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The efficacy of aqueous leaf extracts of *Hyptis suaveolens* (L.) Poit and *Ocimum basilicum* (L.) in the control of *Meloidogyne* spp infecting Onion, *Allium cepa* (L.)

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Abstract

The efficacy of aqueous leaf extracts of *Hyptis suaveolens* and *Ocimum basilicum* in the control of *Meloidogyne* spp infecting *Allium cepa* L (Onion) was investigated using sterile soil that was filled into polythene planting bags. Two varieties of onion (Safari and Belami) and three concentrations (60 mg/ml, 45 mg/ml and 30 mg/ml) each of *H. suaveolens* and *O. basilicum* leaf extracts were used. Furadan at 7.7 mg/ml was used as a positive control and the negative control was untreated. Each treatment was replicated three times in a completely randomized design (CRD). Onion raised in steam-sterilized soil were infected with 2000 juveniles of root-knot nematodes four weeks after planting. Two weeks thereafter, the different concentrations of the leaf extracts of the test plants and furadan were applied. The plants were then monitored for growth and yield parameters at harvest. Phytochemical analysis of the aqueous leaves extracts of the two plants were carried out. The result showed that growth and yield data of infected but treated onions were higher than their infected but untreated counterparts. Onions treated with *O. basilicum* at 60 mg/ml had the highest performance: plant height (39.90 cm), dry bulb weight (16.70 g) among others followed by those treated with *H. suaveolens* at 60 mg/ml, plant height (36.30 cm), dry bulb weight (13.20 g) then furadan, plant height (36.17 cm), dry bulb weight (14.93 g) while the untreated onions had the least growth and yield data, plant height (24.40 cm), dry bulb weight (8.40 g). Statistical analysis revealed that the various treatments significantly increased plant height, collar girth and leave number ($p< 0.05$) as compared to the untreated control. Variety Safari generally performed better than variety Belami. Statistical analysis also showed significant difference ($p< 0.05$) for all parameters. Phytochemical analysis showed presence of some bioactive compounds such as saponins, alkaloid, tannins among others. The study revealed nematicidal efficacy of the aqueous leaves extracts of the test plants, therefore, they can be viable alternatives to synthetic nematicides in nematode management.

Keywords: Efficacy, Nematicidal, *Hyptis suaveolens, Ocimum basilicum*, *Allium cepa*

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INTRODUCTION

Onion (*Allium cepa* L.) is one of the major bulb vegetable crops of the world (Roopa *et al*., 2014). The World onion production is steadily increasing so that it is now the second most important horticultural crop after tomatoes (Bankole *et al.,* 2004). Onion is one of the most important and familiar spice crops throughout the world. It's nutritional and medicinal values such as sugar, vitamins, minerals, electrolytes, protein and dietary fibre cannot be over emphasized (Onuorah and Ifeanyi, 2015). *Allium cepa* is also an important vegetable crop in Nigeria based on consumption and economic values to farmers (Ole *et al.,* 2004).

Globally, annual losses resulting from infestations caused by plant parasitic nematodes are huge. In economic terms, nematodes cause an estimated loss of about $ 157 billion annually to world agriculture (Singh *et al*., 2015). The root-knot nematodes, *Meloidogyne* spp has been reported as the most important plant parasitic nematodes since they infest majority of the economically important plant species in the world (Abad *et al*., 2008, Ekpenyong *et al*., 2016).

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions. They defend against attack from predators such as insects, fungi, nematode and herbivorous mammals. At least 12,000 of such compounds have been isolated so far; a number estimated to be less than 10% of the total **(**Tapsell *et al***.,** 2006**)**. These botanical extracts have been found to contain phytochemicals such as alkaloids, tannins, saponins, flavonoids, diterpenes, glucosinolates, acetylenes and thienyls (Chitwood, 2002). These phytochemicals are effective against plant parasitic nematodes (Khan *et al*., 2017, Singh, 2015). Some members of the family Lamiaceace such as *Hyptis suaveolens* (L.) and *Ocimium basilicum* (L.) have been reported to be rich in phytochemicals such as flavonoids, alkaloids, carbohydrates, tannins, saponins and phenol hence potentially nematicidal (Devi, 2015, Khair-ul-Bariyah, *et al*., 2012). Farmers generally have relied on the use of synthetic nematicides over the years and they are currently the most effective means of pest control. This has resulted to its excessive and unsafe usage (Taniwiryono, *et al.,* 2007).

Indiscriminate use of synthetic nematicides for the control of nematodes leads to phytotoxicity, environmental pollution, nematodes resistance and affects many other non-target organisms. On the other hand, its unsafe usage may result in poisoning of humans especially in developing countries like Nigeria (Ojo, 2016, Anwar *et al*., 2009). To this effect, it is of importance to find alternative control strategies which are as effective as synthetic nematicides, environmentally friendly and relatively available at low cost (Ekpenyong *et al.,* 2016). It is against this backdrop that this study was done with the aim of determining the efficacy of *H*. *suaveolens* and *O. basilicum* in the control of *Meloidogyne* spp infecting onions. The specific objectives were i) To determine the nematocidal effects of different concentrations of extracts of *H. suaveolens* and *O. basilicum* on *Meloidogyne* species infecting onion cultivars. ii) To determine the effect of the treatment on the growth and yield of Onion and iii) To evaluate the proximate composition of onions bulbs from all treatments

MATERIALS AND METHODS

Experimental Site and Soil sterilization.

The study was conducted at the Botanical Nursery Unit of the Federal College of Forestry, Jos, Plateau state. The soil used for this investigation was heat sterilized at 65 ˚C for 90 minutes using electric soil sterilizer and allowed to cool before packing into perforated polythene planting bags.

Source of onion Seeds

Seeds of onion varieties Safari and Belami used for this study were obtained from Agric Tropic Limited (Technism Office, Jos branch, Plateau State, Nigeria).

Source of Inoculum and Extraction of Root Knot Nematodes

Tomato plants were sampled from different farms within Jos and environs and examined for the presence of galls on their roots (Gowen *et al.*, 2005). Roots showing the presence of galls were brought to the laboratory for extraction of nematodes. The modified Baermann funnel method of nematode extraction (Southey,1970) was used for the nematode extraction. The galls from the roots were placed in Petri dishes, 2 pipette drops of distilled water was added to moisten them, then teased apart. The root samples and distilled water in the Petri dishes were poured into a Baermann funnel with a short

piece of rubber tubing attached to the stem. A test-tube filled with water was then attached to the end of the rubber tube and made airtight at both the point of attachment with the funnel and the test-tube using masking tape. The funnel was lined with a thin layer of cotton wool and supported in an upright position. The root samples in the funnel were then watered to prevent them from drying and to allow for free movement of the nematodes. Twenty (20) sets of the Modified Baermann funnel were prepared. The set-ups were allowed to stand for 48 hours. Nematode juveniles that were hatched in the water pass through the cotton wool and were collected at the bottom of the test-tubes. Nematodes were identified to species level based on morphological features as described by Hooper *et al*., (2005) and the University of Nebraska Lincoln nematode identification website available at http://nematode.unl.edu/konzlistbutt.htm
Identification was based on adult female nematodes. Nematode population estimation was done by counting the number of active juveniles in 1ml of homogenized suspension of the inoculum on a nematode counting dish using a binocular research microscope at X40 magnifications. The enumeration was done 5 times and the average nematode count was determined. One (1) ml of the suspension was found to contain 125 juveniles of nematodes.

Preparation of Plant Extracts

Fresh leaves of *Hyptis suaveolens* and *Ocimium basilicum* were collected from Maza, Rusol and Angwa Rukuba villages in Jos and air-dried before they were blended to powder using VTCL Speedo Mixer Grinder. The pulverized leaves were soaked in water for 72 hours and filtered to remove the residue. The filtrate was concentrated to powder by boiling using a hot plate at 100 ^{o}C. The different concentrations of the plants were then prepared by dissolving variable weights_of the powdered residue (using digital weighing balance) in distilled water as follows:
0.30 g of extract in 10 ml of water = 0.030 g/ml =30 mg/ml; 0.45 g of extract in 10 ml of water = 0.045 g/ml =45 mg/ml; 0.60 g of extract in 10 ml of water = 0.060 g/ml =60 mg/ml

Phytochemical Analysis of *H. suaveolens* and *O. basilicum* leave extracts

The phytochemical screening for qualitative detection of flavonoids, tannins, alkaloids, saponins, phlobatannin and cardiac glycosides among others were performed on the extracts of both plants as described by AOAC (2007).

Experimental Design.

The experiment was laid out in a completely randomized design (CRD) in an open field using poly bags with 2 onion varieties, 2 plant extracts with the extracts applied in 3 different concentrations (30, 45 and 60 mg/ml) furadan (standard nematicide) and a negative control (inoculated but untreated). All treatments were replicated three (3) times.

Pot experiments

Onion seeds were broadcasted on sterile soil in containers and allowed to grow, then maintained in the nursery for six weeks (42 days) after germination. Apparently healthy seedlings were then transplanted into 48 polythene bags (one per polythene bag) filled with sterile soil. Four weeks after transplanting, the plants were inoculated using the modified method of *Hagop et al.* (2012). This was done by pouring 16 ml of the nematode suspension containing 2,000 nematode juveniles into holes around the root of seedlings and 2 weeks afterwards 30 mg/ml, 45 mg/ml and 60 mg/ml of extracts were added to pots separately. Furadan at an application rate of 4 kg/ha which was equivalent to 7.7 mg/ml based on the area of poly bags used for this research was positive control while untreated pots served as negative control. Growth parameters or agronomic characters per plant such as shoot length, root length, root weight and root galls were collected and recorded at harvest. Number of galls per plant was determined and recorded. Also gall indices were calculated on a scale of 0-5 and Resistant Ratings determined as described by Taylor and Sasser (1978), where: 0 galls = immune, 1-2 galls =1 (Highly Resistant), 3-9 galls = 2 (Moderately Resistant), 10-30 galls =3 (Moderately Susceptible), 31-100 galls =4 (Susceptible), 100 and above galls =5 (Highly susceptible)

Data Analysis

All data generated were analyzed using Statistical Package for Social Sciences (SPSS Statistics version 23). The two-way analysis of variance (ANOVA) at 0.05 level of probability was the analytical used while Means were separated using the Least Significant Difference (LSD) test.

RESULTS

The root-knot nematodes species were found to be *Meloidogyne incognita* and *Meloidogye Javanica*.

Phytochemical Screening

The phytochemicals present in the leaf extracts of *H. suaveolens* and *O. basilicum* included flavonoids, alkaloids, carbohydrates, tannins, saponins, phenol and glycosides (Table 1).

Effect of Treatments on Growth Parameters

The result revealed that onion height increased with increase in concentration of the extracts. The highest shoot height was recorded at concentration 60 mg/ml for both extracts i.e 39.90 cm and 36.17 cm for *O. basilicum* and *H. suaveolens* respectively. Generally, *O. basilicum* extract accounted for higher onion height than *H.suaveolens* extract across the various concentrations (Table 2). All treated plants had higher plant height than their untreated controls. Statistical analysis revealed that, the various treatments significantly increased onion height ($P< 0.05$) as compared to the untreated control (Table 2). Among inoculated and treated onions as well as untreated ones, variety Safari had the highest plant height (36.23 and 24.0) respectively (Table 2). Number of leaves per plant increased with increase in concentration of extracts for both plants.Generally, onion treated with *H. suaveolens* extract at 60 mg/ml had the highest number of leaves (8.33) as compared with all other treatments. Number of leaves were higher for variety Safari than Belami. Statistical analysis revealed that the number of leaves produced by onion plants treated with the extracts were significantly ($p<0.05$) higher than those produced by the untreated controls (Table 3).

Table 1: Phytochemical constituents of *H. suaveolens* and *O. basilicum* aqueous leaf extracts

Phytochemical Constituent	*H.suaveolens*	*O.basilicum*
Flavonoids	-	++
Tannins	+++	+
Saponins	++	+++
Phenol	+	_
Glycoside	+	++
Carbohydrate	+	+
Alkaloid	-	+++

Key: - = not present, ++ = present in moderate concentration (amount), +++ = present in high concentration (amount)

Table 2: Effect of the different concentrations of test plants extracts and the controls on plant height (cm) of onion infected with nematodes

Treatment	Concentration (mg/ml)	Varieties		$LSD_{0.05}$
		Safari	Belami	
H. suaveolens	30	29.50	28.50	0.435
	45	31.07	32.23	
	60	36.23	34.67	
O. basilicum	30	32.50	33.00	
	45	32.37	31.83	
	60	35.00	39.90	
Furadan2		34.50	36.17	
Untreated		24.00	23.80	
$LSD_{0.05}$	0.163			

Table 3: Effect of the Different **Concentrations of Test Plants Extracts and the Controls** on number of leaves of Onion infected with nematodes.

Treatment	Concentration (mg/ml)	Varieties		$LSD_{0.05}$
		Safari	Belami	
H. suaveolens	60	8.33	8.00	
	45	8.33	8.00	0.247
	30	7.00	7.67	
O. basilicum	60	7.67	7.33	
	45	7.67	7.00	
	30	7.67	7.67	
Furadan	2	8.00	7.00	
Untreated		6.70	6.67	
$LSD_{0.05}$	0.177			

Collar girth increased with increase in concentration of extracts for both plants. Onion plants treated with *O. basilicum* extract at 60 mg/ml had higher collar girth (4.73 cm) than all others (Table 4). The treated plants had higher collar girth than their untreated counterparts. Statistically, the treatments significantly ($P<0.05$) influenced collar girth as compared with the untreated control (Table 4).

Effect of Treatments on yield data

The fresh and dry weights of onion showed that the weight increased with concentration of extracts for both plants.

Onion treated with *O. basilicum* at 60 mg/ml had the highest dry weight (16.70 g), followed by those treated with Furadan (14.93 g), then *H. suaveolens* at 60 mg/ml (13.20 g) while the untreated control had the least (8.40 g). Variety Safari had higher fresh and dry weight than variety Belami (Table 5)

Effects of treatments on Number of Galls, Gall Index and Resistant Rating

Number of galls was highest in untreated onions (18.33) followed by onions treated with 30mg/ml (2.0) then 45 mg/ml (1.33) while onions

Table 4: Effect of the different concentrations of test plants extracts and the controls on collar girth (cm) of onion infected with nematodes

Treatment	Concentration(mg/ml)	Varieties		$LSD_{0.05}$
		Safari	Belami	
H. suaveolens	60	3.56	3.40	
	45	3.32	3.20	0.658
	30	3.03	3.00	
O. basilicum	60	4.73	3.60	
	45	3.27	3.23	
	30	3.17	3.17	
Furadan2		3.10	3.60	
Untreated		2.50	2.87	
LSD	0.383			

Table 5: Effect of concentrations of test plants extracts and the controls on fresh weight (g) and dry weight (g) of the two varieties of onion

Treatment	Concentration (mg/ml)	Safari		Belami		LSD
		Fresh weight	dry weight	Fresh weight	dry weight	
H. suaveolens	60	28.18	10.17	21.57	13.20	1.23
	45	26.30	9.80	19.83	9.07	
	30	25.67	9.63	19.18	8.67	
O. basilicum	60	32.50	13.90	26.27	16.70	
	45	31.80	13.63	25.43	13.53	
	30	26.67	12.20	23.67	10.53	
Furadan	2	34.03	14.93	28.30	12.43	
Untreated		18.90	8.40	23.03	14.53	
LSD	2.06					

treated with concentration 60 mg/ml had the least number of galls (0.33). Onions treated with *O. basilicum* extract at 30 mg/ml had higher number of galls (3.33) than those treated with *H. suaveolens* extract at 30 mg/ml (2.00). Variety Belami had higher number of galls across treatments except among the untreated Onions where Variety Safari had 18.33 while Variety Belami had 14.00 galls (Table 6). Resistance rating showed variation in the plants' response to the varying treatments. The untreated plants were moderately susceptible with gall index of three (3). Belami variety treated with *O. basilicum* at concentration 30mg/ml was also moderately susceptible with gall index of 3.33. *H. suaveolens* treated at concentration 30mg/ml was moderately resistant with gall index of 2. All other plants were highly resistant with gall index of one (1) except Belami variety treated with *O. basilicum* extract at concentration 45mg/ml which was moderately resistant with gall index of 2 (Table 6)

Table 6: Effects of the varying concentrations of *H. sauveolens* and *O. basilicum* on resistance rating of onion cultivars**.**

Variety	Treatment	Concentration (mg/ml)	Number of Galls	Gall index	Level of Resistance
Safari	*H. sauveolens*	30	1.67	2	Moderately resistant
		45	1.00	1	Highly resistant
		60	0.33	1	Highly resistant
	O. bacilicum	30	1.67	2	moderately resistant
		45	1.33	1	Highly resistant
		60	0.33	1	Highly resistant
	Furadan		1.33	1	Highly resistant
	Untreated		18.33	3	Moderately susceptible
Belami	*H. sauveolens*	30	2.0	2	moderately resistant
		45	1.33	1	Highly resistant
		60	0.67	1	Highly resistant
	O. basilicum	30	3.33	3	Moderately susceptible
		45	2.33	2	moderately resistant
		60	1.00	1	Highly resistant
	Furadan		1.00	1	Highly resistant
	Untreated		14.00	3	Moderately susceptible

Discussion

The phytochemical analysis revealed that the leaves extracts of *H. suaveolens* and *O. basilicum* contain some bioactive constituents such as flavonoids, alkaloids, carbohydrates, tannins and saponins. The presence of these phytochemicals in the extracts of the two plants may have been responsible for the observed improved growth and yield of treated onion cultivars as compared to their untreated controls. Khan *et al.* (2017) reported the presence of alkaloids, flavonoids, tannins and saponins in weeds from India and further said they could be responsible for the observed mortality of *Meloidogyne* population *in vitro*. Chitwood (2002) also reported that bioactive compounds such as Alkaloid, Tannin, Saponin, Flavonoid and Steroid among others have nematicidal activity. These, therefore, explains the nematicidal activities of the leaf extracts in the present study.Growth and yield data of infected onions treated with extracts of *H. suaveolens* and *O. basilicum* increased as compared to their untreated controls. This is indicative of the nematocidal potential of the extracts which may have ameliorated the effects of the nematodes hence the improved growth and yield in treated plants. This finding is similar to that of Khan *et al.,* (2019) who reported that nematode infestation led to stunted growth and treatment with extract led to increase in growth and yield. They attributed their findings to the presence of the bioactive components in the extracts that suppressed the nematodes' activities. They also said higher performance of extract treated plants compared to the untreated controls may have been due to increased organic matter content to the plant. Singh (2015) reported similar findings and further said that bioactive constituents in plants extracts used as treatments accounted for the improved yield of nematode infected plants compared to their untreated counterparts. Growth and yield of extract treated onions increased with application rate. This may mean that more bioactive components with nematocidal activity were available in the soil. It could also be that more organic matter was available to the onions to improve their performance. Singh (2015) while evaluating nematocidal potency of botanical biopesticides on *Meloidogyne incognita* infecting

chickpea reported that the efficacy of the biopesticides increased with application rate.

M. incognita and *M. javanica* were the species of root knot nematodes identified in this study. It is very likely that these species are more adapted to the Jos soil environment than other species of the root knot nematodes Yao *et al*. (2017) reported that *M. incognita* and *M. javanica* are the most dominant root knot nematodes species in Nigeria. Olowe (2004) working on distribution of root knot nematodes in cowpea fields in Nigeria also reported these two species as the most prevalent.

Number of galls and gall index indicated variability in the variety and the various treatments. The improved resistance ratings of treated plants over untreated plants suggests that the treatment improved the onions response to the nematode infection and that the extract of the test plants must have been toxic to juveniles of nematodes thus reducing nematode population density as well as galling. Similar results have been reported by other workers (Zhou *et al*. 2012, Oka *et al.,* 2007). Variation among the varieties may be due to inherent factors. Onions that were inoculated and un-amended performed poorly, this was expected. It implies that the un-amended onions were readily attacked by nematodes as evident by the highest number of galls and the least growth and yield data. This finding is in line with the report of Khan *et al.,* (2019) who said nematode infection of plants led to stunted growth.

Conclusion

The findings of this study have shown that the leaf extracts of *H. suaveolens* and *O. basilicum* have nematicidal activities. The 60 mg/ml concentration outperformed the standard nematicides, furadan. It is therefore, concluded that the plant extracts are viable alternatives to chemical nematicides in the control of root-knot nematodes and are therefore recommended for use as bio-pesticides.

Conflict of interest

Authors have no conflict of interest to declare.

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