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THE EFFICACY OF AQUEOUS LEAF EXTRACTS OF MORINGA AND BITTER LEAF IN THE CONTROL OF *MELOIDOGYNE* SPP INFECTING CARROT

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ABSTRACT

The efficacy of aqueous leaf extracts of *Moringa oleifera* in the control of *Meloidogyne* spp infecting carrot was investigated. Two varieties of carrot (Touchon and Nantes) infected with 2000 juveniles of RKN, three concentrations (150 mg/ml, 100 mg/ml and 50 mg/ml) each of Moringa and Bitter leaf extracts, Furadan (7.7 mg/ml) and the untreated sample were the treatments. Each treatment combination was replicated 5 times. Growth and yield data were collected at harvest. Phytochemistry of the extracts of test plants were determined. Data collected were subjected to two-way analysis of variance. Growth and yield of infected but treated carrot were higher than their infected but untreated counterparts. Carrots treated with *M. oleifera* at 150 mg/ml had the highest performance in plant height (41.48 cm), fresh tuber weight (89.67 g) among others while the untreated carrots had the least performance. Statistical analysis revealed that the various treatments significantly increased plant height and fresh tuber weight ($p < 0.05$) as compared to the untreated control. Phytochemistry showed presence of flavonoids, saponins, alkaloid among others. The study revealed nematicidal efficacy of the aqueous leaf extracts of the test plants; therefore, they can be viable alternatives to synthetic nematicides in nematode management.

Keywords: Efficacy; nematicidal**;** *Moringa oleifera***;** *Vernonia amygdalina; Daucus carota*
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INTRODUCTION

Carrots (*Daucus carota* L.) are succulent vegetables cultivated worldwide and can be produced fresh throughout the year (Schaller and Schnitcher, 2000). Global production of carrot is estimated at 23,321 metric tonnes. China is reportedly the leading producer of carrot with an estimated output of 8,395,500 metric tonnes, while Europe contributes an estimated total of 7,484 metric tonnes. Africa's global share of production is estimated at 1,054 metric tonnes (World Carrot Museum, 2013). In Nigeria, carrot is commonly grown in the Northern part of the country, particularly during the dry season between February and May (Simon, 2000). The estimated production in Nigeria was reported to have stood at 23,500 metric tonnes from 27,500 hectares of land (FAO, 2003). Among succulent vegetables, carrot ranks third in world production. This is because the crop which is easy to grow has a long period of harvest, transports well and stores for a relatively long period of time at low temperatures (Simon, 2000). Carrot ranks tenth in nutritional value among various fruits and vegetables (Acharya *et al.,* 2008). Carrots contain pro-vitamin A carotene which maintains good eye health. Carrot is a good source of dietary fibre and of the trace mineral molybdenum, rarely found in many vegetables. Molybdenum aids in metabolism of fats and carbohydrates and is important for absorption of iron. It is also a good source of magnesium and manganese. Magnesium is needed for bone, protein, making new cells, activating B vitamins, relaxing nerves and muscles, clotting blood and energy production; secretion and functioning of insulin also require magnesiun (Bartlett and Eperjesi, 2008). Carrot has been reported to possess antioxidant properties (Hocine, 2012, Kamlesh *et al.,* 2012). Carrot has been used for the treatment of leukaemia in traditional medicine. It contains polyacetylenes which could be useful in the development of new leukemic therapies (Ziani *et al.,* 2012, Radulovic *et al.,* 2011). Also, carrots are believed to improve memory, cause wound-healing including stomach ulcer as well as possessing anti-inflammatory activity (Metzger *et al.,* 2008; Vasudevan *et al.,* 2010; Patil *et al.,* 2012). Carrot, like most crops grown in the tropics and sub-tropics, has not escaped the hazards associated with pests and diseases. Plant parasitic nematodes are important pathogens on most food, vegetable, horticultural and fiber crops and without adequate control can cause loss of yield and quality (Sikora and Fernandez, 2005). However, the most destructive of them are the root-knot nematodes belonging to the genus *Meloidogyne* (Luc *et al*., 2005). The root-knot nematodes are important economic pests of many cultivated crops (Lamovšek *et al.,* 2013). Root-knot nematodes reduce yield and quality of agricultural products thereby causing economic losses to the agricultural industry (Perry *et al*., 2009). Globally, losses associated with root-knot nematodes are as high as $157 billion dollars annually (Singh *et al*., 2015). To combat these effects, efficient control methods are required for these pathogens. Chemical control using nematicides, which are the most effective approach for the management of nematodes, is being slowly phased out or restricted due to high toxicity and hazard to the environment (Collange *et al*., 2014).

Plant extracts have been found effective for the control of plant parasitic nematodes (Khan *et al*., 2017). Plant extracts have the advantages of cheapness, ready availability as well as health and environment friendliness (Singh, 2015). This study was aimed to evaluate the nematicidal potential of leaf extracts of *Moringa oleifera* and *Venonia amydalina* on root-knot nematodes (*Meloidogyne* sp.) infecting carrot (*Daucus carota*), and to determine the phytochemicals present in *M. oleifera* and *V. amygdalina.*

MATERIALS AND METHODS

The study was carried out in Jos North LGA of Plateau State. Jos, a region in the middle belt of Nigeria, is located between latitude 7^0 – 11^0 North and Longitude 7^0 – 8^0 East at an altitude of about 1,200 m above sea level. The topography of the area lies south of guinea savannah of Nigeria with temperature ranging between 10^0 and 32^0C and mean annual rainfall of about 140 mm (Deshi *et al*., 2018).

Fresh plant leaves of *Moringa oleifera* (Lam) and *Vernonia amygdalina (Del)* were collected from Pil-Gani, Langtang North, Plateau State. The plant materials were dried under shade and then ground into fine powder with VTCL Speedo Mixer Grinder. The powdered materials were stored in air-tight containers until the time of use.

Fifty (50) g of each leaf powder was soaked in 500 ml of water and then kept in orbital shaker for 48 hrs at room temperature. After 48 hrs, the mixture was filtered through a clean muslin cloth. The filtrate was filtered again by using a Whatmann No.1 filter paper and the extracts were concentrated to dryness using a water bath. After evaporation, the dried extracts were stored at 4°C until further use.

Phytochemical screening for identification of bioactive constituents in leaf extracts of *Moringa oleifera* and *Vernonia amygdalina* was carried out using standard procedures described by Chhabro *et al*. (1984) and Trease and Evans (1989), to ascertain the presence or absence of some specific secondary metabolites such as resin, tannins, saponins, flavonoids, alkaloids, steroids, glycosides and phenol.

Nematodes inoculum was extracted from the roots of tomato (*Lycopersicum esculentum* Mill) infected by root-knot nematodes from farms around Jos North LGA, Plateau state. Nematodes in galls on roots of infected plants were extracted using the modified Baermann funnel method as described by Southey (1970).

Nematodes were identified to generic level based on morphological features as described by Hooper *et al*., (2005) and the University of Nebraska Lincoln nematode identification website (http://nematode.unl.edu/konzlistbutt.htm). Identification was based on adult female nematodes. Estimation of nematodes was carried out by counting the number of active juveniles in 1 ml of homogenised suspension of the inocula using the software vividia lenscope™ , a Hp laptop computer connected to the microscope via Vividia Lens™ hardware. 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.

Seeds of 2 varieties of carrot (Touchon and Nantes) sourced from Farin Gada market were sown into 60 pots (30 cm^2 each) containing sterile soil. The plants were thinned to 1 per pot after germination. Fifty (50) of the plants were inoculated by pouring 16 ml volume of nematode suspension containing 2000 nematode juveniles around the root of the seedlings at two (2) weeks after germination. A week after, different concentrations of leaf extracts of *M. oleifera* and *V. amydalina* (50.0 mg/ml, 100.0 mg/ml, 150.0 mg/ml) were added to the pots separately. While 7.7mg/ml Furadan was used as standard nematicide, un-inoculated and untreated carrots served as positive control; inoculated but untreated carrots served as negative control. The treatments were replicated 5 times. The plants were watered daily and weeds were removed by hand picking.

The experiment was laid out in a Completely Randomised Design (CRD) using 60 pots, 2 varieties of carrot, 6 treatment combinations in 5 replicates. Agronomic characters per plant such as number of branches, plant height, weight of leaves, root length, root diameter and fresh tuber weight were measured and recorded. Number of galls per plant was counted and recorded. Gall index was calculated on a scale of 0-5 as described by Taylor and Sasser (1978). Where 0 galls = Immune, 1 (1-2 galls) = Highly resistant, 2 (3-9 galls) = Moderately resistant, 3 (10-30 galls) = Moderately susceptible, 4 (31-100 galls) = Susceptible, 5 (100- above galls) = Highly Susceptible.

RESULTS

Effects of Treatments on Number of Branches

The result revealed that number of branches increased with increase in concentration of the two extracts. The highest number of branches was recorded at 150 mg/ml for both extracts and varieties. For *Moringa oleifera leaf* extract, it was 10.0 and 10.4 for varieties Touchon and Nantes, respectively; while for *Vernonia amygdalina* extract, it was 9.0 and 9.8 for Touchon and Nantes, respectively (Table 1). All treated carrot varieties had higher number of branches than the untreated control. Statistical analysis revealed that the various treatments significantly increased plant branches ($p < 0.05$) as compared to the untreated control (Table 1).

Effects of Treatments on Plant Height

Plant height increased with increase in concentration of the two plants extracts. The highest carrot height was observed at 60 mg/ml for both extracts and varieties. For *Moringa oleifera leaf* extract, it was 41.48 cm and 41.36 cm for varieties Touchon and Nantes, respectively; while for *Vernonia amygdalina* extract, it was 39.52 cm and 36.74 cm for Touchon and Nantes, respectively (Table 2). All treated carrot varieties had higher plant height than their untreated control. Statistical analysis showed that the various treatments significantly increased plant height ($p < 0.05$) as compared to the untreated control (Table 2).

Effects of Treatments on Mean Fresh weight of Leaves

Fresh weight of carrot leaves increased with increase in concentration of extracts. Carrot varieties treated with *Moringa oleifera* had higher leaf weight than those treated with *Vernonia amydalina*. Among the extract-treated carrots, Variety Touchon performed best in fresh weight of leaves (Table 3).

Effects of Treatments on Mean Fresh weight of Tubers

Fresh weight of carrot tubers increased with increase in concentration of extracts. Carrots treated with *Moringa oleifera* had higher tuber weight than those treated with *Vernonia amygdalina* leaf extract. The Nantes variety generally had higher fresh tuber weight than the variety Touchon. Treated carrots had higher tuber weight than their inoculated but untreated control (Table 4).

TABLE 1: Effects of different extracts on mean number of branches per plant infected with nematodes

Treatment	Concentration (mg/ml)	Plant Varieties (cm)		LSD
		Touchon	Nantes	(0.05)
Moringa oleifera	150.0	10.00	10.4	0.17
	100.0	9.4	9.8	
	50.0	7.4	7.4	
Vernonia amygdalina	150.0	9.0	9.8	
	100.0	9.0	9.0	
	50.0	7.0	7.0	
Furadan		7.6	9.0	
Uninoculated		8.8	9.0	
Inoculated but Untreated		5.2	5.0	
LSD (0.05)	**0.35**			

Each value is a pair of means of 5 replicates. Pairs of means that differ by more than the LSD value are significantly different at 0.05 level of probability

Table 2: Effect of the different extracts on mean plant height of carrot

Treatment	Concentration	plants height (cm)		LSD
	(mg/ml)	Touchon	Nantes	(0.05)
Moringa oleifera	150.0	41.36	41.48	3.1
	100.0	39.64	39.5	
	50.0	33.67	32.0	
Vernonia amygdalina	150.0	39.52	36.74	
	100.0	36.14	34.2	
	50.0	32.20	31.8	
Furadan		38.22	30.84	
Uninoculated		30.4	30.2	
Inoculated but untreated		24.2	23.97	
LSD (0.05)	**6.4**			

Each value is a pair of means of 5 replicates. Pairs of means that differ by more than the LSD value are significantly different at 0.05 level of probability

Table 3: Effects of different extracts on mean fresh leaf weight (g) of carrot

Treatment	Concentration	Fresh leaf weight		LSD
	(mg/ml)	Touchon	Nantes	(0.05)
Moringa oleifera	150.0	34.95	34.94	0.51
	100.0	32.70	32.57	
	50.0	27.31	27.55	
Vernonia amydalina	150.0	32.99	33.57	
	100.0	28.62	30.99	
	50.0	26.89	26.72	
Furadan		33.03	31.31	
Uninoculated		31.10	34.00	
Inoculated and Untreated		16.76	17.16	
LSD (0.05) 0.66				

Each value is a pair of means of 5 replicates. Pairs of means that differ by more than the LSD value are significantly different at 0.05 level of probability

Table 4: Effects of the different extracts on mean fresh weight of tubers of carrot

Treatment	Concentration	Fresh weight of tubers (g)		LSD
	(mg/ml)	Touchon	Nantes	(0.05)
Moringa oleifera	150.0	68.39	89.67	11.11
	100.0	60.76	78.81	
	50.0	52.49	69.65	
Vernonia amygdalina	150.0	62.41	76.64	
	100.0	46.58	70.01	
	50.0	45.08	45.82	
Furadan		31.31	29.03	
Uninoculated		50.51	64.18	
Inoculated and Untreated		29.94	40.63	
LSD (0.05) 5.2				

Each value is a pair of means of 5 replicates. Pairs of means that differ by more than the LSD value are significantly different at 0.05 level of probability

Effects of Treatments on Mean Tuber Length

The highest length of tuber was recorded for both extracts and varieties at the highest concentration (150 mg/ml). *Moringa oleifera* leaf extract had 13.36 cm and 18.72 cm for varieties Touchon and Nantes, respectively while *Vernonia amygdalina* extract had 11.78 cm and 18.28 cm for varieties touchon and Nantes, respectively

(Table 5). The treated carrot had higher tuber length than the inoculated but untreated counterparts. Statistically, the treatment significantly increased the length of tubers ($p<0.05$) as compared to the untreated control (Table 5).

Effects of Treatments on Number of Galls and Gall Index

The result on Table 6 revealed that the number of galls was highest among the inoculated but untreated carrots followed by carrots treated with concentration 50 mg/ml, then 100 mg/ml while those treated with 150 mg/ml had the least number of galls. Carrots treated with *Vernonia amygdalina* leaf extract had a higher number of galls than those treated with *Moringa oleifera* leaf extract. Variety Nantes generally had a higher number of galls than Touchon.

The final nematode populations were highest among the inoculated but untreated carrots followed by carrots treated with concentration 50 mg/ml, then 100 mg/ml while those treated with 150 mg/ml had the least final nematode population. Carrots treated with *Vernonia amygdalina* leaf extract had higher final nematode population than those treated with *Moringa oleifera* leaf extract. Variety Nantes generally had higher final nematode population than Touchon (Table 6).

Gall index showed variation in the response of the carrot varieties to the treatments. The inoculated but untreated carrots were moderately susceptible with Gall Index of 3 for both varieties. Varieties Touchon and Nantes treated with *V. amygdalina* at 50.0 mg/ml were also moderately susceptible. Touchon and Nantes varieties treated with *Moringa oleifera* at 50.0 mg/ml were moderately resistant with Gall Index of 2; the same rating was observed in Variety Nantes treated with furadan. All other carrots were highly resistant with Gall index of 1.

TABLE 5: Effects of different extracts on Mean Tuber Length (cm) of carrot varieties

Treatment	Concentration (mg/ml)	Plant varieties(cm) Touchon	Nantes (0.05)	LSD
Moringa oleifera	150.0	13.36	18.72	0.78
	100.0	11.52	18.14	
	50.0	11.17	14.90	
Vernonia amygdalina	150.0	11.78	18.28	
	100.0	11.18	15.08	
	50.0	9.24	12.34	
Furadan		11.13	15.18	
Uninoculated		10.34	14.96	
Inoculated and Untreated		8.05	13.52	
LSD		1.65		

Each value is at pair of means of 5 replicates. Pairs of means that differ by more than the LSD value are significantly different at 0.05 level of probability

Table 6: Effects of treatments on resistance rating of *carrot*

Variety	Treatment	Concentration mg/ml	Mean Number of galls	Gall index	Final Nematode Population	Resistance rating	LSD **(0.05)**
Touchon	*M. oleifera*	150.00	1.4	1	800	HR	0.45
		100.00	1.8	1	1000	HR	
		50.00	5.0	2	1400	MR	
	V. amygdalina	150.00	1.4	1	800	HR	
		100.00	2.2	1	1300	HR	
		50.00	13.0	3	1620	MS	
	Furadan		2.4	1	1240	HR	
	Inoculated + Untreated		24.3	3	1700	MS	
Nantes	*M. oleifera*	150.00	1.4	1	820	HR	
		100.00	2.3	1	1120	HR	
		50.00	8.0	2	1600	MR	
	V. amygdalina						
		150.00	2.0	1	1180	HR	
		100.00	2.3	1	1120	HR	
		50.00	14.0	3	1640	MS	
	Furadan		3.4	2	1320	MR	
	Inoculated but untreated		26.40	3	1760	MS	
	LSD (0.05)	1.07					

KEY: HR-highly resistant, **MS-**Moderately resistant, **MS-** Moderately susceptible, **S**- susceptible, **HS-Highty** susceptible, **I**- immuned

Effects of treatments on nematode reproduction in *carrot*

Nematode Reproduction Factor was least (0.4) in Touchon variety treated at 150 mg/ml of *Moringa oleifera,* followed by Nantes treated with 150 mg/ml (0.41) of *M. oleifera;* Nantes infected but not treated had the highest (0.88). Generally, carrots treated with varying concentrations of bitter leaf extract had higher Nematode Reproduction Factor than those treated with Moringa extracts. Also, variety Nantes generally accounted for higher Nematode Reproduction Factor than variety Touchon (Table 7).

Proximate analysis

The proximate analysis showed variation in the nutritional content of carrot. The variety Touchon had a higher nutritional content than the variety Nantes. The result revealed that carrot varieties treated with *Moringa oleifera* leaf extract had the highest nutritional content, followed by the un-inoculated and untreated control while the inoculated and the untreated control had the least (Table 8).

Table 7: Effects of treatments on nematode reproduction in *carrot*

Variety	Treatment	Concentration Mg/ml	Mean Number of galls	Gall indexing	Final Nematode Population	Nematode Reproduction Factor	LSD **(0.05)**
Touchon	*M. oleifera*	150.00	1.4	1	800	0.4	0.45
		100.00	1.8	1	1000	0.5	
		50.00	5.0	2	1400	0.70	
	V. amygdalina	150.00	1.4	1	800	0.40	
		100.00	2.2	1	1300	0.65	
		50.00	13.0	3	1620	0.81	
	Furadan		2.4	1	1240	0.62	
	Inoculated + Untreated		24.3	3	1700	0.85	
Nantes	*M. oleifera*	150.00	1.4	1	820	0.41	
		100.00	2.3	1	1120	0.61	
		50.00	8.0	2	1600	0.70	
	V. amygdalina						
		150.00	2.0	1	1180	0.59	
		100.00	2.3	1	1120	0.61	
		50.00	14.0	3	1640	0.82	
	Furadan		3.4	2	1320	0.66	
	Inoculated but untreated		26.40	3	1760	0.88	
	LSD (0.05)	1.07					

KEY: **HR-**highly resistant, **MS-**Moderately resistant, **MS-** Moderately susceptible, S- Susceptible, **HS-** Susceptible, **I-** Immuned

Table 8: Phytochemical analysis of leaf Extracts of *V. amygdalina and M. oleifera*

Pytochemical	***V. amygdalina***	***M. oleifera***
Saponin	+++	+++
Tanin	-	-
Flavonoid	+	+++
Steroid	++	+++
Terpernoid	+	-
Alkaloid	+	+++
Glycoside	++	+
Resin	+	-
Polyphenol	+++	++

Key

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

Plate 1: Inoculated but treated carrot tuber

Plate 2: Root galls on infected but untreated *carrot* tuber

DISCUSSION

The phytochemical analysis showed that the leaf extracts of *Vernonia amygdalina* and *Moringa oleifera* contain bioactive compounds such as flavonoids, alkaloids, steroids 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 carrot varieties as compared to the untreated control. Khan *et al.* (2017) reported the presence of alkaloids, flavonoids and saponins in weeds from India and suggested that they could be responsible for the observed mortality of *Meloidogyne* population *in vitro.* Chitwood (2002) also reported that bioactive compounds such as alkaloid, saponin, flavonoid and steroid among others have nematicidal activity. These may be responsible for the nematicidal activities of the leaf extracts in the present study.

Growth and yield data of infected carrots treated with extracts of *V. amygdalina* and *M. oleifera* increased when compared with their untreated control. This is suggestive of the nematicidal capability 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 nematode activities. They also suggested that the higher performance of extract- treated plants compared to the untreated control might have been due to increased organic matter content. Singh (2015) reported similar findings and concluded that the bioactive constituents in plant extracts used as treatments accounted for the improved yield of nematode-infected plants compared to the untreated counterparts.

Growth and yield of extract-treated carrots increased with the rate of application, indicating that more bioactive components with nematicidal action were available in the soil. It could also be that more organic matter was available to the carrots to improve their performance. Singh (2015), while evaluating nematicidal potency of botanical biopesticides on *Meloidogyne incognita* infecting chickpea, reported that the efficacy of the biopesticides increased with the rate of application

The number of galls and gall index showed variability in the carrot varieties and the various treatments. The improved resistance rating of extract-treated carrots over the untreated ones suggests that the treatment improved the carrot's response to the nematode infection and that the extract of *V. amygdalina* and *M. oleifera* must have been toxic to juveniles of nematodes, thus reducing nematode population density as well as galling. Similar findings have been reported by other workers (Oka *et al.*, 2012, Zhou *et al*. 2007). Variation among the varieties may be due to inherent factors. Carrots that were inoculated and un-amended performed poorly. The carrots suffered both nutrient deprivation and nematode attack. It also implies that the un-amended carrots 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 noted that nematode infection of plants led to stunted growth.

CONCLUSION

The findings in this study have shown that the leaf extracts of *V. amygdalina* and *M. oleifera* have nematicidal activities. The 150 mg/ml concentration out-performed the standard nematicide, furadan. It is, therefore, concluded that the plant extracts are viable alternatives to chemical nematicides in the control of root-knot nematodes and are recommended for use as bio-pesticides.

REFERENCES

Acharya, U. R., Mishra, M., Patro, J. and Panda, M. K. (2008). Effects of vitamins C and E on spermatogenesis in mice exposed to cadmium. *Reproductive Toxicology, 25:* 84-88.

Bartlett, H.E. and Eperjesi, F. (2008). Nutritional supplementation for type 2 diabetes: A systematic review. *Ophthalmic Physiological Optics,* 28 (6); 503-523.

Chhabro, S. C., Uiso, F. C. and Mshin, E. N. (1984). Phytochemical screening of Tanzanian medicinal plants, *Journal of Ethnopharmacology*, 11: 157-159.

Chitwood, D. J. (2002). Phytochemical-based strategies for nematode control. *Annual Review of Phytopathology*, 40:221–249.

Collange, B., Navarrete, M., Montfort, F., Mateille, T., Tavoillot, J., Martiny, B. and Tchamitchian, M. (2014). Alternative cropping systems can have contrasting effects on various soil-borne diseases: Relevance of a systemic analysis in vegetable cropping systems. *Crop Protection*, ***55***, 7–15.

Deshi, K. E., Obasi, M., Nanbol, K., Sirajo, S. and Okechalu, B. (2018). The effects of growth and environments on growth and yield of onion *(Allium cepa* L.) in Jos Plateau State, Nigeria. *Journal of Natural Science*, (6); 6: 66-74.

FAO (2013). Production Year Book. Food and Agriculture Organisation (FAO) of the United Nations, Rome, Italy. pp 67 – 103.

Hocine, A. (2012) Evaluation of antioxidant activity of essential oil and extracts from Algerian *Daucus carota* L. aerial part. *Global Journal of Pharmaceutical Research, 1* 1121-1129.

Hooper, D. J., Hallmann, J. and Subbotin, S. (2005). Methods of extraction, processing and detection of plant and soil nematodes. In: Luc, M., Sikora, R. A. and Bridge, J. (Eds.). Plant Parasitic Nematodes in Subtropical and Tropical Agriculture, 2nd Edition (Pp 53-86). Wallingford.

Kamlesh, S., Nisha, S., Anish, C. and Ashish, M. (2012) *In vivo* antioxidant and hepatoprotective activity of methanolic extracts of *Daucus carota* seeds in experimental animals. *Asian Pacific Journal of Tropical Biomedicine,* **2**(5) 385-388.

Khan, R., Hussain, S., Khan, A., Ullah, S., Rashid, M. and Siddique, I. (2019). Phytochemical management of root knot nematodes (*Meloidogyne incognita)* by *Artemisia* spp in tomato. *Brazilian Journal of Biology*, 10:590 – 600.

Khan, A., Asif, M., Tariq, M. and Rehman, B. (2017). Phytochemical investigation of Nematostatic and Nematicidal potential of weed extracts against *Meloidogyne incognita in vitro*. *Asian Journal of Biological Sciences*, 10 (2):38 – 46.

Lamovšek, J., Urek, G. and Trdan, S. (2013). Biological Control of Root-Knot Nematodes (*Meloidogyne* spp.): Microbes against the Pests. *Acta Agriculturae Slovenica*, *101*(2); 263–275.

Luc, M., Sokora, R.A. and Bridge, J. (2005). *Plant-Parasitic Nematodes in Subtropical and Tropical Agriculture*, 2nd edn. CAB International, Wallingford. 871p.

Metzger, B. T., Barnes, D. M. and Reed, J. D. (2008). Purple carrot (*Daucus carota* L.) polyacetylenes decrease lipopolysaccharide-induced expression of inûammatory proteins in macrophage and endothelial cells *Journal of Agriculture and Food Chemistry,* 56 3554-3560.

Oka, Y., Tkachi, N., Shuker, S. and Yerumiyahu, U. (2007). Enhanced Nematicidal activity of Organic and Inorganic Ammonia-releasing Amendments by *Azadirachta indica* Extracts. *Journal of Nematology, 39*:9-16.

Patil, M. V. K., Kandhare, A. D. and Bhise, S. D. (2012). Anti-inflammatory effect of *Daucus carota* root on experimental colitis in rats. *International Journal Pharmaceutical Science, 4:* 337-343

Perry, R. N., Moens, M. and James, L.S. (2009). Meloidogyne Species: *A Diverse Group of Novel and Important Plant Parasites. In Root-knot Nematodes Wallingford UK. pp.*1-17.

Radulovic, N., Dord-Evic, N. and Stojanovic, Z. (2011). Volatiles of the Balkan endemic *Daucus guttatus* ssp. *zahariadii* and cultivated and wild growing *D. carota* – A comparitive study. *Food Chemistry,* 125: 35-43.

Schaller, R.G. and Schnitzer, W. H. (2000). Nitrogen nutrition and flavour compounds of carrots (*Daucus carota* L.) cultivated in Mitscherlich. *Journal of the Science of Food and Agriculture,* 80: 49-56.

Simon, P. W. (2000). Domestication, historical development and modern breeding of carrot. *Plant Breeding Review,* 19: 157-190.

Singh, S., Singh, B. and Singh, A. P. (2015). Nematodes: A Threat to Sustainability of Agriculture. *Procedia Environmental Sciences*, *29;* 215–216.

Singh, R. (2015). Evaluation of nematicidal potency of botanical biopesticides in combination with triazophos gainst root-knot nematode, *Meloidogyne incognita* infestation on chickpea, *Cicer arietinum* L. *International Journal of Applied. and Natural Science, 4* (3): 75-82.

Sikora, R.A. and Fernandez, E. (2005). Nematode Parasites of vegetables. In: Plant Parasitic Nematodes in Sub-tropical and tropical Agriculture E. Luc, M. Sikora, R.A. and Bridge, J. *CABI Publishing, Wallingford, UK*, pp319-392.

Southey, J. F. (1970). *Laboratory Technique for Work with Plant and Soil Nematodes*. Tech bill to her Majesty Stationary Office London, 148p.

Taylor, A. L. and Sasser, J. N. (1978). *Biology, identification and control of plant – knot Nematode*, M*eliodogyne*. North California State University Graphics 22p.

Trease, B. E. and Evans, W. A. (Eds.). (1989). *Preliminary screening of plants for their chemical constituents*, CAB International. 104p.

Vasudevan, M., Milind, P., Kalavathy, R., Abu, B. and Abdul, M. (2010). Anti-dementia potential of *Daucus carota* seed extract in rats. *Pharmacology online* 1, 552-565 World Carrot Museum, Carrot Production Statistics, 2013, http://www.carrotmuseum.co.uk/statistics.htm (accessed on 6th December, 2013)

Zaini, R. G., Brandt, K., Clench, M. R. and Le Maitre, C. L. (2012). Effects of bioactive compounds from carrots (*Daucus carota* L.), polyacetylenes, beta-carotene and lutein on human lymphoid leukaemia cells *Anticancer Agents in Medicinal Chemistry*, 12 640-652.

Zhou, L., Wang, J., Wang, K., Xu, J., Zhao, J., Shan, T. and Luo, C. (2012). Secondary Metabolites with antinematodal activity from higher plants. *Chemistry*, 37: 67-114.