Official publication of Pakistan Phytopathological Society
Pakistan Journal of Phytopathology
ISSN: 1019-763X (Print), 2305-0284 (Online)
http://www.pakps.com

PHYTOCHEMICAL AND NEMATICIDAL SCREENING ON SOME EXTRACTS OF DIFFERENT PLANT PARTS OF EGYPTIAN *MORINGA OLEIFERA* L.

[a] Eman A. A. H. Alam, [b]Ahmed S. M. El-Nuby

[a] *Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Nasr City, Cairo, Egypt.*
[b] *Plant Protection Department, Desert Research Centre, Mattariya, Cairo, Egypt.*

ABSTRACT

Aqueous and organic (methanol and ethanol) extracts of different plant parts of *Moringa oleifera* were investigated phytochemically, additionally, nematicidal activities of these extracts against root-knot nematode, *Meloidogyne incognita* were estimated also. Results of preliminary phytochemical screening on boiling water extracts of different plant parts of *Moringa oleifera* revealed the presence of all investigated phytochemicals including; flavonoids, anthraquinones, steroids and/triterpenoids, tannins, saponins, alkaloids and/or nitrogenous bases, carbohydrates and/or glycosides, cardiac glycosides, chlorides, sulphates, iridoids and sublimable substances in most samples. Regarding extracts of fresh samples; it was found that both extracts of mature and immature seeds and fruits, in addition to stems are the richest extracts regarding all investigated phytochemicals. Meanwhile, concerning extracts of dry samples; it was noticed that both extracts of leaf blades and immature fruits are the richest extracts regarding all investigated phytochemicals, followed by stems in this regard. It was noticed also that all samples are devoid of iridoids. On the other hand, results of preliminary phytochemical screening on methanol extracts of different plant parts revealed the presence of all investigated phytochemicals in all samples under investigation (except extracts of both fresh and dry leaf blades, leaf petioles and stems are devoid of saponins). Concerning extracts of fresh samples; it was found that both extracts of mature and immature seeds and fruits are the richest extracts regarding all investigated phytochemicals. Meanwhile, regarding extracts of dry samples; it was noticed that both extracts of mature and immature fruits, leaf blades and leaf petioles are rich in all investigated phytochemicals, followed by stems in this regard. Preliminary phytochemical screening on ethanol extracts of different plant parts revealed that all of these extracts contained all investigated phytochemicals, but in the least amounts compared to other extracts (except extracts of both fresh and dry leaf blades, leaf petioles and stems are devoid totally of saponins). Regarding extracts of fresh samples; it was found that both extracts of mature and immature seeds and fruits are the richest extracts regarding all investigated phytochemicals. Meanwhile, regarding extracts of dry samples; it was noticed that both extracts of mature and immature fruits are rich in all investigated phytochemicals. Concerning total phenolic contents, it was found that regarding extracts of fresh samples; the highest amount was recorded to be in both leaf blades and stems (20.243±0.025 and 20.083±0.020, respectively), the least amount was recorded to be in immature fruits (5.251±0.005). In this regard; total flavonoidal contents reached its maximum in mature fruits, followed by leaf blades (5.481±0.005 and 5.326±0.006, respectively), the least amount was recorded to be in mature seeds (0.050±0.001). On the other hand, concerning total phenolic contents in case of extracts of dry samples; the highest amount was recorded to be in both leaf petioles and stems (22.000±0.023 and 21.451±0.025, respectively), the least amount was recorded to be in immature fruits (8.029±0.015). In this regard also; total flavonoidal contents reached its maximum in leaf blades (6.111±0.012), the least amount was recorded to be in immature seeds (0.093±0.001). Results also indicated that most extracts adversely affect the second stage juveniles (J_2s) survive as they recorded mortality percentages ranged between 2.7 to 100%. Aqueous extracts in general were achieved the best results, extracts of all investigated seven parts can kill more than 50% of J_2s. Extraction with methanol showing efficacy lower than water, but the fresh Immature fruit showing mortality similar to aqueous extract, meanwhile the rest parts of the plant gave mortality percentages not exceed than 33.7%. the lowest antinematodal activity was belonging to ethanol extracts of fresh and dried parts of the plant compared with other solvents. In this respect, the efficient extracts are water extracts, followed by methanolic and ethanolic extracts, those contain the lower amounts of these investigated phytochemicals. These results of nematicidal activities of all tested extracts are confirming those of phytochemical analyses. It was concluded that, nematicidal activity of those investigated extracts are positively correlated with the presence of considerable amount of phytochemicals.

Keywords: *Moringa oleifera* L., Nematicidal activity, Plant extracts, Total flavonoidal contents, Total phenolics.

INTRODUCTION

It is well known that, the most plenteous animals on planet are Nematodes indisputably, they included plant, animal, human, insect parasites and free living forms those feed on other microorganisms and occupy various habitats (Britannica, 2020). Nematodes are soil borne microorganisms attack many host plants globally. About five thousand species of plant pathogenic nematodes are identified, they represent a huge challenge to the agricultural production, causing an economics yield deficit more than $125 billion by year universally (Mesa-Valle *et al.,* 2020). Root-knot nematodes- RKN-

Submitted: August 28, 2022
Revised: November 12, 2022
Accepted for Publication: December 12, 2022
* Corresponding Author:
Email: aalam.eman@gmail.com

(*Meloidogyne spp.*) are considered to be the most destructive genus of all phtyonematodes (Ali *et al*., 2021), the most abundant species in tropical areas are *M. incognita, M. javanica* and *M. arenaria* (Feyisa, 2021), these three species are found in Egypt and infected various plants (Ibrahim and Mokbel, 2009), as Egypt lies within the arid tropical, in south, and subtropical climate zone, in north. RKNs are protected with plant tissues and spend their life cycle inside the host roots as they totally endoparasitic and sedentary forms. They can infect more than five thousand plants, in another expression they are adversely affecting approximately all plants in the world, the damage may be singular, either from the nematode itself, or in association with other soil pathogens causing great damage to plants and economic yield losses (Abad *et al*., 2003; Hu *et al*., 2020; El-Nuby and Alam, 2020; Forghani and Hajihassani, 2020).

Chemical nematicides are common approaches for combating phytonematodes in the past, as they represent the easiest and effective manner, to keep nematode under the economic threshold. In the last two decades' methyl bromide was phased out, potent fumigant for the elimination of nematodes, also many of nematicides were banned due to their high and far-ranging toxicity and harmful effects on the environment (Chitwood, 2003). Accordingly, attention turned towards preserving the environment, which prompted scientists to adopt different control methods to minimize based mainly on natural products or get rid of, in some cases, application of synthetic nematicides. On the other hand, discovering and registration of new effective compounds against nematodes need high cost and extended research, so it become a big issue for potential new chemical nematicides.

Biopesticides are particular types of pesticides produced from natural resources e.g., plants, animals, fungi, bacteria and certain minerals (EPA, 2022). They considered to be practical and promised alternatives to combat plant pathogens, because their applications' can eliminate or alleviate the well-known hazardous effects of agrochemicals on human health and conserve the ecosystem integrity and also because these chemical nematicides (those used in combating nematodes) are very expensive and not affordable for many framers (Danahap and Wonang, 2016). Using extracts of different plants or other biocontrol agents can change the indigenous microbial communities of the rhizosphere in an approach that leads to augment soil suppressiveness to soil borne nematode and other fungal or bacterial pathogens in the rhizosphere, also application of phytochemicals-based nematicides are generally ecofriendly and safe to human beings (Vargas-Ayala *et al.,* 2000; Shaukat Siddiqui, 2001; Chitwood, 2002).

Secondary metabolites in plants are small molecules with variable chemical formula and bioactivities, they have not any important role in plant growth, but they participate in many process like resistance to biotic stresses, such as pests, and tolerance to different abiotic stresses, but according to the advancement in plant researches, some exceptions of secondary metabolites' roles have been discovered. Plants produce many important secondary metabolites, around two hundred thousand, the most known antinematodal phytochemicals are; Alkaloids, benzoxazinoids, glucosinolates, organosulfur compounds, Phenolic compounds, saponins, terpenoids and many other compounds (Desmedt *et al*., 2020). Extraction form various plants, either wild or cultivated and plants, wastes as well as sea weeds, have been accomplished by many researchers to evaluate their efficacy against nematodes (Abdel-Rahman, *et al*., 2017; Alam and El-Nuby, 2019; El-Nuby *et al*., 2020; El-Nuby *et al*., 2021).

Moringa (*Moringa spp.*) was utilized by the ancient Egyptians, Greeks and Romans; it is now widely cultivated and has become naturalized in various locations in the tropics (Fahey, 2005). This genus is the sole member of the family. It is a small deciduous tree (family Moringaceae). Moringaceae's members of are woody, often quite stout-stemmed shrubs or trees containing one genus, *Moringa*, with twelve species, native to tropical Asia, growing in Madagascar, northeast and southwest Africa and Arabia, with three species, they consider natural plant in Africa and tropical America (Britannica, 2018). *Moringa* have many various species across the globe, those are known for their valuable uses e.g., *Moringa ovalifolia, Moringa drouhardii, Moringa longituba, etc*., (Leone *et al*., 2015). Thirteen species of *Moringa* have been widely cultivated throughout Africa and Asia for their valuable uses (Abd Rani *et al*., 2018). Many studies have been carried out on this genus in order to study its biological activities, with special reference to *M. oleifera,* since it is considered to be the most vastly cultivated species of the genus *Moringa* (Mahmood *et al*., 2010; Fakayode and Ajav 2016). Besides, this plant was used in the treatment of dracunculiasis, filariasis, leishmaniasis, malaria, schistosomiasis and

trypanosomiasis (Fahey, 2005). Many researches revealed that different parts e.g., leaf, stem, seed, flower, seed and root of *Moringa oleifera* produced, by different extraction methods isothiocyanates those have long been known for their bactericidal, fungicidal, antiviral, antiparasitic, herbivore deterrent, nematicidal and allelopathic properties. A similar point of view said that: most biological activities of *Moringa* are due to their high contents of flavonoids, glucosides and glucosinolates (Wang *et al.*, 2016; Abd Rani *et al*., 2018; Fahey *et al*., 2018). *Moringa oleifera* is considered to be one of the most important magical Indian plants, due to its high medicinal importance. However, there is still a real need to understand its phytochemical compositions and variations in their extracts due to the usage of different solvents (with different polarities), there is still a real need to understand their potential properties and to establish their applications in various important fields (Bhalla *et al*., 2021).

Anthelminthic efficacy of *Moringa oleifera* was previously studied, Rastogi *et al*. (2009) studied the anthelmintic activity of various concentrations of ethanolic extracts of *Moringa oleifera* against Indian earthworm; *Pheritima posthuma*, they found that *M. oleifera* caused paralysis of worm, followed by complete death. Tayo *et al*. (2014) showed that all three extracts of *M. oleifera* leaves, extracted using ethanol and water, exhibited potential larvicidal activities against first and second stage juveniles of *Haemonchus contortus* and also adversely affected on either embryonated or fresh eggs. In Ghana, Aboagye *et al.* (2015) stated that *M. oleifera* possessed an anthelmintic efficacy in the control of parasites in *Achatina achatina* snail. They also suggested that addition of the fresh foliage of *M. oleifera* to the feed of reared edible snails may be useful in enhancing its resistance to parasitic helminthes. In Philippines, Cabardo and Portugaliza (2017) evaluated the anthelmintic conceivable of aqueous and ethanolic extracts of seeds of *Moringa oleifera* against *Haemonchus contortus* third stage juveniles and eggs; they found that all tested concentrations are able to suppress hatching of egg and kill juveniles. Recently, investigation was carried out to test the anthelmintic activity of various *Moringa* extracts against parasitic nematodes. The aqueous, ethanolic and methanolic extracts of *M. oleifera* leaf possessed anthelmintic effects against gastrointestinal nematodes; *Haemonchus, Trichuris* and *Trichostrongylus*, this inhibition was maximized by increasing concentrations. They suggested that *Moringa* extracts' can be used instead of chemical anthelmintics to treat the worm infections in animals (Rafique *et al*., 2022).

Antinematodal properties of *Moringa olifera* against phytonematodes was previously documented in some articles; Murslain *et al*. (2014) reported that water extracts of leaves of *M. oleifera* caused significant reduction in egg hatching and juvenile mortality of *Meloidogyne javanica*, they also found that the *in vivo* application of extracts reduced nematode populations and enhanced eggplant growth'. Salles *et al*. (2014) noticed that the nematicidal potential of seeds of *M. oleifera* contained different phytochemicals, especially those with low molecular weights. Youssef *et al*. (2014) evaluated the efficacy of *Moringa oleifera* aqueous extracts of dry leaves were used at 5% concentrations; they noticed a reduction in galls numbers and nematodes' reproduction, besides improving in growth criteria. *Moringa* aqueous extracts were reduced M. incognita infecting sugar beet as well as improve plant growth in pot experiment (El-Nagdi and Youssef, 2015). The toxicity of *Moringa* seeds and their protein fractions were reported by El-Ansary and Al-Saman (2018), they concluded that, the use of the first precipitate of proteins of *M. oleifera* (fraction A) in 50% saturated ammonium sulphate, possessed the maximum antinematodal effect on *M. incognita* on banana plants. El-Mesalamy (2018) documented that, leaves aqueous extracts of *M. oleifera* caused significant inhibition of hatching of eggs and also achieved mortality of m. incognita juveniles compared to control. The aqueous extracts of seeds and leaves of *M. oleifera* were added to cucumber in a field trial, results revealed that *Moringa* extracts were active against the *Meloidogyne incognita* and also seeds extracts' showed significant suppression in nematodes than leaves extracts (Olajide *et al*., 2018). Another investigation reported an inhibitory effect of different concentrations of leaf extracts of *Moringa oleifera* on egg hatching of *M. incognita,* they also concluded that, *M. olifera* can be used for the combating root knot nematodes of susceptible plants (Ladi *et al*., 2019). It was observed that, leaf and seed aqueous extract of *Moringa oleifera*, which applied to infected tomato as soil drench couple days after inoculation with *M. incognita* and repeated every week, achieving significant reduction in all nematode parameters, also increase in tomato yields (Oluwatayo *et al*., 2019). Significant

suppression of *M. incognita* populations and improvement in plant growth of eggplant after the treatment with dry leaf powder and various extracts of *M. olifera* had been observed. Also these extracts were more effective than leaf power in reducing final populations. The effect of aqueous extracts of leaf of *Moringa oleifera* in the control of *Meloidogyne spp* infecting carrot was investigated, results showed that, the concentration of 150 mg/ml possessed the lowest gall number and positively affect the carrot growth (Okechalu *et al*., 2021)

This study was performed to evaluate the nematotoxicity of different extracts (boiling water, methanolic and ethanolic extracts) of both dry and fresh plant parts of *M. oleifera* against *M. incognita* infective stage juveniles. Additionally, a survey using preliminary phytochemical screening to detect the richest extract of certain plant part amongst all investigated plant parts was performed in this study. Moreover, the richest extract belonging to the best plant part regarding their total phenolics and total flavonoidal contents was selected also.

MATERIAL AND METHODS

Collection of Samples: Different plant parts (leaf petioles, leaf blades, immature and mature seeds and fruits, stems) of *Moringa oleifera*, were obtained as a scientific gift from both Prof. Dr. Aboelfetoh Mohamed Abdalla and Ass. Prof. Dr. Mohamed Ibrahim Ezzo. Scientific Egyptian Association of *Moringa*, Agricultural and Biological Research Institute, National Research Centre, Dokki, Giza, Egypt.

Preparation of Successive Extracts: Different plant parts (leaf petioles, leaf blades, immature and mature seeds and fruits, stems) of *Moringa oleifera* tree, were cleaned, air dried, extracted by using different solvents in a descending successive extraction manner according to their polarity gradients (boiling water, followed by absolute methanol then ethanol "80%") then filtered; these extracts (1 ml of each extract is belonging to 50 mg/g Dry or Fresh Weight of each sample) will be screened phytochemically and both total phenolic and flavonoidal contents will be estimated also according to Alam (2019).

Preliminary Phytochemical Screening: Flavonoids were screened by following the method of Mabry *et al*. (1970).

Screening of Anthraquinones were done according to Farnsworth *et al.* (1969) method.

Unsaturated sterols and/or Triterpenes were screened according to a-Liebermann- Burchardt's test (Claus, 1967) and b-Salkowiskit's test (Schmidt, 1964).

Tannins were screened according to the method of Trease and Evans (1978).

Saponins were determined according to the method of Hungund and Pathak (1971) via a-Forth test and b-Blood hemolysis test.

Alkaloids and/or Nitrogenous bases were checked according to the method of Shellard (1957).

Carbohydrates and/ or Glycosides were detected according to the method of Stank *et al.* (1963).

Cardiac glycosides screening was carried out according to Balbaa *et al*. (1981). a-Killer –Kiliani test. b-Kedde's reaction. c- Libermann's reaction.

Chlorides and Sulphates were determined according to the method of Islam *et al*. (1993).

Irodoids were checked according to the method obtained by Weiffering (1966).

Sublimation: The presence and accumulation of sublimable substances in different investigated extracts were detected using the method of Afifi (1972).

Assay for total phenolics: Total phenolics were estimated in all extracts by following the method of Gursoy *et al.* (2009).

Assay for total flavonoids: Total flavonoids were determined in all extracts also using the method of Gursoy *et al.* (2009).

Source of Nematodes: Culture of root knot nematode, *Meloidogyne incognita,* was prepared to be used in the current study, this culture was reared on eggplants grown in plastic posts and maintained inside glasshouse. To prepare freshly hatched second stage juveniles' suspension; the infected plants were carefully uprooted then washed under stream of water to remove the soil particles. Egg masses were hand-picked using sterilized forceps from heavily infected roots. Collected egg masses were washed with sterile distilled water and then filtered by using small sieve having tissue paper above its net, then it is poured in Petri dishes containing water just deep enough to contact the egg masses to allow releasing of hatching juveniles to the bottom of plates passing through the sieve, the count of hatched juveniles were observed each six hours and the collected nematode suspension was adjusted to about five hundreds juveniles per 1ml then preserved in refrigerator, it will be ready to be used within few hours of preservation in the refrigerator (El-Nuby and Alam, 2020).

Determination of Nematotoxicity of different extracts: The toxicity of investigated parts' extracts was tested in terms of nematode' immobility leading to confirmed death. Five milliliters of water suspension containing ≃100 second stage juveniles (J_2S) of *M. incognita* were poured in petri dishes containing different extracts (boiling water, methanol, ethanol extracts) of various plant parts; leaf blades, leaf petioles and stems (El-Nuby and Alam, 2020). These Petri dishes were kept at 28°C, there were four replicates / treatment. The immobilized J_2S were counted after 24 hours of the exposure period by the aid of microscope (Meiji 100X). Recovery test was done by empting the dishes and replacing the extracts with distilled water, the immobile juveniles were considered dead (permanent death) if they remain immovable after touching them with a fine needle (Cayrol *et al*., 1989). The mean percentage of mortality was calculated. Mortality percentage (M%) was calculated by the ratio of the dead nematodes/number of total nematodes multiplied by 100 i.e. (M %) = [(No. of dead J_2S)/ (No. of total J_2S)] x 100. The experiment was conducted twice typically to previous protocols.

STATISTICAL ANALYSIS

Nematicidal Activity Studies: In current study experiments completely randomized design was followed. All results were undergone to analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) at 5% significance level (Duncan's, 1955) was used for comparing between treatments means. This analysis was done using statistical program of CoStat version 6.303, copyright (1998-2004), CoHort software statistical packages.

Chemical Studies:

Statistical analyses were performed using Fisher analysis of variance methodology. A least significant difference test was applied at 5 and 1% probability levels, in order to determine the differences among means of all treatment (Steel and Torrie, 1984). The CO-STAT computerized package program was undergone to the regular statistical analyses of variance (Nissen *et al.,* 1985), using 2 designs -1- Anova-1 completely randomized design (CRD) -2- Factorial implemented in completely randomized design. Each reading = mean of four replicates ± SE for all experiments.

RESULTS

Phytochemical Analyses: Results of preliminary phytochemical screening on boiling water extracts of different plant parts of *Moringa oleifera* revealed the presence of all investigated phytochemicals including; flavonoids, anthraquinones, steroids and/triterpenoids, tannins, saponins, alkaloids and/or nitrogenous bases, carbohydrates and/or glycosides, cardiac glycosides, chlorides, sulphates, iridoids and sublimable substances in most samples. Regarding extracts of fresh samples; it was found that both extracts of mature and immature seeds and fruits, in addition to stems are the richest extracts regarding all investigated phytochemicals. Meanwhile, concerning extracts of dry samples; it was noticed that both extracts of leaf blades and immature fruits are the richest extracts regarding all investigated phytochemicals, followed by stems in this regard. It was noticed also that all samples are devoid of iridoids (Table. 1 a, b).

Table 1.a. Preliminary Phytochemical Screening on boiling water extract of different plant parts of *Moringa oleifera* (Fresh samples).

Experiment	A. Fresh Samples						
	Leaf blades	Leaf Petioles	Stems	Immature Fruits	Immature Seeds	Mature Seeds	Mature Fruits
1-Flavonoids	+	+	++	++	++	++	++
2-Anthraquinones	+	+	++	++	++	++	++
3-Unsaturated sterols and/or Triterpenoids	+	+	++	++	++	++	++
4-Tannins	+	+	++	++	++	++	++
5- Saponins	++	+	+	+	+	+	+
6-Alkaloids	+	+	++	++	++	++	++
7-Carbohydrates and/or Glycosides	+	+	++	++	++	++	++
8-Cardiac Glycosides	+	+	+	+	+	+	+
9-Chlorides	+	+	++	++	++	++	++
10-Sulphates	+	+	++	++	++	++	++
11-Iridoids	-	-	-	-	-	-	-
12-Sublimable substances	+	+	++	++	++	++	++

Table 1.b. Preliminary Phytochemical Screening on boiling water extract of different plant parts of *Moringa oleifera* (Dry samples).

Experiment	B. Dry Samples				
	Leaf blades	Leaf Petioles	Stems	Immature Fruits	Mature Fruits
1-Flavonoids	+++	+	++	+++	+
2-Anthraquinones	+++	+	++	+++	+
3-Unsaturated sterols and/or Triterpenoids	+++	+	++	+++	+
4-Tannins	+++	+	++	+++	+
5- Saponins	++	++	++	++	+
6-Alkaloids	+++	+	++	+++	+
7-Carbohydrates and/or Glycosides	+++	+	++	+++	+
8-Cardiac Glycosides	+	+	+	+	+
9-Chlorides	+++	+	++	+++	+
10-Sulphates	+++	+	++	+++	+
11-Iridoids	-	-	-	-	-
12-Sublimable substances	+++	+	++	+++	+

Results of preliminary phytochemical screening on methanol extracts of different plant parts revealed the presence of all investigated phytochemicals in all samples under investigation (except extracts of both fresh and dry leaf blades, leaf petioles and stems are devoid of saponins). Concerning extracts of fresh samples; it was found that both extracts of mature and immature seeds and fruits are the richest extracts regarding all investigated phytochemicals. Meanwhile, regarding extracts of dry samples; it was noticed that both extracts of mature and immature fruits, leaf blades and leaf petioles are rich in all investigated phytochemicals, followed by stems in this regard. (Table. 2 a, b).

Preliminary phytochemical screening on ethanol extracts of different plant parts revealed that all of these extracts contained all investigated phytochemicals, but in the least amounts compared to other extracts (except extracts of both fresh and dry leaf blades, leaf petioles and stems are devoid totally of saponins). Regarding extracts of fresh samples; it was found that both extracts of mature and immature seeds and fruits are the richest extracts regarding all investigated phytochemicals. Meanwhile, regarding extracts of dry samples; it was noticed that both extracts of mature and immature fruits are rich in all investigated phytochemicals (Table. 3 a, b).

Table 2. a. Preliminary Phytochemical Screening on methanol extract of different plant parts of *Moringa oleifera* (Fresh Samples).

Experiment	A. Fresh Samples						
	Leaf blades	Leaf Petioles	Stems	Immature Fruits	Immature Seeds	Mature Seeds	Mature Fruits
1-Flavonoids	+	++	+	++	++	++	++
2-Anthraquinones	+	++	+	++	++	++	++
3-Unsaturated sterols and/or Triterpenoids	+	++	+	++	++	++	++
4-Tannins	+	++	+	++	++	++	++
5- Saponins	-	-	-	++	++	++	++
6-Alkaloids	+	++	+	++	++	++	++
7-Carbohydrates and/or Glycosides	+	+++	+	+++	+++	+++	+++
8-Cardiac Glycosides	+	++	+	++	++	++	++
9-Chlorides	+	+	+	+	+	+	+
10-Sulphates	+	++	+	++	++	++	++
11-Iridoids	+	++	+	++	++	++	++
12-Sublimable substances	+	++	+	++	++	++	++

Table 2. b. Preliminary Phytochemical Screening on methanol extract of different plant parts of *Moringa oleifera* (Dry Samples).

Experiment	B. Dry Samples				
	Leaf blades	Leaf Petioles	Stems	Immature Fruits	Mature Fruits
1-Flavonoids	++	++	+	++	++
2-Anthraquinones	++	++	+	++	++
3-Unsaturated sterols and/or Triterpenoids	++	++	+	++	++
4-Tannins	++	++	+	++	++
5- Saponins	-	-	-	++	++
6-Alkaloids	++	++	+	++	++
7-Carbohydrates and/or Glycosides	+++	+++	+	+++	+++
8-Cardiac Glycosides	+++	+++	+	+++	+++
9-Chlorides	++	++	+	++	++
10-Sulphates	++	++	+	++	++
11-Iridoids	++	++	+	++	++
12-Sublimable substances	++	++	+	++	++

Table 3. a. Preliminary Phytochemical Screening on ethanol extract of different plant parts of *Moringa oleifera* (Fresh Samples).

Experiment	A. Fresh Samples						
	Leaf blades	Leaf Petioles	Stems	Immature Fruits	Immature Seeds	Mature Seeds	Mature Fruits
1-Flavonoids	+	+	+	+	+	+	+
2-Anthraquinones	+	+	+	+	+	+	+
3-Unsaturated sterols and/or Triterpenoids	+	+	+	+	+	+	+
4-Tannins	+	+	+	+	+	+	+
5- Saponins	-	-	-	++	++	++	++
6-Alkaloids	+	+	+	+	+	+	+
7-Carbohydrates and/or Glycosides	+	+	+	+	+	+	+
8-Cardiac Glycosides	+	+	+	+	+	+	+
9-Chlorides	+	+	+	+	+	+	+
10-Sulphates	+	+	+	+	+	+	+
11-Iridoids	+	+	+	+	+	+	+
12-Sublimable substances	+	+	+	+	+	+	+

Table 3. b. Preliminary Phytochemical Screening on ethanol extract of different plant parts of *Moringa oleifera* (Dry Samples

Experiment	B. Dry Samples				
	Leaf blades	Leaf Petioles	Stems	Immature Fruits	Mature Fruits
1-Flavonoids	+	+	+	+	+
2-Anthraquinones	+	+	+	+	+
3-Unsaturated sterols and/or Triterpenoids	+	+	+	+	+
4-Tannins	+	+	+	+	+
5- Saponins	-	-	-	++	++
6-Alkaloids	+	+	+	+	+
7-Carbohydrates and/or Glycosides	+	+	+	+	+
8-Cardiac Glycosides	+	+	+	+	+
9-Chlorides	+	+	+	+	+
10-Sulphates	+	+	+	+	+
11-Iridoids	+	+	+	+	+
12-Sublimable substances	+	+	+	+	+

According to results represented in Table. 4. concerning total phenolic contents, it was found that regarding extracts of fresh samples; the highest amount was recorded to be in both leaf blades and stems (20.243±0.025 and 20.083±0.020, respectively), the least amount was recorded to be in immature fruits (5.251±0.005). In this regard; total flavonoidal contents reached its maximum in mature fruits, followed by leaf blades (5.481±0.005 and 5.326±0.006, respectively), the least amount was recorded to be in mature seeds (0.050±0.001). On the other hand, concerning total phenolic contents in case of extracts of dry samples; the highest amount was recorded to be in both leaf petioles and stems (22.000±0.023 and 21.451±0.025, respectively), the least amount was recorded to be in immature fruits (8.029±0.015). In this regard also; total flavonoidal contents reached its maximum in leaf blades (6.111±0.012), the least amount was recorded to be in immature seeds (0.093±0.001).

Table 4. a. Total phenolic and flavonoidal contents (mg/g) in different plant parts of *Moringa oleifera* (Fresh Samples).

Experiment	A. Fresh Samples						
	Leaf blades	Leaf Petioles	Stems	Immature Fruits	Immature Seeds	Mature Seeds	Mature Fruits
Total phenolic contents (mg/g)	20.243±0.025	11.463±0.010	20.083±0.020	5.251±0.005	17.412±0.015	16.444±0.014	19.524±0.016
Total flavonoid contents (mg/g)	5.326±0.006	2.733±0.002	0.051±0.001	0.950±0.002	0.089±0.015	0.050±0.001	5.481±0.005

Table 4. b. Total phenolic and flavonoidal contents (mg/g) in different plant parts of *Moringa oleifera* (Dry Samples).

Experiment	B. Dry Samples				
	Leaf blades	Leaf Petioles	Stems	Immature Fruits	Immature Seeds
Total phenolic contents (mg/g)	21.333±0.025	22.000±0.023	21.451±0.025	8.029±0.015	18.213±0.020
Total flavonoid contents (mg/g)	6.111±0.012	4.700±0.012	0.101±0.002	0.121±0.001	0.093±0.001

Nematicidal Activity of water, methanol and ethanol extracts of different parts of *M. olifera* on *M. incognita* juvenile mortality:

Aqueous, methanolic and ethanolic extracts of different fresh and dry plant parts (leaf petioles, leaf blades, immature and mature seeds and fruits, stems) of *Moringa oleifera* tree were *in vitro* tested for their nematicidal activity against *M. incognita* juveniles. Results indicated that, all tested extracts have nematotoxic effects, since they achieved mortality percentage varies between 2.7 to 100%. Boiling water extracts showed the highest killing potentials, in general, dry immature fruit extract achieved 100% mortality and also the other five parts possessed mortality more than 50%. Methanol extracts followed the water in toxicity to nematode, the maximum killing potential (76.7%) achieved by fresh immature fruit mortality also, and meanwhile the remaining parts gave mortality not reached to 35%. In this regard, ethanolic extracts of both fresh and dried parts of the plant showed the least nematicidal activity and subsequently the juveniles' mortality was ranged between 3- 26.7% compared to water and methanol extracts. Generally, water extracts were the most effective compared with control, followed by methanolic extracts, while the ethanolic extracts possessed lower toxicity to root-knot nematode juveniles. The untreated nematode juveniles (control those received distilled water only instead of extract) were mobile and live till the end of bioassay, while in treated vials the dead larvae were immobile and have straight body (Figure 1).

%M= Mortality percentage, F, D = fresh and dry samples, respectively, D. W.= distilled water

Within the same column values followed by similar letter are not significantly different according to Duncan's Multiple Range test (DMRT) at 5% significant level.

DISCUSSION

Root knot nematodes (RKNs) are a very famous group of plant parasitic nematodes, as they are found in all the globe, particularly they occurred in warm regions. RKNs represent the greatest challenge in agricultural production, as they attack over five thousands of plants and embed inside the root tissue during the life span of the plant, and secure food supplies for human being universally. Non-chemical control tactics, especially biological control of nematodes, become urgent need and most feasible substitutes for using pesticides, to reduce the pollution of the earth, improving human health and mitigate the impacts of climatic changes as well as combating desertification. Various approaches are integrally used in nematode control, this strategy call integrated nematode management (INM), but the biological control would be the most enviable choice particularly after the banned of the most effective nematicides. Recently synthetic nematicides application has declined globally, according to their wide spectra toxicity included; mammals and beneficial organisms along with the low degradation in the ecosystem. Consequently, many researches seeking some alternatives; such as soil amendment using organic matters which achieved varyied degrees of suppression of nematodes, plant allelochemicals found to be effective against root knot nematode. The focus was increased on the development of botanical-based products to manage phytonematodes as well as conserve the sustainability of natural resources. Various plants have been evaluated for their nematicidal potential. Utilizing plant extracts for suppressing nematode pests was previously investigated (El-Nuby *et al*., 2021; Alam and El-Nuby, 2019; Abdel-Rahman *et al.,* 2017).

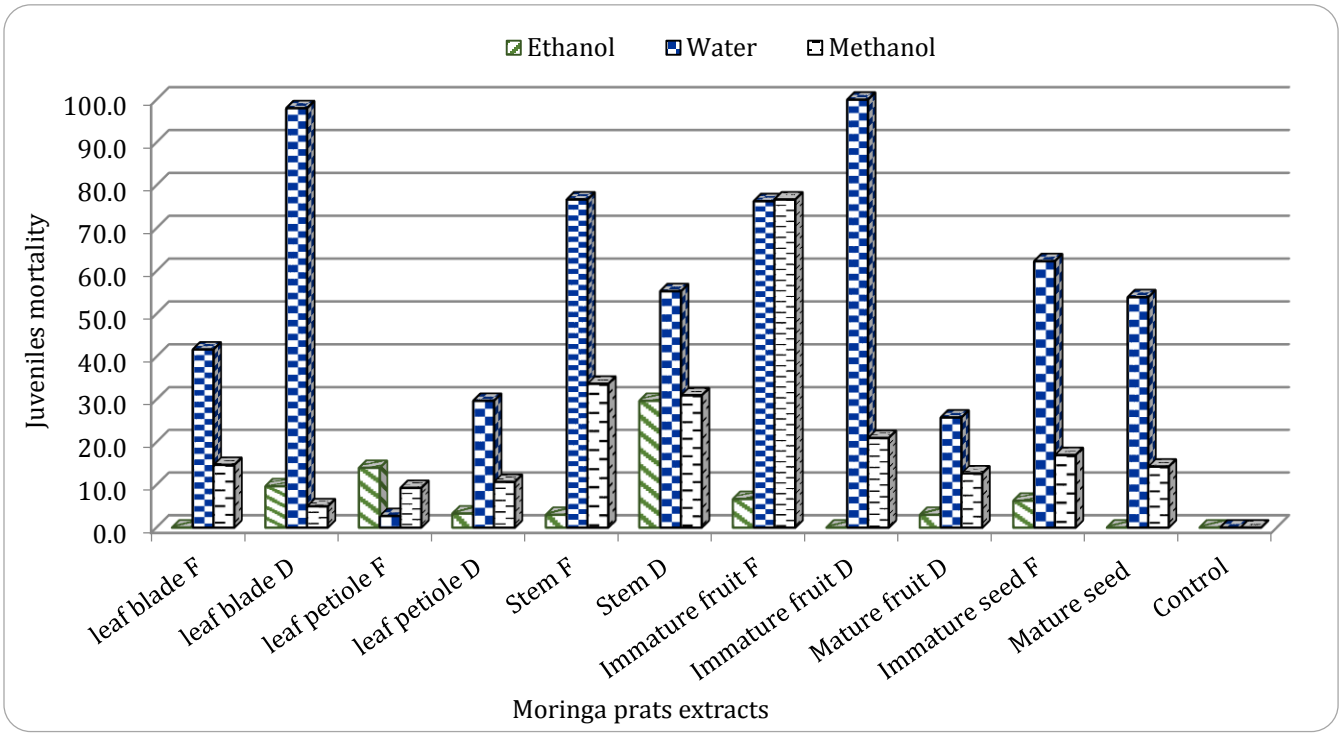

Figure 1. Effect of diffenrt extacts of different plant parts of *M .olifera* on *M. incognita* juveniles mortality. F, D = fresh and dry samples, respectively.

To preserve the various elements of the environment and mitigate the effects of climate change, this research was conducted to assess the nematicidal potential of *M. olifera* different extracts towards root knot nematode. Results showed that, extracts of various parts of *M. olifera* possessed toxicity with different degrees and the efficacy was affected by the part of the tree used and/or solvent used in extraction. The highest efficacy against nematodes was achieved by aqueous extract compared to methanol and ethanol solvents. These results in accordance with some studies; water leaves extract of *M. oleifera* caused significant reduction in egg hatching and *Meloidogyne javanica* juvenile mortality (Murslain *et al*., 2014). Also, El-Mesalamy (2018), stated that, aqueous extracts of *M. oleifera* leaves caused significant imbibition of hatching of eggs and causing mortality of *M. incognita* juveniles, the antinematodal efficacy extended to eggplant infected with *M. incognita* in greenhouse

resulted in the suppression of nematodes populations and the improvement of plant growth. Sowley *et al*. (2018) found that, the addition of *Moringa* leaf powder after one week of planting, has an extended efficacy in decreasing populations of root-knot nematodes of cowpea. It is well known that some plants have antinematodal properties, some plants have antinematodal properties either nematostatic or nematicidal action. Many of nematotoxic compounds those secreted as secondary metabolites in plants have been documented (Kim *et al.,* 2008). The inhibitory activity of varied concentrations of extracts of *M. oleifera* leaves on hatching of *M. incognita* eggs had been reported by Ladi *et al*. (2019). Recently, Khairy *et al*. (2021) detected a high suppression of root gall nematode reproduction and a clear increase in eggplant growth after the addition of leaf powder and some extracts of *M. olifera*, also they observed that drenching extracts were better than amending soil with dry leaves in the suppression of nematodes.

The antinematodal activity of *M. olifera* may be due to their phytochemical components. *M. olifera* contains high amounts of specific oxygenated compounds those are characterized by their lipotrophic attributes that facilitate them to dissolve the cytoplasmic membrane of cells of nematodes and their functional groups and to interpose with the structure of protein that constitute an enzyme (Knobloch *et al*.,1989). Also Ladi *et al*. (2019) suggested that, inhibiting egg hatching of *M. incognita* may be due to some nematicidal properties of some phytochemicals such as flavonoids, alkaloids, amides and ketones found in *M. oleifera.* Other phytochemicals like lectins are responsible for nematotoxicity of *Moringa* seed extracts (Santos *et al*., 2009). It was found that the nematicidal efficacy of seeds of *M. oleifera* is related to the presence of various compounds, in particular those with low molecular weights (Salles *et al*., 2014).

Preliminary phytochemical screening on all screened extracts of *M. olifera* revealed the presence of all investigated phytochemicals including; flavonoids, anthraquinones, steroids and/triterpenoids, tannins, saponins, alkaloids and/or nitrogenous bases, carbohydrates and/or glycosides, cardiac glycosides, chlorides, sulphates, iridoids and sublimable substances in most samples. In this concern, results of preliminary phytochemical screening on all studied extracts of all plant parts indicated that, the richest extracts regarding these studied phytochemicals are boiling water extracts of all plant parts, followed by their methanolic extracts, meanwhile ethanolic extracts contained the lowest amounts of screened phytochemicals. Also, results of estimation of both total phenolics and total flavonoidal contents of these extracts of different plant parts showed that they are rich in both phenolics and flavonoids. These results revealed a clear correlation between phytochemical compositions and nematicidal activities of these examined extracts (Ladi *et al*., 2019). These finding introduced non-traditional tools for combating nematodes from *M. olifera.* Further studies for the determination of the best extract including the most potent fraction and main compounds responsible for nematotoxicity but less toxic to plant and human are highly required.

CONCLUSION

According to the obtained results of preliminary phytochemical screening on different extracts (aqueous, methanol and ethanol extracts) of both fresh and dried plant parts (leaf petioles, leaf blades, immature and mature seeds and fruits, stems) of *M. oleifera,* it was found that, all extracts of all investigated plant parts are rich sources of all examined phytochemicals. Additionally, these extracts are rich also in their total phenolics and total flavonoidal contents. These results will encourage more studies for *in vitro* production of these secondary metabolites to improve this phytochemical productivity of such an important medicinal plant. Various *Moringa* parts' extracts showed nemaototoxic potentials towards juveniles of *M. incognita* and there is positive correlation between the phytochemical composition and the nematicidal activity of each tested extract. Results revealed the high nematotoxic efficiency of aqueous extracts of different parts of M. *oleifera* are positively correlated with their chemical compositions. The examined extracts can be an untraditional alternative for many synthetic pesticides. Advanced research should be focused on the determination of nematicidal compounds in details, also the appropriate extraction protocols, along with the detection of the targeted compound which is required for both *in vitro* and *in vivo* nematotoxicity studies, in order to detect the responsible compound on this activity. Until that, the use of *M. olifera* is highly recommended for managing nematodes as a soil addition, if possible, or as a water extract by integration with other control practices. These findings are introducing uncommon approaches for combating nematodes and

considered to be a step in the road of production of bio-nematicides. Furthermore, it is advisable to offer these materials in low costs to be affordable for smallholder farmers in desert areas. *In vivo* evaluation of *M. oleifera* singly or in combination with other tools should be carried out in the future in order to optimize its application in field.

REFRENCES

Abad, P., B Favery, M. N. Rosso and P. Castagnone-Sereno. 2003. Root-knot nematode parasitism and host response: Molecular basis of a sophisticated interaction. Molecular Plant Pathology, 4: 217-224

Abd Rani, N.Z., K. Husain and E. Kumolosasi. 2018. *Moringa* Genus: A Review of Phytochemistry and Pharmacology. Frontier in Pharmacology, 16: 108-112.

Abdel-Rahman, A.G., H.A. Hashem, H.A. Kassem and N. F. Abdel Aziz. 2017. Allelopathic activity of some desert plants against plant pathogenic bacteria and nematodes. Journal of Environmental Sciences, 37: 15-35.

Aboagye, I. F., D. Mensah and F. Boadu. 2015. Anthelmintic Effect of *Moringa oleifera* Lam. in Wild caught *Achatina achatina* Linnaeus, 1758 from the Sefwi Wiawso District, Ghana. West African Journal of Applied Ecology, 23: 27-33.

Afifi, M. 1972. Pharmacological studies on some genera of Polygonaceae and Cucurbitaceae grown in Egypt. Ph.D. Thesis, Pharmacology Department, Faculty of Pharmacy, Cairo University, Egypt, 1972: 68.

Alam, E.A. 2019. Natural Phytochemical Products and Our Life (Herbal Medicine from the Land to the Hand), Osiris Publisher, Cairo, Egypt.

Alam, E.A. and A.S.M. El-Nuby. 2019. Phytochemical and Antinematodal Screening on Water Extracts of Some Plant Wastes against *Meloidogyne incognita*. International Journal of Chemical and Pharmaceutical Sciences, 10: 1-14.

Ali T., M. Mubeen Y. Jamil U. Ahmad, H. A. Khan, A. S. Iqbal, Y. Baber, F. Hassan H. M. Usman M. A. Sohail and A. Abbas, 2021. An overview of root-knot nematodes and their management. Journal of Entomology and Zoology Studies, 9: 35-40.

Balbaa, S.I., H.H. Sayed and Y.Z. Ashgan. 1981. Medicinal plant constituent, General organization for university and school books, 3rd ed. 190-255.

Britannica. 2018. The Editors of Encyclopaedia. "*Moringa*". *Encyclopedia Britannica*, 27 Jul. 2018.

Britannica, 2020. The Editors of Encyclopaedia. "nematode". *Encyclopedia Britannica*, 19 Feb. 2020.

Cabardo, D.E. Jr. and H.P. Portugaliza. 2017. Anthelmintic activity of *Moringa oleifera* seed aqueous and ethanolic extracts against *Haemonchus contortus* eggs and third stage larvae. International Journal of Veterinary Science and Medicine, 5: 30-34.

Chitwood, D. J. 2003. Nematicides, In Plimmer, J.R. (Ed.), Encyclopedia of agrochemicals, Vol. 3, New York: John Wiley & Sons, 1104-15.

Chitwood, D.J. 2002. Phytochemical-based strategies for nematode control. Annual Review of Phytopahtology, 40: 221-249.

Claus, E.P. Pharmacognosy, Henery Krimpton, London, 5th ed.,1967: 168.

Danahap, L. S. and D. L. Wonang. 2016. Antinematicidal efficacy of root exudates of some crotalaria species on *Meloidogyne incognita* (root-knot nematode) (kofoid and white) chitwood isolated from infected *lycopersicum esculentum* (Tomato) plant. International Journal of Scientific and Technology Research, 5: 79-84.

Desmedt, W., S. Mangelinckx T. Kyndt and B. Vanholme. 2020. A Phytochemical Perspective on Plant Defense Against Nematodes. Frontier in Plant Science, 13: 602079.

Duncan's, D.B. 1955. Multiple ranged multiple F-tes. Biometrics, 11: 1- 47.

El- Mesalamy, A. F. 2018. Efficacy of some aqueous plant extracts on egg hatching and juveniles mortality of the root knot nematode, *Meloidogyne incognita* under laboratory conditions. Journal of Plant Protection and Pathology, 9: 759-761.

El-Ansary, M. S. M. and M. A. Al-Saman. 2018. Appraisal of *Moringa oleifera* crude proteins for the control of root-knot nematode, *Meloidogyne incognita* in Banana. Rendiconti Lincei. Scienze Fisiche e Naturali, 29: 631-637.

El-Nagdi, W.M.A. and M.M.A. Youssef. 2015. Nematicidal effect of some aqueous extracts of botanicals and a commercial bacterial byproduct for biocontrolling root knot nematode, *Meloidogyne incognita* infecting sugar beet. Scientia Agriculturae Bohemica, 10: 55-58.

El-Nuby, A. S. M., A. I. Afia and A. A. Alam. 2021. In vitro evaluation of the toxicity of different extracts of some marine algae against root-knot nematode (*Meloidogyne incognita*). *Pakistan Journal of*

Phytopathology, 33: 55-66.

El-Nuby, A. S. M. and E. Alam. 2020. Phytochemical and nematicidal activity studies of some extracts of different plant parts of *Leucaena leucocephala*against *Meloidogyne incognita*. International Journal of Chemical and Pharmaceutical Sciences, 11: 1–17.

El-Nuby, A. S. M., S. A. Montasser and I.A. El-Khadrawy. 2020. Control of root-knot nematodes using wild plants colonized sinai, Egypt. Pakistan Journal of Nematology, 38: 67- 80.

EPA. 2022. U.S. Environmental Protection Agency. 2022. What are Biopesticides?

Fahey, J. W., M. E. Olson, K. K. Stephenson, K. L. Wade, G. M. Chodur, D. Odee, W. Nouman, M. Massiah J. Alt ,P. A. Egner and W. C. Hubbard. 2018. The Diversity of Chemoprotective Glucosinolates in Moringaceae (*Moringa spp.*), 8: 7994-7999.

Fahey, J.W. 2005. *Moringa oleifera*: a review of the medical evidence for its nutritional, therapeutic and prophylactic properties. Trees for Life Journal, 1: 1-15.

Fakayode, O.A. and E.A. Ajav. 2016. Process optimization of mechanical oil expression from *Moringa* (*Moringa oleifera*) seeds. Industrial Crops and Products, 90:142-151.

Farnsworth, N.R., H.H. Fong, R.N. Blomster and F.G. Draus. 1969. Studies on *Vinca major* (Apocyaceae). Journal of Pharmaceutical Science, 51: 217-224.

Feigl, F. 1960. Spot tests in organic analysis. Elsevier Publishing Co, New York, 6th ed.,1960: 29-59.

Feyisa, B. 2021. A review on root knot nematodes (RKNs): Impact and Methods for Control. Journal of Plant Pathology and Microbiology, 12: 547-553.

Forghani, F. and A. Hajihassani. 2020. Recent advances in the development of environmentally benign treatments to control root-knot nematodes. Frontier in Plant Science, 11: 1125-1131.

Gursoy, N., C. Sarikurikcu, M. Cengiz and M.H. Solak. 2009. Antioxidant activities, metal contents, total phenolics and flavonoids of seven Morchella species. Food and Chemical Toxicology, 47: 2381-2388.

Hu, W., K. Kingsbury S. Mishra and P. DiGennaro. 2020. A comprehensive transcriptional profiling of pepper responses to root-knot nematode. Genes, 11: 1507-1512.

Hungund, B.L. and C.H. Pathak. 1971. USDA forest Service Research Paper: 201.

Ibrahim, K. A. and A. A. Mokbel. 2009. and distribution of the root-knot nematodes *Meloidogyne* spp. and their host range in northern egypt. Egyptian Journal of Experimental Biology, 5: 125 -129.

Islam, A.M., E.A. Hassan and I.B. Hannout. 1993. Manual of practical chemistry 1993, Dar AlMaaref, Egypt, 2nd ed.: 19-39.

Khairy, D., A. R. Refaei. and F.A.M. Mostafa. 2021. Management of *Meloidogyne incognita* infecting eggplant using moringa extracts, vermicompost, and two commercial bio-products. Egyptian Journal Agronematology, 201: 1-16.

Kim, J., S.M. S.G. Seo Lee, S.C. Shin and I.K. Park. 2008. Nematicidal activity of plant essential oils and components from coriander (*Coriandrum sativum*), oriental sweetgum (*Liquidambar orientalis*), and valerian (*Valeriana wallichii*) essential oils against pine wood nematode (*Bursaphelenchus xylophilus*). Journal of Agricultural and Food Chemistry, 56: 7316-7320.

Knobloch, K, A. Pauli, N. Iberl, N. Weigand and H.M. Weis. 1989. Antibacterial and antifungal properties of essential oil components. Journal of Essential Oil and Research, 1: 119-128.

Ladi, B. Y., A. K. Muhammad, B. Joy, N. M. Alake and S. O. Jumoke. 2019. Inhibitory effect of neem (*Azadirachta indica*) and *Moringa* (*Moringa oleifera*) leaf extracts on egg hatch of root knot nematode *Meloidogyne incognita* World Journal of Advanced Research and Reviews, 1: 28-33.

Leone, A., A. Spada, A., Battezzati, A. Schiraldi, J. Aristil, S. Bertoli. 2015. Cultivation, genetic, ethnopharmacology, phytochemistry and pharmacologyof *Moringa oleifera* leaves: an overview. International Journal of Molecular Science, 16: 12791–12835.

Mabry, T.T., K. R Markhan and M.B. Thomas. 1970. The systemic identification of flavonoids 1970. Springer, 46-54.

Mahmood, K. T., T. Mugal, and I. U. Haq. 2010. *Moringa oleifera*: a natural gift - a review. Journal of Pharmaceutical Sciences and Research, 2: 775-781.

Mesa-Valle C. M., J. A. Garrido-Cardenas, J. Cebrian-Carmona, M. Talavera and F. Manzano-Agugliaro. 2020. Global Research on Plant Nematodes. Agronomy, 10: 1148-1155.

Murslain, M., N. Javed, S. A. Khan, H. U. Khan, H. Abbas and M. Kamran. 2014. Combined Efficacy of *Moringa*

oleifera Leaves and a Fungus, *Trichoderma harzianum* Against *Meloidogyne javanica* on Eggplant Pakistan Journal of Zoology, 46: 827-832.

Nissen, O., S.P. Eisensmith, R. Freed, E.H. Everson, V. Smail, M. Weber, J. Tohme, J. Anderson, K. Rorick, G. Portice, D. Rittersdorf, P. Wolberg, B. Bricker, and T. A. Heath. 1985. Microcomputer program for the design, management and analysis research experiments. Version 4, Michigan State University and Agriculture University of Norway, USA, 4.

Bhalla, N., I. Nitin, V. Srilakshmi and D. Patri Haranath. 2020. Phytochemical analysis of *Moringa Oleifera* leaves extracts by GC-MSand free radical scavenging potency for industrial applications. Saudi Journal of Biological Sciences, 28: 6915–6928.

Okechalu, O. B., T. P. Danbong, D. L. Wonang and L. S. Danahap. 2021. The efficacy of aqueous leaf extracts of *Moringa* and Bitter leaf in the control of Meloidogyne spp. Infecting Carrot. *Nordic Journal of Botany***,** 34: 215-227.

Olajide, M.C., N.B. Izuogu, R.A. Apalowo, H.S. Baba. 2018. Evaluation of the nematicidal and antifungal activity of aqueous extracts of *Moringa olifera* leaves and seed in cucumber field. Cercetări Agronomice în Moldova 4: 47-59.

Oluwatayo, J. I., C. I. Jidere and A. Nwankiti. 2019. Nematicidal Effect of Some Botanical Extracts for the Management of *Meloidogyne incognita* and on Growth of Tomato Asian Journal of Agricultural and Horticultural Research, 4: 1-8.

Purena, R., R. Seth, R. Bhatt. 2018. Protective role of *Emblica officinalis* hydroethanolic leaf extract in cisplatin induced nephrotoxicity in Rats. Toxocology Reports, 5: 270–277.

Rafique, A., M. S. Mahmood, R. Z. Abbas, S. Ali, A. Mian, and A. Rizwan. 2022. Anthelmintic activity of *Moringa oleifera* and *Azadirachta indica* against gastrointestinal nematodes of wild sheep. Journal of the Hellenic Veterinary Medical Society, 73: 3989-3996.

Rastogi, T., V. Bhutda, K. Moon, P. B. Aswar and S. S. Khadabadi. 2009. Comparative Studies on Anthelmintic Activity of *Moringa Oleifera* and Vitex Negundo. Asian Journal of Research in Chemistry, 2: 181-182

Salles, H.O., A.C.L. Braga, M.T.C. Nascimento, A.M.P. Sousa, A. R. Lima, L.S. Vieira, A.C.R. Cavalcante, A.S. Egito and L.B.S. Andrade. 2014. Lectin, hemolysin and protease inhibitors in seed fractions with ovicidal activity against *Haemonchus contortus*. Revista Brasileira de ParasitologiaVeterinária, 23: 136-143.

Santos, A.F.S., L. A. Luz A.C.C. Argolo, J.A. Teixeira, P.M.G. Paiva, L.C.B.B. Coelho, 2009. Isolation of a seed coagulant *Moringa oleifera* lectin. Process Biochemistry, 44: 504-508.

Schmidt, J. 1964. Textbook of Organic Chemistry 1964. Olivar and Poyed ed., London: 673.

Shaukat, S. S. and I. A. Siddiqui. 2001. Effect of some weed extracts on mortality and egg hatching of *Meloidogyne javanica* (Treub) Chitwood. Pakistan Journal of Biological Sciences, 4: 1251-1252.

Shellard, E.J. 1957. Practical plant chemistry. Pitman 1957, Medicinal publishing Co., LTD, London, 53-54.

Sowley, E. N. K., F. Kankam and H. Dasmana. 2018. Effect of time of application of *Moringa oleifera* L. leaf powder on root-knot nematode (*Meloidogyne* spp.) infecting cowpea (*Vigna unguiculata* L. walp). Advances in Agricultural Science, 6: 32-41.

Stank, J., M. Cerny, J. Kocoursk and J. Pacok. 1963. The monosaccharides 1963, Publishing House of the Czechosolovak, Academy of Sciences, Prague: 22-100.

Steel, R.G.D. and J.H. Torrie. 1984. Principles and procedures of statistics, Mc Graw Hill Book Co. Inc, New York, USA, 2nd ed.

Tayo, G.M., J.W. Pone, M. C. Komtangi, J. Yondo, M.N. Alidou and M. Mbida. 2014. Anthelminthic activity of *Moringa oleifera* leaf extracts evaluated in vitro on four developmental stages of *Haemonchus contortus* from goats. American Journal of Plant Sciences, 4:1702-10.

Trease, G.T. and W.C. Evans. 1978. Text book of Pharmacognsy 1978, Bailliere Tindall and Cox, London, 11th Ed, 536.

Vargas-Ayala R., R. Rodriguez-Kabana, G. Morgan-Jones, J. A. McInory and J. W. Kloepper. 2000. Shifts in soil micro flora induced by velvet bean (*Mucuna deeringiana*) in cropping systems to control root-knot nematodes. Biological Control, 17: 11-22.

Wang L., X. Chen and A. Wu. 2016. Mini Review on Antimicrobial Activity and Bioactive Compounds of *Moringa oleifera*. Medicinal Chemistry, 6: 578-582.

Weiffering, J.H. 1966. Aucubin artigeglucoside und verwandte heteroside als systematische merkmale. Phytochemistry, 5: 1053-1059.

Youssef, M. M. A.; W. M. A. El-Nagdi and M.F.M. Eissa. 2014. Population Density of Root Knot Nematode, *Meloidogyne incognita* Infecting Date Palm under Stress of Aqueous Extracts of Some Botanicals and a Commercial Bacterial Byproduct. Middle East Journal of Applied Sciences, 4: 802-805.

Contribution of Authors:		
Eman A. A. H. Alam	:	All preparations of extracts and all phytochemical examinations of these prepared extracts, participation in the writing and editing processes before and after the submission of the manuscript till publication as a corresponding author, general supervision on the study
Ahmed S. M. El-Nuby	:	*In vitro* nematicidal studies of the prepared extracts, participation in the writing process