Toxicity of Aqueous and Powdered Sparrow grass, *Asparagus africanus* to *Meloidogyne incognita* on egg plant.

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ABSTRACT

The efficacy of aqueous plant extracts and dry powder from sparrow grass, *Asparagus africanus* in the control of root-knot nematode, *Meloidogyne incognita* was investigated in an experiment using potted plants of *Solanum melongena var.* black beauty. The experimental eggs plants were inoculated with two nematode population levels (0 and 20,000 eggs) and treated with three forms of application (aqueous extract dry powder and a combination of the two at three different doses (0% /0t/ha, 50%/ 1.5t/ha, 100%/3.0t/ha) of sparrow grass. Standard chemical tests for the bioactive ingredients were carried out plus infrared spectroscopy of extracts of the leaves, roots and stems of *A. africanus*. The results revealed that the different forms and levels of the test plant used reduced the incidence and adverse effects of *Meloidogyne* spp. on the growth and yield of egg plant. All the measured parameters; vegetative and root parameters were significantly better in treated plants than for the untreated control. However, the combination of the higher levels of treatment 100% (aqueous)/3.0t/ha (powder) were significantly more effective than the others, either singly on in combination. Phytochemical screening showed that all the parts examined contained tannin, alkaloids and sterols. Infrared spectra showed that they contained mixture of similar chemical compounds.

Keywords: Root knot nematode, toxicity, plant extract, phyto-chemical, egg plant.

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INTRODUCTION

Egg plant, *Solanum melongena* belonging to the family of Solananceac is a fruit vegetable grown as an annual crop. It is a crop of warm season, and requires continuous long warm weather during growth and fruit maturation. The crop grows in light sandy soils in the early spring and in loam soils for later production (Yamaguchi, 1983). Attempts to cultivate egg plant have been hampered by pest and disease problems among which are thrips and flea beetle, leave spot caused by *Cercospora deightonri* and anthracnose of fruit caused by *Colletotrichum gloesporiodes* (Messiaen, 1992). Root-knot infection has also been reported in this plant (Abid and Maqbool, 1991).

Root-knot disease in South-Western Nigeria has increased tremendousy due possibly to increasing and intensified vegetable production in the area coupled with an environment which favours disease development and proliferation. Distribution and abundance of plant parasitic nematodes are dependent on the environment (Adekunle et al; 2006). Andrewatha and Birch (1954) defined environment as consisting of all things that might influence their chances to survive and reproduce. Egg plant being a warm season crop which thrives well in light sandy soils, provides a conducive environment for development and multiplication of root-knot nematodes, *Meloidogyne* spp. Nematode control measures which had been employed over the years include the use of chemical nematicides, resistant varieties, cultural practices and crop rotations (Dahlber, 1991). Chemical control method, which had proved to be the most effective, are costly and environmentally unfriendly among others (Izuogu, 2009). The increasing public and governmental concerns over the harmful effects of some chemical pesticides have led to their withdrawal or reduced use (Adekunle and Aderogba, 2008) hence the quest for the use of botanicals which are pesticidal in nature, biodegradable, eco-friendly and cheap for control of the root-knot nematode pests of the egg plant.

In view of the increasing importance of egg plant due to its nutritional value and considerably low cost as substitutes to other fruit crops such as apple, pineapple, paw-paw etc, the present study was therefore undertaken to investigate the efficacy of the aqueous extract and dry powder of sparrow weed, *Asparagus africanus* in the control of root-knot nematode on egg plant.

MATERIALS AND METHODS

Preparation of Plant Extract:

Sparrow grass, *A. africanus* was collected and air-dried at room temperature of 28^0C. Two kilograms of the test plant was weighed out and ground into powder from which 1kg was soaked in 2litres of methanol for 24 hours and thereafter sieved with muslin cloth. The extract was concentrated using a rotary evaporator and the resulting semi-solid extract was diluted with 2 litres of distilled water to make 100% stock solution. Serial dilutions were made from this and distilled water served as control. Different forms and rates of the test plant were used for the trials. They include dry powder, aqueous extract and powder + aqueous extracts. The rates were 0.0t/ha, 1.5t/ha and 3.0t/ha for powder and 0% 50% and 100% for aqueous extracts.

Soil Sterilization and Planting of Seeds:

Sandy-loam soil was sterilized using the method described by Gautam and Goswani (2002). After cooling, the soils were packed into 36 perforated experimental pots at the rate of 10kg per pot and were placed on raised slab to avoid nematode re-infestation. Seeds of egg plant variety black beauty which were previously soaked in water overnight were planted 2cm deep in all the pots. The pots were watered regularly until germination. The seedlings were later thinned to one vigorous plant per stand.

Extraction of root-knot nematode eggs and inoculation:

Using the method described by Hussey and Barker, 1973, root-knot nematode eggs were extracted from galled roots of *Celosia argentea* such that one ml suspension was standardized to contain 1000 eggs. Fourteen days of planting, each plant was inoculated with approximately 20,000 eggs of *Meloidogyne incognita,* 1 cm holes around the base of the seedling using a syringe.

Treatment application and experimental design:

Seven days after inoculation, treatments were applied at the rate of 0g, 7.5g and 15g/pot for the test plant powder. These are equivalent to 0t/ha, 1.5t/ha and 3.0t/ha. For the aqueous plant extract, 0%, 50% and 100% were applied by pouring 50ml each around the base of the seedling. The 0t/ha and 0% plant extract were the control. The experimental design was 2x3x3 factorial experiment in a randomized complete block design.

Data collection: Data were collected on the following parameters: plant height, number of leaves, number of branches, fresh shoot weight (g), dry shoot weight and fruit weight (fresh and dry). Additional data were collected on the final nematode population from the roots and the soils after harvest.

All numerical data were subjected to Statistical Analysis of Variance. Where necessary treatment means were partitioned using Duncan's Multiple Range Test at P=0.05.

Phytochemical screening and Biochemical food test:

Standard chemical tests for the bioactive ingredients were carried out plus infrared spectroscopy of extracts of the leaves, roots and stems of *A. africanus*. Tests were also conducted on the biochemical food components of treated egg plants.

RESULTS AND DISCUSSION

Tables 1 and 2 show the main effects of treatment materials on the vegetative and yield parameters measured; plant height, number of leaves, fresh shoot weight, dry shoot weight and fruit weight. The results show that generally from the 6th week after planting, there were significant difference between uninoculated control plants and inoculated plants. All the parameters measured were significantly higher in the uninoculated control plants than in the inoculated plants.

In most cases, the forms (aqueous extract, dry powder or a combination of aqueous extract and dry powder) in which the test plant material (sparrow grass) was applied did not significantly affect the vegetative growth and yield of the treated egg plant, *Solanum melongena*. However, the treatment combination of aqueous extract and dry powder of test plant was superior to the powder treatment in most cases. The result further showed that the treatments were significantly superior to the control while in most cases, the higher doses (100% or 3.0t/ha) of treatment were not significantly better than the lower doses (50% or 1.5/ha).

The uninoculated fresh root weight were significantly higher than the nematode inoculated root weight of the egg plants (Table 3). The forms which the treatment material was applied did not significantly affect the performance or effectiveness of test plant while the highest dose at which the treatment material was applied was superior to the lower dose. Treatment combination

also recorded significantly higher fresh root weight at the highest dose (100% or 3.0t/ha).

The main effects on the final soil nematode population (Table 3) showed that the highest levels of treatment either singly or in combination significantly reduced the soil nematode population than the lower levels. The form of treatment material used did not significantly affect the performance. The same trend of result goes for the final population of nematodes in 10g root of treated plants (Table 3). All the inoculated but untreated plants gave the least result. Biochemical food components as reflected in table 4 show that the treatments did not significantly affect the chemical composition of the egg plant fruit.

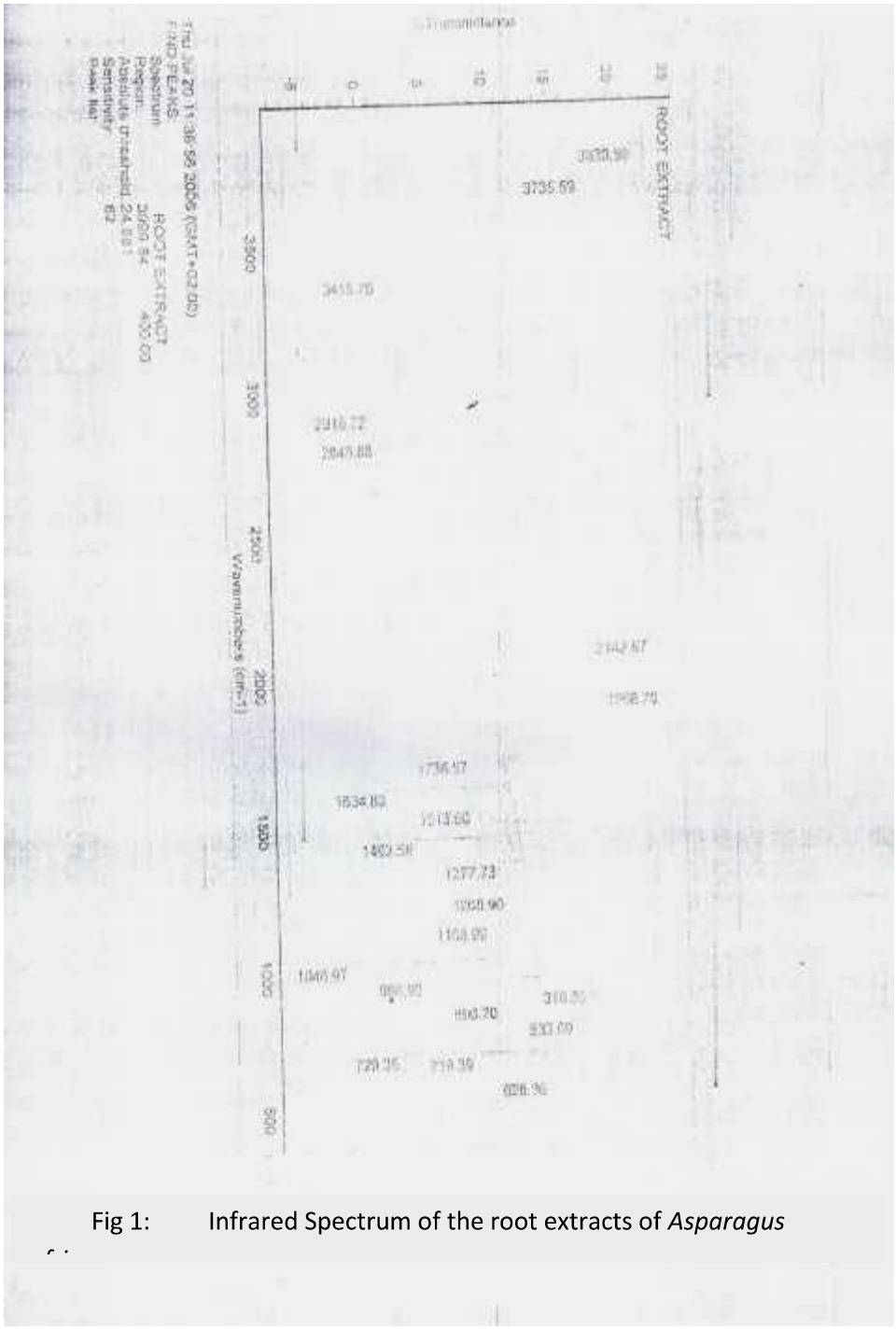

Fig 1: Infrared Spectrum of the root extracts of *Asparagus*

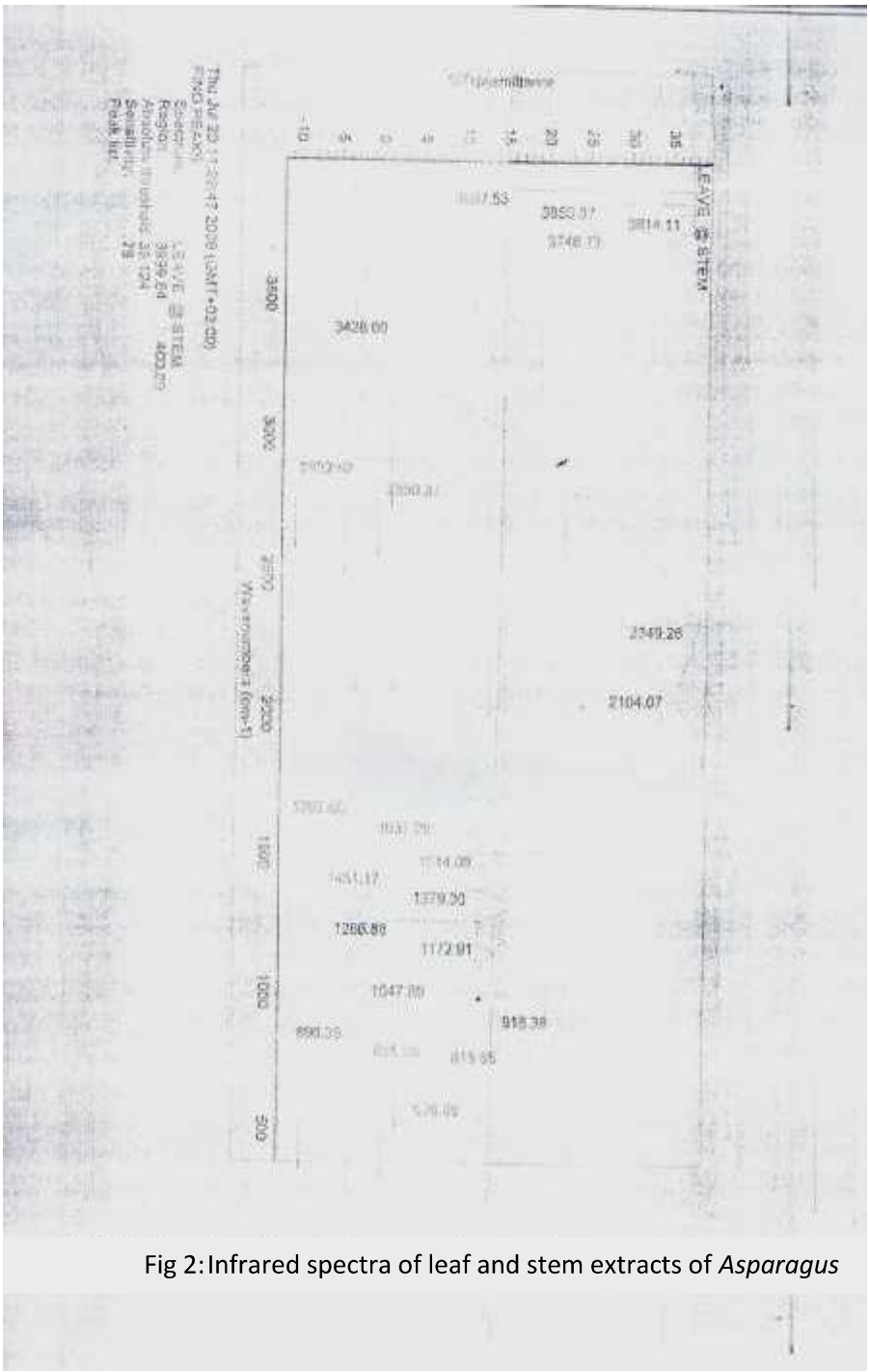

Fig 2: Infrared spectra of leaf and stem extracts of *Asparagus*

Table 1: The main effects of nematode population, treatment materials (aqueous extract and dry powder and a combination of aqueous extract and dry powder) and material levels on the plant height and number of leaves of *Solanum melongena*

Nematode population (egg)	Plant height (cm) at weeks after planting (WAP)						Number of leaves at weeks after planting(WAP)					
	2	**4**	**6**	**8**	**10**	**12**	**2**	**4**	**6**	**8**	**10**	**12**
0	4.5	11.1	22.1a	31.9a	43.1a	66.0a	3.3	4.9	7.8	18.9a	39.0a	68.6a
20,000	4.3	10.1	20.7b	28.0b	45.5b	58.6b	3.2	4.7	7.0	14.3b	33.8b	59b
S.E	0.667	0.966	1.577	2.403	2.883	3.964	0.107	0.256	0.478	1.318	2.759	3.743
	N S	N S					N S	N S	N S			
A. africanus (plant material)												
Extract	3.2	10.4	19.7	34.7	49.6	66.1	3.2ab	4.6ab	7.9	13.5	35.3ab	65.7
Powder	3.2	10.3	20.2	29.1	47.6	65.7	3.0b	4.4b	6.6	13.2	33.0b	64.6
Extract and Powder	6.5	11.1	21.2	36.1	53.6	58.7	3.5a	5.4a	8.5	17.1	41.9a	73.3
S.E.	0.817	1.183	1.932	2.944	2.796	2.405	0.131	0.314	0.586	1.615	3.379	4.586
	N S	N S	N S	N S	N S				N S	N S		N S
A. africanus levels												
0% / 0 t/ha	4.4	7.2b	13.5b	23.2b	41.6b	62.7b	2.8b	4.1b	5.7b	9.1b	22.7b	56.3b
50% / 1.5 t/ha	4.6	12.0a	22.3a	36.6a	53.3a	66.6ab	3.4a	4.8a	8.3a	15.3a	40.0a	71.2a
100% / 3.0 t/ha	4.8	12.5a	25.3a	40.1a	55.9a	71.1a	3.5a	5.5a	9.1a	19.3a	43.6a	76.0a
S. E	0.817	1.183	1.932	2.944	2.7960	2.405	0.131	0.314	0.586	1.615	3.379	4.586

Table 2: The main effects of nematode population, treatment materials (aqueous extract and dry powder and a combination of aqueous extract and dry powder) and material levels on the fresh shoot weight and mean fruit weight of *Solanum melongena.*

Nematode Population weight (g)	Mean Fresh Shoot weight (g)	Mean Fruit
0	124.79a	34.07a
20,000	108.09b	292.13b
S.E	7.831	31.289
Asparagus africanus Extract	112.58	297.45
Powder	111.34	291.85
Extract and Powder	112.38	319.99
S.E.	2.591	33.321
	N S	N S
Levels *Asparagus africanus*		
0% / 0 t/ha	88.40b	168.99b
50% / 1.5 t/ha	113.73ab	330.32a
100% / 3.0 t/ha	132.18a	409.99a
S. E	4.571	38.321

Each value is a mean of three replicates. Figures with same letter(s) in the same column are not significantly different using Duncan's Multiple range test at P = 0.05 (S. A. S 1997 Statistical Package).
N. S = Not Significant

Table 3: The main effects of nematode population, treatment materials (aqueous extract and dry powder and a combination of aqueous extract and dry powder) and material levels on the fresh root weight and final nematode populations in the soil and root of *Solanum melongena at* 15 weeks after planting *(*WAP*)*

Nematode population	Mean Fresh root weight (g)	Mean final Nematode pop. in 200ml of soil	Mean final Nematode pop. in 10g root
0	34.34a	0.00a	0.00a
20,000	29.73b	13.22b	13.78b
S.E	3.598	0.684	0.412
Asparagus africanus			
Extract	29.31	12.22	12.83
Powder	30.70	13.11	13.06
Extract and Powder	36.01	12.44	11.89
S.E.	4.406	0.838	0.505
	N S	N S	N S
Levels *Asparagus africanus*			
0% / 0 t/ha	21.81b	22.61c	24.39c
50% / 1.5 t/ha	28.39b	10.78b	9.22b
100% / 3.0 t/ha	45.89a	4.39a	4.39a
S. E	4.406	0.838	0.505

Each value is a mean of three replicates. Figures with same letter(s) in the same column are not significantly different using Duncan's Multiple range test at P = 0.05 (S. A. S 1997 Statistical Package).
N. S = Not Significant

Table 4: The main effects of nematode population, treatment materials (aqueous extract and dry powder and a combination of aqueous extract and dry powder) and material levels on the biochemical food components of treated egg plant fruits

Nematode Population	Moisture content	Dry matter	Crude fat	Crude protein	Total ash	Crude fibre
0	5.16	95.13	4.44	13.47	7.34	3.51
20,000	5.38	94.87	4.29	13.31	7.34	3.45
S.E	0.123	0.220	0.128	0.152	0.122	0.092
	N S	N S	N S	N S	N S	N S
Plant material of *A. africanus*						
Extract	5.22	94.94	4.37	13.75	7.45	3.50
Powder	5.42	94.92	4.31	13.39	7.32	3.38
Extract and Powder	5.17	95.13	4.41	13.03	7.26	3.57
S.E.	0.151	0.269	0.157	0.187	0.150	0.113
	N S	N S	N S	N S	N S	N S
Material levels						
0% / 0 t/ha	5.27	94.70	4.25	13.38	7.51	3.59
50% / 1.5 t/ha	5.19	95.06	4.49	13.59	7.27	3.51
100% / 3.0 t/ha	5.35	95.24	4.35	13.20	7.25	3.35
S. E	0.151	0.269	0.157	0.187	0.150	0.113
	N S	N S	N S	N S	N S	N S

Each value is a mean of three replicates. Figures with same letter(s) in the same column are not significantly different using Duncan's Multiple range test at P = 0.05 (S. A. S 1997 Statistical Package).
N. S = Not Significant

The results obtained from the phytochemical screening of the root, stem and leaves of sparrow grass plant revealed that all the plant parts examined contain Tannins, Alkaloids and sterols. Saponins were present only in the roots while Flavonoids were detected only in the stems and leaves. Infrared spectra of all the parts of the test plant (Figs. 1 and 2) showed that they probably contain mixture of same or similar chemical compounds as the spectra of the extracts were similar. The major diagnostic features is the very strong absorption bands in the regions ~ 3400 cm^{-1} region which is believed to belong more to amino (-NH) stretching bands. The absence of any significant stretching bands in the 3500cm^{-1} excludes the probability of the extracts containing the steroids of Terpene class to any appreciable level. The absence of any band attributable to the hydroxyl (0-H) bonding in the 1410- 1260 cm^{-1} region further supports the exclusion of steroids and Terpenes as components of the extracts. The presence of alkaloids is a major component of the extracts. The other major diagnostic stretching mate in these spectra is the ~ 1630cm^{-1} found in the spectrum of each extract. These are attributable to the carbonyl (C-

O) group which is a common feature of plant extracts including those of the alkaloid family.

The study has shown that root-knot nematodes adversely effected the growth and yield of infected and untreated egg plants, *S. melongena* when compared with inoculated but treated plants. Root-knot nematode infection in crops is known to result in soil nutrient-uptake impairment and nutrient diversion by the nematode pests. These lead to poor growth and yield in affected crops. Oyedunmade (1998) and Babatola (1990) reported loss in fruit weight of cowpea and okra respectively infected by *Meloidogyne incognita.*

The effectiveness of aqueous extracts of sparrow grass in the control of root-knot nematodes, *M. incognita* as reflected in improved egg plant growth and yield, reduced nematode population densities in the soil and roots could be attributed to inhibitory effect of test plant on nematode population densities. This inhibitory effect could be due to the toxic materials of tannins, alkaloids sterols, saponins and flavonoids defected in the roots, stems and leaves. This result is in agreement with Jacobson (1988) who demonstrated the inhibition of development of nematode egg by the leaves, seeds and other parts of neem which he ascribed to toxic substances present in them. Subramaniyan and Vadivelu (1990) had similar reports on nematicidal properties of *Crotalaria spectabilis* leaves and roots. Tahmid et al. (2002) demonstrated the suppression of nematode activities in the root of the egg plant treated with *Azolla pinnata* extract, ultimately confirming the low galling of root-knot nematode in the egg plant, *S. melongena.*

The superiority of the highest dose (100% or 3.0t/ha) of *Asparagus africanus* over the lower levels is in line with Adegbite and Adesiyan (2005) who reported that hundred percent of root extracts of Siam weed and neem gave the maximum inhibition of egg hatching (100%) followed by lemon grass and castor bean with 95 and 93.2% inhibition respectively. Similar observations were made by Sharma and Prasad (1995) and Ahmed et al (1990).

The observed increase in the growth and yield of *S. melongena* may be due to the nematicidal effect of aqueous extract and dry powder of *A. africanus* against the root-knot nematodes. The treated plants were able to carry out their normal physiological functions which resulted in the production of more number of leaves and branches and improved plant height as compared to the untreated control plant. This corroborates the findings of Fatoki (2002) who reported that the water and ethanol extracts of the leaf and root of neem and Siam used prevented *M. incognita* egg hatch and juvenile survival invitro. Tahmid et al. (2002) also reported that the plant extract of *Azolla pinnata,* apart from reducing the galling in roots, improved the growth of egg plant.

The proportional increase in both plant height and number of treated

plants could be attributed to the fact that treated plants were less attacked by nematodes. As a result, more nutrients were available in their system to carry out the normal physiological functions including leaf development, photosynthesis, replacement and regeneration of new branches. This agrees with report of Routary and Das (1988).

The results of the phytochemical studies buttressed earlier findings of Sofowora (1982) who reported that alkaloids, flavonoids, saponins and tannins are principal bioactive agents generally contained in medicinal plants. Adegbite (2002) reported that extracts of Siam weed, neem, lemon grass and castor oil contained alkaloids, flavonoids, saponins, amides benzamides and ketones that singly and in combination inhibited hatching of nematode eggs. Similar result was recorded by Izuogu and Oyedunmade (2009) that saponin and tannin were present in leaf extracts of brimstone, cassia, chanca piedra and lemon grass while crude alkaloid was absent in all the leaf extracts and present only in cassia.

The use of plant materials has consistently shown that it is one of the most feasible alternatives in the control of plant parasitic nematodes. Aqueous extract and dry powder of sparrow grass as demonstrated in the present study holds great promise for the future control of plant parasitic nematodes.

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