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Optimized Nematodes Isolation Protocol for Soils from Tissue Culture Banana Field

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Abstract: Nematodes are one of the most economically important pests affecting banana production worldwide. Studies on how nematodes affect banana production involve isolating the nematodes from the banana fields for further analysis laboratory. Different protocols have been developed however, the protocols have not been fine-tuned to save on time and increase efficiency. The time required for isolation therefore needs to be optimized so that the optimal number of nematodes is isolated. This study reports optimization of nematodes isolation time from soil samples in a tissue culture banana plantation at 5 and 9 months. The nematodes harvesting experiments were set at different times (12, 20, 24, 30, 35 and 48 hours). It also reports the influence of harvesting time at 5 and 9 months when bananas were growing in the field. The study recorded the highest number of nematodes at 48 hours which was significantly different from all the other hours evaluated. It also found that harvesting time trends at 5 and 9 months did not have significant differences. The results imply that laboratories isolating nematodes from soils where tissue culture bananas are planted can use 48 hours as optimal time for extraction of nematodes.

Keywords: Nematodes, Tissue Culture Bananas, Optimum Nematodes Isolation Time

1. Introduction

Bananas are important to millions of people around the world for consumption and trade [1, 2]. Research in banana breeding programs has been going on to support banana production. One of the significant area of banana improvement programs is use of biotechnology to produce tissue culture bananas [3]. Laboratory produced tissue culture bananas have a wide range of advantages to farmers. They are disease free and therefore helps significantly in controlling diseases [4]. Research has been carried out about tissue culture bananas especially on nematodes. Such studies includes how nematodes affects bananas production especially the parasitic nematodes. However protocol for isolation of nematodes for tissue culture bananas soils has not been optimized for time required to isolate optimum number of nematodes. This status therefore becomes a challenge in nematology studies for bananas yet this branch of study is important in banana production.

Nematodes studies in tissue culture bananas have been utilizing isolation protocols such as maceration and centrifugal flotation, enzymatic digestion and baermann funnel/Oostenbrik dish [5]. Among these, the most used method especially in the developing countries is the baermann nematodes extraction protocol. The protocol is based on the principle that during isolation, nematodes moves from the soil sample to the water where they are then counted and identified under a microscope [6, 7]. Baermann is the oldest active method of extraction and has the advantage of being simple and does not require complicated equipment.

The baermann's isolation protocol operates in the principle of the migration of the nematodes from the soil sample to the water. Nematodes movement is through the undulatory propulsion during which a projection of dorsol-ventral waves are passed from the head to the tail [8-10]. The waves are formed through very well coordinated relaxation and contraction of the longitudinal muscles on the dorsol and ventral side of the body. While doing this the nematode has to

exert pressure against all the obstacles to produce a forward movement. Soil factors like structure and texture affect nematodes movement [11, 12]. Very compacted soil will restrict movement of nematodes due to small spaces between soil particles. Soil moisture is also important as more water will lessen the force required by nematodes to move forward [13, 14]. The movement of nematodes during extraction is a factor of time. The time required by the nematodes to migrate from the soil sample to water is of great interest as it determines the numbers of nematodes moving from the soil sample to the basin to be isolated [15, 16].

This study provides optimal time required to isolate highest number of nematodes from soil samples from tissue culture bananas. The protocol can be used for nematode isolation for field studies involving bananas produced through tissue culture.

2. Materials and Methods

Tissue culture banana plantlets of Gros Michel and William Cavendish varieties were acquired from National horticultural research institute (NHRC), Thika Kenya. The planting hole size used was 2.5 by 2.5 by 2.5 feets. Compound fertilizer NPK (17:17:17), 20 kg of well composted manure and 20 litters of water was applied to every plantlet. At 5 and 9 months soil samples were collected at 30 cm away from the banana and processed for nematode extraction. Half a Kg of soil sample was placed in a small meshed bowl that had a paper towel at the base. The bowl was then placed on a plate, water was then poured into the meshed bowl containing soil so that the nematodes would move from the soil sample to the plate containing water through the paper towel. The experiment was left to run for different times (12, 20, 24, 30, 35 and 48 hours). The nematodes upon elapse of the hours were retrieved and counted under micro-scope. The data was entered into the spread sheets and the variance of nematodes numbers at 5 and 9 months was analyzed via One-Way ANOVA. The variance in nematodes numbers at 5 months was compared to 9 months. The means were separated using Tukeys family test at $p \leq 0.05$.

3. Results and Discussions

At 5 months the number of nematodes harvested during 12, 20, 24, 30, 35 and 48 hours had significant differences ($p \leq 0.05$) (Table 1). At 48 hours the number of nematodes extracted at 5 months was the highest at 263 while the lowest number of nematodes extracted was 63 at 12 hours. This implies that time influenced the number of nematodes collected because as time increased the nematode number increased. At 9 months the number of nematodes harvested during 12, 20, 24, 30, 35 and 48 hours had significant differences ($p \leq 0.05$) (Table 1). The nematodes number was highest at 48 hours of harvesting at 300 while the lowest was 12 hours. The observation was that as time increased the number of nematodes increased too implying that time influenced the nematodes number collected.

Table 1. *Number of nematodes harvested at 5 and 9 months using different time in hours.*

Time (Hrs)	Months	Mean
12	5	63.3^{c}
20	5	116.67^{bc}
24	5	123.33^{bc}
30	5	146.7^{b}
35	5	180^{b}
48	5	263.3^{a}
12	9	83.3^{d}
20	9	123.33^{cd}
24	9	133.3^{cd}
30	9	163.33^{bc}
35	9	223.3^{b}
48	9	300^{a}

Means that do not share the same superscript letter in the same number of months are significantly different at $p \leq 0.05$) according to Turkeys HSD test.

The comparison between nematode numbers at 5 months and 9 months of same time in hours did not have significant ($p > 0.05$) (Table 2). This implied that the number of hours used to collect nematodes did not influence the number of nematodes collected at 5 and 9 months. For instance at 12 hours nematodes collected at 5 months were 63 while at 9 months the nematodes were 83. The 63 and 83 nematodes collected were not significantly different (Table 2). Previous nematodes isolation studies laid emphasis on the isolation techniques as opposed to taking into consideration the duration of time required by the nematodes to move from the soil sample to the nematodes collection vessel [17, 18]. As demonstrated by the findings of this study, time taken by the nematode to migrate through the soil sample has implication to the number of nematodes isolated.

Table 2. *Number of nematodes harvested at 5 and 9 months.*

Time (Hours)	Month of collection	Means
12	5 months	63.3^{f}
12	9 months	83.3^{ef}
20	5 months	116.67^{def}
20	9 months	123.33^{def}
24	5 months	123.33^{def}
24	9 months	133.3^{de}
30	5 months	146.7^{de}
30	9 months	163.33^{cd}
35	5 months	180^{cd}
35	9 months	223.3^{bc}
48	5 months	263.3^{ab}
48	9 months	300^{a}

Means that do not share the same superscript letter within the same time in hours are significantly different at ($p \leq 0.05$) according to Turkeys HSD test.

4. Conclusion

In summary, time allocated for the nematodes to migrate from the soil and settle in water influences the number of nematodes collected. The best time in hours for nematodes collection according to this study was 48 hours as more nematodes were harvested compared to all the other hours. The other observation is that time for collection at either 5

months or 9 months was not influential to the number of nematodes collected. This protocol is the first for optimal nematodes isolation for tissue culture bananas and can be used by laboratories that are dealing with nematodes isolation from soils where tissue culture bananas have been planted. Application of this protocol for nematodes isolation for nematology studies will contribute to increased banana production and in extension increased income and food security to millions who depend on bananas.

Conflict of Interest

The authors declare no conflict of interest.

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