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Phytochemical and Nematicidal Activity Studies of Some Extracts of Different Plant Parts of *Leucaena leucocephala* against *Meloidogyne incognita*

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ABSTRACT

Water, methanolic and ethanolic extracts of both fresh and dried parts (leaf blades, leaf petioles, stems, fruits, seeds and flowers) of *Leucaena leucocephala* (English name: white lead tree) were tested for their *in vitro* antinematodal activity against juveniles of *Meloidogyne incognita*, also phytochemical screening was carried out on these extracts, additionally total phenolics and total flavonoids were estimated in all studied parts of this plant. Results revealed that, all tested extracts possessed nematotoxic effects as they achieved mortality percentage varied between 28.54 to 100%. Water extracts of all tested samples showed 100% mortality except dried samples of both stem (69.00 %) and mature fruit (38.7 %). Methanol extracts of fresh samples of leaf blades, leaf petioles, stems, immature fruits, immature seeds and flowers showed 100% mortality; meanwhile the remaining parts gave only above than 50% mortality. In this regard, ethanol extracts of fresh and dried parts of the plant showed the least antinematodal activity (compared to both water and methanol extracts) except dried samples of both immature fruit (62.30 %) and flower (96.80 %). Preliminary phytochemical screening on all studied extracts of all plant parts revealed the presence of carbohydrates and/or glycosides, tannins, flavonoids, anthraquinones, steroids and/triterpenoids, sublimable substances, saponins, alkaloids and/or nitrogenous bases, cardiac glycosides, coumarins, chlorides, sulphates and iridoids in all samples. In this regard, results of preliminary phytochemical screening on all studied extracts of all plant parts showed that, the richest extracts in these studied phytochemicals are belonging to water extracts of all plant parts, followed by methanolic extracts of all plant parts, meanwhile ethanolic extracts of all plant parts were found to contain the least amounts of phytochemicals under investigation, respectively. Phytochemical content, total phenolics and total flavonoids are positively correlated with antinematodal activity.

Keywords: *Leucaena leucocephala,* Plant extracts, *Meloidogyne incognita***,** Total phenolics, Total flavonoids, Preliminary phytochemical screening, Nematotoxicity.

1. INTRODUCTION

Plant feeding nematodes are one of the most destructive pathogens that threat agriculture production all over the globe individually or combined with other soil pathogenic microorganisms causing tremendous losses. Approximate yield losses due to plant parasitic nematodes have been estimated to be $ 100 billion worldwide yearly. The root-knot nematodes-RKN- (*Meloidogyne spp*.) represent the most polyphagous genus of phytoparasitic nematodes, over 100 species were recognized

under this genus and the major species are *M. arenaria, M. hapla, M. incognita* and *M. javanica*. They are considered the most important group of plant-attacking nematodes worldwide invading nearly every crop and affecting quantity and quality of the crop production (Karajeh, 2015; Elling, 2013; Jones *et al*., 2013; Pavaraj *et al.,* 2012; Ibrahim *et al.,* 2010 and Adekunle and A. Akinlua, 2007). *Meloidogyne spp.* being the most common and widespread group of Root Knot Nematodes in the world, increase the severity of soil borne diseases such as *Fusarium* wilt in watermelon. Plant growth impairment caused by *Meloidogyne spp.* to vegetable crops is influenced by nematode species and physiological race as well as the initial nematode population density in the soil at sowing or transplanting. The impact of the *Meloidogyne* species become huge by their wide host ranges of more than five thousands plant species (Aissani, 2013 and Trudgill and Blok, 2001).

Synthetic chemicals are the main control method for controlling plant parasitic nematodes (PPN) since more than forty years ago, as they are considered the easiest tool but it is not a promising tool because of their hazardous effects in ecosystem, expensive cost, a few nematicides are available and are economically viable only for high value crops, rather than many effective compounds were banned or restricted in many countries, in addition, the economic cost of research and registration of new chemicals are big obstacles for prospective new chemical nematicides (Fosu-Nyarko and Jones, 2015 and Tsay *et al.,* 2004).

In this concern, biological control of plant pathogens becomes highly significant in agricultural field in recent decades. There is a great stimulant to discover biologically active natural products from higher plants to act as bio-pesticides, which are better than synthetic agrochemicals and are much safer from a health and environmental point-of view. searching for new compounds of plant origin, low cost and eco-friendly, are future and urgent need to alternate or complementing the synthetic harmful and expensive chemicals in combating nematode pathogens at present (Danahap and Wonang 2016 and Adekunle and Aderogba, 2008). Biocidal compounds, naturally occurring as products of plant secondary metabolism, may represent a large source of biocompatible nematicides have been tested for their nematicidal activities for managing nematode pests were used before this study. Plants produce a wide diversity of secondary metabolites (e.g., phenylpropanoids, flavonoids, terpenoids, alkaloids and others) and many of these compounds play a crucial role in the interaction of plants with their environment. It is important to note that several phenolic compounds have been found to be toxic for plant-parasitic nematodes, and they are frequently associated with plant resistance to different pathogens. In this regard, the *in vitro* nematicidal activity of extracts of 20 native wild plants was tested against second-stage juveniles (J2) of *N. aberrans*, and in addition, the total phenolic and flavonoid contents were determined (Alam and El-Nuby, 2019; Abdel-Rahman, *et al.,* 2017; D'Addabbo, *et al.,* 2017; Laquale , *et al.,* 2016; Akyazi, 2014; Aydinli and Mennan, 2014; Pavaraj *et al.,* 2012 and Abdel-Rahman and Saleh, 2006).

The family Leguminoase is of considerable agricultural utility and agronomic potential however its biological activities remain unexplored. The ethanolic extracts of seeds of selected legumes (*Cicer arietinum, Vigna unguiculata, Phaseolus mungo, Vigna radiate* and *Lens culinaris*) were screened for their antifungal activity and their activity against the root-knot nematode *Meloidogyne* species (Shakeel *et al.,* 2010).

Phenolic compounds such as; terpenoids, flavonoids, tannins, coumarins and steroidal alkaloids from many leguminous plants, can also play a relevant role in the formulation of innovative nematicidal products. Using green manures or crop rotations with indigenous (*Medicago spp., Trifolium spp., Vicia spp., Lupinus albus*) and tropical plants of Leguminosae (*Mucuna spp., Crotalaria spp.*) species, as well as soil amendments with their formulated plant biomass, have been reported to achieve a satisfactory nematode suppression, due to the high content of bioactive metabolites (phenols, alkaloids, alcohols and mainly saponins) of these plants. Essential oils from a wide range of aromatic and medicinal plants and their volatile bioactive components (terpenes, aldheydes, ketones and more) have been largely acknowledged for a high nematicidal activity, both in vitro studies and as soil treatments in water solution or by fumigation.(Vázquez-Sánchez *et al.,* 2018 and Laquale *et al.,* 2016).

Leucaena, white lead tree, is a genus of about twenty four species of leguminous trees and shrubs belongs to the family Mimosaceae (Mabberley, 1997). *Leucaena leucocephala* (Lam.) de Wit (Fabaceae) is a small tropical tree and one of the fastest-growing leguminous trees in drought-prone and semi-arid areas (Sethi and Kulkani, 1995 and Adekunle and Aderogba, 2008). The shrub which is freely available in Ibadan, South-west Nigeria is native to Mexico and Central America (Hill, 1971), but now widely distributed throughout the tropics. Different Uses *of Leucaena leucocephala* have been reported; it is planted as a

shade tree for coffee, cacao and other cash crops; for soil fertility improvement; erosion control; site preparation in reforestation and used for a variety of other purposes including timber and fuel wood (Rushkin, 1984 and Whitesell, 1974). The protein-rich leaves and legumes are widely used as fodder for cattle, water buffalo, and goats (Sethi and Kulkani, 1995). The leaves and seeds are used as human food in Central America, Indonesia, and Thailand but are not recommended for extensive human consumption because of the mimosine toxic component (Rushkin, 1984). Also it is used for a variety of purposes in agriculture, land management and homeopathic medicine, also it have been reported that various parts of it have various medicinal properties (Hassan *et al.,* 2014 and Adekunle and Aderogba, 2008).

Anthelminthic activity of *L. leucocephala* was previously reported, in Senegal, the seed is also reported to be useful in expelling *Ascaris* species worms (Adebowale, 1993). Farmers in Nigeria commonly use fresh seeds of *Leucaena leucocephala* to deworm their animals. A paste made by pounding 50 to 100 seeds is administered orally, with 200 ml of water (Ademola and Idowu, 2006). Ademola, *et al.,* (2005) found that, fraction D (contains polar polyphenols) from *L. leucocephala* seeds were significantly more active than all the other fractions. Fraction D contains polar polyphenols, thus it can use aqueous extract as anthelmintic therapy. Ademola and Idowu, (2005) reported the anthelmintic effect of an aqueous extract of *L. leucocephala*. Aqueous extraction of the pounded fresh seeds carries a certain risk of mimosine toxicity. Mimosine is well-known toxic non-protein amino acid that is found in the seeds of *L. leucocephala*, is slightly soluble in water (The Merck Index, 2001). Mimosine, due to polar fragments in its chemical structure, will also exhibit a degree of solubility in water. It is reported to be slightly soluble in water and much less soluble in methanol and ethanol (The Merck Index, 2001). Recently, Rivero-Perez *et al.*, (2019) stated that *L. leucocephala* pod hydroalcoholic extract was inhibited the eggs hatching of gastrointestinal nematodes in sheep as it inhibited egg hatching.

Antinematodal activity against phytoparasitic nematodes also documented before this investigation; crude water extracts of roots and leaves of *Leucaena leucocephala* have been found to be suppressor to egg forming and toxic to juveniles of *Meloidogyne incognita* in okra plants leading to reduce nematode reproduction (Adekunle and Akinlua, 2007). The flavonoid compound, Quercetin, was isolated and characterized from extract of leaves, using different organic solvents of *L. leucocephala* showed high toxicity to eggs and juveniles of *M. incognita in vitro* (Adekunle and Aderogba, 2008). Crude extracts of roots and leaves also, reduced populations of *M. incognita*, *Pratylenchus* spp., *Paratylenchus* spp. and *Hoplolaimus spp.* were recorded when okra varieties were planted in alleys of *L. leucocephala* (Adekunle, 2008).

Depending on the abundance and distribution of *Leucaena leucocephala* in many regions of Egypt, this study is aimed to evaluate the nematicidal activity of water, methanolic and ethanolic extracts of different parts of *L. leucocephala* against *Meloidogyne incognita,* in addition to investigate preliminary phytochemical screening of these extracts, besides, total phenolics and total flavonoids of all parts of *L. leucocephala* parts.

2. MATERIAL AND METHODS

2.1. Sample collection

Different parts (leaf blades, leaf petioles, stems, fruits, seeds and flowers) of *Leucaena leucocephala* tree, freely grown, were collected from Desert Research Center (DRC), Cairo, Egypt. *L. leucocephala* was identified by aid of Taxonomy unit, Plant Ecology and Ranges Department and a voucher herbarium specimen was deposited in DRC herbarium.

2. 2. Preparation of different extracts

Plant materials (leaf blades, leaf petioles, stems, fruits, seeds and flowers) were cleaned, air dried, extracted following descending successive extraction methods (using hot water followed by methanol then ethanol) then filtered, these extracts (1 ml of each extract = 50 mg/Dry Weight or Fresh Weight) were studied for their nematicidal activity and preliminary phytochemical screening were carried out on these extracts also (Alam, 2019).

2.3. Nematicidal activity of *L. leucocephala* extracts

To assay the antinematodal effect of water, methanolic and ethanolic extracts of different parts of *L. leucocephala* on mortality of root knot nematode larvae*,* pure cultures of *Meloidogyne incognita* were maintained on tomato (*Lycopersicon esculentum* Mill.) roots in pots kept in the greenhouse of Plant Protection Department (Desert Research Center, Cairo, Egypt). Second-stage larvae (L_2S) were obtained from hatched eggs by incubating handpicked egg masses in sterile distilled water at 28°C. Then 2 ml of each extract was poured in a 5 cm diameterZA-Petri dishes and about 100 freshly hatched L_2S of *M. incognita* were placed in each petri plate. Juveniles kept in sterile distilled water served as check

(control). All dishes were incubated at 27°C in low temperature incubator in dark, after 24 hours of exposure dead and alive nematodes in each plate were counted by the aid of stereo microscope (Meiji 40X) also microscope (150X) was used for detailed examination of static or immobile L_2S. The mortality percentage of nematodes larvae was calculated according to this formula; Mortality percentage (M %) = [(No. of killed larvae)/(No. of total larvae)] x 100. The larvae were considered dead if they remain static or immobile (paralyzed) after probing them with a fine needle (Cayrol *et al.,* 1989; Abbasi *et al.,* 2008 and Akyazi, 2014). The recovery test was done by transferring larvae to distillated water then their mobility was checked after 24 hrs, the immobile larvae were considered dead (permanent death). Each treatment was replicated four times and the experiment was repeated twice as described above without any modification.

2.4. Preliminary Phytochemical Screening

2.4.1. Carbohydrates and/ or Glycosides

The ethanolic extract (5ml) was mixed with 0.5 ml of ethanolic α- naphthol reagent, then 1ml of sulphuric acid was carefully poured on the walls of the test tube. A violet ring was formed at the interface indicating the presence of carbohydrates and/or glycosides (Stank *et al.,* 1963).

2.4.2. Saponins

Saponins were determined according to the methods adopted by Hungund and Pathak, (1971).

a-Forth test

About 3 grams of the dried sample were extracted with boiling water then filtered. After cooling, the aliquot was shacked vigorously until forth was obtained, then allowed to stand for 15-20 minutes and classified according to their saponin contents (No forth means negative, forth less than 1Cm height = weakly positive and forth 1-2 Cm or higher means positive).

b-Blood hemolysis test

About 5 grams of the dried sample were extracted with hot ethanol (95%). One ml aliquot portion was added to 10 ml of 1:4 suspensions of erythrocytes in physiological saline solution and hemolysis was observed indicating the presence of saponins.

2.4.3. Tannins

*A*bout 5 grams of the dried sample were extracted with ethanol (50%) and filtered. The addition of ferric chloride reagent to the filtrate gave a green color then changed to a bluish black color or precipitate indicates the presence of tannins (Trease and Evans, 1978).

2.4.4. Unsaturated sterols and/or Triterpenes

The alcoholic extract (corresponding to 2 grams of the dried sample) was evaporated. The residue was treated with anhydrous chloroform (10 ml) and filtered; the filtrate was divided into two portions and subjected to the following reactions:

a-Liebermann- Burchardt's test

To the first portion, 1 ml of acetic anhydride was added, followed by 2 ml of H_2SO_4 down on the wall of the test tube. If a reddish - violet ring was produced at the junction of two layers, then the solution become bluish- green in color in the acetic acid layer it indicates the presence of unsaturated sterols and / or triterpenes (Claus, 1967).

b-Salkowiskit's test

To the second portion, an equal volume of sulphuric acid was added; if a red color was produced it indicates the presence of unsaturated sterols and/or triterpenes (Schmidt, 1964).

2.4.5. Alkaloids and/or Nitrogenous bases

About 10 grams of the dried sample were extracted with 100 ml of dilute hydrochloric acid. The acidic extract was filtered, adjusted to be alkaline with ammonium hydroxide solution and extracted with chloroform. The chloroformic extract was evaporated to dryness and the residue was dissolved in about 2 ml of hydrochloric acid. The acidic solution gave faint brown precipitate with Wagner's reagent {1.3 grams of Iodine, 2 grams of Potassium Iodide, dissolved in 100 ml dist. water} and very slight yellow precipitate with Mayer's reagent {1.36 grams of Mercuric chloride, 5 grams of Potassium Iodide, dissolved in 100 ml dist. water }(Shellard, 1957).

2.4.6. Cardiac glycosides

About 2 grams of the dried sample were boiled with 15 ml of 70 % methyl alcohol for five minutes and filtered. The filtrate was diluted with distilled water and 0.5 ml of concentrated solution of lead acetate was added (to remove chlorophyll and other pigments) and filtered, to remove the excess of lead acetate, H_2SO_4 (10%) was added drop wise until no further precipitate was formed, then filtered. The filtrate was extracted with 10 ml chloroform. The chloroform extract was evaporated to dryness and the following tests were carried out according to Balbaa *et al*., (1981).

a-Killer –Kiliani test

About 1ml of ferric chloride solution (3.5 %) in glacial acetic acid was added to one portion of chloroform residue and left, concentrated sulfuric acid was added carefully down the wall of the test tube. On standing, a brown or red layer appeared at the interface (due to the aglycone) and the upper acetic acid layer becomes blue to green (due to desoxy sugar).

b-Kedde's reaction

To another portion of the chloroform residue, 3.5-dinitrobenzoic acid (2%) in 90% methanol and one drop of NaOH (2%) were added. The solution acquired a violet color on standing.

c- Libermann's reaction

The third portion of the chloroform residue was dissolved in glacial acetic acid, then acetic anhydride (2 ml) was added. Concentrated H_2SO_4 was added carefully down the wall of the test tube. On standing, two layers were afforded, pink color (upper layer) and green color (lower layer).

2.4.7. Flavonoids

About 5 grams of the dried sample were soaked for one day with 150 ml of 1% HCl and filtered. The filtrate was tested for flavonoid compounds as follows:

About 10 ml of the filtrate were adjusted to be alkaline with sodium hydroxide. The formation of a yellow color indicates the presence of flavonoids. About 5 ml of the filtrate were mixed with 5ml HCl and small pieces of magnesium metal (0.5 g). The formation of red color after 3 minutes indicates the presence of flavonoids (Mabry *et al*., 1970).

2.4.8. Anthraquinones

About 2 grams of the dried sample were boiled for few minutes with 0.5 N KOH (10 ml) to which 1ml of diluted H_2O_2 was added. After cooling, the mixture was filtered and acidified, then extracted with benzene (10 ml). The benzene extract was shacked with NH_4OH (5ml). The presence of anthraquinones was indicated by the formation of red color in the alkaline layer (Farnsworth *et al.,* 1969).

2.4.9. Coumarins

A small amount (5 g) of the moistened dried sample was placed in a test tube that covered with a filter paper moistened with diluted NaOH (0.1 N) solution. The tube was then removed and examined under U.V. light and any fluorescence is indicated for the presence of coumarins (Feigl, 1960).

2.4.10. Irodoids:

About 2 grams of fresh samples were cut into small pieces and placed in a test tube with 5 ml of 1% aqueous HCl. After 3-6 hours, 0.1 ml of the macerate was decanted into another tube containing 1 ml of the Trim and Hill reagent (10 ml acetic acid, 1 ml 0.2 % Cu SO_4 in water and 0.5 ml conc. HCl). When the tube is heated for a short time on a flame, a blue color is produced if a certain irodoid is present (Weiffering, 1966).

2.4.11. Chlorides and Sulphates

Chlorides and Sulphates were determined according to the methods adopted by Islam *et al*., (1993).

a-Chlorides

Silver nitrate solution gives with a solution containing chlorides a white flocculent precipitate of silver chloride which dissolves in ammonium hydroxide solution and does not dissolve in dilute nitric acid. Note: The color of the precipitate changes gradually in direct sunlight to violet.

b-Sulphates

Barium chloride solution gives a white precipitate of barium sulphate which does not dissolve in mineral acids.

2.4.12. Sublimation

One gram of each sample was carefully subjected to microsublimation in dry crucible, covered with a clean slide. Dark yellowish-brown fumes were evolved and condensed on the lower surface of a slide as a dark brown oily condensate which dissolved in potassium hydroxide solution producing red color indicating the presence of anthraquinons (Afifi, 1972).

2.5. Assay for total phenolics

Total phenolics were estimated using the method of Gursoy *et al.,* (2009) involving Folin–Ciocalteu reagent and Gallic acid as standard. 1 ml of ethanol extract (contains 50 mg Dry Weight) was added to 1 ml Folin–Ciocalteu reagent in a volumetric flask then 45 ml distilled water was added. The flask was shaken vigorously. After 3 minutes, a 3 ml of Na_2CO_3 (2%) solution was added and the mixture was allowed to stand for 2 hours by intermittent shaking. Each sample was done in triplicate. Absorbance was measured at 760 nm (by using UV 2401 Pc, UV-VIS recording spectrophotometer, Shimazu, Germany). Concentrations of phenolic compounds were calculated according to the following equation

that was obtained from the standard Gallic acid graph. The calibration curve of reference standard (Gallic acid) was made using four different concentrations.

Absorbance = 0.0167 Gallic acid (µg) + 0.017 (R^2 = 0.997).

2.6. Assay for total flavonoids

Total flavonoids were estimated using the method of Gursoy *et al.,* (2009) as following: 1ml of methanol extract of different plant materials (contains 66.7 mg F.W.) was added to the same volume of 2% $AlCl_3$ in methanol extract. Each sample was done in triplicate. Absorbance was measured at 415 nm (by using UV 2401 Pc, UV-VIS recording spectrophotometer, Shimazu, Germany) after 10 minutes against blank sample containing 1 ml extract with 1 ml methanol without $AlCl_3$. Concentrations of flavonoid contents were calculated according to the following equation that was obtained from the standard Quercetin graph.

Absorbance = 0.0228 Quercetin (µg) – 0.0045 (R2 = 0.918).

2.7. Statistical analysis

2.7.1. Nematicidal Activity Studies

A completely randomized design was used; the data obtained were analyzed by means of an analysis of variance (ANOVA). The differences between means were tested using Duncan's Multiple ranged test at the 5% significance level or confidence level of 95%, (Duncan's, 1955) using CoStat version 6.303 (CoHort Software)statistical packages.

2.7.2. Chemical Studies

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

3. RESULTS

3.1. Nematicidal Activity Studies of water, methanol and ethanol extracts of different parts of *L. leucocephala* on *M. incognita* larval mortality

Water, methanolic and ethanolic extracts of both fresh and dried parts (leaf blades, leaf petioles, stems, fruits, seeds and flowers) of *Leucaena leucocephala* (English names: white lead tree) were tested for their *in vitro* antinematodal activity against juveniles of *Meloidogyne incognita*. Results in Table: 1 indicated that, all tested extracts possessed nematotoxic effects as they achieved mortality percentage varies between 28, 54.00 to 100%. Water extracts of all tested samples showed 100% mortality except dried samples of both stem (69.00 %) and mature fruit (38.70 %). Methanol extracts of fresh samples of leaf blade, leaf petiole, stem, immature fruit, immature seed and flower showed 100% mortality also; meanwhile the remaining parts gave only above than 50% mortality. In this regard, ethanol extracts of fresh and dried parts of the plant showed the least antinematodal activity (compared to both water and methanol extracts) except dried samples of both immature fruit (62.30 %) and flower (96.80 %). In general water extract was the most effective compared with control followed by methanolic extracts, while the ethanolic extracts were less toxic to examined nematodes. All juveniles in control treatment (distilled water) still mobile and live till the experiment ended, in treated plates the dead larvae were immobile and have straight body (Fig. 1).

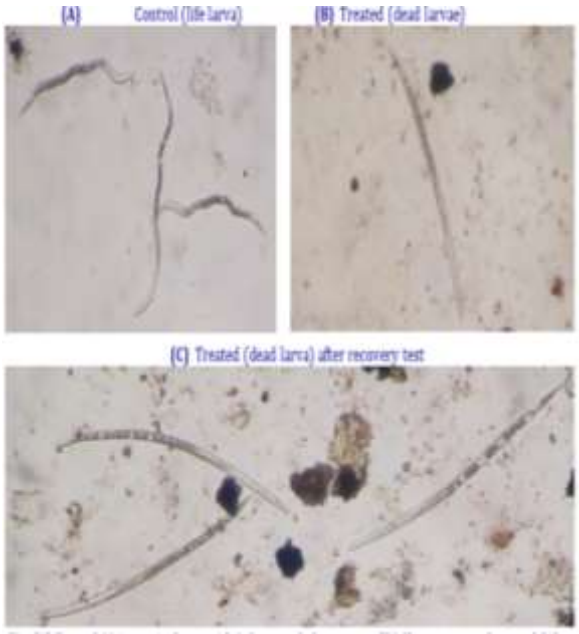

Figure -1: Treated *M. incognita* larvae with *L. leucocephala* extracts (B&C) vs. untreated control (A).

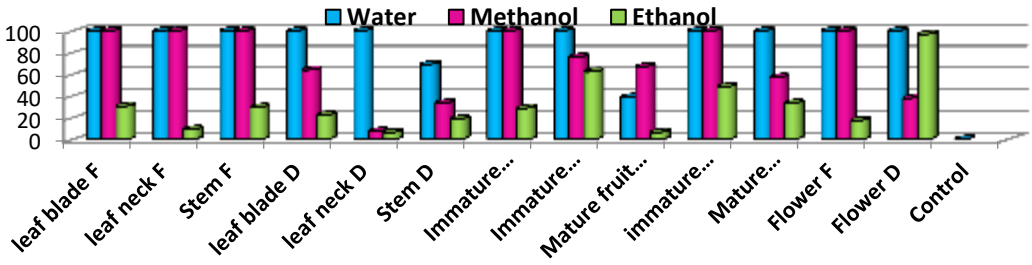

Fig. (2) Effect of diffenrt extacts of *L.* leucocephala on mortality of M. incognita larva
F= fresh samples, D= dried samples (except mature fruits and mature seeds –naturally dry).

Table (1) Effect of water, methanol and ethanol extracts of different parts of *L. leucocephala* on *M. incognita* larval mortality.

Parts used	Water extracts		Methanolic extracts		Ethanolic extracts	
	Dead larva	M%	Dead larva	M%	Dead larva	M%
Leaf blades F	100.0 a	100	100.0 a	100	31.0 e	30.4
Leaf blades D	100.0 a	100	64.0 d	64.0	22.3 g	22.2
Leaf petioles F	100.0 a	100	100.0 a	100	9.0 i	9.0
Leaf petioles D	100.0 a	100	7.3 h	7.3	6.3 j	6.2
Stems F	100.0 a	100	100.0 a	100	29.3 f	29.3
Stems D	69.0 b	69	33.3 g	33.3	18.7 h	18.7
Immature fruit F	100.0 a	100	100.0 a	100	28.7 f	28.6
Immature fruits D	100.0 a	100	75.7 b	75.7	62.3	62.3
Mature fruits D	38.7 c	38.7	67.0 c	67.0	6.3 j	6.3
Immature seeds F	100.0 a	100	100.0 a	100	48.3 c	48.3
Mature seeds D	100.0 a	100	57.0 e	57.0	33.3 d	33.3
Flowers F	100.0 a	100	100.0 a	100	17.3 h	17.3
Flowers D	100.0 a	100	38.0 f	38.0	96.7	96.8
Control (H_2O)	0 d	-	0 i	-	0 k	-

% M= Mortality percentage, F= fresh samples, D= dried samples.

Within the same column values followed by similar letter are not significantly different according to DMRT at 5% significant level.

3.2. Chemical Investigation of Different Parts of *L. leucocephala:*

3.2.1. Preliminary Phytochemical Screening on Water, Methanol and Ethanol Extracts of Different Parts of *L. leucocephala:*

Preliminary phytochemical screening on all studied extracts of all plant parts revealed the presence of carbohydrates and/or glycosides, tannins, flavonoids, anthraquinones, steroids and/triterpenoids, sublimable substances, saponins, alkaloids and/or nitrogenous bases, cardiac glycosides, coumains, chlorides, sulphates and iridoids in all samples. In this regard, results of preliminary phytochemical screening on all studied extracts of all plant parts showed that, the richest extracts in these studied phytochemicals are belonging to water extracts of all plant parts, followed by methanolic extracts of all plant parts, meanwhile ethanolic extracts of all plant parts were found to contain the least amounts of phytochemicals under investigation (Tables: 2-4).

3.2.2.Total Phenolics of Different Plant Parts of *L. leucocephala*:

Estimation of total phenolic contents in all plant parts under investigation (Table: 5) showed that, all investigated samples of different plant parts are rich in phenolics with special reference to both fresh and dried samples of seeds (25.571±0.003 and 28.438±0.003 respectively).

3.2.3. Total Flavonoids of Different Plant Parts of *L. leucocephala*:

Estimation of total flavonoids contents in all plant parts under investigation (Table: 6) showed that, all investigated samples of different plant parts are rich in flavonoids with special reference to both fresh and dried samples of stem (5.947±0.001 and 5.940±0.001, successively), leaf blade (5.865±0.001 and 5.829±0.001, consequently), seeds of the plant (5.894±0.001 and 5.904±0.001, respectively), flowers (5.899±0.001 and 5.904±0.001, respectively) and leaf petiole (5.686±0.001 and 5.978±0.001, consequently).

Results of phytochemical screening, total phenolics and total flavonoids are in strong positive relationship with those of in vitro antinematodal activity against juveniles of *Meloidogyne incognita.*

Table. 2: Preliminary phytochemical screening of water extracts of different fresh and dried plant parts of *L. leucocephala.*

Water extracts (Fresh samples)							
Experiment	Leaf blades	Leaf petioles	Stems	Immature fruits	Mature fruits	Flowers	Immature seeds
1-Carbohydrates and/or Glycosides	+++	+++	+++	+++	++	+++	+++
2-Tannins	+++	+++	+++	+++	++	+++	+++
3-Anthraquinones	+++	+++	+++	+++	++	+++	+++
4-Sublimation	+++	+++	+++	+++	++	+++	+++
5-Flavonoids	+++	+++	+++	+++	++	+++	+++
6-Unsaturated sterols and/or Triterpenoids	+++	+++	+++	+++	++	+++	+++
7-Alkaloids	+++	+++	+++	+++	++	+++	+++
8- Saponins	+++	+++	+++	+++	++	+++	+++
9-Cardiac Glycosides	+++	+++	+++	+++	++	+++	+++
10-Iridoids	+	+	+	+	+	+	+
11-Chlorides	+++	+++	+++	+++	++	+++	+++
12-Sulphates	+++	+++	+++	+++	++	+++	+++
Water extracts (Dried samples)							
Experiment	Leaf blades	Leaf petioles	Stems	Immature fruits	Mature seeds	Flowers	Immature seeds
1-Carbohydrates	+++	+++	++	+	+++	+++	+++
2-Tannins	+++	+++	++	+	+++	+++	+++
3-Anthraquinones	+++	+++	++	+	+++	+++	+++
4-Sublimation	+++	+++	++	+	+++	+++	+++
5-Flavonoids	+++	+++	++	+	+++	+++	+++
6-Unsaturated sterols	+++	+++	++	+	+++	+++	+++
7-Alkaloids	+++	+++	++	+	+++	+++	+++
8- Saponins	+++	+++	++	+	+++	+++	+++
9-Cardiac Glycosides	+++	+++	++	+	+++	+++	+++
10-Iridoids	+	+	+	+	+	+	+
11-Chlorides	+++	+++	+++	+	+++	+++	+++
12-Sulphates	+++	+++	+++	+	+++	+++	+++

Table. 3: Preliminary phytochemical screening of methanolic extracts of different fresh and dried plant parts of *L. leucocephala.*

Methanolic extracts (Fresh samples)							
Experiment	Leaf blades	Leaf petioles	Stems	Immature fruits	Mature fruits	Flowers	Immature Seeds
1-Carbohydrates and/or Glycosides	++	++	++	++	++	++	++
2-Tannins	++	++	++	++	++	++	++
3-Anthraquinones	++	++	++	++	++	++	++
4-Sublimation	++	++	++	++	++	++	++
5-Flavonoids	++	++	++	++	++	++	++
6-Unsaturated sterols and/or Triterpenoids	++	++	++	++	++	++	++
7-Alkaloids	++	++	++	++	++	++	++
8- Saponins	++	++	++	++	++	++	++
9-Cardiac Glycosides	++	++	++	++	++	++	++
10-Iridoids	+	+	+	+	+	+	+
11-Chlorides	++	++	++	++	++	++	++
12-Sulphates	++	++	++	++	++	++	++
Methanolic extracts (Dried samples)							
Experiment	Leaf blades	Leaf petioles	Stems	Immature fruits	Mature seeds	Flowers	Immature seeds
1-Carbohydrates	++	+	+	++	++	+	++
2-Tannins	++	+	+	++	++	+	++
3-Anthraquinones	++	+	+	++	++	+	++
4-Sublimation	++	+	+	++	++	+	++
5-Flavonoids	++	+	+	++	++	+	++
6-Unsaturated sterols	++	+	+	++	++	+	++
7-Alkaloids	++	+	+	++	++	+	++
8- Saponins	++	+	+	++	++	+	++
9-Cardiac Glycosides	++	+	+	++	++	+	++
10-Iridoids	+	+	+	+	+	+	+
11-Chlorides	++	+	+	++	++	+	++
12-Sulphates	++	+	+	++	++	+	++

Table. 4: Preliminary phytochemical screening of ethanolic extracts of different fresh and dried plant parts of *L. leucocephala.*

Ethanolic extracts (Fresh samples)							
Experiment	Leaf blades	Leaf petioles	Stems	Immature fruits	Mature fruits	Flowers	Immature Seeds
1-Carbohydrates and/or Glycosides	+	+	+	+	+	++	+
2-Tannins	+	+	+	+	+	+	+
3-Anthraquinones	+	+	+	+	+	+	+
4-Sublimation	+	+	+	+	+	+	+
5-Flavonoids	+	+	+	+	+	+	+
6-Unsaturated sterols and/or Triterpenoids	+	+	+	+	+	+	+
7-Alkaloids	+	+	+	+	+	+	+
8- Saponins	+	+	+	+	+	+	+
9-Cardiac Glycosides	+	+	+	+	+	+	+
10-Iridoids	+	+	+	+	+	+	+
11-Chlorides	+	+	+	+	+	+	+
12-Sulphates	+	+	+	+	+	+	+

Ethanolic extracts (Dried samples)							
Experiment	Leaf blades	Leaf petioles	Stems	Immature fruits	Mature seeds	Flowers	Immature seeds
1-Carbohydrates	+	+	+	++	+	++	+
2-Tannins	+	+	+	++	+	++	+
3-Anthraquinones	+	+	+	++	+	++	+
4-Sublimation	+	+	+	++	+	++	+
5-Flavonoids	+	+	+	++	+	++	+
6-Unsaturated sterols	+	+	+	++	+	++	+
7-Alkaloids	+	+	+	++	+	++	+
8- Saponins	+	+	+	++	+	++	+
9-Cardiac Glycosides	+	+	+	++	+	++	+
10-Iridoids	+	+	+	+	+	+	+
11-Chlorides	+	+	+	++	+	++	+
12-Sulphates	+	+	+	++	+	++	+

Table. 5: Total Phenolics of Different Plant Parts of *L. leucocephala* (mg/ml).

Plant part	Total phenolics	
	Fresh samples	Dried samples
Leaf blades	28.530±0.003	13.292±0.001
Leaf petioles	10.035±0.001	19.533±0.002
Stems	20.683±0.003	20.629±0.003
Immature fruits	16.181±0.002	20.117±0.003
Mature fruits	22.933±0.002	-
Flowers	16.381±0.001	22.171±0.003
Immature Seeds	25.571±0.003	28.438±0.003

Table. 6: Total Flavonoids of Different Plant Parts of *L. leucocephala* (mg/ml).

Plant part	Total flavonoids	
	Fresh samples	Dried samples
Leaf blades	5.865±0.001	5.829±0.001
Leaf petioles	5.686±0.001	5.978±0.001
Stems	5.947±0.001	5.940±0.001
Immature fruits	5.894±0.001	1.471±0.001
Mature fruits	5.208±0.001	-
Flowers	5.899±0.001	5.904±0.001
Immature Seeds	5.894±0.001	5.904±0.001

4. DISCUSSION

Now a days the usage of conventional nematicides has declined internationally because their toxicity to human and non-target organisms and their persistence in the environment. Consequently, several groups of nematologists are trying to develop plant-based products for effective and sustainable nematode management. Alternative control techniques, such as organic amendment have been used with some success (El-Nuby, 2002) also allelochemicals have been used (Abdel-Rahman *et al.,* 2017). Many plants have been tested for their nematicidal activities. Using plant extracts for managing nematode pests was previously used (Alam and El-Nuby, 2019; Abdel-Rahman *et al.,* 2017 and Abdel-Rahman, and Saleh, 2006). The nematicidal phyto-compounds in the form of substances such as alkaloids, glucosides, isothiocyanates, phenolics, thianins, thiophenics and fatty acids have been identified (Fatoki and Fawole, 2000 and Gommers 1973). As new strategies are currently being sought to control PPN, this study was carried out to determine the nematicidal activity of *L. leucocephala* different extracts against root knot nematode *M. incognita*. Obtained results found that different parts of *L. leucocephala* showed nematotoxic properties ranged between low to high toxic (complete death of juveniles) which differ depending on the used part of the tree. Our finding supports the allelopathy strategy for combating RKN. Water extract showed maximum efficacy against nematodes compared to other organic solvents. These results in accordance with Adekunle and Akinlua (2007), they reported allelopathic potential against *M. incognita* of crude water extracts of roots and leaves of *L. leucocephala* and recorded suppression in nematode final population on RKN-infected okra plants. The antinematodal activity of *L. leucocephala* may be due to their chemical components viz., falvonoids, polyphenols and another compounds, these theory are confirmed with finding of Adekunle and Aderogba (2008), They isolated and characterized a flavonoid compound called Quercetin from extract of leaves of *L. leucocephala* and confirmed its nematicidal activity for eggs and juveniles of *M. incognita in vitro*. Allelopathic activity against RKN may also be correlated with chemical composition of the plant; phytochemical analysis revealed that, *L. leucocephala* leaves showed the presence of phenolic compound, aromatic amide and carboxylic acid, while the roots showed the presence of phenolic compounds and carboxylic acid (Adekunle and A. Akinlua, 2007).

Some researchers tried the allopathic ability of crude extracts of roots and leaves in reducing populations of *M. incognita*, *Hoplolaimus* spp., *Pratylenchus* spp. and *Paratylenchus spp.* when planting some okra varieties in alleys of *L. leucocephala* (Adekunle, 2009). It was found that, protein extracts from the seeds, shell and cotyledon of *L. leucocephala* had a detrimental effect on nematode eggs (Soares, *et al.,* 2015). Methanolic extract in our study showed high nematotoxicity and this in coincidence with the finding of Rivero-Perez *et al*. (2019) as they found inhibition on eggs hatching of nematodes and viability of infective larvae after using pod extract of *L. leucocephala***.**

Ademola, *et al.,* (2005) found that fraction D of *L. leucocephala* was significantly more active than all the other fractions. Fraction D contains polar polyphenols (flavonoids and tannins), thus providing a scientific justification for the use of aqueous extract in traditional practice. The polyphenol fraction of *L. leucocephala* seed could help in finding application in anthelmintic therapy in veterinary practice. They also stated that, absence of alkaloids in the most active fraction means the most potent anthelmintic principles of the seed which can be easily obtained without the risk of mimosine toxicity (Ademola, *et al.,* 2005). Hassan *et al.,* (2014) were identified flavonoidal constituents isolated from extract of aerial parts of *L. leucocephala* as Caffeic acid, Chrysoeriol, Isorhamnetin, Isorhamnetin 3-O-galactoside, Kaempferol-3-O-rubinoside, Luteolin-7-glucoside and Quercetin-3-O-rhamnoside. Recent investigations reported the bioactivity of *L. leucocephala,* as crude or isolated compounds, against various pathogens and in medicine purposes (Zayed and Sallam, 2018). Ademola & Idowu (2005) reported the anthelmintic effect of an aqueous extract of *L. leucocephala*. Aqueous extraction of the pounded fresh seeds carries a certain risk of mimosine toxicity. Mimosine is well-known toxic non-protein amino acid that is found in the seeds of *L. leucocephala*, is slightly soluble in water. Mimosine, due to polar fragments in its chemical structure, will also exhibit a degree of solubility in water. It is reported to be slightly soluble in water and much less soluble in methanol and ethanol (The Merck Index, 2001)

Preliminary phytochemical screening on all studied extracts of all plant parts revealed the presence of carbohydrates and/or glycosides, tannins, flavonoids, anthraquinones, steroids and/triterpenoids, sublimable substances, saponins, alkaloids and/or nitrogenous bases, cardiac glycosides, coumains, chlorides, sulphates and iridoids in all samples. In this regard, results of preliminary phytochemical screening on all

studied extracts of all plant parts showed that, the richest extracts in these studied phytochemicals are belonging to water extracts of all plant parts, followed by methanolic extracts of all plant parts, meanwhile ethanolic extracts of all plant parts were found to contain the least amounts of phytochemicals under investigation. These results demonstrated a relationship between the phytochemical composition and nematicidal activity of these studied extracts. Estimation of total phenolic contents in all plant parts under investigation showed that, all investigated samples of different plant parts are rich in phenolics with special reference to both fresh and dried samples of seeds (25.571±0.003 and 28.438±0.003, respectively). Estimation of total flavonoids contents in all plant parts under investigation showed that, all investigated samples of different plant parts are rich in flavonoids with special reference to both fresh and dried samples of stems (5.947±0.001 and 5.940±0.001, respectively), leaf blades (5.865±0.001 and 5.829±0.001, respectively), seeds of the plant (5.894±0.001 and 5.904±0.001, successively), flowers (5.899±0.001 and 5.904±0.001, consequently) and leaf petioles (5.686±0.001 and 5.978±0.001, respectively). Results of phytochemical screening, total phenolics and total flavonoids are in strong positive relationship with those of *in vitro* antinematodal activity studies against juveniles of *Meloidogyne incognita***.**

Our finding introduced a non-conventional tool as a bio-nematicide from *L. leucocephala* (unused or less employed tree especially it is widely distributed in Egypt), and also reduced the burning of *L. leucocephala* tree which is accumulating wastes in the surrounding environment. Detailed studies for the determination of the best extract including the most potent fraction and main phytochemicals responsible for nematotoxicity, but less toxic to plant and human are highly required to be performed in the future. *In vivo* evaluation of various extracts is needed to ensure their effects on nematode suppression. As previous reports had suggested that, *Leucaena* is resistant to *Meloidogyne spp.*, but Stirling *et al.,* (1992) reported that, *L. leucocephala* was resistant to *M. javanica* and *M. incognita* and susceptible to *M. arenaria*. Also it was reported that *L. diversifolia* and *L. leucocephala* showed galls in their roots, but they did not accumulate high nematode populations in either the soil or the roots, besides they possessed the lowest total of other parasitic nematodes and this finding will support the high reproduction (Desaeger, and RAO, 1999). So intercropping *L. leucocephala* plants with other susceptible plants are valuable procedure in suppressing RKN, the majority of *Meloidogyne* species in Egypt are *M. incognita* then *M. javanica* but *M. arenaria* are rarely recorded according to the available literature. 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 needed.

5. CONCLUSION

Water, methanolic and ethanolic extracts of both fresh and dried parts (leaf blades, leaf petioles, stems, fruits, seeds and flowers) of *L. leucocephala* (English names: white lead tree) are rich sources of nematotoxic agents against juveniles of *Meloidogyne incognita* and there is positive relationship between the phytochemical components and the nematicidal activity of each extract of each plant part under investigation. Results showed high nematotoxic potential of *L. leucocephala* extracts particularly water extracts. The evaluated extracts can be an unconventional alternative for chemical nematicides. Further research should be conducted to determine the nematicidal effects of *L. leucocephala* extracts, as well as the active mechanism(s) and elucidation of the chemical structures of the active components, specially tannins and flavonoids, of these extracts, to discover new approaches for combating PPN. These findings are promised and novel strategy for combating PPN to produce free-pesticides plants, also the economic dimension must be considered (simple methods) to stimulate the adoption of this protocol by small farmers in rural or desert areas with available and easy handled resources. *In vivo* evaluation of *L. leucocephala* extracts is recommended to confirm their suppression impacts of PPN before expanding application at the field level.

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