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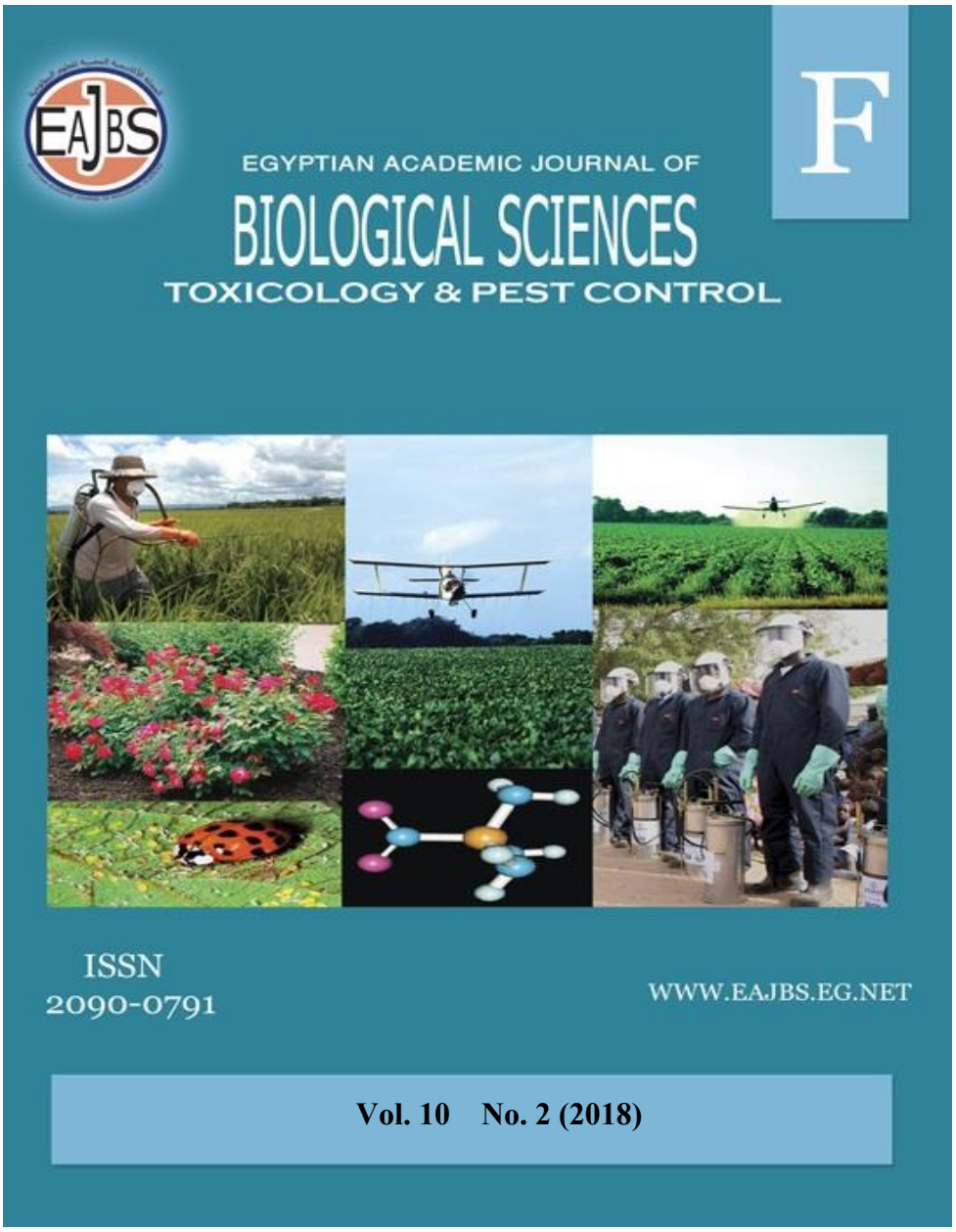

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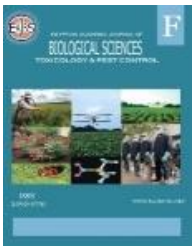

Phytochemical Analysis of Some Aqueous Leaf Extracts and Their Nematicidal Activity Against *Meloidogyne incognita* on Pepper

Abdelhadi A. I. Ali, Ramadan M. El-Ashry and Mahmoud M. Ramadan
Plant Protection Department, Faculty of Agriculture, Zagazig University, Egypt
E-mail: mahmoudram2010@gmail.com

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ABSTRACT

Since chemical pesticides have numerous negative effects on the environment**,** the study aimed to evaluate the bionematicidal effect of aqueous leaf extracts of chinaberry, datura, marigold and oleander at concentrations 0.1 g/ml (10% *w/v*) on root-knot nematode, *Meloidogyne incognita* egg hatching and juvenile mortality *in vitro* besides *in vivo* efficiency of different rates and times of soil drenching application in suppress nematode infection progress on pepper plants.

Phytochemical analysis of aqueous plant extracts revealed that the highest contents of flavonoids, phenolics, saponins and tannins presented in chinaberry and marigold followed by oleander responsible for their highest effect as bionematicide, whereas the least content was found in datura extract.

In vitro, the standard aqueous extract of marigold gained the highest ovicidal effect followed by datura then chinaberry and oleander and with percent, egg masses hatching inhibition 91.43, 59.78, 56.88, and 55.48% after 5 days of exposure, respectively. Marigold extract showed high hatching inhibition on treated egg masses even with half concentration (5 % *w/v*), with an increase in efficiency of other plant extracts against hatchability of free eggs.

The descending order of plant extracts efficacy against J2 treated with 10 % (w/v) was marigold followed by oleander, datura and chinaberry recorded 75.60, 65.60, 54.20, and 52.20 % mortality, respectively after 5 days post-treatment and the larvicidal effect was directly proportioned to concentration and exposure time.

In vivo, plant extracts significantly increased biomass of infected pepper plants and decreased reproduction of *M. incognita* as measured by IJs/100 g, egg masses numbers beside the number of galls and size as compared to the infected plants. Among this, oxamyl treatment surpassed composted chicken manure followed by marigold extract treatment. Twice application achieved significant amending plant growth and decreased nematode reproduction particularly in marigold extract treatment.

INTRODUCTION

Biological origins compounds isolated from naturally occurring plants extensively played an essential role as the primary source of pesticides in controlling several plant diseases other than their contributions for new toxophores from plants resistant to pests.

Biopesticides have a wide range of different biocontrol agents that include pest pathogenic organisms, whether protozoa, nematode, fungi, bacteria, viruses, as well as botanical extracts and culture filtrates also plant-incorporated-protectants or genetically modified organisms. Despite biopesticide's insufficiency, varying field performance, and high cost but they introduce a safe eco-friendly alternative for synthetic pesticides (Glare *et al.,* 2012), some biopesticides showed a promising future in pest control e.g. baculoviruses (Szewczyk *et al.,* 2006), *Bacillus thuringiensis* (Schnepf *et al.,* 1998) whether bacteria or genetically modified plant or bioactive enterotoxin and abamectin, a toxin secreted by *Streptomyces avermitilis* (Liu and Sengonca, 2003). Identifying the bioactive components responsible for effectiveness accelerates the pace of improving activity spectra, delivery options, the persistence of effect subsequently produced on a large scale as in the case of natural original substances e.g. azadirachtin, nicotine, pyrethrins, rotenone, ryanodine, sabadilla (Guleria and Tiku, 2009).

Hardly to define a single bioactive compound, but the biological effect is attributed to the presence of several compounds responsible for the toxic effect e.g. natural pyrethrum (Casida, 1973) and neem extract (Stark and Walter, 1995). Plant products are characterized by low adverse effects on non-target organisms, no risk of pest resistance development, no phytotoxicity, and low cost because of easily available (Prakash and Rao, 1996). The botanical extracts showed potency on different pest groups as insecticide, miticides, nematicides, rodenticides (Mamun and Ahmed, 2011).

Some plants showed antagonistic interaction with nematode presence in root zone reducing population densities (Sukul, 1992) e.g. marigold varieties showed resistance against plant-parasitic nematode (Bridge, 1996; Tyler, 1938)**.** Also, castor bean, chinaberry tree, datura, oleander, lantana, and marigold leaves significantly reduced the nematode in the soil and tomato root galling compared to the untreated control (Radwan *et al.,* 2007). They believed that antagonistic interaction involved allelopathy with nematicidal properties such as aterthienyl (Gommers and Bakker, 1988) that has a role in nematode suppression. The bioactive plant origin may be a multi-effect substance that occurs their effect as attractants, repellents, hatching stimulants or inhibitors, and nematotoxicants (Chitwood, 2002). Successive chemical explorations confirmed the existence of oxygen radicals formed by heterocyclic sulfur-containing thiophenes, such asaterthienyl (Castro and Munoz, 1982). So, botanical extracts introduce nematotoxic potential extractants with higher concentration can be delivered easily to the infection site to stop nematode progress. Also, the high variation in extract yield depends on extraction technique components included solvent, time, and temperature of extraction (Mohammed and Al-Saddi, 2003). Aqueous plant extraction is a common technique and easy-to-implement method besides the ease of delivering the extract to the root zone of the soil, but it is faulty for its ability only to extract materials of high polarity however, it has proven effective against nematodes (Altemimi *et al.,* 2017).

Therefore, this work aimed to clarify the efficiency of plant extracts (marigold, oleander, datura, and chinaberry tree) on root-knot nematode,

Meloidogyne incognita (Kofoid and White) Chitwood and the relationship between the biological activity of repeated-application of plant extracts and phytochemical compounds available in their water extracts.

MATERIALS AND METHODS

Pesticides Used:

Vydate 24% SL (oxamyl) is an approved commercial formulation of nematicide available in Egypt was obtained from the Central Laboratory of Pesticides, Dokki, Giza. Oxamyl was used as standard nematicide at an application rate of 5 liters/feddan.

Nematode Inoculum Preparation:

The identified pure inoculum of root-knot nematode (RKN) *Meloidogyne incognita*, previously isolated by El-Deeb *et al*. (2018) was used. The culture was maintained in the greenhouse on the tomato, *Solanum lycopersicum* L., susceptible cultivar Super Strain B.

Egg masses of equal size needed to study the effect of the tested extracts on *M. incognita* egg hatching. The selected egg masses were collected using forceps from galls on the infected tomato roots obtained from RKN pure culture. The selected egg masses were surface sterilized in an aqueous solution of 0.2 % (*v/v*) NaOCl for 5 min. (Haseeb *et al.*, 2005).

Free eggs and second-stage juveniles needed for experiments were prepared by cutting infected tomato roots into 2 cm pieces then transferred to a flask containing 200 ml (0.5 % NaOCl). The tightly capped flask was shaken for 3 minutes to dissolve the gelatinous matrix partially to free eggs from the egg masses (Hussey and Barker, 1973). The eggs suspension poured through sieves (200 mesh nested upon a 500 mesh). The collected eggs were washed immediately to get rid of sodium hypochlorite residue under sterilized water and incubated in Petri dishes at 25±2 °C until hatching. The newly hatched juveniles (J2) were collected by using a micropipette.

Aqueous Extract Preparation:

Fresh leaves of *Melia azedarach* (Chinaberry tree), *Datura stramonium L.* (datura), *Tagetes erecta* L. (marigold), and *Nerium oleander L.* (oleander) were collected from the yard of the Faculty of Agriculture, Zagazig University, Sharkia Government, Egypt. The leaves were shade dried, ground to a fine powder, and kept in cool dark conditions until used. The aqueous extraction was done by soaking 100 g of each single plant powder in one liter of water. The extraction was carried out at 25 °C with constant stirring overnight (24 h) in dark to allow the auto influx of plant metabolites to solvent extraction (Moosavi, 2012). The mixtures were filtered using filter paper (Whatman no.1) to get the stock solution with a concentration of 0.1 g/ml equal to 10 % (*w/v*) which used for the preparation of lower concentrations in bioassays.

Quantitative Phytochemical Analyses:

The phytochemicals present in the tested leaves extracts were determined and quantified by standard procedures included:

1.Total alkaloids were determined according to Harborne, 1973.

2.Total flavonoids content was determined according to the procedure described by Ordonez *et al*., 2006.

3.Total phenolic compounds were determined using Folin-Ciocalteau reagent by the method of Siddhuraju and Becker, 2003.

4.Total saponins content was determined based on vanillin-sulphuric acid colorimetric reaction with some modifications (Makkar et al., 2007).
5.Tannins content was estimated by the method of Siddhuraju and Manian, 2007.

Laboratory Bioassay:

Effect of the Tested Extracts on Egg Masses and Free Eggs Hatching:

Five fresh and uniform size egg masses were transferred to Petri dishes (6 cm diam.) contained 10 ml of 10 and 5 % (*w/v*) of the tested four plant extracts. The egg suspension concentrate was adjusted to about 2000 eggs/ml. In each treatment, 0.1 ml of egg concentrate (Approximately 200 free eggs) was transferred to a Petri dish (6 cm diam.) and complemented to 10 ml of 10 and 5 % (*w/v*) of the tested plant extracts. Control treatment prepared using 10 ml distilled water containing the same number of egg masses or free eggs. All treatments were replicated five times and incubated at 25±2˚C. The newly hatched juveniles enumerated periodically at 1, 2, 3, 5, 7- and 10-days post-treatment using a research microscope. The percentage of hatching inhibition was calculated according to the following equation:

$$\text{Egg hatching inhibition } \% = \left(\frac{\text{Control}-\text{Treatment}}{\text{Control}}\right) \times 100$$

Effect of the Tested Extracts on Juvenile Survival:

Suspension juveniles were adjusted to 10^3 J2/ml. 0.1 ml (100 J2) transferred to the Petri dish and complemented to 10 ml volume of the tested plant extracts to obtain the desired concentration (10 and 5 % (*w/v*)). While the control treatment was complemented to 10 ml with only distilled water. The treatments were kept in an incubator at 25±2 ˚C. Periodically examination of J2 was conducted at 1, 2, 3, 5- and 7-days post-treatment. In the microscopic examination, Inactive straight posture J2 or did not show up any response after prodding were counted in mortality (Nardo and Grewal, 2003). The mortality percentages were estimated by:

Mortality (%) = (No. of dead juveniles/Total number of juveniles) x100

The Potential Effect of The Tested Botanical Extracts Against *M. incognita in Vivo*:

Sterilized pots (20 cm diam.) with formalin solution (5%) were filled with about 1700 g soil (2:1 *v/v* sandy soil: clay soil) with formalin solution (5%). Each pot was transplanted with one pepper seedlings (*Capsicum annuum* L.) var. local. When pepper seedlings were nearby 17 cm in height and have four leaves, each seedling was inoculated with 1000 IJs of *M. incognita.* The RKN inoculum suspension (2 ml) was distributed in several holes near the root system then backfilled sandy soil. The investigated treatments included: healthy plants without J2 infection (negative control), infected plants with J2 (positive control), treatments received plant extracts (marigold, oleander, datura, and chinaberry extracts) with 10 ml volume of stock solution (10 % (*w/v*)/plant, oxamyl nematicide (0.2 ml formulation/plant) diluted in 10 ml water and poured near the stem. In comparing with composted chicken manure (CCM) treatment as a traditional cultural control method, 3 g/plant was incorporated with the upper 5 cm of soil around the pepper plant. The pepper plants received different treatments after adding nematode inoculum.

Moreover, a parallel experiment was conducted to measure the effectiveness of the repeated application of tested extracts on infection of pepper plants with RKN. The pepper plants received one, two, and three applications in successive periods 0, 3, and 7 days after RKN J2 inoculation. The plants in the greenhouse incubated at 24±4°C., and all received similar horticultural treatments.

The pepper growth responses and RKN pathogenesis-related parameters were investigated after 60 days. Plant growth parameters included shoot and root fresh weight, stem diameter, and leaves number/plant whereas, the nematode parameters

included number of galls, egg masses, and IJs /250 g soil. Also, root galls and egg masses index (RGI and EI) and reproduction factor (RF) were evaluated. To calculate the reproduction factor (RF) the equation of RF =P_{intial}/ P_{final} was used in 100 g soil and nematode population included (no. of eggs and J2 in soil). The RKN account in soil samples was conducted by a combination of sieving and Baermann trays extraction technique (Hooper, 1990)**.** Gall diameter intervals (< 2, 2:4, and > 4 mm) were assessed according to El-Deeb *et al.* (2018)**.** The root-knot index was assessed using the scale proposed by (Taylor and Sasser, 1978)**.** All uprooted pepper plants were covered with tissue paper to prevent water loss until the end of recording the various measurements. The parameters changing (%), increase or reduce assigned to negative or positive control values, and the current equations were used. $\text{Reduction}\,(\%) = ((\text{Control} - \text{Treated})/\text{Control}) \times 100$ and $\text{Increase}(\%) = ((\text{Treated} - \text{Control})/\text{Control}) \times 100$

Statistical Analysis:

A completely randomized design was implemented in the experiment. data were subjected to one-and two-way analysis of variance (ANOVA) using CoStat V 6.451. Means were compared by Duncan's multiple range test at $P \leq 0.05$ probability.

RESULTS

Quantitative phytochemical analysis of leaf aqueous extract of tested plants exhibited the presence of many bioactive compounds involved in plant resistance against pest attack. Aqueous extracts contained alkaloids, flavonoids, phenolics, saponins and tannins (Figs. 1; a and b). The presence of these substances is an indicator of the plant leaves rich in active substances besides the efficiency of the extraction process with water influxing of these materials from the leaf powder to solvent in varying quantities according to the plant. Despite the presence of these substances, but some haven't an annihilating effect against pests, as well as their efficient action mechanism inside the plant requires a qualitative and quantitative harmony besides the releasing order into the plant to cause systemic acquired resistance and induced systemic resistance based on elicitor type (Vallad and Goodman, 2004).

Total alkaloids content in tested leaf extracts were in the range of 0.6 - 0.9% (600 - 900 µg/100 g) with surpassing oleander extract in total alkaloids content (900 µg/100 g) and equality datura and chinaberry extracts (700 µg/100 g) and lowest content in marigold extract (900 µg/100 g). The highest saponins content observed in chinaberry extract followed by oleander then datura and finally, marigold extras recorded 1.94, 1.68, 1.52 and 1.19 mg DE/100 g, respectively, as elucidated in Fig. (1, a).

Moreover, flavonoids content was higher in chinaberry (417.45 µg QE/100 g extract) and marigold (394.87 µg QE/100 g extract), while oleander extract contained 301.32 µg QE/100 g extract and the least flavonoids content observed in datura (93.26 µg QE/100 g extract).

The same trend was observed in the total phenolic compounds in chinaberry (336.14), marigold 9306.67), oleander (264.16) and datura (237.59 µg GAE/100g extract). Tannins content trend was the same total phenolic compound content as derived from phenols but differed in free phenols which were higher in oleander extract as elucidated in Fig. (1, b).

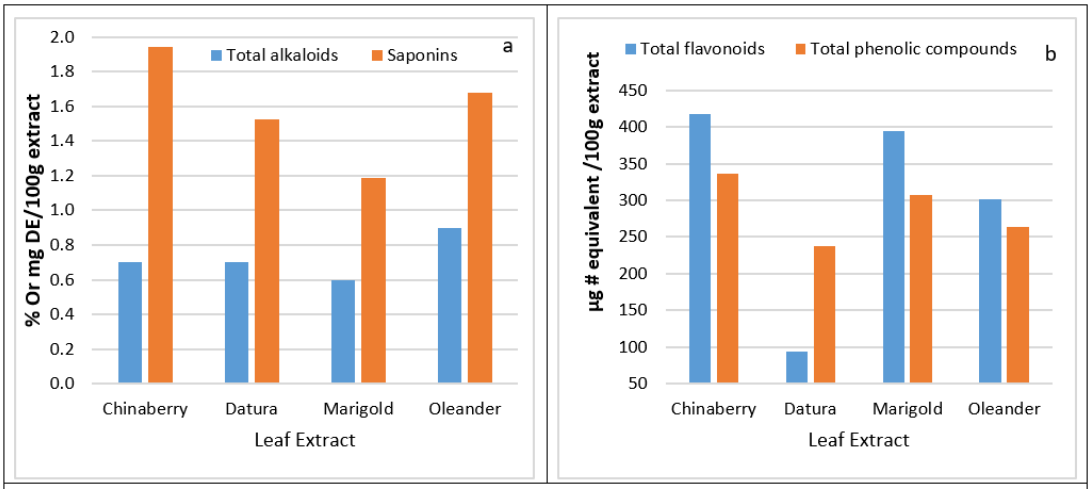

Fig. 1 a) Total alkaloids (%) and saponins contents (mg Diosgnin equivalent/100 g extract) in different leaf aqueous extracts of tested plants*;* **b)** Total flavonoids (µg Quercetin equivalent /100 g extract), and total phenolics compounds (µg Gallic acid equivalent /100 g extract) in different leaf aqueous extracts of tested plants.

Effect of the Tested Aqueous Plant Extracts on Egg Hatching:

The Response of Egg-Masses Hatching:

Exposure of *M. incognita* egg masses to the stock solution 10% (*w/v*) and half-stock solution 5 % (*w/v*) of tested plant extracts (marigold, oleander, datura**,** and chinaberry**)** were significantly ($P \leq 0.05$) inhibited egg hatching when used to judge the ovicidal effect. All the tested plant extracts were found to exhibit different levels of ovicidal effect at different times of exposure is illustrated in Table1. Marigold aqueous extract of leaves has the highest ovicidal effect and gained the lowest number of emerged juveniles followed by oleander, datura**,** and chinaberry at the standard, 10 % (*w/v*) and half standard solutions 5 % (*w/v*) after one, three, five, and ten days of treatment. In stock solution, 10 % (*w/v*), numbers of emerged juveniles in treatments of marigold, oleander, datura**,** and chinaberry after one day of treatment were 25.67, 103.67, 114.67, and 140.67 juveniles, respectively with percentages inhibition 88.67, 54.26, 49.41 and 37.94%.

The same trend was observed after 3 days post-treatment and the larvicidal effect of the tested plant extracts was expanded to reach a relatively higher value at 10 and 5 % (*w/v*) *i.e.,* marigold, (89.10 and 89.35%), oleander (47.37 and 60.26%), datura (41.56 and 37.78%), and chinaberry (40.78 and 10.54%), respectively whereas the parallel values after 5 days were (91.43 and 90.21), (55.48 and 44.40), (59.78 and 43.22) and (56.88 and 18.45%).

With all tested plant extracts, as dilution increased to 5 % (*w/v*), numbers of hatched juveniles were increased and percent inhibition decreased to reach 154.70 (87.04), 620.70 (48.00), 723.00 (39.43), and 888.33 (25.58%) juveniles after 7 days post-treatment, respectively.

After ten days, the number of emerged J2 in distilled water reached to1354.70 compared to 205.70, 899.70, 856.70, and 708.00 juveniles in treatments of marigold, chinaberry, datura, and oleander, respectively at the hundred percent concentrations (10 % (*w/v*)).

Moreover, with the most tested concentrations, the ovicidal effect noticeably increased from 3 to 7 days after treatment. In the control treatment, significantly ($P \leq 0.05$) highest numbers of emerged juveniles were observed when compared with all tested plant extracts at different times of treatment.

Among the tested plant extracts, marigold displayed significantly higher influence followed by oleander with insignificant variations with datura while chinaberry showed a significantly lower effect.

Table 1 Egg hatching inhibition percentages of half- and stock solution of tested plant extracts on egg masses hatching of *M. incognita in vitro*.

Concentration (*w/v*)	Exposure time (Day)	Plant extract				
		Control	Chinaberry	Datura	Marigold	Oleander
Stock solution **10 %**	**1**	226.67^{a}	140.67^{b} (37.94)	114.67^{bc} (49.41)	25.67^{d} (88.67)	103.67^{c} (54.26)
	2	433.33^{a}	277.33^{b} (36.00)	228.33^{bc} (47.30)	47.33^{d} (89.07)	198.67^{c} (54.15)
	3	596.67^{a}	353.33^{b} (40.78)	348.67^{b} (41.56)	65.00^{c} (89.10)	314.00^{b} (47.37)
	5	930.00^{a}	401.00^{bc} (56.88)	374.00^{c} (59.78)	79.67^{d} (91.43)	414.00^{b} (55.48)
	7	1205.00^{a}	752.70^{b} (37.53)	754.70^{b} (37.36)	120.70^{c} (89.98)	591.00^{b} (50.95)
	10	1354.70^{a}	899.70^{b} (33.58)	856.70^{b} (36.76)	205.70^{d} (84.81)	708.00^{c} (47.73)
Half- stock solution 5 %	**1**	226.67^{a}	191.67^{b} (15.44)	142.33^{c} (37.20)	30.67^{e} (86.76)	106.00^{d} (53.23)
	2	531.00^{a}	433.33^{b} (18.39)	191.00^{c} (64.03)	62.00^{d} (88.32)	159.33^{c} (69.94)
	3	667.00^{a}	596.67^{ab} (10.54)	415.00^{bc} (37.78)	71.00^{d} (89.35)	265.00^{c} (60.26)
	5	930.00^{a}	758.33^{b} (18.45)	528.00^{c} (43.22)	91.00^{d} (90.21)	517.00^{c} (44.40)
	7	1193.70^{a}	888.33^{b} (25.58)	723.00^{c} (39.43)	154.70^{d} (87.04)	620.70^{c} (48.00)
	10	1346.30^{a}	993.00b (26.24)	749.70^{c} (44.31)	205.70^{d} (84.72)	744.00^{c} (44.73)

*Reported numbers represent means of 5 replicates; **Figures in parenthesis are percentages of egg hatching inhibition in comparison with control of distilled water; ***Different letters in the same column indicate significant differences ($P \leq 0.05$) according to Duncan`s multiple range test.

The Response of Free Egg Hatching:

Plant extracts similarly showed a significant ($P \leq 0.05$) effect against free eggs of *M. incognita* after exposure to 10 and 5 % (*w/v*) concentrations. Free eggs viability was influenced by type, time of exposure, and concentrations of tested plant extract under laboratory conditions. Results of Table 2 revealed that marigold exhibited the highest effect followed by oleander while chinaberry extract was the least effective one. After one and three days of treatment, numbers of hatched juveniles in marigold, oleander, datura, and chinaberry were (13.40 and 91.20), (27.20 and 122.00), (22.80 and 106.60), and (46.40 and 154.40) while the percentages of hatching inhibition were (98.00 and78.25), (84.07 and 70.91), (86.65 and 74.58) and (72.83 and 63.18 %) at 10 % (*w/v*) concentration, respectively. Hatched juveniles of free eggs after 7 days of exposure and % hatching inhibition were 174.40 (72.80), 244.40 (61.88), 202.00 (68.49), and 276.00 (56.95) in mentioned plant extracts at 10 % (*w/v*), respectively. At the end of the experiment, when plant extracts dilution decreased to 5 % (*w/v*), hatched J2 increased and percent of egg inhibition decreased. On the tenth days of treatment, the number of emerged J2 and percent of egg inhibition in Petri dishes treated with 10 % (*w/v*) recorded 255.20 (66.95%), 371.80 (51.85%), 281.20 (63.58%), and 388.60 (49.76%) with

marigold, oleander, datura, and chinaberry whereas, the parallel values in Petri dishes treated with 5 % (*w/v*) were 386.80 (49.90), 465.20 (39.75), 434.00 (43.79) and 491.80 (36.31%), respectively.

Among which, tested plant extracts, marigold showed significantly higher influence followed by oleander with significant variations by datura, while chinaberry displayed a significantly lower effect.

Generally, the nematocidal effect of the tested plant extracts on *M. incognita* eggs was directly proportional to concentration and exposure time.

Table 2 Eggs hatching inhibition percentages of half- and stock solution of tested plant extracts on free eggs hatching of *M. incognita in vitro.*

Concentration (*w/v*)	Exposure time (Day)	Plant extract				
		Control	Chinaberry	Datura	Marigold	Oleander
Stock solution 10 %	1	170.80^{a}	46.40^{b} (72.83)	22.80^{c} (86.65)	13.40^{d} (98.00)	27.20^{c} (84.07)
	2	280.00^{a}	97.40^{b} (65.21)	78.00^{c} (72.14)	58.80^{d} (79.00)	86.80^{bc} (69.00)
	3	419.40^{a}	154.40^{b} (63.18)	106.60^{cd} (74.58)	91.20^{d} (78.25)	122.00^{c} (70.91)
	5	568.00^{a}	186.80^{b} (67.11)	126.40^{d} (77.74)	110.00^{d} (80.63)	150.80^{c} (73.45)
	7	641.20^{a}	276.00^{b} (56.95)	202.00^{d} (68.49)	174.40^{e} (72.80)	244.40^{c} (61.88)
	10	772.20^{a}	388.60^{b} (49.76)	281.20^{c} (63.58)	255.20^{c} (66.95)	371.80^{b} (51.85)
Half- stock solution 5 %	1	178.00^{a}	123.00^{b} (30.89)	60.80^{d} (65.84)	40.80^{e} (77.07)	87.00^{c} (51.12)
	2	568.00^{a}	330.00^{b} (41.90)	272.80^{d} (51.97)	206.40^{e} (63.66)	304.80^{c} (46.33)
	3	280.00^{a}	205.00^{b} (26.78)	141.20^{d} (49.57)	122.00^{e} (56.42)	189.20^{c} (32.42)
	5	419.40^{a}	293.60^{b} (29.99)	207.60^{c} (50.50)	152.20^{d} (63.71)	260.00^{b} (38.00)
	7	641.20^{a}	432.00^{b} (32.62)	364.80^{c} (43.10)	274.00^{d} (57.26)	406.80^{b} (36.55)
	10	772.20^{a}	491.80^{b} (36.31)	434.00^{d} (43.79)	386.80^{e} (49.90)	465.20^{c} (39.75)

*Reported numbers represent means of 5 replicates; **Figures in parenthesis are percentages of egg hatching inhibition in comparison with control of distilled water; ***Different letters in the same column indicate significant differences ($P \leq 0.05$) according to Duncan`s multiple range test.

The ovicidal effect of aqueous plant extracts on egg masses and free eggs at two concentrations (10 and 5 %*(w/v)*) revealed the effectiveness of marigold as compared with datura, oleander, and chinaberry (Fig.2). The nematicidal effect of plant extracts were more effective against free eggs than egg masses at two tested concentrations as well as, hatching inhibition of *M. incognita* eggs was directly proportional to concentrations.

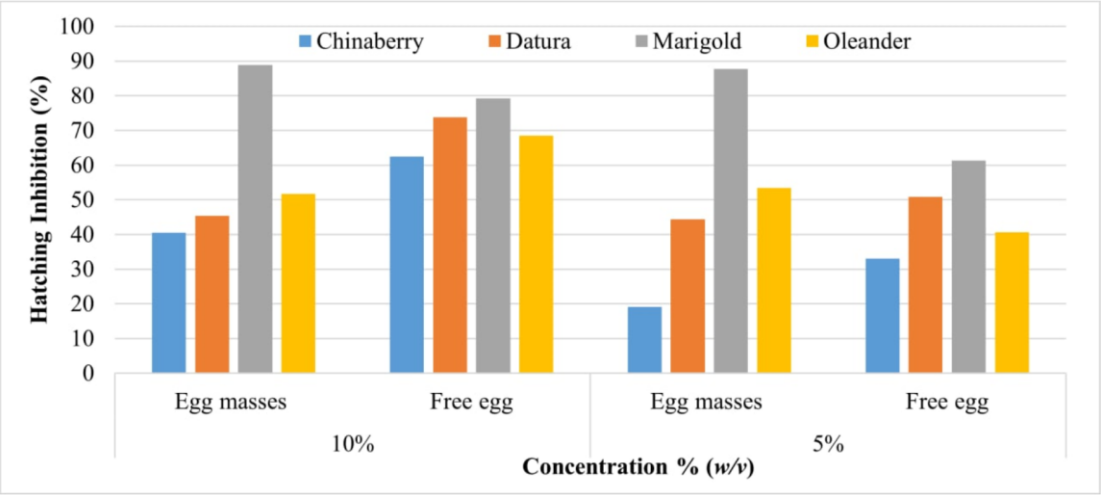

Fig. 2 Mean hatching inhibition percentages of M. incognita egg masses and free eggs exposed to 10% and 0.5 % (w/v) of the tested extract**.**

Larvicidal Activity of Aqueous Plant Extracts on Second Juveniles *in vitro*:

The aqueous plant extracts of marigold, oleander, datura, and chinaberry were subjected to nematicidal activity against the mortality of the 2nd stage juveniles of *M. incognita in vitro* and J2 mortality was recorded after different time intervals such as 1, 3, 5, 7, and 10 days.

All aqueous plant extracts were found to be significantly ($P \leq 0.05$) effective against J2 of *M. incognita* and mortality rates in J2 were increased as the concentration and exposure times increased from 1 to 10 days after treatment at 10 and 5 % (*w/v*) concentrations (Table 3). Among which, marigold and oleander were the most toxic extracts followed by datura while chinaberry showed the least nematicidal activity against the *M. incognita* juveniles. Larvicidal activity increased gradually as time intervals increased to reach the highest mortality value of J2 that which detected in Petri dishes treated with 10 % (*w/v*) of marigold after 10 days of exposure (95.60%) followed by oleander (84.60%) and datura (77.40 %) while chinaberry scored 69.40 compared with 4.00% in the treatment of distilled water. The respective values for current plant extract at a half-stock solution, 5 % (*w/v*) after the same exposure time was 84.40%, 81.60%, 73.40%, and 56.20 %, respectively. Generally, the nematicidal effect of the tested plant extracts on *M. incognita* juveniles was directly proportioned to concentration and exposure time.

Table 3 Mortality percentage of *M. incognita* juveniles exposed to half- and stock solution of tested plant extracts at different exposure periods *in vitro*.

Plant extract	Concentration % (*w/v*)	Exposure period (day)				
		1	3	5	7	10
Chinaberry	10	14.60^{d}	30.60^{e}	52.20^{d}	63.00^{d}	69.40^{f}
	5	11.40^{e}	18.40^{d}	33.80^{e}	37.60^{a}	56.20^{g}
Datura	10	18.40^{c}	35.20^{c}	54.20^{d}	65.00^{d}	77.40^{d}
	5	16.40^{cd}	34.00^{c}	53.20^{d}	63.20^{d}	73.40^{e}
Marigold	10	26.80^{a}	57.00^{a}	75.60^{a}	83.40^{a}	95.60^{a}
	5	17.80^{c}	42.00^{bc}	67.20^{b}	74.80^{bc}	84.40^{bc}
Oleander	10	21.60^{b}	45.00^{b}	65.60^{b}	78.20^{b}	84.60^{b}
	5	17.40^{c}	40.00^{c}	58.80^{c}	73.20^{c}	81.60^{c}
Distilled water		0.20^{f}	1.20^{f}	2.00^{f}	2.80^{f}	4.00^{g}

*Reported numbers represent means of 5 replicates.; **Different letters in the same column indicate significant differences ($P \leq 0.05$) according to Duncan`s multiple range test**.**

Evaluation of Aqueous Plant Extracts *in vivo*

Comparison Changes in Infected Pepper, Capsicum annuum L. (cv. local) Biomass and Nematode Parameters Treated with Plant Extracts, Composted Chicken Manure (CCM) and Oxamyl Under Greenhouse Conditions.

Changes in biomass of infected pepper plants and soil parameters related to *Meloidogyne incognita* reproduction like the number of IJs /100 g, egg masses numbers beside the number of galls and size treated with aqueous plant extracts (marigold, oleander, datura, and chinaberry), composted chicken manure (CCM) compared to the recommended dose (RD) of oxamyl after two months of the application were illustrated in Table 4.

Table4. Biomass, soil parameters and galls number changes in infected pepper, Capsicum annuum L. (cv. local) by J_2 of Meloidogyne incognita treated with plant extracts in comparison with composted chicken manure (CCM) and oxamyl under greenhouse conditions.

Treatment	Plant growth parameter (Increasing %)			Nematode reproduction parameter (Decreasing %)			Root gall size		
	Fresh root weight	Fresh shoot weight	No. of leaves/ plant	No. IJs /100 g soil	No. egg masses	No. of galls	>4 mm	4:2mm	<2 mm
Healthy plant	3.78^{a} (34.92)	6.37^{a} (57.45)	4.60^{a}	0.00^{f}	0.00^{f}	0.00^{f}	0.00^{c}	0.00^{e}	0.00^{f}
Infected plant with RNK	2.46^{e}	2.71^{f}	2.40^{d}	91.20^{a}	17.00^{a}	28.60^{a}	0.80^{a}	11.80^{a}	14.00^{a}
***M. incognita* +Chinaberry**	2.80^{de} (13.82)	3.17^{ef} (16.97)	2.60^{cd}	63.40^{b} (30.48)	11.60^{cd} (31.76)	19.80^{bc} (30.76)	0.40^{abc}	7.60 ^{cd}	11.80^{ab}
***M. incognita* +Datura**	3.03^{cd} (23.17)	3.62e (33.57)	2.60cd	54.40^{c} (40.35)	12.60^{bc} (25.88)	19.40^{bc} (32.16)	0.40^{abc}	8.20^{bcd}	10.80^{bc}
***M. incognita* +Marigold**	3.35^{abc} (36.17)	4.47^{c} (64.94)	3.20^{bc}	35.80^{d} (60.74)	9.40^{d} (44.70)	14.80^{d} (48.25)	0.00^{c}	6.00^{d}	8.80^{cde}
***M. incognita* +Oleander**	3.14^{bcd} (27.64)	4.15^{c} (53.13)	2.80^{cd}	49.80^{c} (45.39)	11.00^{cd} (35.29)	18.00^{cd} (37.06)	0.20^{bc}	7.40^{d}	9.60b^{cd}
***M. incognita* +Chicken manure**	3.62^{a} (47.15)	4.08^{cd} (50.55)	3.20^{bc}	17.60^{e} (80.70)	5.00^{e} (70.58)	10.00^{e} (65.03)	0.00^{c}	1.60^{e}	8.40^{de}
***M. incognita* +Oxamyl**	3.53^{ab} (43.49)	5.25^{b} (93.72)	3.60^{b}	13.60^{e} (85.08)	2.60^{ef} (84.70)	7.20^{e} (74.82)	0.00^{c}	0.40^{e}	6.80^{e}

*Each value is a mean of five replicates.
Means in each column followed by the same letter (s) are not significantly different at the 5% level of probability according to Duncan's multiple range test.

All tested materials significantly ($P \leq 0.05$) increased the biomass of pepper plants as compared to the control treatment (infected plants). Among which, treatment of oxamyl surpassed those treated with CCM and aqueous plant extract of marigold whereas, pots treated with chinaberry extract showed the least percent of the increase. In concerning fresh root weight, it was evident that pots treated with CCM (47.15%) increased fresh root weight with insignificant differences with healthy plants and oxamyl (43.49%) followed by marigold plant extract (36.17). Conversely, highly significant differences were detected between oxamyl and plant extracts (marigold and oleander) treatments compared with the minimum percentage increase in treatments of chinaberry and datura. However, following healthy plants, a high number of leaves / plants was detected in plants treated with oxamyl (3.60) followed by CCM and marigold (3.20 for each).

Regarding the effectiveness of aqueous plant extracts when compared with chemical nematicide, oxamyl, and amendment with composted chicken manure on *M. incognita* reproduction (IJs/100 g, number of galls and egg masses), obtained results presented that oxamyl (85.08%), CCM (80.70%) marigold (60.74%), oleander (45.39%), datura (40.35%) and chinaberry (30.48%) significantly minified numbers of IJs/100g as compared to infected plants with *M. incognita* under greenhouse conditions.

Effectiveness of tested materials against egg masses and galls numbers, % reduction in pots treated with oxamyl, CCM, marigold, oleander, datura and chinaberry were 84.70,74.82; 70.58,65.03; 44.70,48.25; 35.29, 37.06; 25.88, 32.16 and 31.76, 30.76, respectively.

Moreover, plants in pots received RD of oxamyl, CCM, and marigold extract exhibited the least number of >4mm galls as compared with other treatments and insignificantly ($P \leq 0.05$) with healthy plants. As well as the number of galls showing < 4-2 mm and < 2 mm diameter were significantly decreased with all treatments.

Generally, it could be concluded that the curative application of oxamyl and CCM significantly reduced gall formation and egg mass production of *M. incognita* in pepper plants. Moreover, oxamyl and CCM of all tested treatments gave good results as compared to aqueous plant extracts.

Results of Table 5 illustrate the effect of multiple applications of aqueous plant extracts at different time intervals on total plant fresh weight and root-knot nematode reproduction (egg mass numbers and EI, galls number, and GI besides the number of J2 in pot soil).

Table 5. Numbers of galls and egg masses of *Meloidogyne incognita* on infected pepper *Capsicum annuum* L. (cv. local) roots and pot soil parameters after multiple applications with plant extracts**.**

Parameter	Applications time*	Plant extract					Mean	Interaction
		control	Chinaberry	Datura	Marigold	Oleander		
No. of galls	1	28.60	19.80	19.40	14.80	18.00	**20.12^{a}**	ns
	2	28.60	17.60	16.40	10.00	14.00	**17.32^{b}**	
	3	28.60	15.80	14.20	9.20	12.40	**16.04 ^{b}**	
	Mean	**28.60^{a}**	**17.73^{b}**	**16.67^{bc}**	**11.33^{d}**	**14.80^{c}**		
Root gall index (RGI)	1	3.00	3.00	3.00	3.00	3.00	**3.00^{a}**	*
	2	**3.00**	3.00	3.00	2.40	2.80	**2.84^{b}**	
	3	3.00	3.00	3.00	2.20	2.80	**2.80^{b}**	
	Mean	**3.00^{a}**	**3.00^{a}**	**3.00^{a}**	**2.53^{b}**	**2.87^{a}**		
Egg masses	1	17.00	11.60	12.60	9.40	11.00	**12.32^{a}**	ns
	2	17.00	10.40	11.00	4.80	9.00	**10.44^{b}**	
	3	17.00	9.80	9.80	4.00	7.80	**9.68^{b}**	
	Mean	**17.00^{a}**	**10.60^{b}**	**11.13^{b}**	**6.07^{c}**	**9.27^{b}**		
Egg masses index (EI)	1	3.00	2.80	3.00	2.40	2.60	**2.76^{a}**	ns
	2	3.00	2.40	2.60	2.00	2.20	**2.44^{b}**	
	3	3.00	2.20	2.40	2.00	2.00	**2.32^{b}**	
	Mean	**3.00^{a}**	**2.47^{bc}**	**2.67^{b}**	**2.13^{d}**	**2.27^{cd}**		
Js Reduction (%)	1	91.20	63.40	54.40	35.80	49.80	**58.92^{a}**	ns
	2	91.20	55.60	53.20	27.40	43.00	**54.08^{ab}**	
	3	91.20	54.80	54.20	25.60	37.80	**52.72^{b}**	
	Mean	**91.20^{a}**	**57.93^{b}**	**53.93^{b}**	**29.60^{d}**	**43.53^{c}**		
Plant weight increase (%)	1	5.18	5.98	6.66	7.83	7.32	**6.59^{c}**	**
	2	5.18	6.63	6.75	8.84	8.31	**7.14^{b}**	
	3	5.18	7.22	7.35	9.63	9.12	**7.70^{a}**	
	Mean	**5.18^{d}**	**6.61^{c}**	**6.92^{c}**	**8.77^{a}**	**8.25^{b}**		

* 1: applied during nematode inoculation; 2 applied at 0 and 3 days of nematode inoculation; 3: applied at 0, 3, and 7 days of nematode inoculation.

**Each value is a mean of five replicates.

***The same letter (s) in rows or columns indicates no significant differences at $P \leq 0.05$ according to Duncan's multiple range test.

For total plant fresh weight, multiple applications with marigold, oleander, datura, and chinaberry improved root condition and significantly ($P \leq 0.05$) increased the plant growth compared with the control plants infected with J2 during once

application (at nematode inoculation), twice applications (at nematode inoculation and 3 days post J2 inoculation) and triple applications (at nematode inoculation also, 3 and 7 days post nematode inoculation).

In concerning gall numbers, the general mean showed insignificant differences ($P \leq 0.05$) between twice and triple applications. However, the marigold displayed the lowest gall numbers (11.33) as compared with oleander (14.80), datura (16.67), and chinaberry (17.73). Similarly, root gall index (RGI) decreased significantly in pots treated with marigold as compared to infected plants and with insignificant differences with other plant extracts.

The number of egg masses and egg masses index (EI) showed insignificant differences after twice and triple applications. Twice applications exhibited the least number of egg masses and EI in pots treated with marigold (6.07, 2.13) and oleander (9.27, 2.27) followed by chinaberry (10.60, 2.47) and datura (11.13, 2.67).

Regarding the efficiency of aqueous plant extracts on decreasing infective juveniles of *M. incognita* in the soil of treated pots, insignificantly differences were found between multiple application treatments and the number of J2/100 g soil significantly minified to reach 29.60 in soil pots treated with marigold extract followed by oleander (43.53) and datura (53.93) compared to the infected plants in the control treatment (91.20).

Generally, it could be concluded that multiple curative application of aqueous plant extracts significantly enhances total plant fresh weight and reduced gall formation and egg mass production of *M. incognita* in pepper plants. Moreover, twice applications were more effective than triple applications in all tested plant extracts under greenhouse conditions.

DISCUSSION

The biopesticides are pest pathogenic organisms or natural bioactive compounds produced by different organisms contribute to causing reducing economic damages attributed to low pest activities. These bioactive compounds are sustainable naturally produced active substances that play a cryptic role in the environment to decrease pest population or preventing damages in other areas. Exploring and understanding these compounds, produce organisms, toxic effects, and selectivity will reduce dependence on synthetic pesticides and avoid their toxic effects. Also, the indigenous soil fauna will play a role in pest control and will reduce the use of cost chemical pesticides. Plants are a produced organism, some resistant plants to pest infestation produced a toxic bioactive secondary metabolite as alkaloids, phenolics, terpenoids, flavonoids, tannins, coumarins and minor secondary chemicals (Laquale *et al.*, 2016). Many plant species reported to possess cidal effects against some deleterious organisms such as chinaberry, chrysanthemum, datura, derris, lantana, marigold, moringa, neem, oleander, tobacco, and many others can be grown with minimum expense. The botanical materials showed efficiency in controlling delicate insects e.g. aphids, jassid, and thrips, etc. as well as spider mite and nematodes (Mamun and Ahmed, 2011). Available indigenous plants with nematicidal potency can be applied with different application techniques e.g. intercrop, green-manuring, and soil amendment (El-Gindi *et al.*, 2005; Hooks *et al.*, 2010; Jankowska *et al.*, 2012; Pant and Narayan, 2015) besides application plant extraction. Using calendula proposed for dealing with root-knot nematodes *M. incognita* problem (Betancourth Garcia *et al.*, 2011).

Plant samples subjected to extraction has a special concern because it

defines the nematicidal yield extraction. The nematicidal of marigold extraction was increased using old age plants (Walia and Gupta, 1997). Also, plant species showed significant differences such as *Datura stramonium*, *D. innoxia*, and *D. tatula* extracts (LC_{50} = 75.1-486.8 mg/ml and 52.5-113.0 mg/ml for aqueous and ethanol extracts, respectively) (Babaali et al., 2017). The variation in marigolds nematicidal efficiency is attributed to the application method, intervals between application, age of the marigold plant, and species or races of target nematodes (Hooks *et al.,* 2010). Besides, host plant infectivity with RKN (Alijani *et al.,* 2015). *Datura metel* leaves extract subjected for total alkaloids determination showed hyoscine as effective nematicidal effective only against *Hoplolaimus indicus*, causing 90% mortality (Qamar *et al.,* 1998).

Besides, the plant part sample used for extraction (bulbs, fruits, inflorescences, leaves, roots, seeds, and spikes) showed varying efficiency when using the same extraction method (Babaali *et al.,* 2017; Vineuza *et al.,* 2006). Different plant extracts of *D. stramonium* showed varied potency as the aqueous extract from the seeds was more effective against nematodes than the aqueous extract from the leaves (Al-Saba *et al.,* 2001).

The applicable indigenous extraction method is the challenge face using botanical materials extraction because it wheels the quality and quantity of compounds extracted. Most extraction methods used water and methanol or ethanol as common solvent extraction able to influx the nematicidal bioactive compound from plant tissue to the liquid phase. Some solvents extract may cause phytotoxicity and plant growth retardation (Stephan *et al.,* 2001). The variation between solvent extraction and nematicidal effect ascertained (Al-Saba *et al.,* 2001 and Babaali *et al.,* 2017) with datura (*D. stramonium*) exhibited solvent efficiency and hence extract efficiency without bias for a solvent. Using accelerated extraction means as microwave, sonication, hot water, and enzymes can enhance extraction yield disparately (Chaudhary et al., 2013). In addition to the ability to concentrate the plant extract and eliminate nematode toxic solvents showed a clear variability and inaccuracy in the expression of the concentrations of the extracts used for the bioassay was observed in most of the previous studies.

Finally, the difference in sensitivity of nematode species to the same plant extract as exhibited in susceptibility to *M. incognita* and *H. indicus* compared to *Tylenchorhynchus vulgaris* populations to transplant *Tagetes erecta* and *T. patula* under field conditions (Prasad *et al.,* 1992)

The aqueous marigold extract (0.5%) caused 94.8% hatching inhibition of RKN after 14 hours (Kaur and Katoch, 2012). Aqueous extract (10%) of *Tagetes* sp. decreased 37.5-60.0% egg hatching of the nematode (Beni *et al.,* 2016) with irreversible toxic effect in stopping hatching (Walia and Gupta, 1997). Also, *Datura stramonium* extract inhibited egg hatching (Mennan *et al.,* 2000). while *Melia azedarach* leaf extracts inhibited hatching of *M. incognita* eggs (associated with a delay in embryonic development), reduced nematode motility, and killed the larvae (Lee, 1987). All the tested aqueous extracts showed nematotoxicity depended on concentration and exposure time (Ahmad *et al.,* 1991; Chaudhary *et al.,* 2013; Devi, 2008; Ganaie and Khan, 2016; Ramakrishnan *et al.,* 1999). The same trend was observed with the larvicidal effect of the tested extracts. *D. stramonium* was effective in causing a knockdown effect/mortality of *M. incognita* juveniles (Sharma, 1996). The speed of the ovicidal or larvicidal effect will depend on the quality and quantity of the toxic bioactive secondary metabolite in the extract and the final yield in crude extract and the above-mentioned factors affecting it. As noticed in infected marigold,

Calendula officinalis inoculated with *Meloidogyne javanica* raised the flavonoid, triterpenoid compounds, and ursolic acid levels (Alijani *et al.,* 2015).

Intercropping of *Tagetes erecta* and *T. patula* in eggplants field decreased all phytonematodes in the soil and the population reduces gradually (Prasad *et al.,* 1992). The population reduction resulted from antagonistic marigold to nematode in infested fields (Ying e*t al.,* 2016). All root exudates were nematotoxic, the mortality of the nematodes increasing with increasing concentration of exudates and exposure period (Stephan *et al.,* 2001; Wani and Ansari, 1993). The nematode population was also affected adversely in soils treated with the root extract of marigold *(Aggarwal et al.,* 2005).

Nematodes infection was suppressed by applying 10 ml and 20 ml/plant of extracts, enhanced tomato plant development (Mennan *et al.,* 2000). While immersion in any of the extracts (*Tagetes minuta*) for 2 days was sufficient to kill all *Meloidogyne* J2 for *Paratylenchus* spp. and *Xiphinema* spp. (Toida, 1972). The aqueous extract of *Calendula* (marigold) caused a reduction of nematode females and egg masses (78.30 and 70%) (Montasser *et al.,* 2012).

Aqueous extracts in most previous studies proved that polar constituents were responsible for the nematicidal activity (Verma e*t al.,* 1989). Water as the most common solvent used in irrigation and necessary for nematodes survival is the optimum for delivery of polar constituents of the extract to the infection zone. The aqueous seed extract was also more effective in reducing both gall formation and nematode populations in the soil. The effect of pre-planting treatment with either extract was more considerable than post-planting treatments. The efficiency increased with raising the extract concentration (Olabiyi, 2006). The aqueous plant extract also increased the tomato vegetative growth parameters e.g. *Calendula* (Montasser *et al.,* 2012), *Tagetes erecta* treatment (Kamali and Karegar, 2016; Olabiyi, 2006), and *Melia azedarach* (Stephan *et al.,* 2001). but Maximum RKN reduction was obtained in marigold followed by datura (Singh and Devi, 2012). The increase in yield might be attributed to a reduction of nematode densities in soil by marigold. Also, marigold plant materials may serve as organic manure and provide nutrients for rice growth (Polthanee and Yamazaki, 1996).

Conclusion

From current *in vivo* investigation, aqueous plant extracts of chinaberry, datura, marigold and oleander possessed potential nematicidal activity against root-knot nematode, *M. incognita* and might be attributed to the presence of secondary metabolites i.e. flavonoids, phenolic and saponins which determined quantitatively. While a future qualitative analysis will explain their nematicidal effect against eggs and infective juveniles of *M. incognita* and improvement biomass of pepper plants under greenhouse conditions. Although the aqueous plant extracts are ineffective enough to surpass livestock animal manures, might be considered an inexpensive method with less feasibility and used in organic farms or with medicinal and aromatic plants.

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ARABIC SUMMARY

التحليل الكيميائي النباتي لبعض مستخلصات الأوراق النباتية المائية ونشاطها الإبادي ضد نيماتودا تعقد الجذور *Meloidogyne incognita* على الفلفل

عبد الهادي عبد الحميد إبراهيم علي - رمضان محمد العشري – محمود محمد رمضان
قسم وقاية النبات - كلية الزراعة - جامعة الزقازيق – مصر

نتيجة تعدد التأثيرات السلبية للمبيدات الكيماوية على البيئة، استهدفت الدراسة تقييم التأثير النيماتودي المحتمل لمستخلصات الأوراق المائية تركيز 0.1 جم/مل (10% وزن/حجم) من الزنزلخت، الداتورة، القطيفة، الدفلة على فقس بيض نيماتودا تعقد الجذور *Meloidogyne incognita* وأيضا موت الأطوار اليرقية معمليا، إلى جانب تقييم كفاءة تلك المستخلصات في قمع نيماتودا تعقد الجذور على نباتات الفلفل المعداه والمعاملة بطريقة غمر التربة بمحلول المستخلص بمعدلات وأوقات تطبيق مختلفة في الصوبة. أظهر التحليل الكيميائي النباتي وجود مركبات الفلافونويدات والفينولات و السابونينات والتانينات في جميع المستخلصات النباتية بمستويات مختلفة وسجل أعلي محتوي من هذه المواد النباتية في مستخلصي القطيفة والزنزلخت يليهما مستخلص الدفلة وأقل محتوى في مستخلص الداتورة.

معمليا حقق المستخلص المائي القياسي للقطيفة أعلى تأثير كمانع لفقس بيض نيماتودا تعقد الجذور يليه مستخلص الداتورة ثم الزنزلخت ثم الدفلة مسجلا 91.43 و59.78 و56.88 و55.48% بعد 5 أيام من التعرض على التوالي. أظهر مستخلص القطيفة قدرة تثبيطيه عالية كمانع للفقس ضد كتل البيض حتى مع انخفاض التركيز بمقدار النصف (5% وزن/حجم) على عكس باقي المستخلصات التي أظهرت تفوقها على البيض المفرد. كان الترتيب التنازلي لفاعلية المستخلصات هو القطيفة ثم الدفلة ثم الداتورة والزنزلخت مسجلا 75.60 و65.60 و54.20 و52.20% موت علي التوالي بعد 5 أيام من المعاملة بتركيز 10 %، ولقد توافق التأثير القاتل على الأطوار اليرقية للمستخلصات بشكل مباشر مع التركيز ووقت التعرض.

تحت ظروف الصوبة، أحدثت المستخلصات زيادة معنوية في وزن نباتات الفلفل المصابة وانخفاض في تكاثر نيماتودا تعقد الجذور (عدد الأطوار اليرقية/100 جم تربة)، وأعداد كتل البيض بجانب عدد العقد الجذرية وحجمها مقارنة بالنباتات المصابة. ومن بين المعاملات المختلفة، تفوق تأثير الأوكساميل يليه روث الدجاج المتحلل ثم مستخلص القطيفة. وحققت المعاملة التي استخدم فيها مستخلص القطيفة مرتين تحسين معنوي في نمو النباتات وتقليل تقدم الإصابة بالنيماتودا.