

EVALUATION OF CULTURING METHODS FOR THE YAM NEMATODE *Scutellonema bradys*

ABSTRACT

The study evaluated eight culturing methods for *Scutellonema bradys*. The methods evaluated were *in vitro* yam plantlets (tissue culture), kenaf callus, yam disc inoculated the same day, yam disc inoculated five days after disc preparation, yam disc placed on water agar, whole yam tuber and yam setts planted in potted soil (control). Each method was fitted into a completely randomised design with five replicates and inoculated with fifty individuals of *S. bradys*. After eight weeks of incubation, *S. bradys* were extracted from the media and plant tissue collected from each method. Final population numbers were estimated and nematode reproductive factor (RF) was calculated. The highest RF value was recorded for the whole yam tuber method and tissue culture method with no significant differences ($p = .05$) between the two. *S. bradys* did not multiply in the yam disc methods evaluated. The findings from the study show that the whole yam tuber method is the best option for culturing *S. bradys* while the use of tissue culture plantlets may hold some promise.

Key words: Callus, culturing, inoculum, population, reproductive factor, tissue culture,

INTRODUCTION

Cultivated yam belongs to the family Dioscoreaceae and to the genus *Dioscorea* L. [1]. This genus is reported to comprise 600 species [2]. The most important edible varieties are believed to have originated in the tropical areas of South East Asia, South America and Africa [2]. Yam is the second most important root and tuber crop in Africa and contributes more than 200 calories daily for 60 million people, particularly in the yam belt [3]. It is a valuable source of carbohydrate in the diet of West Africans and parts of South East Asia, India, Islands of the South Pacific, the Caribbean and part of Brazil [4]. Yam is becoming increasingly important as the major source of saprogenic precursors of cortisone[4]. It is composed mainly of starch, with a small amount of proteins, lipids [5], all the vitamin C required by consumers [6], and the crop is rich in minerals [7].

31 Yam production is adversely affected by several factors, which include limited availability
32 and cost of planting material, high cost of labour for operations such as land preparation,
33 staking, weeding, harvesting, storage, pests and diseases [8]. Yam is prone to infection by
34 fungi, viruses and nematodes from the seedling stage through to harvesting and even in
35 storage [9]. It is reported that post harvest losses of tubers could be as high as 80% [10], and
36 25% of this could be due to diseases. To a large extent, parasitic attacks directly reflect the
37 biocoenosis of the soil, in that they often include a complex of bacteria, fungi, nematodes and
38 insects [11]

39 Among the nematodes associated with yam cultivation, three are considered to be major
40 constraints namely root-knot nematodes (*Meloidogyne* spp.), the lesion nematode
41 (*Pratylenchus coffeae*) and the yam nematode (*Scutellonema bradys*) [12]. The yam
42 nematode is found in the rhizosphere of soil around yam but mainly occurs as a migratory
43 endoparasite of roots and tubers [13]. In the tuber, the nematode is primarily confined to the
44 sub-dermal, peridermal and underlying parenchymatous tissues in the outer 1 to 2 cm of
45 tubers [14] where it feeds intracellularly, rupturing of cell walls and forming cavities [10]. It.
46 The nematode continues to feed and reproduce in yam tubers stored after harvest [15] [16]
47 causing severe damage of dry rot [17]. The females lay eggs singly within the tubers and eggs
48 hatch and emerging juveniles feed and go through three subsequent moults to emerge as
49 adults [18]. The duration of the life cycle is about 26 days in a good host [19] Several
50 reproduction cycles in the tuber can yield up to 6000 nematodes per gram of tuber [20]

51 Yam is grown mainly as a subsistence crop in Africa [2]. In this system, farmers have largely
52 relied on natural variation for their selection of suitable varieties of yam to cope with the
53 damage caused by plant- parasitic nematode rather than the use of pesticides. Nematode
54 resistant yam cultivars can be one of the most useful, economical and effective means of
55 managing nematodes for resource-poor farmers. The process of identifying resistance
56 involves screening of existing and developing germplasm. Hence the need for a screening
57 protocol that is highly efficient, time saving and economical. When screening for resistance,
58 or undertaking routine studies with a nematode pest, an important prerequisite is a readily
59 available and consistent supply of the nematode inoculum. Currently no suitable artificial
60 media or culturing mechanism has been identified for the culturing and production of *S.*
61 *bradys*. Though nematode species can be artificially cultured on various media such as carrot
62 discs, callus tissue, tomato roots [21] [22], the only documented method that has provided

63 successful multiplication of *S. bradys* was found by Kwoseh [23], who recorded an increase
64 of 240 fold five months after inoculating yam tuber slices with 30 nematodes.

65 Infected tubers are not always available during the whole year and can also be difficult to
66 maintain for nematode multiplication. Moreover, yam tuber deterioration due to invasion by
67 fungi, bacteria and mites after nematode infection reduces nematode populations on the
68 tubers [10]. It would therefore be useful to have methods that provide a continuous supply of
69 nematodes for experiments during those periods of the year when tubers infected with *S.*
70 *bradys* will not be available and alternatively provide a pure source of inoculum throughout
71 the year. It was the objective of this study, therefore, to evaluate various culturing methods
72 for *S. bradys* on yam: using *in vitro* yam plantlet (tissue culture), kenaf callus, yam disc
73 inoculated the same day, yam disc inoculated five days after disc preparation, yam disc
74 placed on water agar, whole yam tuber, and yam sett planted in potted soil.

75

76 **MATERIALS AND METHODS**

77 **Source of yam tubers**

78 Healthy yam tubers of *Dioscorea rotundata* cv *Amula* were collected from the yam barn of
79 the International Institute of Tropical Agriculture (IITA) at Ibadan, Nigeria. Selected yam
80 tubers were seed yam size of 12-15 cm length and 4-6 cm diameter. The tubers used were
81 selected as uniformly as possible. Yam tubers showing symptoms of dry rot, and flaking off
82 of tuber skin characteristic of *S. bradys* infection, were also obtained from IITA Ibadan and
83 the major yam market in Ibadan, Nigeria.

84 **Extraction of *Scutellonema bradys***

85 Nematodes were extracted from infected yam tubers showing symptoms of dry rot disease,
86 using the extraction tray method, according to Coyne *et al.* [24]. The part of the yam showing
87 symptoms of dry rot was peeled, chopped into 3 - 5 mm pieces and placed in the extraction
88 set-up. The chopped diseased yam pieces were then evenly distributed on the tissue. Water
89 was added to the set-up through the area between the sieve and the plate. The set up was left
90 for 72 hours, with daily collection of extracted nematodes and addition of more water. The
91 extracts were all poured into a large 2-l beaker, then later concentrated by sieving (38µm).
92 The concentrated nematodes were rinsed out into a 250 ml capacity beaker

93 **Estimation of nematode population in inoculum**

94 In order to get 50 *S. bradys* per 1 ml, 10 ml of the extract was taken out at each time and
95 dispensed into a separate beaker. A syringe was used to draw 1 ml of the 10 ml extract and
96 transferred into a counting slide and counted. Water was then added to the beaker to dilute
97 the extract to give a concentration of about 50 individuals of *S. bradys* per ml.

98 **Sterilization**

99 The glassware were sterilized in an oven at 160°C for 2 hours. Scalpels and cock borers were
100 autoclaved at 121°C for 15 min before use, and were immersed into 70% alcohol then flamed
101 between use. The working surface was wiped with 70% alcohol. Soil was sterilised at 120°C
102 for two hours in the soil sterilizing chamber of the Department of Crop Protection and
103 Environmental Biology, University of Ibadan

104 The *S. bradys* inoculum to be sterilized was poured in a suspension on to a 38 µm sieve from
105 the beaker. The sieve containing nematodes was immersed into a 150 mm petri-dish
106 containing 0.01% mercury chloride for 2 min and rinsed in three changes of sterile distilled
107 water. The nematodes were then rinsed out of the sieve into a sterile test tube with sterile
108 distilled water. Streptomycin sulphate solution (6000 mg/l) was prepared and added to the
109 nematode suspension at a ratio of 1 (streptomycin) : 2 (sterile distilled water) [20]. This was
110 left to stand overnight. The supernatant was siphoned off with a sterile pipette, leaving the *S.*
111 *bradys* at the base of the test tube and replaced with sterile distilled water.

112 **Evaluation of culturing methods**

113 **Yam disc method**

114 Yam tubers were washed thoroughly with soap and rinsed thoroughly with distilled water,
115 then surfaced sterilised by rinsing in 1% sodium hypochlorite. A sterile knife was used to cut
116 1-2 cm off both ends of the yam tuber. Tubers were then peeled, and cut into 0.5 cm
117 thickness, with a diameter of approximately 4 cm, then placed in petri dishes under the
118 lamina flow chamber. The knife was dipped in ethanol and flamed before each contact. Each
119 yam disc was surface sterilized by dipping it in 10% sodium hypochlorite, then in 70%
120 alcohol and flaming slightly. The yam discs were thereafter placed into sterile glass petri
121 dishes (9 cm diameter) [25].

The yam disc method procedure was varied to give three different yam disc methods. In the first yam disc method, the yam discs were inoculated with *S. bradys* immediately after preparation as described by Kwoseh [23]. For the second yam disc method, the yam discs were allowed a period of incubation of five days before inoculation following the procedure for carrot disc culturing method [22]. In the third method, yam discs were placed in agar and then inoculated [26].

Fifty individuals of *S. bradys* were inoculated on each sterile yam disc under the lamina flow hood, and the petri dishes were sealed with parafilm. The inoculated yam discs were kept at room temperature for 60 days, after which the nematodes were extracted for population estimation using the extraction tray method. After extraction, the nematode suspension was concentrated to 50 ml, and the *S. bradys* population was counted from 2 ml from the homogenized nematode suspension, in a counting dish. Three representative samples were taken from the suspension for counting and the mean value was calculated. This value was used to estimate the total nematode population extracted per yam disc. Reproductive factor (RF) was calculated using the formula $RF = Pf/Pi$, where Pf is the final population and Pi is the initial population. This was done for each of the experiments.

Whole yam tuber method

Yam tubers were washed thoroughly with soap and rinsed properly with distilled water, then surfaced sterilized by rinsing in 1% sodium hypochlorite. The nematode inoculum was sterilized using the method of dela Cruz *et al.* [25] as previously described. A sterile cork borer was used to bore three holes of about 10 mm depth and 5 mm diameter into the tuber and the discs were placed in a sterile petri dish and later used to cover the hole after inoculation with nematodes.

Each hole was inoculated with 50 individuals of *S. bradys* using a sterile syringe. The water was allowed to be absorbed by the tuber, the hole was plugged by the disc removed and then sealed with parafilm and labelled. Tubers were placed in a clear plastic container maintained at room temperature in the laboratory for two months. The experiment was arranged in a completely randomised design and replicated five times. The inoculated yam tubers were kept at room temperature for 60 days, after which the nematodes were extracted for population estimation, using the extraction tray method.

After extraction, the nematode suspension was concentrated to 50 ml , from which nematodes were assessed as previously described. Following this, the total number of nematodes and RF were computed as previously described.

Kenaf callus as a medium for culturing nematode

Seeds of kenaf, NHC 15 cultivar were cultured in modified MS medium [27]containing 4.43 g MS medium, 30 g sucrose (sugar), 0.1 g myo-inositol, and 8 g agar per litre. The pH of the medium was measured and adjusted to 5.7 when necessary. The medium was heated in a microwave oven for 8 min allowing the constituents to dissolve before dispensing 10 ml into test tubes. The test tubes containing the medium were then sterilized in an autoclave. The medium was left to solidify and also left for 3 days in order to ensure lack of contaminants. Seeds were surfaced sterilized by immersing them in 1% sodium hypochlorite for 3 sec, rinsed in distilled water, then placed into the growth medium inside the laminar air flow chamber and incubated at room temperature till germination.

Pieces of leaves and stems of the *in vitro* germinated seedlings were cultured in modified medium [27] containing 4.43 g MS medium, 30 g sucrose, 0.1 g myo-inositol, 0.1 mg 2, 4-dichlorophenoxy acetic acid, 0.5 mg kinetin and 8 g Agar per litre with pH adjusted to 5.7 then autoclaved as previously described. The medium was dispensed in to Petri-plates inside the laminar flow hood. The explants were aseptically placed in the medium and incubated at room temperature till visible callus formation.

After callus tissue had developed, each callus was inoculated with 50 individual of *S. bradys*, using a sterile syringe. Plates were maintained at room temperature in the laboratory for 60 days after which the nematodes were extracted for population estimation, using the extraction tray method. The experiment was arranged in a completely randomised design and replicated five times.

***In vitro* yam plantlet method (tissue culture)**

Solid and liquid media were used for this experiment with yam plantlets. Cotton wool was used as a support for the yam roots in the liquid media. The liquid media contained 4.43 g Murashige and Skoog basal medium, 100 g myo-inositol 100, 30 g Sugar, 1 g kinetin, 20 g l-cysteine per litre while the solid media contained in addition 2.4 g gelrite.

In vitro yam plantlets of *D. rotundata* cv *Amula* were collected from the genetic laboratory of Crop Protection and Environmental Biology, Ibadan and sub-cultured in the prepared solid and liquid media. The pH was measured and adjusted to 5.7 when necessary and 10 ml distributed into the autoclavable plastic containers (4 cm base diameter and 5 cm height). These were autoclaved at 121°C for 15 min and the preparation transferred to the lamina flow hood where the sub-culturing was carried out. The plants were removed from the old culture and placed in the new medium. The culture was left for 5 days to allow for acclimatization. Sterilized nematodes were inoculated into the media very close to the plant root using a sterile syringe.

After inoculation, the *in vitro* yam plantlets, in both the liquid and solid media, were left for 60 days to allow for two reproductive cycles of the nematode. Plants were thereafter removed from the medium and nematodes were extracted from the yam roots and the medium using the extraction tray method following Coyne *et al.* [24]. After extraction, the nematode suspension was concentrated to 50 ml, from which nematodes were assessed as previously described.

Yam plants in pot method

The yam tubers were cut into 50 g setts and planted in saw dust filled into a basket, plants were watered every 2 days until setts sprouted. The sprouted yam plants were removed and transplanted in 5 kg pots filled with sterilized soil; this was replicated five times. At two weeks after transplanting, the soil was inoculated with 500 *S. bradys* very close to the plant.

The yam was harvested at 90 days after inoculation when the tubers were taken to the nematology laboratory for extraction of yam nematode. Nematode extraction was carried out on the roots, tuber peels and soil taken from the yam rhizosphere using the extraction tray method. Reproductive factor (RF) was calculated using the formula $RF = Pf/Pi$, where Pf is the final nematode population and Pi is the initial nematode population.

Data analysis

Data collected on nematode counts in root, tubers media or soil were summed per treatment and replicate to give the final nematode population. The data was submitted for analysis of variance and means were separated using the New Duncans multiple range test at $p = .05$. Standard error was used to differentiate among the methods for nematode reproductive factor.

RESULTS

Effectiveness of culture media in culturing *Scutellonema bradys*

Scutellonema bradys were not recovered from yam disc methods that were inoculated on the same day of its preparation and five days after preparation. The population of *S. bradys* was significantly higher in *in vitro* yam plantlets in solid media than on yam plantlets in liquid media as presented in Table 1.

The reproductive factor of *S. bradys*, at eight weeks after inoculation, reached a value of 2 in two of the culturing methods evaluated in this study. Yam setts planted in pot had a low reproductive factor of 0.56 at the time of evaluation. There was no significant difference between the reproductive factor of *S. bradys* cultured in *In-vitro* yam plantlet (solid media) and whole yam tuber as shown in Figure 1. The use of kenaf callus as a culturing method for *S. bradys* attained a reproductive factor of 0.8. The yam disc immersed in agar gave a reproductive factor of 0.46 and a final population of 23, a value lower than the initial population.

Table 1: Population of *Scutellonema bradys* extracted from plant material and media using different culturing methods

Culturing method	Final population
Tissue culture (SM)	103.00±5.61a
Tissue culture (LM)	71.00±5.10 b
Kenaf callus	40.00±2.24 c
Yam disc (SD)	0.00± 0.00 e
Yam disc (5D)	0.00± 0.00e
Yam disc (agar)	23.00±4.06 d
Whole tuber	105.00±2.80 a
Yam sett (pot)	28±5.98d

Means±standard error followed by the same letter are not significantly different at $p=0.05$ using Duncans's multiple range test; no of replicates =5

SM = Solid media; LM = Liquid media; SD = Same day inoculation; 5D = inoculation 5 days after preparation; Agar = yam disc placed in agar

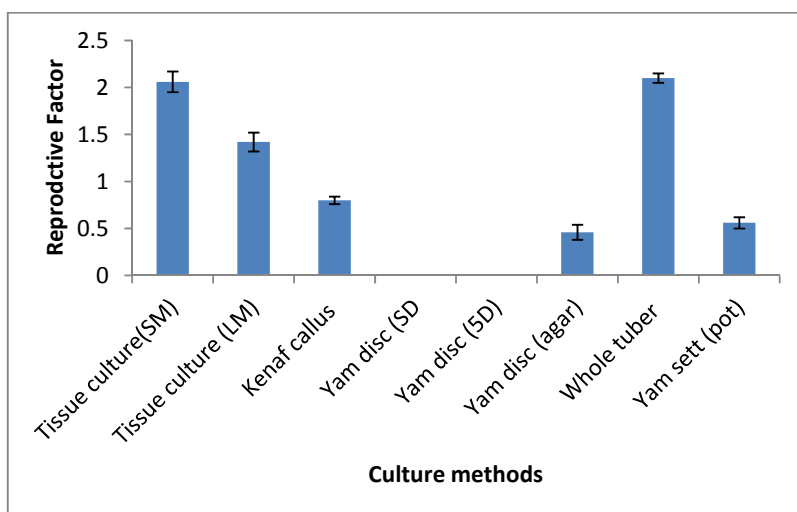


Fig 1: Reproductive factor of *Scutellonema bradys* in various culturing media

Values are means of five replicates; bar = standard error; Reproductive factor = Pf/Pi where Pf is final nematode population and Pi is initial population (applied inoculum); SM = Solid media; LM = Liquid media; SD = Same day inoculation; 5D = inoculation 5 days after preparation; Agar = yam disc placed in agar

DISCUSSION

The study showed that *S. bradys* could survive and reproduce in most of the media used in this investigation. The nematodes did not survive in any of the yam discs that were not immersed in agar because the yam discs dried out and could not provide a suitable nutrient and environment for the nematode survival. This was also observed by Kwoseh [23] where the yam slice used rotted. The nematode population doubled at eight weeks after inoculation in the yam sett in pot method and the yam tissue culture (solid media). Baimey [28] indicated that a reproductive value of 1 means that *S. bradys* increase two fold. Part of the initial population may have been lost during sterilization or change in environmental conditions. The surviving sterilised population will then further start a new generation. This may have been the reason for the low population of *S. bradys* at the end of the experiment, especially given the shorter duration of the experiment compared to Kwoseh [23]. When kenaf callus was used for culturing *S. bradys* it was observed that *S. bradys* could multiply in the callus as *S. bradys* was recovered from the medium after two months. Yam callus was not part of the methods evaluated as Baimey [28] reported that culturing *S. bradys* on yam callus was unsuccessful.

The yam disc method, where discs were placed directly in petri dishes, did not support *S. bradys* survival. Nematodes was not recovered from the yam disc inoculated on the same day of disc preparation nor from inoculation five days after yam disc preparation. The five days interval between disc preparation and inoculation was to cure the slice and determine if it provide a better environment and substrate for *S. bradys*. The yam disc culturing method did not support *S. bradys* multiplication as the yam disc dried out. This was also observed by Kwoseh [23]who reported a high level of loss of tuber slices which affected their potential use as a multiplication medium. However, when the yam discs were placed on agar, *S. bradys* was extracted at two months after inoculation. The agar appeared to have provided a better environment for the nematode activities by providing moisture for the yam disc through the culturing period, or because the agar provided additional nutrients for the preservation of the yam disc. This was the only yam disc method where the nematode reproduced. Ways in which the yam disc culturing method can be optimized include peeling in such a way that the preferred peridermal layer in which the nematodes usually thrive is not removed with the skin. The tuber skin could also be left without peeling if an effective sterilization procedure is conducted. *S. bradys* is reported to be confined to the first 2 cm tissue beneath the yam skin [12] [29].

The yam sett culturing method gave a RF value of less than 1, indicating that the final population is lower than the initial population. Factors affecting nematodes in locating and penetrating the plant root tissue may be the cause for the low population retrieved [28]. Also high populations are usually observed during storage and since tubers were not stored, the multiplication cycles were reduced. Only whole yam tuber and one *in vitro* yam plantlet method allowed for considerable reproduction of *S. bradys* by two months after inoculation, indicating the two options as the bests method for culturing *S. bradys* in this study. Although tissue culture seems to be effective in culturing *S. bradys*, the protocol cost is very high. The use of whole tuber for culturing *S. bradys* is a the preferred method as it is efficient, cheap and space conserving. Obtaining thousands of *S. bradys* from the whole tuber method is possible as Kwoseh [23]obtained a 240 fold increase of the *S. bradys* population after five month of storage. This can be achieved if more cores are made for inoculation on the tubers and with additional storage time. However, when tuber availability is a limiting factor, the *in vitro* yam plantlet method can be explored.

CONCLUSION

Among the methods used culturing on whole yam tubers and *in vitro* yam plantlet on semi solid media were the most effective. Ways of improving or optimising the yam disc or callus tissue method can improve the nematode yield from them. These methods can be used to produce sufficient quantities of pure cultures of *S. bradys* for experimental purposes.

REFERENCES

- [1] Coursey DG. *Yams. An account of the nature, origins, cultivation and utilisation of the useful members of the Dioscoreaceae*. Longmans: London, UK, 1967
<https://www.cabdirect.org/cabdirect/abstract/19722000939> (accessed 16 Jul 2018).
- [2] Orkwor GC, Asiedu R, Ekanayake IJ. *Food yams: advances in research*. IITA: Ibadan, Nigeria, 1998 <https://cgspace.cgiar.org/handle/10568/95901> (accessed 16 Jul 2018).
- [3] Nweke FI, Ugwu BO, Asadu CLA, Ay P. Production costs in the yam based cropping systems of Southeastern Nigeria. International Institute of Tropical Agriculture: Ibadan, Nigeria, 1991 <https://idl-bnc-idrc.dspacedirect.org/handle/10625/11174> (accessed 16 Jul 2018).
- [4] FAOSTAT. <http://www.fao.org/faostat/en/#home>. 2016.
<http://www.fao.org/faostat/en/#home> (accessed 27 Jun 2018).
- [5] Wanasundera JPD, Ravindran G. Effects of cooking on the nutrient and antinutrient contents of yam tubers (*Dioscorea alata* and *Dioscorea esculenta*). *Food Chemistry* 1992;**45**(4):247–250.
- [6] Wanasundera JPD, Ravindran G. Nutritional assessment of yam *Dioscorea alata* tubers. *Plant Food Hum Nutr* 1994;**46**(1):33–39.
- [7] Terry ER, Doku EV, Arene OB, Mahingu NM. *Tropical Root Crops: Production and Uses in Africa: Proceedings of the second Triennial Symposium of the International Society for Tropical Root Crops — Africa Branch held in Douala*. International Society for Tropical Root Crops: Douala, Cameroon, 1984.
- [8] Degras L. *The yam: a tropical root crop*. MacMillan: London, UK, 1993
<https://www.cabdirect.org/cabdirect/abstract/19936792103> (accessed 16 Jul 2018).
- [9] Amusa NA, Adegbite AA, Muhammed S, Baiyewu RA. Yam diseases and its management in Nigeria. *African Journal of Biotechnology* 2003;**2**(12):497–502.
- [10] Adesiyan SO (Univ I (Nigeria)), Odihirin RA, Adeniji MO. Histopathology studies of the yam tuber (*Dioscorea rotundata*) Poir infected with *Scutellonema bradys* (Steiner + Le Hew). *International Biodeterioration Bulletin (UK)* 1975. <http://agris.fao.org/agris-search/search.do?recordID=XE7528977> (accessed 16 Jul 2018).
- [11] Ekanayake IJ, Asiedu R. Problems and Perspectives of Yam-Based Cropping Systems in Africa. *Journal of Crop Production* 2003;**9**(1–2):531–558.

- 327 [12] Bridge J, Coyne DL, Kwoseh CK. Nematode parasites of tropical root and tuber crops
328 (excluding potatoes). In: Luc M, Sikora RA, Bridge J, editors. *Plant parasitic*
329 *nematodes in subtropical and tropical agriculture*. CABI: Wallingford, 2005: 221–258.
- 330 [13] Kwoseh C, Plowright RA, Bridge J. The yam nematode: *Scutellonema bradys*. *Plant*
331 *resistance to parasitic nematodes* 2002;:221–228.
- 332 [14] Adesiyan SO, Adenji, M. O. Studies on some aspects of yam nematode (*Scutellonema*
333 *bradys*). *Ghana Journal of Agricultural Science* 1975;8.
- 334 [15] Bridge J, Coyne D, Kwoseh CK. Nematode Parasites of Tropical Root and Tuber Crops
335 (Excluding Potatoes)*. In: Luc M, Sikora RA, Bridge J, editors. *Plant parasitic*
336 *nematodes in subtropical and tropical agriculture*. CABI Pub: Wallingford,
337 Oxfordshire, UK ; Cambridge, MA, 2005.
- 338 [16] Claudius-Cole AO, Asiedu R, Fawole B. Tropical cover crops for the management of
339 the yam nematode, *Scutellonema bradys*. *International Journal of Pest Management*
340 2016;62(1):85–91.
- 341 [17] Kwoseh CK, Plowright RA, Bridge J, Asiedu R. Search for *Scutellonema bradys*
342 Resistance in Yams (*Dioscorea* spp.). *Journal of Science and Technology*
343 2007;27(3):23–42.
- 344 [18] Adesiyan SO, Caveness FE, Adenji, M. O., Fawole B. *Nematode pests of tropical crops*.
345 Heinman Educational Boks: Ibadan, Nigeria, 1990.46pp
- 346 [19] Claudius-Cole AO, Fawole B. Reproduction and biology of *Scutellonema bradys* in
347 roots of tropical cover crops. 2016;46(1):9.
- 348 [20] Bridge J. Nematodes as pests of yams in Nigeria. *Mededelingen Fakulteit*
349 *Landbouwwetenschappen* 1973;38(3):841–852.
- 350 [21] Inserra RN, Duncan LW, Coyne DL, Brentu FC, Troccoli A, Waeyenberge L, et al.
351 *Pratylenchus speijeri* n. sp. (Nematoda: Pratylenchidae), a new root-lesion nematode
352 pest of plantain in West Africa. *Nematology* 2012;14(8):987–1004.
- 353 [22] Speijer PR, Waele DD. *Screening of Musa Germplasm for Resistance and Tolerance to*
354 *Nematodes*. IITA, 1997.
- 355 [23] Kwoseh CK. *Identification of resistance to major nematode pests of yams (Dioscorea*
356 *spp.) in West Africa*. 2000. <http://ethos.bl.uk/OrderDetails.do?uin=uk.bl.ethos.340023>
357 (accessed 16 Jul 2018).
- 358 [24] Coyne DL, Nicol JM, Claudius-Cole B. *Practical plant nematology: a field and*
359 *laboratory guide*. International Institute of Tropical Agriculture: Cotonou, Benin, 2007.
- 360 [25] dela Cruz FS, van den Bergh I, De Waele D, Hautea DM, Molina AB. *Towards*
361 *management of Musa nematodes in Asia and the Pacific - Technical manual of the*
362 *training workshop on enhancing capacity ofr nematode management in small scale*
363 *banana cropping systems*. IPGRI, Italy INIBAP, Philipines: Los Banos Philipines, 2005.

- 364 [26] Chitamba JJ. Carrot Discs Nematode Culture. Nemaplex.ucdavis.edu. 2003.
 365 <http://nemaplex.ucdavis.edu/Methods/CarrotDisc.htm> (accessed 17 Jul 2018).
- 366 [27] Murashige T, Skoog F. A Revised Medium for Rapid Growth and Bio Assays with
 367 Tobacco Tissue Cultures. *Physiologia Plantarum*;15(3):473–497.
- 368 [28] Baimey H. *Scutellonema bradys* as a pathogen of yam in Benin. Ph.D Thesis, University
 369 of Pretoria, SouthAfrica. 2006.
 370 <http://repository.up.ac.za/bitstream/handle/2263/29022/Complete.pdf?sequence=3>
 371 (accessed 27 Feb 2015).
- 372 [29] Adesiyan SO. Penetration and multiplication of *Scutellonema bradys* in yams
 373 (*Dioscorea* spp.). *Nematologia Mediterranea* 1977;5:313–317.

374