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Original Article

Phytochemical management of root knot nematode (*Meloidogyne incognita*) kofoid and white chitwood by *Artemisia spp.* in tomato (*Lycopersicon esculentum* L.)

R. Khan[a], *I. Naz*[a], *S. Hussain*[a], *R. A. A. Khan*[b]*, *S. Ullah*[a], *M. U. Rashid*[c] and *I. Siddique*[a]

[a]Department of Plant Pathology, The University of Agriculture, Peshawar, Khyber Pakhtunkhwa, Pakistan

[b]Institute of Vegetable and Flowers, Graduate School of Chinese Academy of Agricultural Sciences, Beijing, P.R. China

[c]Department of Chemistry, Faculty of Arts & Basic Sciences, Balochistan University of Information Technology, Engineering and Management Sciences, Pakistan

*e-mail: asadraja@aup.edu.pk

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(With 3 figures)

Abstract

In vitro and screen house experiments were conducted to investigate the effectiveness of thirteen phytochemicals from *Artemisia elegantissimia* and *A. incisa* on root knot nematode, *Meloidogyne incognita* in tomato (*Lycopersicon esculentum* L.) cv. Rio Grande. A positive control (Carbofuran) and negative control (H_2O) were also used for comparison. Effectiveness of phytochemicals against juveniles (J2s) mortality and egg hatch inhibition were evaluated after 24, 48 and 72 hours of incubation at three concentrations viz; 0.1, 0.2 and 0.3 mg/mL in vitro conditions. Amongst thirteen phytochemicals, Isoscopletin (Coumarin), Carbofuran and Apigenin (Flavonoid) showed the highest mortality and egg hatch inhibition of *M. incognita* at all intervals. Inhibition of eggs and J2s mortality were the greatest (90.0%) and (96.0%) at 0.3 mg/mL concentration. Application of phytochemicals caused reduction in number of galls, galling index, and egg masses on tomato plant and enhanced plant growth parameters under screen house conditions. Gall numbers (1.50), galling index (1.00), number of juveniles (4.83) and egg masses (4.00) were greatly reduced and plant growth parameters such as; plant height (28.48 cm), fresh (72.13 g) and dry shoot weights (35.99 g), and root fresh (6.58 g) and dry weights (1.43 g) were increased significantly by using Isoscopletin. In structure activity relationship, juveniles of *M. incognita*, exhibited variations in their shape and postures upon death when exposed to different concentrations of phytochemicals of *Artemisia* spp. The present study suggests that *Artemisia* based phytochemicals possess strong nematicidal effects and can be used effectively in an integrated disease management program against root knot nematodes.

Keywords: Artemisia spp., *Meloidogyne incognita*, tomato, phytochemicals, eco-friendly management.

Manejo fitoquímico do nematoide do nódulo de raiz (*Meloidogyne incognita*) *kofoid* e do *chitwood* branco por *Artemisia spp.* em tomate (*Lycopersicon esculentum* L.)

Resumo

Experimentos *in vitro* e de triagem foram conduzidos para investigar a eficácia de treze constituintes fitoquímicos de *Artemisia elegantissimia* e *A. incisa* no nematóide de galhas, *Meloidogyne incognita* em tomateiro (*Lycopersicon esculentum* L.) cv. Rio Grande. Um controle positivo (carbofuran) e controle negativo (H_2O) também foram utilizados para comparação. A eficácia dos fitoquímicos contra a mortalidade juvenil (J2s) e a inibição da eclosão de ovos foram avaliadas após 24, 48 e 72 horas de incubação em três concentrações, tais como: 0,1; 0,2 e 0,3 mg/mL em condições *in vitro*. Dentre os treze fitoquímicos, isoscopletina (cumarina), carbofurano e apigenina (flavonoide) apresentaram a maior mortalidade e a inibição da eclosão de ovos de *M. incognita* em todos os intervalos. A inibição da mortalidade dos ovos e J2s foi a maior (90,0%) e (96,0%) na concentração de 0,3 mg/mL. A aplicação de fitoquímicos causou redução no número de galhas, índice de fricção e massa de ovos no tomateiro e melhorou os parâmetros de crescimento das plantas em condições de triagem. Números de galhas (1,50), índice de insetos galhadores (1,00), número de juvenis (4,83) e massas de ovos (4,00) foram bastante reduzidos e os parâmetros de crescimento das plantas, como altura da planta (28,48 cm), peso fresco (72,13 g) e seco (35,99 g), raiz fresca (6,58 g) e peso seco (1,43 g) foram significativamente aumentados usando isoscopletina. Na relação atividade estrutura, juvenis de *M. incognita*, exibiram variações em sua

forma e posturas após a morte quando expostos a diferentes concentrações de fitoquímicos de *Artemisia* spp. O presente estudo sugere que os fitoquímicos à base de artemísia possuem fortes efeitos nematicidas e podem ser usados eficazmente em um programa integrado de controle de doenças contra nematóides de galhas.

Palavras-chave: Artemisia spp., *Meloidogyne incognita*, tomate, fitoquímicos, gestão ecoamigável.

1. Introduction

Tomato (*Lycopersicon esculentum* L.) belongs to family Solanaceae. It is universally edible crop after potato and cultivated in fields, greenhouses and net houses of the world. Tomato is an important kitchen crop of Pakistan and is cultivated on 53.1 thousand hectares having an output of 536.2 tons with standard yield of 10.1 tons per hectares (Pakistan, 2012). Yield, growth and quality of the plants are affected directly or indirectly by certain biotic and abiotic factors. Reduction in yield of tomato is due to its susceptibility to many pathogens including nematodes, fungi, bacteria, and viruses. Nematode parasitism is greatly destructive and unmanageable issue. Root knot nematodes (RKNs) (*Meloidogyne* spp.) are the most destructive obligate semi-endoparasites (Fuller et al., 2008) and cause major problems by reducing yield. Losses of US$ 125 billions occurred annually due to RKNs (Moens et al., 2009). There are more than 100 described species of Meloidogyne that cause great destruction in cultivated crops (Van Hoenselaar and Karssen, 1998). *Meloidogyne incognita*, *M. javanica*, *M. hapla* and *M. arenaria* are of great significance for the reason of their wide spread distribution (Castagnone-Sereno, 2002). Specific symptoms caused by root knot nematodes on tomato include swelling/knots and galls all over the root system and affected plants may be killed in case of severe infestation (Sikora and Fernandez, 2005; Youssef, 2001). Controlling plant-parasitic nematodes through nematicides of organic synthesis has posed serious threats to humans, domestic animals and help in buildup of resistance in these pests (Veremis and Roberts, 1996). As such eco-friendly control methods are required to be employed. The use of phytochemicals in crop protection offers attractive potential as nematicidal agents that are well-known in several higher plants (Chitwood, 2002). Nematicidal extracts or phytochemicals are environment friendly. These compounds include attractants, repellents, nematotoxicants, and hatch inhibitors or stimulants either formed when nematode is present or constitutive (Chitwood, 2002). Researchers evaluated alternative management of plant parasitic nematodes by using plant extracts (Akhtar and Mahmood, 1994; Adegbite and Adesiyan, 2005). For example, studies have shown that Neem oil based formulations controlled the population of RKNs in tomato and chickpea (Khan et al., 2008; Javed et al., 2008). Gomes et al. (2018) reported 100% mortality of *M. incognita* by using *Synadenium grantii* latex proteases.

Researchers reported nematicidal activity of ethanol fraction from rhizomes of *Artemisia vulgaris* on hatching and mortality of *Meloidogyne* spp. and reported reduction in galling indices when extract was applied in a dose dependent manner (Costa et al., 2003). In an in-vitro study, the essential oil of worm wood (*A. annua*) leaves showed toxic effects on second stage juvenile of root knot nematodes, *M. incognita* (Shakil et al., 2004). Aqueous extracts of dried aerial parts caused mortality in more than 90% of juveniles (J2s) of *M. incognita* after a 24-hour contact (Dias et al., 2000). Extracts of dried foliage of *A. vulgaris* reduced the number of juveniles of *M. megadora* on *C. sativus* when applied to infested soil.

Artemisia spp. belongs to the family Asteraceae, commonly known as Mugwort, with about 500 species of which 38 species have been identified in Pakistan (Muhammad et al., 2010). More than 1000 biodynamic compounds and different types of secondary metabolites like polyacetylenes, phenolic hydrocarbons, oxygenated aliphatic hydrocarbons, Furans, Flavonoids, Alkamides and Coumarins have been reported from *Artemisia* spp. (Saadali et al., 2001). Most of the *Artemisia* compounds have pharmacologically revealed insecticidal, anti-ulcerogenic, anti-hyperglycemic, anti-spasmodic, anti-fungal, anti-cancer, anti-malarial, anti-bacterial, anti-oxidant, anti-histamine, anti-helmintic and anti-allergic activities (Bora and Sharma, 2011). Researchers reported that Coumarin-based nematicides caused alterations in structures of juveniles of *M. incognita* upon death when applied at different concentrations (Pan et al., 2016).

In Pakistan, information regarding nematicidal potential of phytochemicals against plant parasitic nematodes is scanty. Therefore, the current research was initiated to determine the nematicidal effect of phytochemicals from *Artemisia spp*., to achieve the following objectives; 1) To investigate nematicidal activity of phytochemical compounds from *A. elegantissima* Pamp and *A. incisa* Pamp against juvenile's mortality and eggs hatch inhibition of *Meloidogyne incognita* in-vitro and 2) To investigate screening of phytochemical compounds of *Artemisia* spp. against *M. incognita* in- vivo in tomato.

2. Material and Methods

2.1. In vitro experiments

2.1.1. Preparation of pure culture

Thirteen pure phytochemical compounds (Figure 1) previously isolated from *A. elegantissima* and *A. incisa* were obtained from Balochistan University of Information, Science Technology Engineering and Management (BUISTEM) (Rashid et al., 2017). Earthen pots (15 cm diameter) were filled with sterilized potting mixture containing clay and sand in a ratio of 2:1 v/ v. Three weeks old tomato seedlings were inoculated with mature egg mass

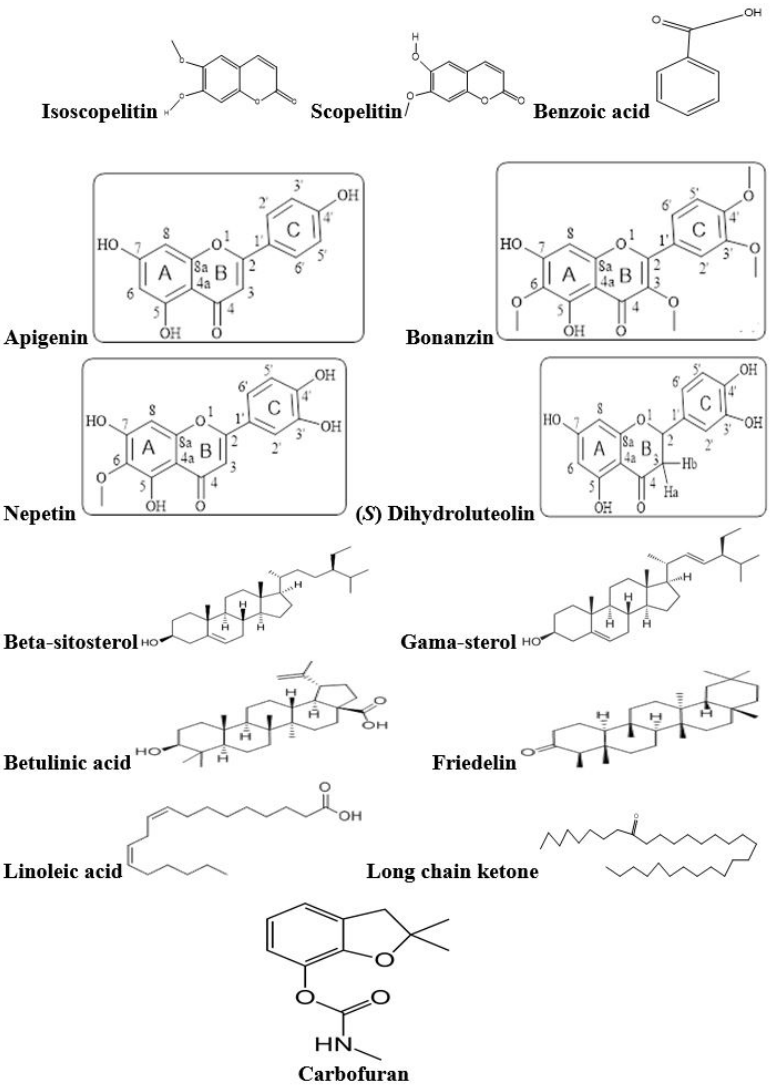

Figure 1. Structures of carbofuran and phytochemicals from *Artemisia elegantissima* and *Artemisia incisia*.

of the known *Meloidogyne* species (*M. incognita*) in pots (Naz et al., 2013a). Fresh tomato seedling of the same cultivar were inoculated with 11-15 egg masses obtained from the pure culture to prepare sub culture in order to obtain sufficient inoculum for the screen house experiment on tomato (Naz et al., 2013a). Eggs of *M. incognita* were separated from the galled roots of tomato and J2s were obtained by incubating eggs in distilled water at room temperature for 24 hours (Hussey, 1973). Nematode species was identified by perineal pattern morphology (Jepson, 1987). Ten to fifteen females were dissected and identified using standard procedure and nematode identification key (Naz et al., 2013a).

2.1.2. Preparation of pytochemical dilutions

Phytochemicals (5.0 mg each) of *Artemisia* spp. were diluted in dimethylsulphr oxide (99% DMSO, Merck) and stock solutions (1:1 v\v) were prepared following standard methods (Naz et al., 2013a). The stock solutions were diluted in simple distilled water (SDW) and three final concentrations viz, 0.1, 0.2 and 0.3 mg/mL were formed from each stock solution with following formula i.e $V_1C_1 = V_2C_2$ (Naz et al., 2013b). Juveniles (50) and eggs (100 ±10) of *M. incognita* were exposed to each phytochemical concentration in separate and simultaneous in-vitro experiments. Treatments were arranged in a completely randomized design (CRD) with five repetitions. Carbofuran (Furadan 3G) dissolved in 1% DMSO was used as positive control, whereas inoculum treated with simple distilled water (SDW) dissolved in DMSO (H_20: DMSO) was regarded as negative control. Bioassay plates were incubated in the dark at 27 °C (Naz et al., 2013b). Eggs and J2s were shifted to SDW plates after 72 hours of exposure to observe any change in juveniles. Juveniles without any movement or appearing straight were regarded as dead (Pan et al., 2016). Data on J2s mortality and hatch inhibition

were recorded after 24, 48 and 72 hours according to the following formulae: Percentage J_2 mortality = No. of J_2s killed/Total No. of juveniles × 100; Percentage egg hatch inhibition = No. of eggs inhibited/Total No. of eggs × 100.

2.2. In planta experiments

Nursery of tomato was developed in February-March, 2016. Sand and soil in a specified ratio (2:1; v/v) were used to prepare potting mixture. This mixture was autoclaved for 45 minutes at 121 °C in heat resistant plastic bags (Sharon et al., 2001). Pure potting mixture was added to clay pots each 15cm in diameter. Pots were drained and kept for overnight. Appropriate amount of water was added to each pot in order to protect seedlings from damage and root decaying. Healthy and uniform seedlings of four-week old tomato were transplanted. Young seedlings were inoculated with water suspension (100mL) containing J2s of *M. incognita* (@ 3000 J2s /mL). Inoculation was done after seven days of transplantation when seedlings were established (Sharon et al., 2001). Thirteen phytochemicals were dissolved in DMSO (99% pure and (1:1v/v) distilled water and adjusted to a fixed concentration of 1mg/1000mL. Phytochemicals, each at a concentration of 0.1 mg/mL, were applied to experimental units per pot 5- days post inoculation as a root drench. The experiment was conducted under screen house at ambient temperature (30 ± 5 °C) and relative humidity of 70.0% (Naz et al., 2013b). After inoculation, all plants were allowed to grow for 50 days following which these were removed cautiously. Roots were cleaned gently with tap water (Naz et al., 2013a, b). Data were recorded on fresh and dry shoot weight, shoot length, fresh and dry root weight, number of galls, galling index, egg masses / 10 cm of root system and number of juveniles.

The scale of 0-5 (Taylor and Sasser, 1978) for galling index was used as follow; where 0 = No galls on roots, 1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls, 5 = More than 100 galls.

2.3. Statistical analysis

Data on J2s mortality and egg hatch inhibition were analyzed by Analysis of Variance (ANOVA) (Steel et al., 1997). Trapezoidal Integration rule was used to calculate area under cumulative percent mortality (AUCPM) and hatch inhibition (AUCPHI) (Campbell and Madden, 1990). Screen house data were analysed by Statistix (version 8.1; NH Analytical software). Least Significant Difference (LSD) test was used to separate treatment means when these showed significant differences (Steel et al., 1997).

3. Results

3.1. In vitro experiment

3.1.1. *In vitro* nematicidal effect of phytochemicals of *Artemisia spp.* and their concentrations on hatching of *M. incognita*

Phytochemicals of *Artemisia* species and their three concentrations (0.1, 0.2 and 0.3 mg/mL) significantly ($P < 0.01$) increased the percentage hatch inhibition of *M. incognita* eggs over 24, 48 and 72 h of incubation ($P < 0.05$) (see Figure 2A - a, b, c). Increase in concentration resulted in increase of hatch inhibition. The highest egg hatch inhibition (90.66%) was attained with Isoscopletin (C6) followed by Carbofuran and Apeginin (F1) at the highest concentration of 0.3 mg/mL. Control treatments showed minimum egg hatch inhibition. However, the interactive means of phytochemicals and their concentrations were non-significant.

3.1.2. *In vitro* effect of phytochemicals of *Artemisia spp.* and their concentrations on J2s mortality of *M. incognita*

Results revealed significant effect of phytochemicals of *Artemisia* spp against J2s mortality of *M. incognita* at 0.1, 0.2 and 0.3 mg/mL concentrations and 24, 48 and 72 h of incubation ($P < 0.05$). Increase in concentration resulted in increased juvenile's mortality. Phytochemicals of *Artemisia* spp killed maximum J2s of *M. incognita* at the highest concentration of 0.3 mg/mL and 72 h of incubation. Among different phytochemicals the highest J2s mortality was attained with Isoscopletin (C6) followed by Carbofuran and Apeginin (see Figure 2B - a, b, c) at 0.3 mg/mL concentration. The lowest inhibition was attained by control after 24 hours' incubation period. The interactive effect of phytochemicals and there concentrations was non-significant.

3.2. Structure-activity relationships

Juveniles (J2s) of *M. incognita* exposed to different phytochemicals showed variations in their postures. All phytochemicals of *Artemisia* species against J2s behaved differently and induced variable structural changes on juveniles at 72 hrs of incubation. Juveniles of *M. incognita*, when treated with Isoscopletin (C6) at a concentration of 0.3 mg/mL, exhibited circular to semi-circular shape while J2s treated with Apigenin (F1), Benzoic acid and Betulinic acid gave a straight to semi curve shape. Other phytochemicals such as Friedlin showed a posterior ring structure, whereas the anterior bodies of J2s were straight and free. Long chain ketones and Linoic acid showed the circular postures, with double ring structures whereas the standard Carbofuran showed a partially curved structure. In treatments where simple distilled water was employed, the nematodes possessed a natural posture and did not show any specific rings or semi-circular postures (see Figure 3).

3.3. Effect of phytochemicals of Artemisia spp. on M. incognita in tomato and plant growth parameters

Application of phytochemicals of *Artemisia* spp. had an effect ($P < 0.01$) on nematode and plant growth parameters (as shown in Table 1). The treatments significantly caused reduction in number of galls, galling index and egg masses on plant roots. Isoscopletin (C6) exhibited the greatest reduction in nematode galls (1.50), galling index (1.10) and egg masses (4.00) compared with the untreated controls where the number of galls, galling index and number of egg masses were (104.5), (5.21) and (32.33) respectively.

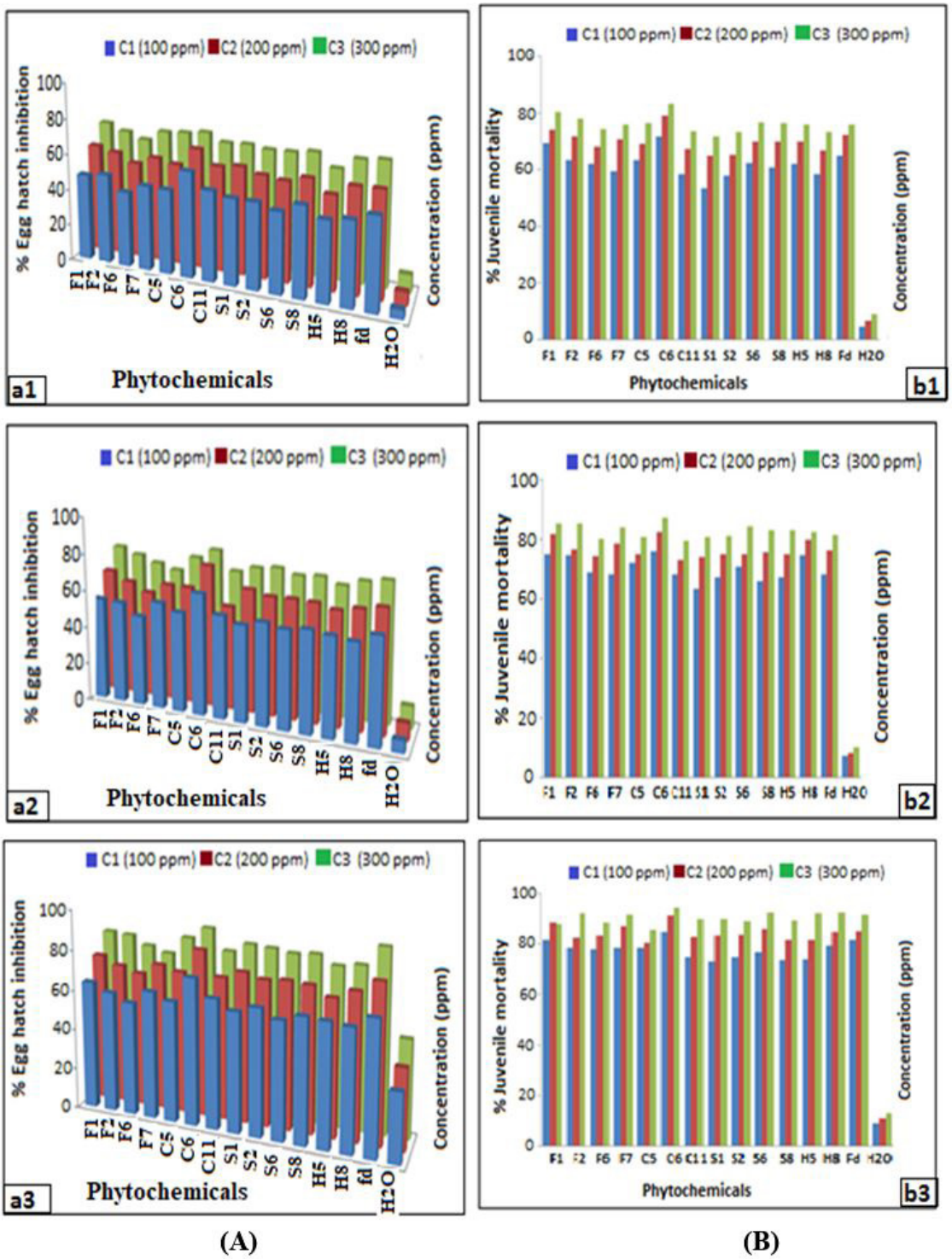

Figure 2. (A) Effect of phytochemicals and their concentrations on percent egg hatch inhibition of *M. incognita* after (a1) 24-hrs, (a2) 48-hrs and (a3) 72-hrs of incubation; (B) Effect of phytochemicals and their concentrations on percent on percent juvenile (J2) mortality of *M. incognita* after (b1) 24-hrs, (b2) 48-hrs and (b3) 72-hrs of incubation. F1: Apigenin; F2: Bonanzin; F6: Nepitin; F7: (S) – dihydroluteolin; C5: Scopletin; C6: Isoscopletin; C11: Benzoic acid; S1: Beta-sitosterol; S2: Gama-sterol; S6: Betulinic acid; S8: Friedlin; H5: Linoleic acid; H8: Long chain ketone; fd: Carbofuran; H20: Control.

The lowest numbers of juveniles were found in the rhizosphere soil of tomato treated with Isoscopletin (C6) (4.83) followed by Carbofuran (13.33) and Apeginin (F1) (11.66). Maximum numbers of J_2s per 100 cm^3 of soil were registered in the control (39.3).

Phytochemicals of *Artemisia* spp. significantly ($P< 0.05$) improved the cucumber plant growth parameters (as shown in Table 1). Data in Table 1 indicated that Phytochemicals enhanced the plant growth parameters as compared with control. Isoscopletin (C6) resulted in maximum fresh shoot weight (72.13 g), dry shoot weight (35.99 g), dry root weight (1.43 g) and shoot length (28.48 cm) as compared to other treatments. This was followed by Carbofuran and Apigenin (F1) while the lowest values for plant growth parameters were exhibited by control.

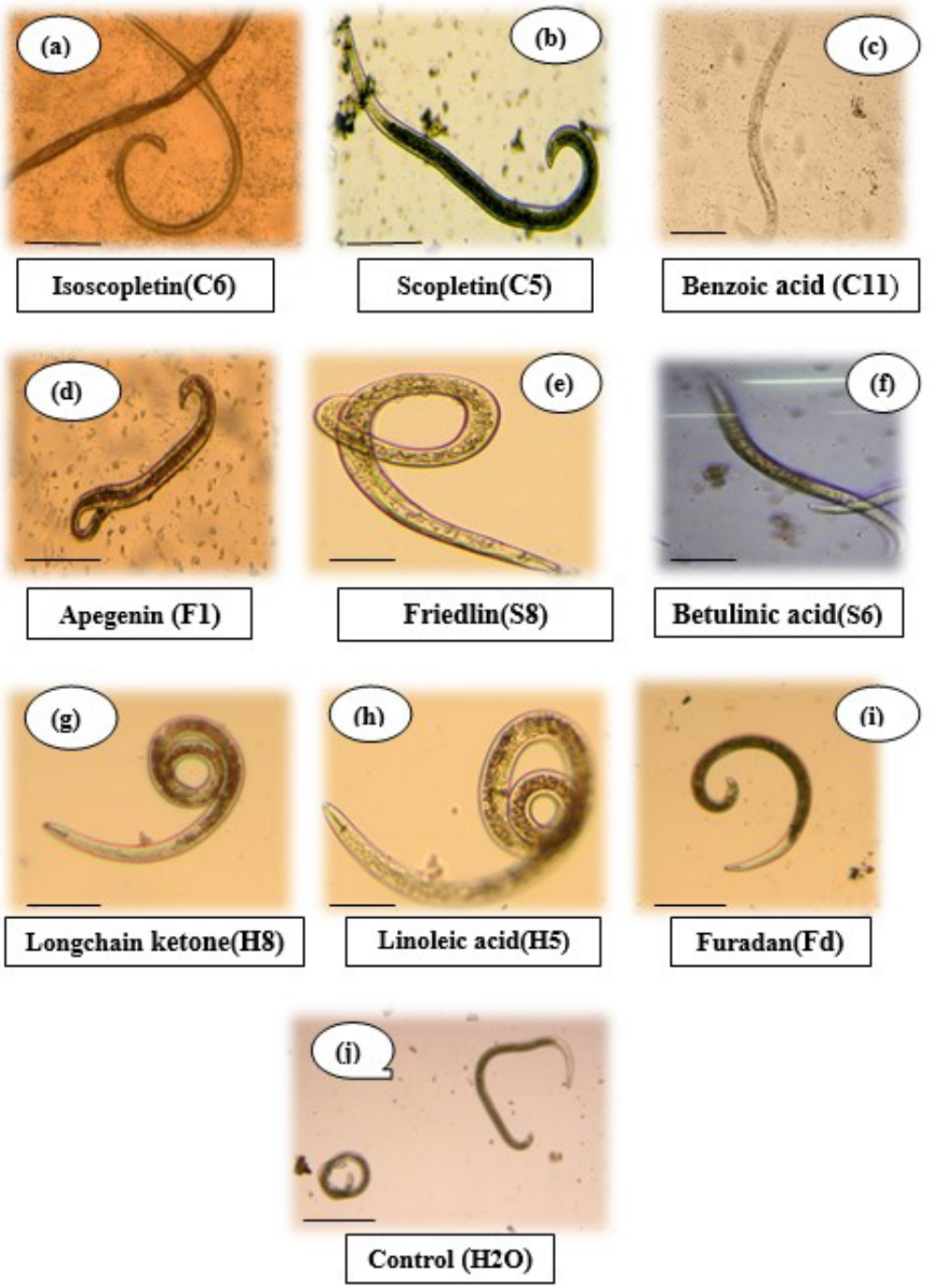

Figure 3. Structure+activity relationships of *M. incognita* influnced by different phytochemicals of *Artemisia* spp. All scale bars = 0.1 mm.

Table 1. Nematicidal effects of phytochemicals of *Artemisia spp.* on *M. incognita* and plant growth parameters of tomato under screen house conditions.

Phyto-chemicals	Nº. of Galls	GI	Nº .of Juvenile (J_2S)	Nº. of Egg Masses	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)	Shoot length (cm)
Apigenin (F1)	4.8 cd	2.7 bc	11.6 cd	9.5 cd	42.5 b	21.2 b	5.0 ab	0.60 b	18.3 bc
Bonanzin (F2)	7.5 bc	2.8 bc	15.3 cd	14.6 cd	37.6 bc	18.6 bc	5.5 ab	0.48 b	15.5 bc
Nepitin (F6)	11.6 ab	3.0 b	30.8 ab	25.6 ab	36.1 bc	17.9 bc	4.2 ab	0.40 b	16.0 bc
(S) – dihydroluteolin (F7)	7.0 c	2.8 bc	17.3 bcd	18.0 bc	39.5 bc	19.6 bc	4.6 ab	0.25 b	16.5 bc
Scopletin (C5)	8.1bc	2.7 bc	18.1 bcd	15.8 bc	29.5 bc	14.8 bc	3.6 ab	0.38 b	16.6 bc
Isoscopletin (C6)	1.5 d	1.1 d	4.8 d	4.0 d	72.1a	35.9 a	6.5 a	1.43 a	28.4 a
Benzoic acid (C11)	4.8 cd	2.8 bc	15.6 cd	11.5 cd	28.7 bc	14.4 bc	2.3 b	0.28 b	14.6 c
Beta-sitosterol (S1)	5.0 cd	2.7 bc	17.3 bcd	12.8 cd	37.1 bc	18.7 bc	3.7 ab	0.38 b	15.4 bc
Gama-sterol (S2)	6.0 c	2.9 b	18.5 bcd	14.8 bcd	31.6 bc	15.6 bc	4.0 ab	0.28 b	15.1 bc
Betulinic acid (S6)	14.3 cd	3.0 b	15.3 cd	11.6 cd	21.6 c	10.8 c	2.4 b	0.23 b	13.2 c
Friedlin (S8)	4.3 cd	2.8 bc	13.8 cd	11.8 cd	36.6 bc	18.3 bc	3.8 ab	0.38 b	14.8 c
Linoleic acid (H5)	5.6 cd	2.7 bc	14.6 cd	11.6 cd	31.0 bc	15.6 bc	3.6 ab	0.55 b	13.6 c
Long chain ketone (H8)	6.8 c	2.8bc	23.1bc	17.3bc	28.5 bc	14.25bc	2.3 b	0.20 b	12.5 c
Carbofuran (fd)	3.8 cd	2.3bc	13.3 cd	8.8 cd	42.7 b	21.36 b	5.3 ab	0.70 b	20.7 b
Control (H20)	104.5 a	5.2 a	39.3 a	32.3 a	20.2 c	10.28 c	6.1 a	1.83 a	16.7 bc
LSD value P ≤ 0.05	4.78	0.5	14.68	10.98	20.30	10.02	3.71	0.55	5.90

Means followed by the same letters in a column do not differ significantly ($p \leq 0.05$) according to Fisher's Protected LSD test.

4. Discussion

Root knot nematodes (RKNs) are the most important and fundamental group of plant parasitic nematodes. Amongst RKNs, *Meloidogyne incognita* is devastating plant parasitic nematode of ornamentals and vegetables throughout the world and mainly prevalent in Pakistan. *M. incognita* has a broad host range of above 3000 plant species (Anwar et al., 2009). Management of root knot nematode is much risky than other pathogen due to their recognized reproductive character. These habitually attack buried parts of plants and therefore, cannot be detected well by the farmers (Sikora and Fernandez, 2005).

Several practices have been used in integrated disease management (IDM) program to overcome these plant parasitic nematodes. Consumption of phytochemicals in agricultural output offers remarkable potential as nematicidal principles that are well-known in a number of higher plants (Chitwood, 2002). The current research was planned to investigate the effectiveness of phytochemicals of *Artemisia* spp viz; *A. incisa* and *A. elegantisima*. The present study involved use of thirteen phytochemicals viz Apigenin (F1), Bonanzin (F2), Nepetin (F6), (S)- dihydroluteolin (F7), Scopelitin(C5), Isoscopelitin(C6), Benzoic acid (C11), Beta-sitosterol (S1), Gama-sterol (S2), Betulinic acid (S6), Friedelin (S8), Linoleic acid (H5) and Long chain ketone (H8) from *Artemisia* species. These were evaluated both in laboratory and in the screen house experiments against *M. incognita*.

In laboratory study, phytochemicals at different concentrations i.e. 0.1, 0.2 and 0.3 mg/mL were tested on juvenile's mortality and egg hatch inhibition at 24, 48, and 72 hrs of incubation. The findings testified that juvenile's mortality and egg hatch inhibition were the highest at the highest concentration (0.3 mg/mL) and at a greater time exposure (72 hrs).

The in vitro examination revealed that Isoscopelitin gave advantageous turnout among all other phytochemicals followed by Carbofuran and Apegenin (F1). Our outcomes are in line with the past studies (Naz et al., 2013b). It was shown that juvenile's mortality and egg hatch inhibition were reduced by applying various concentrations of phytochemicals. Coumarins and Flavonoids (F1) especially offered dreadful effects in the present study. In vitro study demonstrated that the phytochemical compounds of *Artemisia* spp. had vigorous effects on juveniles and eggs of *M. incognita* and these turnouts occurred at a high concentration and exposure time. These findings are analogous to those revealed by other researchers, who applied crude ethanoic extracts from the leaves and shoots of *F. parviflora* and reported more than 50% juvenile's mortality of *M. javanica* (Naz et al., 2013a). Pan et al. (2016) also showed the nematicidal activity of Coumarins against *M. incognita.* Other researchers investigated the promising nematicidal effects of artemisinin, caffeic and chlorogenic acid from *A. annua* on *M. incognita*, potato cyst nematode *Globodera rhostochiensis* and virus- vector dagger nematode *Xiphinema index* (D' Addabbo et al., 2013). Similarly Chin et al. (2018) recently studied the effect of flavonoids against plant parasitic nematodes and reported that these secondary metabolites were involved both in root development and plant defense responses against a range of microorganisms.

In vitro results were further supported by data obtained from in planta experiments, where these phytochemicals significantly influenced the parameters in nematode parasitism (egg masses, juvenile data, galling index, number of galls) and plant parameters (fresh and dry root weight, fresh, and dry shoot weight and plant height). Our in planta results showed that the plants treated with Isoscopletin (Coummarins) had the least number of egg masses, juvenile's, number of galls and galling index as compared with other phytochemicals including the standard Carbofuran. Isoscopletien enhanced root, shoot lengths, fresh root and dry shoot weights, therefore, enhancing plant health. Our results are in line with those of Naz et al. (2013b) who studied the significance of *F. parviflora* on *M. incognita* and reported that the application of phytochemicals of *F. parviflora* improved seedling growth of tomato and number of nematodes were considerably decreased at 0.3 mg/mL concentration of plant extracts.

In structure–activity relationships (SAR), Isoscopelitin (Coumarins) showed the highest nematicidal activity than Scopletin, where OH of Isoscopletin at C6 and C7 position induced significant mortality. These results were strongly supported by Takaishi et al. (2008) and Adfa et al. (2012) whose research confirmed the structure–activity interactions of derivatives of Hydroxycoumarin and recommended that the alterations in the backbone of C4 and C7 position could increase termiticidal, bactericidal activities and tumor cytotoxicity. Structure activity relationships of phytochemicals on juveniles were reported by Pan et al. (2016). These authors reported that Coumarin- based nematicides i.e. joined 7-hydroxy- 4-methyl Coumarin (2) and 4-hydroxycoumarin (1) with alkyl bromides had broad alteration in activity. Juveniles of *M. incognita* showed different type of structures that were circular, semi-circular rounded while some were straight or irregular when exposed to different chemical concentrations of *Artemisia* plant.

In this study, nematicidal activity of long chain ketones was observed. Long chain ketones strongly inhibited juvenile's mortality and egg hatching *M. incognita* (Naz et al., 2013b). Previous studies along with the current findings confirmed that alcohols, aldehydes, and phenols were more reactive nematicides against Bursaphelenchus xylophilus (Choi et al., 2007) whereas an Aliphatic ketone (2-undecanone) showed best results against *M. javanica* and *M. incognita* (Ntalli et al., 2011). In Isoscopletin, the strongest activity against nematodes might be the presence of carbonyl group, as confirmed by many researches where they evaluated that nematicidal activity of the compounds varied with alterations among the functional groups, carbon skeleton and saturation (Kong et al., 2007). In another study on alkanals and 2E-alkenols, it was suggested that compounds with C8−C11 chain lengths revealed 100% nematicidal activity at 0.5mg m−1L concentration against the pine wood nematode (B. xylophilus) (Seo et al., 2010). Many studies have revealed that nematicidal activity of the compound having the hydroxyl group (OH) or methoxy group (OCH3) enhanced activity except the acetyl group (Park et al., 2005).

In planta study also revealed that root and shoot lengths of tomato were markedly increased with the phytochemical concentrations. These results are in line with the work described by other studies, who showed that use of phytochemicals in tomato plants with nematicidal characteristics increased plant growth (Pakeerathan et al., 2009). It was revealed unexpectedly that the fresh root weight was the highest in case of control (6.16g), followed by Isoscopletin (6.58g), and by Apigenin (5g) which could be due to the highest weight of galls on the roots. Kolapo et al. (2009) also discussed the phytochemical concentrations (saponins, alkaloids, tannins, steroids and phenols) varying significantly among parts of plant and this supported our findings towards the concentrations of total Coumarins and Flavonoid contents. It is suggested that secondary metabolites of *Artemisia* spp. have a potential against nematode defense in plants. Similar findings were reported by researchers who found that phytocehmicals especially the flavonoids induce quiescence by slowing down the movement of nematodes and modify their migration towards the roots by repelling them and kill them (Chin et al., 2018). For example quercetin and myricetin slowed down the movement of *M. incognita* even at micromolar concentrations (Wuyts et al., 2006).

In conclusion the present results showed that plant derived natural compounds possess nematicidal activity. Useful effects of phytochemicals reveal an interesting area of nematode management. *Artemisia* spp. has a potential as a phytochemical due to the diversity and richness of compounds having nematicidal effects against *Meloidogyne* spp. Further study is however required on the type of action, mechanisms and structure activity relationship in the suppression of nematodes with phytochemicals of *Artemisia* spp. Current study suggested that since phytochemicals have great nematicidal potential hence these can be applied effectively against root knot nematodes in an integrated disease management program or organic farming

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