are exposed to high humidity and washed by the intermittent water spray. The chances of exposure of nematodes to toxic tannin is avoided which is not possible by maceration and modified Baermann pan.

This results are in agreement with the observations^[9], who vouched that active nematodes emerge from the roots easily due to high oxygenation and the sap and toxic decomposition products are washed away by the water sprayer through the nozzle.

It has also been recorded that recovery of highest number of *Tylenchulus semipenetrans* from citrus roots by root incubation in comparison with double centrifugation, sodium hypochloride method and their combinations^[4].

Highest recovery of *Hirschmanniella oryzae* from rice root was achieved by incubation of the roots for 4 days^[3].

Root incubation technique is more advantageous for extracting the nematodes like *Radopholus similis* and *Pratylenchus coffeae* infesting banana, coconut and arecanut as the roots contain more of tannins and root maceration or keeping the roots bits on a modified Baermann pan will result in poor recovery as the tannin kills the nematodes.

However various methods and their modifications like maceration of roots and centrifugation with MgSO₄ at 1.18 specific gravity^[8] incubation of roots in plastic bags or mason jars^[5] incubation in hydrogen peroxide^[10] have been employed to extract migratory endoparasitic nematodes.

5. Summary

In order to achieve maximum recovery of nematodes, slight modification has been done in existing methods for nematode extraction in the present study. Among the nine methods tried for extraction of nematodes from roots, incubation of split roots for 24 hours recorded the highest recovery of different nematode genera like *Helicotylenchus incisus* (36.42%), *Pratylenchus coffeae* (23.66%) and saprophytes (25.27%). The lowest nematode recovery was recorded in incubation of cut root bits for 24 hours, with different nematode genera like *Helicotylenchus incisus* (-18.43%) and *Pratylenchus coffeae* (-11.11%).

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