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Biocidal and Antinemic Properties of Aqueous Extracts of *Ageratum* and *Coccinia* Against Root-Knot Nematode, *Meloidogyne Incognita In Vitro*

Mohd Asif[1*], Moh Tariq, Amir Khan and Mansoor A. Siddiqui

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ABSTRACT

Plants are the one of the glorious and fascinating source of natural biopesticides. The present investigation was carried out for pytochemical analysis and to assess the nematicidal and nematostatic potential of aqueous extract of some plant parts viz., Ageratum conyzoides and Coccinia grandis against hatching and nematicidal behavior of root-knot nematode, Meloidogyne incognita in vitro conditions. The second stage juveniles (J2) were exposed at 24, 48 and 72 hours in different concentration (S, S/2, S/10, and S/100) of plant extracts. During in vitro condition inhibition of egg hatchability and J_2 juveniles' mortality varied according to the concentration of plant extract. The extract of Ageratum conyzoides leaves and stem exhibited highly promising mortality (98-100%) after 72 hours of exposure periods while the plant extract of C. grandis leaves and fruit showed minimum mortality (56-60%) after 24 hours of exposure periods. There was a gradual decrease in egghatching with an increase in the concentration of aqueous extract of plants. A. conyzoides leaves and stem extract elucidating most effectiveness in reducing egghatching and increase in mortality of J_2 juveniles of M. incognita. Concentration of the extract was directly proportional to the mortality of second stage juveniles and inversely proportional to the egghatching. Phytochemical analysis in various solvent of different polarity such as ethanol, methanol, acetone, chloroform, petroleum and water marked the impressive outcome of alkaloids, saponins, tannins, flavonoids, protein, amines, glycosides, carbohydrate, steroids, mucilage, gums, terpenoids and phlobatannins in the plant extracts. The aqueous extract of Ageratum conyzoides showed maximum egg inhibition and J_2 juvenile mortality due to the presence of phytoconstituents such as alkaloid, tannins, phenol, saponins, glycosides, flavonoids, carbohydrate, protein, mucilage/gum and phlobatannins while Coccinia grandis showed minimum egg inhibition and juvenile mortality.

Keywords: *Plant extract, Egghatching, Juvenile mortality, phytochemical analysis.*

INTRODUCTION

Plants are an important source of naturally occurring pesticides which may serve as safer alternatives for synthetic nematicides of which research in this area has increased manifolds (Ujvary, 2002). About three quarters of the World's population relies on plants and its extracts for health care (Premanathan *et al.,* 2000; Gabhe *et al.,* 2006). Plant extracts containing volatile compounds (Brown and Morra, 1997), especially essential oils, have been found to possess antimicrobial, insecticidal and nematicidal activity (Digrak, 1999; Okoko, *et al*., 1999). Flora of the nature is the reservoir of large number secondary metabolites *viz*., alkaloids, flavonoids, phenol etc to defend themselves against various diseases and pests. A numbers of plants which having nematicidal properties have been investigated. The application of extracts either enabled the plants to resist the nematode invasion or activated directly the defense mechanisms of plants (Mukhtar *et al*., 2013).

[1.] Section of Plant Pathology and Nematology, Department of Botany, Aligarh Muslim University, Aligarh, India. *asifgc2616@gmail.com*

Phytochemistry is concerned with the chemical study of these plant constituents (Evans, 2002). Phytochemicals have been recognized as the basis for global traditional herbal medicine (Lalitha and Jayanthi, 2012).

Plant parasitic nematodes and soil borne pathogen also attack a wide range of vegetables reducing its yield quality and quantity(Nchore *et al*., 2011), where *Meloidogyne incognita* was found constantly associated with the vegetable crops (Asif *et al*., 2016) and population of nematode was influenced by moisture, temperature, clay and silt percentage (Asif *et al*., 2015). The symptoms of root-knot nematode attack on crops include root galling, chlorosis, stunted growth and poor yields and sometimes over all crop failure were also observed. The estimated yearly crop loss due to root-knot nematode is $100 billion worldwide (Oka *et al*., 2000). Estimated overall average annual yield loss on the world's major crops due to damage by plant parasitic nematodes is 12.3 % (Ravichandra, 2008). In India, a loss to the extent of Rs. 21,068.73 million in 24 different crops was estimated (Jain *et al.,* 2007).

Nematodes are difficult to control because of their wide host range and high rate of reproduction, with females capable of producing up to thousand eggs / female and caused the serious yield losses on a wide range of crops (Natarajan *et al*., 2006). The root-knot nematode, *Meloidogyne incognita* were controlled through the use of cultural methods, chemical nematicides, crop rotation (Chitwood, 2002), resistant varieties and biocontrol agents (Mukhtar and Pervaz, 2003).The use of synthetic nematicides has been the most effective method for the management of plant-parasitic nematodes, but their high cost and hazardous effect on human health, environment and ground water contamination create a necessity to search new, cheap, eco-friendly and harmless methods of nematode control (Chitwood, 2003). Therefore, the use of plant extracts and phyto-products is gaining attention due to their availability, cost effectiveness, proven nature of specificity, no biodegradability, low toxicity and minimum residual toxicity in the ecosystem (Maji *et al*., 2005).

A number of organic components of plant origin, including oil-seed cakes, chopped plant parts and plant extracts have been used as nematode control agents (Akhtar and Alam, 1993; Tiyagi *et al.,* 2009a; Parihar *et al.*, 2012). Botanical extracts that contain alkaloids and flavonoids were found to have ovicidal property against *Meloidogyne* eggs (Adegbite, 2003). Aqueous leaf extract of extracts of *Acyranthes aspera* and *Solanum xanthocarpum* was found highly toxic and showed 100% inhibition in the egg hatching and juvenile mortality of *Meloidogyne incognita* (Asif *et al*., 2017). Similarly, aqueous extract of *Datura stramonium* was also found to be more effective on the second stage juveniles of *M. javanica* in tomato plants (Al-Saba *et al.,* 2001). However, the effect of pesticide over use and misuse around the world has led to costly environmental pollution and disruption of the balance of nature (IITA, 2000).

In recent years, studies have shown the importance of natural nematicidal compounds in the plants themselves that have potential to suppress nematode populations (Pavaraj *et al*., 2010; Moosavi, 2012; Nelaballe and Mukkara, 2013; Muniasamy *et al.*, 2010). Many botanical extracts have been found to contain phytochemicals such as alkaloids, tannins, saponins, flavonoids, diterpenes, glucosinolates, acetylenes and thinlys (Chitwood, 2002) which are effective against plant parasitic nematodes (Goswani and Vijayalakshmi, 1986; Adegbite, 2003). Hence the study was conducted with the aim of phytochemical screening of *Ageratum conyzoides* and *Coccinia grandis* and to characterize the main constituents responsible for the inhibition of egg hatching and second stage juvenile (J2) mortality of *Meloidogyne incognita in vitro*.

MATERIALS AND METHODS

Culture for the nematode inoculum

Pure nematode culture was prepared in brinjal. Highly infected roots of brinjal (*Solanum melongena* L.) family- Solanaceae was uprooted gently so that eggmasses don't get detached from the root and washed in distilled water properly until whole of the soil debris get removed then placed in 15 mesh sieves (8 cm in diameter) having crossed layer of tissue and placed in petridishes having water just deep enough to contact the eggmasses which can favour the juvenile hatching.

Preparation of extract

Leaves stem and fruit of two different plants species *viz., Ageratum conyzoides* (Family-Astraceae) and *Coccinia grandis* (Family-Cuccurbitaceae) were collected from Aligarh Muslim University campus, Near Allama Iqbal Hall, thoroughly washed and chopped. Chopped leaves (25g) of each sample were soaked in 75 ml distilled water and kept overnight. The preparation of extract was taken through the grinding of the leaves through mortar and pestle. The prepared paste was passed through muslin cloths to exclude all the plant debris and then filtered through Whatman's filter paper No.1. The filtrate was named as standard extract (S) designated as (100%). This standard extract was diluted to S/2, S/10 and S/100 by adding distilled water (DW). Distilled water served as control.

Mortality test

For mortality experiment, 5 ml of water suspension containing 100 second stage juveniles (J2) of *Meloidogyne incognita* were transferred to 40 mm petridish having different concentrations (S, S/2, S/10 and S/100) of leaf extract of different plant species separately (Alam, 1985). Each treatment contains three replicates. The petridishes were kept at 28 °C in Biological Oxygen Demand (BOD). The immobilized juveniles were counted after 24, 48 and 72 hrs of the exposure period. The death of juveniles was confirmed by transferring immobilized juveniles into water for 1 h and mean percentage mortality was calculated.

Hatching test

Five fresh and uniform size eggmasses were picked from thoroughly washed roots of brinjal infected with root-knot nematode, *M. incognita*. The collected eggmasses were relocated to 40 mm petridishes having 5 ml of leaf extract of different dilutions (S, S/2, S/10 and S/100) separately. Each treatment has three replicates. After 6 days number of hatched juveniles was observed with the help of counting dish under stereoscopic microscope and percent inhibition over control was calculated. Distilled water served as control for hatching.

Sample collection

Ageratum conyzoides and *Coccinia grandis* were collected locally from the campus of Aligarh Muslim University, Aligarh for the purpose of their phytochemical analysis. Fresh and tender parts of selected plants were used for phytochemical analysis. Plant species were selected during present investigation was given in Table 01.

Preparation of plant extract

Selected parts were picked up from the plants and then washed under running tap water to remove dust particles. The leaves, stem and fruit of plants were then air dried and crushed into powder, stored in polythene bags for use. The plant powder was transferred into test tube and required amount of distilled water was added for the soaking of powder properly. The solution was filtered through filter paper and filtered extract of the selected plant were tested further for pytochemical analysis.

Table 01: Ethnobotanical information of selected medicinal plant species for phytochemical analysis

Plants	Local name	Part used	Family
Ageratum conyzoides	Goat weed, White weed	Leaves	Asteraceae
Ageratum conyzoides	Goat weed, White weed	Stem	Asteraceae
Coccinia grandis	Ivy gourd, Scarlet gourd	Leaves	Cuccurbitaceae
Coccinia grandis	Ivy gourd, Scarlet gourd	Fruit	Cuccurbitaceae

Solvent Extraction

5g dried powder of each plant sample were separately immersed in 50ml of each water, ethanol, acetone, methanol, chloroform and petroleum. The solution was kept at room temperature for 24 hrs and filtered with Whatman's No. 1 filter paper. The filtrate was used for the phytochemical screening using the following tests.

PHYTOCHEMICAL SCREENING

Test for Alkaloids (Wagner's reagent)

A fraction of extract was taken in test tube and treated with 3-5drops of Wagner's reagent (1.27g of iodine and 2g of potassium iodide in 100ml of water) and examine for the formation of reddish brown precipitate (or colouration).

Test for Flavonoids (Alkaline reagent test)

2ml of extracts was treated with few drops of 20% sodium hydroxide solution. Formation of intense yellow colour which becomes colourless on addition of dilute hydrochloric acid, indicates the presence of flavonoids.

Test for Phenols (Ferric chloride test)

A small amount of the extracts was treated with aqueous 5% ferric chloride and inspect for formation of deep blue or black colour.

Test for Phlobatannins (Precipitate test)

2ml of extract was added with 1ml of 1% aqueous hydrochloric acid and then boiled formation of red precipitation represents the presence of phlobatannins.

Test for Amino acids and Proteins (1% ninhydrin solution in acetone).

To 2ml of filtrate add 2-5 drops of ninhydrin solution placed in a boiling water bath for 1-2 minutes and examine for the formation of purple colour.

Test for Saponins (Foam test)

To 6 ml of the water 2 ml of the extract was added. The mixture was shaken vigorously and formation of persistent foam confirms the presence of saponins.

Test for Tannins (Braymer's test)

To 2ml of extract 10% alcoholic ferric chloride added. Formation of blue or greenish colour solution confirms the presence of Tannins.

Test for Terpenoids (Salkowki's test)

1ml of chloroform was mixed to 2ml of each extract followed by a few drops of concentrated sulphuric acid. Reddish brown precipitate formation confirmed the presence of terpenoids.

Test for steroids

1mg crude plant extracts was mixed with 10 ml chloroform, followed by equal volume of concentrated sulphuric acid to the test tube by sides. Conversion of upper layer into red and sulphuric acid layer represent yellow with green fluorescence confirmed the presence of steroids.

Test for Carbohydrates (Molisch's test)

To 2ml of the extracts few drops of Molisch's reagent were added. This was followed by addition of 2ml of conc. sulfuric acid to the test tube. This mixture was then allowed to settle for two-three minutes. Presence of a red or dull violet colour at the interphase of the two layers indicates the presence of carbohydrate.

Test for gum and Mucilage

10 ml of distilled water was treated with the extract (100 mg) and then 2 ml of absolute alcohol was added with constant stirring. Formation of white or cloudy precipitate revealed the presence of gums and mucilage.

Test for Glycosides

50 mg of extract was hydrolyzed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate confirmed the presence of positive test.

a. Borntrager's test

To 3 ml of chloroform 2 ml of filtered hydrolysate was added and shaken, chloroform layer is separation take place followed by 10% ammonia solution. Appearance of pink colour represents the positive test.

b. Legal's test

50 mg of extract was mixed with pyridine then sodium nitroprusside solution was added to make alkaline using 10% NaOH. Formation of pink colour indicates the presence of glycoside.

RESULTS

The nematicidal effect of different parts of plants *viz.,* stem, leaves and fruit extract is shown in table 02 and Figure 01. Among the given treatment maximum hatching of second stage juveniles (J2) was observed by *Coccinia grandis* leaves 18, 62, 72 and 94 followed by *Coccinia grandis* fruit 14, 43, 56, 81, *Ageratum conyzoides* stem 8, 44, 58, 64 respectively in different concentration *viz.,* S, S/2, S/10, S/100 and least 4, 38, 49, 52 have been showed by *Ageratum conyzoides* leaves in same concentration. The results indicated that leaf and stem extract of *A. conyzoides* was found to be more effective in comparison of *C. grandis* leaves extract. It showed that as dilutions increases the toxicity in response to egg inhibition decreases. The control recorded 100 % egg hatchability because it contained only distilled water. Maximum hatching was observed in control (340). The percent inhibition of second stage juveniles hatching in comparison to distilled water decreased with increased in the concentration of extracts (S, S/2, S/10, and S/100). The data suggested that standard concentration of leaves extracts of *A. conyzoides* showing 98.82 % inhibition of J2 hatching. Other dilutions *viz.,* S/2, S/10 and S/100 were less effective as compared to standard concentration (S). It was observed that as the extract was diluted, the nematostatic and nematicidal properties of extract was decreased. The inhibition in eggs hatching of *M. incognita* generally increased with increasing in the exposure period and concentrations of extract. Distilled water (C) shows no mortality.

The result present in table 03 albic, and d showed that at 24 hrs of the exposure *A. conyzoides* leaves and stem showed pronounced mortality of J2 of *M. incognita* 72, 59, 50, 30 and 66, 50, 42, 27 respectively and least was observed in *C. grandis* 56, 40, 30, 17 in different concentration *viz.,* S, S/2, S/10, S/100 as compare to distilled water (DW) control 0. Mortality of *Meloidogyne incognita* juveniles depend upon time and concentration of the extract. The highest mortality of second stage juveniles (J2) at 48 hrs of the exposure was depicted by the aqueous extract of *A. conyzoides* leaves 86, 66, 57, 38 and *A. conyzoides* stem 78, 58, 48, 30 and lowest percent mortality was noticed in *C. grandis* leaves 62, 44, 36, 24 in different concentration *viz*., S, S/2, S/10, S/100

as compared to distilled water (DW) control 0. *A. conyzoides* leaves extract showed most prominent mortality of 100, 76, 67, 50 followed by *A. conyzoides* stem 98, 63, 58, 34, *C. grandis* fruit 86, 63, 58, 34 and *C. grandis* leaves 68, 48, 39, 29 respectively in different concentration *viz.,* S, S/2, S/10, S/100 after 72 hours of exposure time. In all the three durations 24hrs, 48hrs and 72hrs of the exposure time, 24 hrs treatment showed minimum mortality while 72 hrs of the exposure showed maximum mortality. Standard (S) concentration of the extracts and 72 hrs exposure periods represented maximum toxicity compare to all dilutions of the extract and exposure period. The mortality rate of J2 of *M. incognita* notably increased with the increase in concentrations of extract and the exposure time. The leaf and stem extract of *A. conyzoides* showed maximum toxicity. It was found that aqueous extract of above weeds *viz., A. conyzoides* and *C. grandis* was found to be toxic in nature in causing mortality of second stage juveniles (J2) of *Meloidogyne incognita*. During the experiment, the *A. conyzoides* leaves extract shows most prominent effect on second stage juveniles, it might be due to the presence of various types of phytoconstituents in different solvent viz., alkaloids, phenols, terpenoids, flavonoids, carbohydrate, protein etc while *Coccinia grandis* was found to be least effective.

Table 02: Effect of aqueous extract of fresh chopped leaves of different weeds species on the

Treatment	Part used	No. of juvenile hatched different concentration at 6 days				
		S	S/2	S/10	S/100	DW
Ageratum conyzoides	Leaves	4 (98.82%)	38 (88.82%)	49 (85.58%)	52 (84.70%)	340 (0%)
Ageratum conyzoides	Stem	8 (97.64%)	44 (87.05%)	58 (82.94%)	64 (81.17%)	340 (0%)
Coccinia grandis	Fruit	14 (95.88%)	43 (87.35%)	56 (83.52%)	81 (76.17%)	340 (0%)
Coccinia grandis	Leaves	18 (94.70%)	62 (81.76%)	72 (78.82%)	94 (72.35%)	340 (0%)

Each value is an average of three replicate
DW=Distilled Water (Control)
Values for percent inhibition in juvenile hatching over control are given in parentheses.

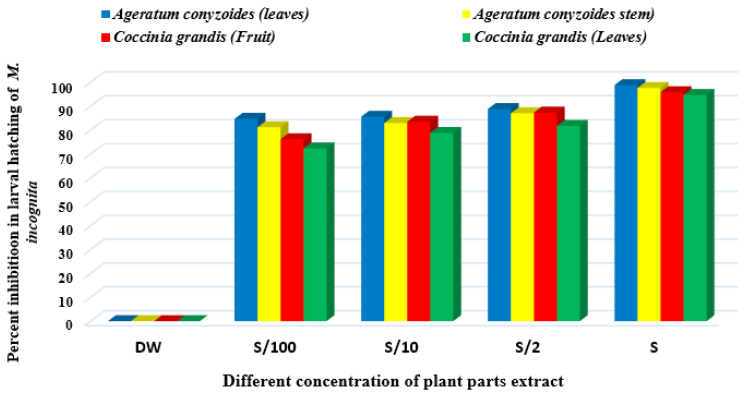

Figure 01: Percentage of inhibition of J2 hatching in aqueous extracts of plant species

Table 03(a): Phytochemical analysis of Coccinia grandis fruit in various solvent of different polarity

Phytochemicals	Ethanol	Methane	Chloroform	Petroleum	Aqueous	Acetone
Alkaloid	+	+	-	-	+	-
Flavonoid	+	+	+	-	-	-
Saponins	-	-	-	-	+	-
Carbohydrates	+	+	-	-	+	-
Gums/ Mucilage	+	-	-	-	-	-
Phenol/ Tannins	+	+	+	-	-	-
Terpenoids	+	+	-	+	+	+
Protein/ Amino	-	-	-	+	+	-
Steroids	-	+	-	-	+	-
Glycosides	+	+	-	-	+	-
Phlobatannins	+	-	-	-	+	-

+ = indicates presence of phytochemicals and
- = indicates absence of phytochemicals

Table 03(b): Phytochemical analysis of *Coccinia grandis* leaves in various solvent of different polarity

Phytochemicals	Petroleum	Chloroform	Ethanol	Aqueous	Methanol	Acetone
Alkaloids	-	+	+	+	+	-
Saponins	-	-	+	-	-	-
Tannins	-	-	+	+	-	+
Flavonoid	-	-	-	+	+	-
Protein / Amino	-	+	+	+	+	-
Glycosides	-	+	-	+	-	+
Carbohydrate	-	-	+	+	+	+
Steroids	+	-	+	+	-	-
Mucilage/ Gums	-	+	-	+	-	-
Terpenoids	-	-	+	-	+	+
Phlobatannins	-	-	+	+	-	-

+ = indicates presence of phytochemicals and
- = indicates absence of phytochemicals

Table 03 (c): Phytochemical analysis of *Ageratum conyzoides* leaves in various solvent of different polarity

Phytochemicals	Methanol	Ethanol	Water	Acetone	Chloroform	Petroleum
Alkaloid	+	-	+	-	+	-
Tannins/phenol	+	-	+	+	-	-
Saponins	+	+	+	+	-	-
Glycosides	+	+	+	+	-	-
Flavonoids	-	+	+	+	-	-
Carbohydrate	+	-	+	-	-	-
Protein	+	+	+	-	+	-
Terpenoids	-	+	-	+	+	+
Mucilage/ gum	-	+	+	-	-	-
Steroids	+	-	-	+	+	+
Phlobatannins	+	+	+	-	-	-

+ = indicates presence of phytochemicals and
- = indicates absence of phytochemicals

Table 03(d): Phytochemical analysis of *Ageratum conyzoides* Stem in various solvent of different polarity

Phytochemicals	Methanol	Ethanol	Water	Acetone	Chloroform	Petroleum
Alkaloid	+	+	+	+	+	-
Tannins/Phenol	+	+	+	-	-	-
Saponins	-	+	+	-	-	-
Glycosides	-	+	-	+	+	-
Flavonoids	+	+	+	+	-	-
Carbohydrate	+	-	-	+	+	+
Protein/ Amino	-	+	+	+	-	-
Terpenoids	+	+	+	-	-	-
Mucilage/ Gum	-	+	-	+	+	-
Steroids	+	+	+	-	-	+
Phlobatannins	-	-	-	+	-	-

+ = indicates presence of phytochemicals and
- = indicates absence of phytochemicals

Table 04: Effect of water extract of chopped leaf of different plant species on the mortality of *Meloidogyne incognita juveniles in vitro* after 24, 48 and 72 hours.

Treatment	Part used	Duration (Hrs.)	Percent mortality in different extracts					Regression Equation
			S	S/2	S/10	S/100	DW	
Ageratum conyzoides	Leaves	24	72 (76.8%)	59 (59.5%)	50 (42.2%)	30 (24.9%)	0 (7.6%)	Ỹ= 42.2+17.3(x-2)
		48	86 (89.4%)	66 (69.4%)	57 (49.4%)	38 (29.4%)	0 (9.4%)	Ỹ = 49.4+20(x-2)
		72	100 (103.8%)	76 (81.2%)	67 (58.6%)	50 (36%)	0 (13.4%)	Ỹ= 58.6+22.6 (x-2)
Ageratum conyzoides	Stem	24	66 (68.0%)	50 (52.5%)	42 (37.0%)	27 (21.5%)	0 (6.0%)	Ỹ= 37+15.5 (x-2)
		48	78 (79.6%)	58 (61.2%)	48 (42.8%)	30 (24.4%)	0 (6.0%)	Ỹ= 42.8+18.4 (x-2)
		72	98 (95.6%)	63 (73.1)	58 (50.6%)	34 (28.1%)	0 (5.6%)	Ỹ= 50.6+22.5 (x-2)
Coccinia grandis	Fruit	24	60 (61.4%)	47 (47.2%)	33 (33.0%)	25 (18.8%)	0 (4.6%)	Ỹ= 33+14.2 (x-2)
		48	69 (73.08%)	55 (56.74%)	48 (40.4%)	30 (24.06%)	0 (7.72%)	Ỹ= 40.4+16.34 (x-2)
		72	86 (90.36%)	63 (69.28%)	58 (48.2%)	34 (27.12%)	0 (6.04%)	Ỹ= 48.2+21.08 (x-2)
Coccinia grandis	Leaves	24	56 (55.6%)	40 (42.1%)	30 (28.6%)	17 (15.1%)	0 (1.6%)	Ỹ= 28.6+13.5 (x-2)
		48	62 (62.0%)	44 (47.6%)	36 (33.2%)	24 (18.8%)	0 (4.4%)	Ỹ= 33.2+14.4 (x-2)
		72	68 (69.36%)	48 (53.08%)	39 (36.8%)	29 (16.52%)	0 (4.24%)	Ỹ= 36.8+16.28 (x-2)

Each value is an average of three replicate; DW=Distilled Water (Control)
Values for percent inhibition in juvenile hatching over control are given in parentheses

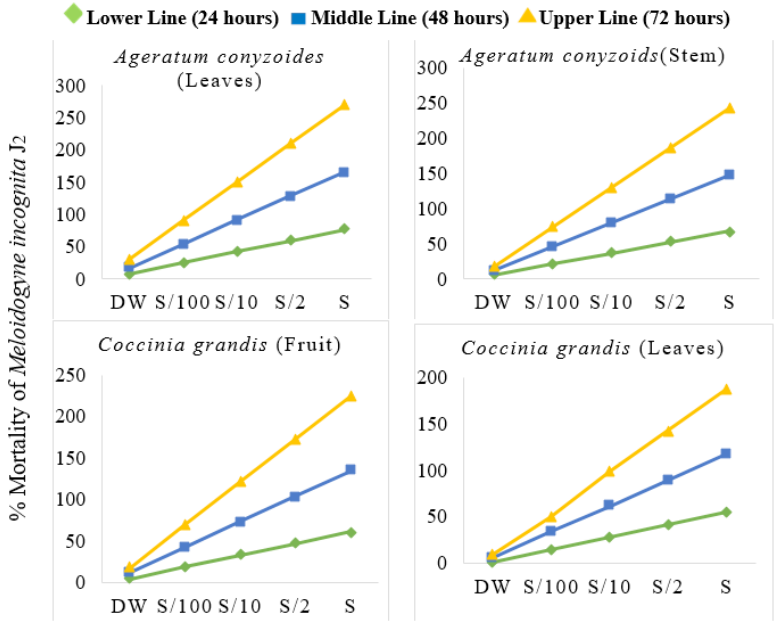

Figure 02: Regression lines showing linear relationship between different concentration of leaf extract of some plants and percent mortality of *Meloidogyne incognita*

DISCUSSION

In the present study, the phytochemical analysis of the extract of *A. conyzoides and C. grandis* revealed the presence of alkaloids, saponins, flavonoids, phenols, tannins, steroids and glycosides. The aqueous extract of *A. conyzoides* shows marked nematostatic as well as nematicidal activity due to the presence of certain chemical constituents in the extract. Pronounced inhibition in egghatching and juvenile mortality may be possible due the presence of secondary metabolites such as phenols, alkaloids, flavonoids, phlobatannins, saponins, tannins, steroids and glycosides. The *A. conyzoides* elucidate the most negative impact against the root knot nematodes *M. incognita* causing 100 % mortality after 72 hrs of exposure time while the *C. grandis* leaves and fruit extract was found to be less inhibitory and toxic to the juveniles. A positive correlation has been setup between the juvenile's mortality, concentration of extract and the exposure time. Our results are in confirmation with Orisajo *et al.,*(2007); Asif *et al*., (2013) and Ganai et al., (2013. Nematicidal activity of *A. mexicana* might be attributed to its contents of alkaloids (berberine, protopine, sarguinarine), amino acids, phenolics, fatty acids (myristic, palmitic, oleic, linoleic acids) and triglyceride (sn-glycerol-1-eicosa-9,12- dienoate-2-palmitoleate-3-linoleate (Facchini, 2001 and Shaukat *et al.*, 2002).

The quantity and quality of bioactive chemicals present in plant parts may vary from one part to another. Leaf extracts in the form of organic additives also released some phenolic compounds/nutrients which accelerated rapid root development and overall plant growth thus help the plants to develop resistance against nematode attack. Plants in the form of byproduct release some secondary metabolites which either enhance the plant growth or reduce the nematode infestation. These compounds are known as alkaloids, diterpenes, fatty acids, glucosinolates, isothiocyanates, phenols, polyacetlenes, sesquiterpenes and thinly (Chitwood, 2002; Bruneton, 1999; Ma *et al*., 1998). *Lantana camara* also contain pentacyclic triterpenoids described as camaric acid, lantanilic acid and olenolic acids shows nematicidal activities against J_2 of *M. incognita* (Shaukat and Siddiqui, 2001). Aoudia *et al*. (2012) observed that phenolic compounds isolated from *Melia azedarach* as gallic acid, ferulic acid, *p*-hydroxybenzoic acid and caffeic acid showed toxicity against *M. incognita*. Cinnamyl acetate purified from *Cinnamomum aromaticum* showed 100% inhibition in *M. incognita* J2 movement after incubation for 50 min with 100 µg/ml (Nguyen *et al*., 2012). Abdalla *et al*. (2008) reported that methanol and hexane extracts depicted nematicidal property against root-knot nematode, *M. incognita* in the laboratory and also noted that extracts that contain alkaloids and flavonoids have ovicidal property against *Meloidogyne* eggs.

The selected plant parts are the source of the secondary metabolites *viz.,* alkaloids, flavonoids, terpenoids, phlobatannins, mucilage, gums, protein, carbohydrate, saponins, tannins, glycosides, terpenoids, phenol and steroids. Above mentioned secondary metabolites posses nematicidal and nematostatic activity. Nematicidal properties of some phytochemicals (saponins, phlobatannins, protein, flavonoids and glycosides) content extracted by these plant leaf or oxygenated compounds that enable them to dissolve the cytoplasmic membrane of the nematode cells and their functional groups interfering with enzyme protein structures of nematodes (Trifonovo and Atanasov, 2009). Cavoski *et al*. (2012) reported that *M. azedarach* fruits containing aldehydes, carboxylic acids and alcohols were used to control *M. incognita* on cucumber. Phytochemical analysis also revealed that plant is rich in alkaloids, phenols, terpenoids, and flavonoids etc have high rate of nematicidal activity (Pavela, 2004). Plant material based phytochemicals are known to possess the effective control of plant-parasitic nematodes (Chitwood, 2002), including root-

knot nematodes (Jourand *et al*., 2004; Hussain *et al*., 2011). Ranjit singh *et al*. (2009) found that plant extracts that contained alkaloid either singly or in combination inhibited egghatch of *Meloidogyne* spp. on Soybean. Pentacyclic alkaloid serpentine isolated from *Catharanthus roseus* root induced death and inhibited hatching of *M. incognita* at 0.2% (Chandravadana *et al*., 1994). The motility of *M. incognita* juveniles was also significantly reduced by exposure to eight different steroid and triterpenoid saponins from plants related to garden asparagus (Chitwood, 2002). In this investigation the extract of *A. conyzoides* and *C. grandis* having a large number of active compounds which include flavonoids, saponins, tannins, gums, glycosides, carbohydrate, steroids and alkaloids, from different plant parts like stem, leaves, and fruits, have been shown to possess nematicidal and nematostatic activity.

The present study gives assurance that aqueous extracts of *A. conyzoides* and *C. grandis* contain biological nematicidal compounds and may be used for the management of the root-knot nematode. Egg inhibition and juvenile mortality in aqueous extract in *A. conyzoides* was an outcome of the presence of secondary metabolites such as alkaloid, tannins, phenol, saponins, glycosides, flavonoids, carbohydrate, protein, mucilage/gum, phlobatannins in the leaves and fruits. Our results also corroborates with Asif *et al,* (2017), Abolusoro, (2005) and Ansari, *et al*., (2016). Further study is necessary to quantify and identify the phytoconstituents so that the exact mechanism can be drawn through the involvement of natural biopesticides formulation for the management of nematodes.

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