NEMATOCIDAL, INSECTICIDAL, ANTI-INFLAMMATORY AND CYTOTOXIC ACTIVITIES OF SELECTED MEDICINAL PLANTS

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Abstract: Medicinal plants play a vital role in human life. The plant-based botanicals are considered to be safer, selective, and effective to conventional and synthetic chemicals used for the same purposes. There are huge number of bioactive plants around the globe which needs to be explored for their potential as herbal alternatives to traditional chemicals. In the present study, the methanolic extracts of *Anaphalis nepalensis* (AN), *Anaphalis virgata* (AV), *Artemisia foetida* (AF), and *Anthemis cotula* (AC) were collected from Gilgit-Baltistan. The main plant extracts were further fractionated into *n*-hexanes (H), dichloromethane (D) and aqueous (A) fractions. All these fractions were analyzed for their nematocidal, insecticidal, anti-inflammatory, and anti-cancer activities. All three fractions of AC, AV, and AF showed excellent nematocidal activity against *Meloidogyne incognita* (90, 94, 95%, respectively) at conc. of 1% after 72 h of the treatment. Similarly, AV-D and AC-D showed excellent anti-inflammatory response with IC_{50} values of 20 and 32 through inhibition of ROS. The cytotoxic activities of AC-H, AC-D, and AC-A against Hela tumor cell lines was found active with IC_{50} values of 22.7, 12.7, and 7.6, respectively.

Key words: Nematocidal; insecticidal; *Meloidogyne incognita*; green pesticides; anti-cancer; anti-inflammatory.

INTRODUCTION

Bioactive compounds can be used as fungicides, nematicides, insecticides, and herbicides [14]. Numerous plant based formulations were also used for the management of pests causing harmful effects to crop and also affect human health [7]. Botanicals can be used as alternatives to control nematodes; in this regard many plant species exhibit promising nematocidal activities [12]. There are above 2000 plant species which have been discovered for their insecticidal action. The insecticidal potential of medicinal plants can be attributed to steroids, alkaloids, terpenoids, phenolics and essential oils present in them. The plant based products were used to control different insect pests since 1920, where DDT was introduced as the most effective synthtic alternative in 1939. These synthetic alternatives are injurious for non-target organisms. Consequently, in 1990s refocus on botanical pesticides was emerged [27]. *Anaphalis nepalensis* used in Chinese preparative method to prevent from bacteria and viruses, removes pathogen, effectively dredge collaterals, promote blood circulation, remove blood stasis, clean germs in ear and remove inflammation and pain and can also be used for the treatment of eardrum inflammation [4]. The plant *Anaphalis virgata* and *Anaphalis nepalensis* known by the same name chhikhi by local communities and is kept in homes, mosques and native caps due to its pleasing smell, and is also used for several remedial purposes [22]. Decoction of *Anaphalis virgata* causes sweating and is used for temperature, respiratory problems, hypertension and cardiac troubles. It is also kept in bread to keep it fresh [29]. Smoke of plants *Anaphalis contorta* and *A. virgata* are used in traditional medicine of Devikund in Indian Himalaya as insect killers [5]. Pesticides are beneficial for crop protection, disease control, food preservation as well as it is risk for human health. Botanical pesticides are natural compounds with insecticidal properties and their use in crop protection is as old as agricultural practice [1, 3]. Although they have been in use for over one hundred years, the advent of synthetic insecticides has unfortunately displaced their use today [9]. Due to fast action, low cost, easy application and efficiency against a wide range of harmful species, synthetic insecticides have become an important part of pest management in modern agricultural systems [30]. The modern agricultural business has led to environmental crisis. Excessive use of pesticides on crops and vegetables to increase the food production may cause in serious environmental issues and diseases [20].

Root knot nematodes, *Meloidogyne* spp. are important plant pathogens found around the globe. Species from the genus caused fall in harvest and even early death of affected plant [6]. Farmers may recognize *Meloidogne* species by the symptoms on affacted plant caused by these species, they mainly cause lesions or lumps on roots of diseased plant [31]. The major familiar species of the root-knot nematode is the *Meloidogyne incognita* and contaminate approximately every cultured plant, which formulate it possibly the most destructive of pathogens [8]. According to an estimate the nematodes cause annually 100 billion dollars loos accros the globe [24]. Nematodes are parasitic agents of plants they affect the quality and quantity of the crop production. They also cause root galling, stunting, nitrogen deficiency and nutrient deficiency [14]. The root-knot nematodes include *Meloidogyne* were found as most destructive nematodes in agriculture [11]. The processes in use to protect against root knot nematodes mainly depend on chemical nematicides, but these chemicals can also take along environmental and health problems related

with their production and use, so it is very necessary to consider the biological method for nematodes control [32]. To control these problems now the scientists are trying to identify natural sources with nematicidal activity like plant volatile compounds, root exudates, endophytic bacteria and especially the plant extracts. The most effective class of the secondary metabolities against nematodes include alkaloids, phenolics, glycosides, isothiacyanate and fatty acids [23].

In the present paper different extract fractions of 4 selected medicinal plants have been tested for their nematocidal, insecticidal, anti-inflammatory, and anticancer activities to find the safe alternatives and environment friendly botanicals.

MATERIALS AND METHODS

Plant material

The plant samples of *Anaphalis nepalensis* (Spreng.) Hand.-Mazz., *Anaphalis virgata* Thomson, *Artemisia foetida* acquem. ex Besser, and *Anthemis cotula* Linnaeus were collected in wet form Deosai, Astore during August 2018. The sample specimens were stored in herbarium for the future reference (Voucher No. 205/18, 206/18, 207/18, 208/18, respectively). The plants material were dried in laboratory, grinded and soaked in methanol. The methanolic extracts were further partitioned with *n-hexane*, dichloromethane and water for biological screening.

Extraction and fractionation

The air dried and ground material of *Anaphalis nepalensis* (AN, 689 g), *Anaphalis virgata* (AV, 250 g), *Artemisia foetida* (AF, 1153 g), and *Anthemis cotula* (AC, 975 g), were soaked in 2-3 L methanol (95%) in separate containers for 2-days and repeated the extraction three times, each by soaking overnight. The combined extracts of each plant sample were filtered separtly followed by evaporation of solvent by using Rota vapor to yield the solid extract reside of AN (57 g), AV (27 g), AF (78 g), and AC (56 g). The crude methanolic extracts were then successively partitioned by solvent-solvent fractionation into three major fractions, *n*-hexane (H), dichloromethane (D), and aqueous (A) (Table 1).

Biological assays

The hexanes, dichloromethane and aqueous solvent fractions of *Anaphalis nepalensis, Anaphalis virgata*, *Artemisia foetida,* and *Anthemis cotula* were subjected to different biological assays including nematocidal [9], insecticidal [2], cytotoxic [18], and anti-inflammatory activities [10].

Nematode culture and collection conditions

A population of molecularly identified root knot nematodes *M. incognita* JQ806341 of Pakistan origin was cultured on eggplant (*Solanum melongena* L.) for 45 days in a glass house at 30 ± 5 °C at the National Nematological Research Center, University of Karachi, Pakistan. Infected roots of 6-12 week old were picked and sliced into 1-1.5 cm long pieces which were shaken vigorously for 2-4 minutes in 1000 mL Pyrex conical flask containing 250 mL of 0.5-1.0 % sodium hypochloride (NaOCl) solution. Freed eggs were collected when NaOCl solution were quickly conceded from a 200 mesh sieve fixed over a 500- mesh sieve. Batches of 10 egg masses were collected and placed in 3 × 3 glass cavity block with water for natural hatching. The cavity block was placed under conditions that promote the development of egg hatching at ambient temperature for 72 h. In next stage, 100 larvae were transfered in a chamber for each dose treatment and replicated thrice to introduce in glass cavity block [2, 9].

Nematicidal assay

For nematicidal effect in laboratory conditions suitable solvent (5% dimethyl DMSO) were used to prepare stock solution (10 mg/mL) of each fraction of 4 different plant species. Three different concentrations of 1%, 0.5% and 0.125% of each compound were applied at a rate of 1 mL in cavity block 3 × 3 with 100 freshly hatched juveniles separately. Nematicide carbofuran was chosen as standard drug while 5% DMSO as a control treatment. The experimental cavity blocks were kept at room temperature 28 ± 2 ºC. Stereoscopic microscope at 4 X was used to observe percentage death rate (mortality) after an interval of 24, 48 and 72 h. Nematodes were considered dead when no movement was detected after mechanical nudge. Then the nematodes bodies were transferred into distilled water for confirmation of irreversible mobility [9].

Insecticidal assay

By using impregnated filter paper *Rhyzopertha dominica* and *Sitophilus oryzae* (insect species) were subjected to the methanolic extract and all solvent fractions [2]. Stock solution was made by mixing sample (200 mg) in methanol (3 mL). With the help of micropipette on petri plates samples (1019.10 µg/cm) were applied to filter paper of size 9 mm. The solvent was evaporated after 24 h break. For test and control from each species 10 insects were set in each plate. For

Table 1. Sample details of different fractions obtained from 4 herbal plants

S. no.	Plant species	Extract fraction	Sample code	Weight obtained (g)
1	*Anaphalis nepalensis*	*n*-hexane	AN-H	14
		dichloromethane	AN-D	19
2	*Anaphalis virgata*	*n*-hexane	AV-H	09
		dichloromethane	AV-D	07
		aqueous	AV-A	11
3	*Artemisia foetida*	*n*-hexane	AF-H	10
		dichloromethane	AF-D	25
		aqueous	AF-A	43
4	*Anthemis cotula*	*n*-hexane	AC-H	10
		dichloromethane	AC-D	14
		aqueous	AC-A	32

positive and negative control 239.5 µg/cm^2 of Permethrin and methanol were used respectively. After maintaining ambient temperature and 50% of humidity in growth chamber the test plates were then incubated in chamber for 24h. Next day, the numbers of survivals were calculated from each species and percentage mortality (%*M*) was determined by applying the formula:

$$\%M = \frac{100 - \text{No. of insects alive in test}}{\text{No. of insects alive in control}} \times 100$$

Statistical analysis

To analyze the treatment differences multifactor analysis of variance was used. By using SPSS statistical software, the obtained data was further submitted to Tukey's test ($P \leq 0.05$). Probit analysis was done under survival analysis for EC_{50} values by SAS, 2000 software.

Cytotoxic assay

Different fractions (11) of the methanolic extracts of 4 herbal plants were studied for cytotoxicity by applying the standard MTT (3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyl-tetrazolium bromide) colorimetric assay on 96-well micro plates [18]. In this process, the fibroblast cells of mouse (3T3) were cultured in Dulbecco's modified eagle medium and mixed with 100 IU/mL of penicillin, 5% of fetal bovsine serum (FBS) and 100 µg/ mL of streptomycin in 75 cm^2 flasks. The flasks were placed in incubator at 37 ^oC having 5% CO_2. Haemocytometer was used to calculate the growing cells and the dilution of cells with medium was also done. Cell cultures (5x10^4 cells/mL) were prepared and these cell cultures were introduced (100 µL/well) into 96 well plates. The medium was removed after 12 h incubation and fresh medium of 200 µL was mixed with different concentrations of samples (1.30 µM). 200 µL MTT (0.5 mg/mL) was added to each well after 48 h and was kept for further incubation of 4h. Later, 100 µL DMSO was mixed to each well. The amount of MTT decreases to Formazan within cells which was then obtained with the help of micro plate reader by determining the absorbance at 540 nm. The cytotoxic activity was noted as IC_{50} for 3T3 cell. At last % inhibition of cells was determined by applying formula:

$$\%\,\text{inhibition} = \frac{100 - (\text{mean of O.D of test compound - mean of O.D of - ve control})}{(\text{mean of O.D of + ve control - mean of O.D of - ve control})} \times 100$$

Anti-inflammatory assay

The sample extracts of plants were tested for anti-inflammatory assay and evaluated the data by the oxidative burst assay using a luminol-amplified chemiluminescence technique as reported earlier [10]. A mixture of 25 µL of diluted whole blood in HBSS^{++} (Hanks balanced salt solution) [14 025, Gibco] and 25 µL of the compound (25 µg/mL) was incubated in a 96 well plate at 37 °C for 15 min. To serve as a control, the cells with HBSS++ and without the test compound were also used. After incubation, each well were added with 25 µL of serum opsonized zymosan (26 701 494, Wako) in tris base NaCl, and 25 µL of the intracellular ROS detecting probe luminol (7×10−5 M) [A14597] in HBSS^{++}, except for the blank wells which containing only the HBSS^{++}. By using the luminometer (Luminoskan RS, Lab system) level of ROS was measured in terms of relative unit (RLU) using Ibuprofen (I4883, Sigma-Aldrich, purity ≥ 98% by HPLC) was used as standard. The % ROS inhibition was calculated by applying the following formula:

$$\text{inhibitory activity}\,(\%) = \frac{\text{Control group RLU - Test group RLU}}{\text{Control group RLU}} \times 100$$

RESULTS

In current study, we evaluated the different solvent fractions of the methanolic extracts of *Anaphalis nepalensis, Anaphalis virgata*, *Artemisia foetida,* and *Anthemis cotula* for bioassays like nematocidal, insecticidal, cytotoxic, and anti-inflammatory activities. The results of all 11 extracts against 4 different bioassays have been presented and described (Figure 1-2 and Table 2-3).

Nematicidal activity

The nematocidal activity of different extracts (11) on larval mortality of *M. incognita* (root-knot nematode) was studied at various concentrations after time internal of 20 minutes, 1, 2, 24, 48, and 72 hours. The nematocidal activities of hexanes (AV-H), dichloromethane (AV-D), and aqueous (AV-A) fractions of *A. virgata* were found to be excellent and more consistent at the conc. of 1, 0.5 and 0.125% with total larval mortality rate of 85-94% after 72 h exposure time. The results presented in figure indicate all three fractions (AV-H, AV-D, and AV-A) of *A. virgata s*howed excellent activities of 94% (Fig. 1). The active fractions of *A. virgata* showed does independent response showing comparable results against root-knot nematodes at all tested concentrations were comparable to each other. The nematocidal activity of *A. nepalensis* extract on larval mortality of *M. incognit*a was studied at various concentrations at different time internal (20 minutes, 1, 2, 24, 48, 72 hours). The nematocidal activities of hexanes (H), and dichloromethane (D) of *A. nepalensis* were more consistent at the conc. of 1, 0.5 and 0.125% with total larval mortality rate of 70-72% after 72 h exposure time. The results presented in figue indicate both the fractions of *A. nepalensis* showed moderate activities of 70% (Fig. 1). We recommend *in-vivo* testing of active extracts, which have been never reported yet to promote the green practices for sustainable agriculture and the protection of the environment.

The nematocidal activity of *A. foetida* extract on larval mortality of *M. incognita* was also studied at various concentrations at different time internals (20 minutes, 1, 2, 24, 48, 72 hours). The nematocidal activities of hexanes (AF-H), dichloromethane (AF-D), and aqueous (AF-A) fractions of *A. foetida* were found to be excellent and more consistent at the all concentration (1, 0.5 and 0.125%), with total larval mortality rate of 75-95% after 72 h exposure time. The results presented in table indicate all three fractions

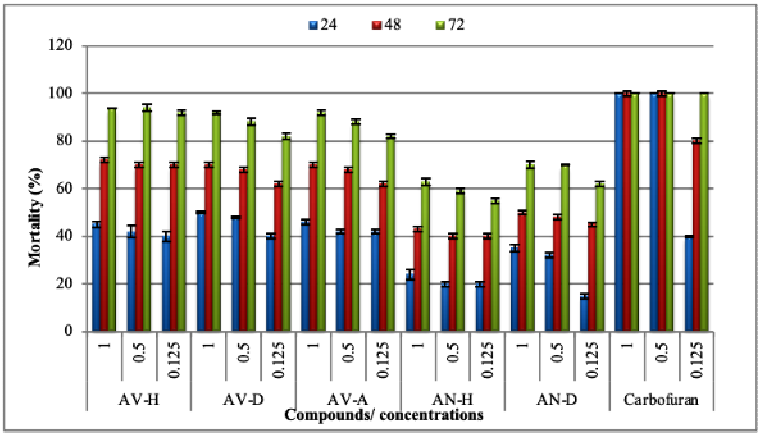

Figure 1. Nematocidal activity of *A. virgata* and *A. nepalensis* against root- knot nematode. Values in columns having same upper-case letters are not significantly different (P< 0.001). Values in rows having same lower-case letters are not significantly different (P< 0.001). Abbreviations: AV= *A. virgata;* AN= *A. nepalensis*; H=hexane fraction; D=dichloromethane; A=aqueous

Table 2. Nematocidal activity of *A. foetida* and *A. cotula* against root- knot nematode

Sample code	Conc. %	24 h	48 h	72h
AF-H	1	45 ± 0.9a	65 ± 0.8a	85 ± 1.0a
	0.5	40 ± 1.4b	60 ± 0.6b	78 ±1.2b
	0.125	40 ± 1.4b	58 ± 1.3b	75± 0.5c
EC_{50} (± SE)		1.92 ± 0.14	0.012±0.26	0.0019 ± 0.02
AF-D	1	38 ± 0.6a	70 ± 0.5a	95 ± 1.5a
	0.5	37 ± 1.6a	68 ± 1.5b	92 ± 1.0
	0.125	35 ± 1.0ab	65 ± 0.6bc	90 ± 1.2b
EC_{50} (± SE)		4.36 ±1.31	0.007 ± 0.9	0.05 ± 0.010
AF-A	1	50 ± 1.8a	80 ± 1.0a	95 ± 0.8a
	0.5	45 ± 1.4b	75 ± 1.5b	94 ± 1.0 a
	0.125	40 ± 1.2c	70 ± 1.0c	90 ± 1.2c
EC_{50} (± SE)		1.161 ± 0.1697	0.0036 ± 0.03	0.0001 ± 0.01
AC-H	1	30 ± 1.0a	52 ± 0.5 a	72 ± 1.1a
	0.5	28 ± 0.5b	48± 1.5b	68 ± 1.0b
	0.125	25 ± 1.2c	42 ± 0.2c	65 ±0.5b
EC_{50} (± SE)		4.06 ± 0.48	0.68 ± 0.06	0.001 ± 0.17
AC-D	1	39 ± 0.5aA	70± 1.0a	90 ± 0.5a
	0.5	37 ± 1.0abA	69 ± 1.2a	89 ± 0.9a
	0.125	35 ± 0.5bA	65 ± 0.5b	89 ± 1.0a
EC_{50} (± SE)		3.30 ± 0.6	0.01 ± 0.16	0.0008 ± 0.00
AC-A	1	46 ± 0.2a	70± 1.2a	89 ± 0.1a
	0.5	40 ± 1.5ab	67 ± 0.2a	85 ± 1.4a
	0.125	39 ± 1.5b	64 ± 0.2b	85 ± 2.0a
EC_{50} (± SE)		1.54 ± 0.3	0.013 ± 0.44	0.0008 ± 0.00
Carbofuran	1	100±0.0a	100±0.0a	100±0.0a
(Furadan)	0.5	100±0.0a	100±0.0a	100±0.0a
	0.125	40±1.5b	80±2.5b	100±0.0a
EC_{50} (± SE)		0.13±0.02	0.07±0.3	0.07±0.3

Values in columns having same letters are not significantly different (P< 0.001).
Abbreviations: AF = *A. foetida;* AC= *A. cotula*; H=hexane fraction; D=dichloromethane

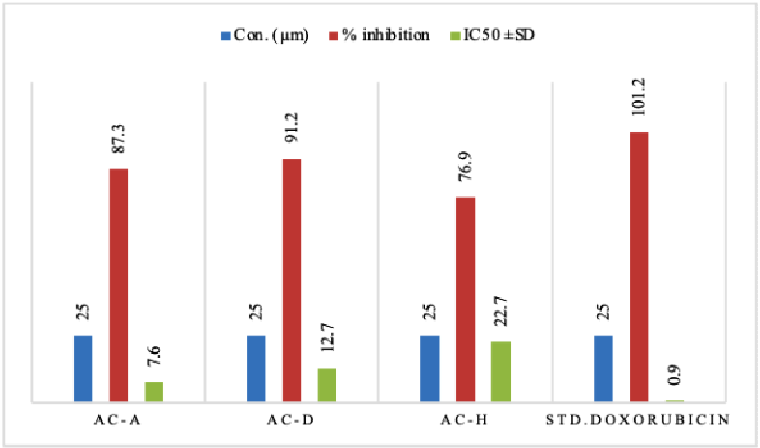

Figure 2. Cytotoxic activity of *A. cotula* against Hela cancer cell lines. Abbreviations*:* AC= *A. cotula*; H: hexanes fraction; D: dichloromethane, A: aqueous fraction.

(AF-H, AF-D, and AF-A) of *A. foetida s*howed excellent activities of 95% (Table 2). The active fractions of *A. foetida* showed dose indepandant activities with simillar mortalities at all concentrations against root-knot nematodes. On the other hand the mortality was increased with increased time of exposure indicating the time dependant response of the active fractions. The nematocidal activity of *A. cotula* extract on larval mortality of *M. incognita* were found to be excellent and more consistent at the conc. of 1, 0.5 and 0.125% with total larval mortality rate of 72-90% after 72 h exposure time indicating dose indepandant response of the extract fraction. The nematocidal activities of hexanes (AC-H), dichloromethane (AC-D), and aqueous (AC-A) fractions of *A. cotula* are presented in table. Both the dichloromethane and aqueous fractions of *A. cotula s*howed excellent activities of 90% (Table 2). The AF-D fraction was found to be the most active (95%) among all other fractions of different pklants extract studied during the present work. The *In-Vivo* studies on active fractins of the tested plant species can futher help to understand the mechanism and efficacy of plant based green nematicides for sustainable agriculture and the protection of environment.

Insecticidal activity

The insecticidal effect of the hexanes, dichloromethane, and aqueous extracts of *Anaphalis nepalensis, Anaphalis virgata*, *Artemisia foetida,* and *Anthemis cotula* were studied against *R. dominica* and *S. oryzae*. The insecticidal activity of different solvent fractions of the methanolic extracts of all 4 herbal plants were carried out with reference to Permethrin as standard drug and +ve control. In general, mortality rate was increased with increasing the concentration of compounds and exposure times same as observed in nematicidal activity. The results of insecticidal activity of the all fractions against *R. dominica* and *S. oryzae* reveal that activity of different solvent fractions remain insignificant at all concentrations.

Cytotoxicity

The cytotoxicity of different solvent fractions (hexanes, dichloromethane, and aqueous extracts) of *Anaphalis nepalensis, Anaphalis virgata*, *Artemisia foetida,* and *Anthemis cotula* were examined at the concentrations of 25 µM, with reference to standard drug Doxorubicin by following the MTT assay protocol. The three solvent fractions of the methanolic extract of *A. cotula* showed excellent cytotoxic activity against the Hela cancer cell lines with IC_{50} values of 7.6, 12.7, and 22.7 for AC-A, AC-D, and AC-H, respectively (Fig. 2).

The extracts of *A. virgata* (AV-H, AV-D, and AV-A), *A. nepalensis* (AN-H and AN-D), and *A. foetida* (AF-H, AF-D, and AF-A) didn't show any considerable action against the Hela cancer cell lines with % inhibition <40.

Anti-inflammatory activity

The ability of the hexanes and dichloromethane and aqueous fractions of the methanolic extracts of *Anaphalis nepalensis, Anaphalis virgata*, *Artemisia foetida,* and *Anthemis cotula* were determined to prevent the release of ROS and also used to measure their anti-inflammatory potential. From the results it was found that the hexane and aqueous extracts were found with no potent inhibitory action on oxidative burst. On the other hand, the dichloromethane factions of both AV and AF showed the moderate inhibition of ROS with IC_{50} values of 20.0 and 32.5, respectively. The ROS inhibitory activity of remaining fractions were in the range <20-45 (Table 3).

Table 3. Anti-inflammatory susceptibility of *A. virgata* and *A. foetida* against standard bacteria

Extract	Conc. (µg/mL)	% Inhibition	IC_{50} ± SD mg/mL
AV-D	1-10	76	20.0 ± 3.1
AF-D	1-10	71	32.5 ± 1.5
Ibuprofen	1	73.2	11.2 ± 1.9

Abbreviations*:* AV-D: *A. virgata* dichloromethane fraction; AF-D: *A. foetida* dichloromethane fraction

DISCUSSION

Carbofuran (Furadan®) used for controlling broad spectrum of insect is highly soluble white crystalline solid chemical and possess low adsorption properties in soil [26]. The standard control, carbofuran gave 100% mortality after 72 h of exposure with Ec_{50} value 0.07 but it is one of the toxic and extremely lethal to the mammals and wildlife. In human it causes reproductive disorders, genotoxic abnormalities, and endocrine disrupting activity [16]. Sustainable methods of controlling insect pests and nematodes to improve the crop yield and quality have become a great challenge to farmers and growers. Selective, effective and eco-friendly plant-based pesticides as benign practices have been already employed in fields for sustainable agriculture [19, 21].

All the tested treatments had nematicidal effects on nematode juveniles; the highest percentage of nematode mortality was achieved by application of *A. foetida* (95%) followed by *A. virgata* (94%), *A. cotula* (90), and *A. nepalensis* (70%). The comparative literature data of highest nematode mortality was in eucalyptus extract (100%) followed by cinnamon (97.1%), nerium (95.6%), ginger (92.7%), neem (91.3%), castor bean (81.1%) and garlic (65.2%) as compared with control (13.0%) [25]. Neem is recognized worldwide as a wonder tree for its unique pest control properties. Leaves of *Artemisia annua* linn. and *Azadirachta indica* were reported to show 100% larval mortality at 200 ppm after 48 h and LC_{50} of 69 ppm at 72 h [13]. Many other plants based natural compounds have been reported in literature and exhibited dose and time dependent pesticidal activity with the least LD_{50} value (0.672 g/L) [17]. The effect varies with insect species and is dose dependent [28]. *Croton tiglium*, *Euphorbia fischeriana*, *Leptopus chinensis* and *Ricinus communis* have been reported with 100% mortalities of the root-knot nematode at 1000 µg/mL for 72 h. The ethanol extract of *E.*

fischeriana exhibited nematicidal activity against *M. incognita* with LC_{50} value of 69.0 µg/mL after 72 h of the treatment [15]. The mortality rate of insect pests and *M. incognita* of all tested plant extracts strongly support the botanical pest control strategies of some other plant species reported in literature [33]. The hexanes, dichloromethane, and aqueous extracts of *Anaphalis nepalensis, Anaphalis virgata*, *Artemisia foetida,* and *Anthemis cotula* found to be active against root knot-nematodes with excellent rate of mortality in parallel to standard carbofuran. We can use these green formulations based on bioactive plants alternative to the harmful conventional nematicides. Therefore, the extract of these biologically active plants can be used as green nematicides whereas further studies can be conducted to find out the active ingredients of the plant. Abamectin, carbofuran and phosphine have been used intensively to control the damage caused by different nematodes and insects but rapid resistance of parasites due to evolutionary and genetic changes require the safe alternatives. The present finding suggest that plant extracts can be used for the development of novel nematicides as pest control agents. The hexanes, dichloromethane and aqueous extracts of *A. cotula* also exhibited excellent cytotoxic activity with more than 80% inhibition against Hela cancer cell lines. Therefore, we conclude that the hexanes, DCM and aqueous extracts of *A. cotula* can be investigated further for its active phytochemical constituents as green nematicide and insecticides.

Conflict of interest. There is no actual or potential conflict of interest in relation to this article.

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