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Comparative Compositional Analysis and Pesticidal Efficacy of Essential Oils from Leaves of *Skimmia Aanquetilia* N.P. Taylor and Airy Shaw

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ABSTRACT

The objective of the current study was to re-examine the chemical components of the essential oil (EO) from the aerial parts of *Skimmia anquetilia* N.P. Taylor & Airy Shaw in two different seasons designated as *Skimmia anquetilia* rainy season essential oil (SKREO) and *Skimmia anquetilia* winter season essential oil (SKWEO). The GC-MS analysis of SKREO and SKWEO resulted in the identification of 42 and 48 constituents, comprising of 95.3 % and 95.4 % of the total composition respectively. Both SKREO and SKWEO varied in their chemical composition in terms of quantity viz: linalyl acetate (15.8% - 17.6%), linalool (13.2% - 13.9%), geijerene (11.6% - 11.7%), α-thujene (11.3% - 11.1%), α-terpineol (6.1% - 6.1%), geranyl acetate (5.0% - 5.1%), α-terpinyl acetate (3.3% - 3.1%), myrcene (3.0% - 3.1%), geraniol (2.6% - 1.9%), α-pinene (2.1% - 2.2%), *trans-β*-ocimene (2.1% - 2.3%), *cis-β*-ocimene (2.0% - 2.2%) and neryl acetate (2.3% - 2.4%). Besides qualitative differences SKREO and SKWEO, both were studied for their pesticidal activities. The study exhibited potent antifeedant activity against *Spodoptera litura* and nematicidal activity against *Meloidogyne incognita.* Based on the present observations, it was found that besides its academic importance, shrub *Skimmia anquetilia* can be a good source of phytochemicals like linalyl acetate, linalool, geijerene, thujene and can be used for the development of herbal source for antifeedant and nematicidal activity after proper clinical trials.

Keywords: Skimmia; Rutaceae; Phytochemicals; Anti-feedant; Nematicidal

ABBREVIATIONS

EO	:	Essential oil
BOD	:	Biological oxygen demand
GC/MS	:	Gas chromatography-mass spectrometry
SKREO	:	*Skimmia anquetilia* rainy essential oil
SKWEO	:	*Skimmia anquetilia* winter essential oil
SPSS	:	Statistical Package for the Social Science
CRD	:	Completely randomised design
MLAC	:	Mean leaf area consumed

1. INTRODUCTION

Humans have acquired life benefits by discovering aromatic and medicinal plants for food and medicine since time immemorial. Traditionally, Indian medicine (ayurveda) has become a part of every civilisation with medicinal and aromatic plants used and applied to sustain life. Natural products have consistently been utilised as nutritional supplements or in the treatment of numerous disorders in comparison to synthetic ones because of their wide range of pharmacological activities, along with better safety and efficacy. Plant-derived natural products have been and will continue to be a major source of pesticides, foods, drugs and other raw materials for mankind.[1,2] *Skimmia anquetilia* N.P. Taylor & Airy Shaw, a Rutaceae family member, is a gregarious aromatic shrub often known as "Nair," "Nairpati," or "Nairpat," that grows in shaded regions and is recognised for its pleasant aroma[3] (Fig. 1). It is found to be distributed from the Himalaya in Kashmir, India and spread right across south-east Asia, Japan and China. It has also been found growing in shrubberies of Afghanistan to Western Nepal.[4] There are 7 species in the genus *Skimmia* reported to be distributed worldwide,[5] three of which (*Skimmia laureola*, *Skimmia anquetilia*, *Skimmia arborescens*) are reported in India. One species (*Skimmia anquetilia*) has been reported in Uttarakhand.[6-8] The *S. anquetilia* plant's roots have

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been reported as remedy to scorpion and snake stings, whereas the plant leaves are used to treat smallpox. In indigenous medicine, the dried leaves have been reported to be used as pesticides, insecticides, food, and flavour in the traditional cuisines of some hill tribes.[9] The local people of the Pir-Panjal Himalayan Range use the plant for paralysis, pneumonia, lung cancer and anaesthesia.[10]

EOs contain terpenoids, which are considered to be responsible for their bioactivity, and modes of resistance.

Figure 1. *Skimmia anquetilia* aerial part.

EOs have been marketed as safe alternatives to synthetic chemical pesticides for pest control because of their low harm to the environment and public health. The EOs of *S. anquetilia* have drawn a lot of interest due to their inherent insecticidal, nematicidal, antifungal, antibiotic, and antiviral capabilities, which protect plants from diseases.[11] The variability in the chemical constituents of the EO of *S. anquetilia* has been ascribed to various components, including geographic conditions, seasonal, climate, harvest time, and distillation method. In a previous study, linalool and linalyl acetate have been reported as the major component of EO extracted from *S. anquetilia.*[12,13,14]

The plant seems to be one of the most promising alternatives to conventional insecticides and pesticides. Plant nematodes are difficult to be controlled by a single strategy, and require a variety of methods as part of an integrated system for managing destructive organisms.[15] Synthetic prescribed nematodes like carbofuran and flupyram have unfavourable effects on the environment, non-target organisms, and plants, leading to a number of environmental and health problems like pesticide poisoning, persistent toxicity in the water, soil, and detrimental effects on beneficial insects.[16,17] As a result, it is necessary to create selective, ecofriendly and safer alternatives approaches for better control of pests. Recently, extensive efforts have been made to screen plants in order to develop new botanical pesticides as an alternatives to the currently used insecticides.[18,19] Botanical insecticides are rapidly biodegradable, having little or no effect on the environment or non-target microorganisms, also are cheap and simple to manufacture, and may inhibit the development of resistance.[20]

A wide range of plant metabolites with nematostatic and nematicidal activities has been thoroughly reported in order to develop eco-friendly alternatives**.** The EOs have been reported to be effective against insects and nematodes.[21,22] The literature search revealed that the plant's antifeedant and nematicidal effect against agricultural pests have not yet been fully investigated. Although the chemical makeup and antioxidant potential of *S. anquetilia* EOs have already been reported from our laboratory[9], we have reinvestigated the seasonal variation in the chemical constituents of EOs extracted from the aerial parts of *S. anquetilia,* and performed pesticidal evaluation with respect to anti-feedant and nematicidal activities.

2. METHODOLOGY

2.1 Plant Sample Collection and Preparation

The fresh aerial plant part material of *Skimmia anquetilia* was collected from Harinagar, District Nainital, Dhari, Uttarakhand, India, situated at the height of 2100m in the months of September 2020 and January 2021. *S. anquetilia* was verified and validated by Dr. D.S. Rawat (Plant Taxonomist), Department of Biological Sciences, CBSH, G.B. Pant University of Agriculture and Technology, Pantnagar with voucher specimen number GBPUH-1027.

2.2 EO Extraction, GC-MS Analysis and Identification of Chemical Compounds

About 950 g of the aerial portions of *S. anquetilia* were subjected to the hydro distillation method in a Clevenger-type equipment for about 3-4 h for EO extraction. EOs were collected neat and desiccated over anhydrous sodium sulphate. In order to facilitate further investigation, the obtained EOs were kept at low temperature (4°C) in the refrigerator. For SKREO and SKWEO, the yield of oils measured on a fresh weight basis was found to be 0.7 % and 0.6 % (v/w), respectively.

Using a GC-MS-QP 2010 Ultra with a 5MS DB-5 silica capillary column (30 m × 0.25 mm and 0.25 µm thickness) and a mass spectrometer (5971 A°) as the detector, volatile EOs were examined for chemo-profiling. Carrier gas He, column flow rate of 1.21 mL/min, injection method: split, pressure of 69 KPa, split ratio of 1:10, inter-phase temperature of 270 °C, and scan range of 50-600 Da were the parameters programmed for the analysis. The temperature was first set at 50 °C for 2 min, then increased to 210 °C (3 °C rate per min), then again decreased for 2 min, then again increased to 240 °C (8 °C rate per minute) and isothermal for 11 min. MS were recorded using a split mode of 1:100 with an injection volume of 0.1 µL under EI conditions (70 eV). By comparing the mass spectra fragmentation pattern with that of actual samples of Kovatt indices and that of the MS library (WILEY8.LIB, NIST14.lib, and FFNSC2.lib) and by comparing the spectra with published data, it was possible to identify the components of the Eos.[23]

2.3 Anti-feedant Activity

The insect anti-feedant activity of SKREO and SKWEO against *Spodoptera litura* was performed under no-choice conditions. EOs at different concentrations of 2.0 µL, 4.0 µL and 8.0 µL were prepared in Tween-20 (1.0 %) solution. Fresh castor leaves were cut into 4×4 square shapes and dipped in each tested oil concentration for 20 sec and then air dried. Similarly, fresh castor leaves were also submerged in Tween 20 (1.0 %) as control setup. The third instar insects were starved for 2-3 h prior to treatment. The treated dried leaves were transferred into petri plates for feeding the insects. Three replicates for each sample were kept for analysis. After 12 and 48 h, the amount of leaf area consumed was plotted on graph paper.[24] Total leaf area consumed was observed after 24 and 48 h and per cent anti-feedant activity was calculated as follows:

$$\% \text{ antifeeding activity} = \frac{\text{Leaf area consumed in control - leaf consumed in treatment}}{\text{Leaf area consumed in control - leaf consumed in treatment}} \text{ X } 100$$

2.4 Nematicidal Activity

2.4.1 Nematode Population Collection

Meloidogyne incognita was raised on capsicum (*Capsicum annuum*) in a glasshouse at 25±2 °C in the Vegetable Research Center of G.B.P.U. A&T, Pantnagar. From the sickly capsicum roots, hand-selected mature egg masses were removed and cultivated in distilled water at 25 °C in a growth chamber. Emerged juveniles (J2) were gathered and kept in storage at 5 °C until needed.[25]

2.4.2 Mortality Assay on J_2 Larvae of M. Incognita

The experiment was carried out to assess the effectiveness of SKREO and SKWEO on the mobility of second-stage larvae (J_2) against *M. incognita*. To separate J_2 larvae, the galled capsicum roots were thoroughly rinsed in running water and then cut into 2 cm small pieces. The small pieces of roots placed in sodium hypochlorite (2 %) solution was shaken for two minutes to separate the organic matter from the eggs. The eggs were sieved on a 38µm-pore, collected, and cleansed after the solution was put through a series of sieves. To collect 2nd stage juveniles from hatched eggs after 48 hrs, the egg suspension was first incubated at 28±1°C. 100 juveniles were taken on Petri dishes containing three concentrations (1.0, 3.0, and 5.0 µL/mL) of EOs. Observations of three replicates were taken at the time intervals of 24, 48, and 72 hrs using a stereo-binocular microscope. The juveniles in Tween 20 (1 %) were treated as control. Every treatment was set up using a CRD (completely randomised design). Larvae were put in water to see if they were still alive (mortality). None of the motionless larvae regained their movement, proving that they had all died. Abbott's algorithm was used to determine the percentage of nematode mortality.

$$\% \, nematode \, mortality = 100 \times (1 - \frac{Nt}{Nc})$$

where, Nt = number of mobile nematodes after the treatment; Nc = number of mobile nematodes in water control.

2.4.3 In-vitro Egg Hatchability Test of M. Incognita

The experiment was conducted to evaluate the effectiveness of SKREO and SKWEO on the emergence of *M. incognita* eggs. To calculate the effect of EOs on the number of emerged eggs from egg masses, root-knot nematodes of an infected capsicum plant were employed. In gridded Petri dishes with SKREO and SKWEO concentrations of 2.0, 6.0, and 10.0 µL/mL, two egg masses of *M. incognita* were taken. The egg masses dipped in a Tween 20 (1.0 %) were used as a control. A BOD incubator was used to keep all treatments set up in triplicate and an entirely random order while maintaining a steady temperature of 27 °C. After 24 h, 48 h, and 72 h, the number of eggs emerged were counted under a microscope (magnification of 40X) to record the findings, respectively.

2.5 Statistical Analysis

The experimental data were expressed as mean±standard deviation, and each treatment was duplicated three times. At a 1 % significance level (p=0.01), ANOVA was used to analyse the experimental data for nematicidal and antifeedant actions. At the appropriate level of significance, it was discovered that the studied data were significantly different. Using the SPSS.16 programme, three factor analysis was performed on the combined data.

3. RESULTS AND DISCUSSION

3.1 Seasonal Effects on Chemical Makeup of EOs

The average yields of SKREO and SKWEO was found to be 0.70 % and 0.60 %, respectively. The GC/MS analysis might reveal the presence of over 42 compounds contributing 95.3 % of the total oil composition in SKREO and over 50 constituents contributing 95.4 % of the total oil in SKWEO. Both SKREO and SKWEO were found to be dominated by monoterpene ester (26.6% and 28.2%), oxygenated monoterpene (23.8% and 23.6%), monoterpene hydrocarbon (23.3% and 23.3%), hydrocarbons (12.7% and 12.7%), oxygenated sesquiterpene (7% and 6.3%), sesquiterpene hydrocarbons (1.4% and 1.0 %) and sesterterpene hydrocarbon (0.5% and 0.3%) respectively. A significant qualitative and quantitative difference in the essential oil composition in present and previous studies by Mathela, *et al*., 1992 and Gondwal *et al.*, 2012 has been observed (Table 1).

Linalyl acetate (15.8%) was the majoritarian component in SKREO followed by linalool (13.2 %), geijerene (11.6%), α-thujene (11.3%), α-terpineol (6.1%), geranyl acetate (5.0%), α-terpinyl acetate (3.3%), neryl acetate (2.3%) and α-pinene (2.1%) whereas in SKWEO the major component remains the same viz. linalyl acetate (17.6%) followed by linalool

Compounds	KI	Classes of compound	% Contribution			
			Present Study		Previous study	
			SKREO	SKWEO	Mathela, *et al.*, 1992	Gondwal, *et al.*, 2012
α-thujene	930	MH	**11.3**	**11.1**	**-**	**t**
α-pinene	939	MH	2.1	2.2	0.2	t
camphene	954	MH	T	t	-	t
sabinene	975	MH	1.4	1.5	0.1	t
myrcene	990	MH	3.0	3.1	0.4	t
cis-*β*-ocimene	1037	MH	2.0	2.2	0.7	0.8
heptyl acetate	1043	ME	-	t	-	-
trans-*β*-ocimene	1050	MH	2.1	2.3	0.5	0.1
γ-terpinene	1059	MH	0.1	t	-	0.6
p-mentha-3,8-diene	1072	MH	0.2	0.1	-	-
cis-linalool oxide	1074	OM	-	t	-	-
terpinolene	1088	MH	0.4	0.2	-	t
linalool	1096	OM	**13.2**	**13.9**	**7.8**	**9.5**
geijerene	1143	Hydrocarbon	**11.6**	**11.7**	**5.0**	**2.0**
β-pinene oxide	1159	OM	0.1	0.1	-	0.6
α-phellandrene	1170	MH	0.7	0.6	-	t
α-terpineol	1188	OM	6.1	6.1	1.8	5.6
nerol	1229	OM	1.3	1.3	0.6	0.1
β-phellandrene	1230	MH	-	-	1.6	1.8
isogeijerene C	1249	Hydrocarbon	-	-	-	0.4
geraniol	1252	OM	2.6	1.9	1.9	-
linalyl acetate	1257	ME	**15.8**	**17.6**	**55.6**	**7.3**
pregeijerene B	1276	Hydrocarbon	-	0.1	-	-
cogeijerene	1285	Hydrocarbon	0.7	0.4	-	-
pregeijerene	1287	Hydrocarbon	0.4	0.5	12.3	0.1
terpinen-4-ol acetate	1299	OM	0.5	0.3	-	0.4
ethyl linalyl acetate	1344	ME	0.2	**-**	**-**	**-**
α-terpinyl acetate	1349	ME	3.3	3.1	0.9	4.2
cis-hydrindane	1351	Ses H	0.5	0.3		-
neryl acetate	1361	ME	2.3	2.4	1.1	0.9
8-epi-dictamnol	1380	OS	-	t	-	-
geranyl acetate	1381	ME	5.0	5.1	2.5	1.8
(Z)-caryophyllene	1408	OS	-	t	-	-
dictamnol	1429	OS	-	0.4	-	-
aromadendrene	1441	SH	-	0.3	-	-

humulene	1454	SH	0.4	-	-	0.4
cabreuva oxide B	1464	OS	0.2	0.1	-	-
epi-cubebol	1494	OS	-	-	-	0.9
bicyclogermacrene	1500	SH	0.2	0.2	-	1.6
α-farnesene	1505	SH	0.4	t	-	-
α-cadinene	1538	SH	-	-	-	0.8
α-copaen-11-ol	1541	OS	T	-	-	-
elemol	1549	OS	1.8	1.3	0.6	-
nerolidol	1563	OS	0.4	0.4	0.4	0.4
germacrene D-4-ol	1575	OS	-	t	-	-
caryophyllene oxide	1583	OS	0.1	t	-	4.4
viridiflorol	1592	OS	-	-	-	0.1
γ-eudesmol	1632	OS	0.3	t	-	3.9
α-acorenol	1633	OS	0.2	-	-	-
guaia-3,9-dien-11-ol	1649	OS	0.2	0.1	-	-
β-eudesmol	1650	OS	-	-	-	0.2
α-cadinol	1654	OS	-	-	-	3.0
selin-11-en-4-α-ol	1659	OS	-	-	-	3.1
bulnesol	1671	OS	-	-	-	0.2
elemol acetate	1680	OS	1.9	1.6	-	-
α-bisabolol	1685	OS	-	-	-	7.2
caryophyllene acetate	1701	SH	0.4	0.5	-	4.4
bicyclovetivenol	1742	OS	-	-	-	1.4
(2E,6E)-farnesol	1743	OS	0.2	-	0.7	-
γ-eudesmol acetate	1784	OS	-	t	-	-
(2E,6E)-farnesyl acetate	1846	OS	1.7	2.4	1.5	-
(Z)-lanceol acetate	1855	SE	-	t	-	-
Total (%)			95.3	95.4	96.2	68.2
Monoterpene Hydrocarbon (%)			23.3	23.3	3.5	3.3
Oxygenated Monoterpene (%)			23.8	23.6	12.1	16.2
Sesquiterpene Hydrocarbon (%)			1.4	1.0	-	7.2
Oxygenated Sesquiterpene (%)			7.0	6.3	3.2	24.8
Sesterterpene Hydrocarbon (%)			0.5	0.3	-	
Monoterpene Ester (%)			26.6	28.2	60.1	14.2
Sesquiterpene Ester (%)			-	t	-	-
Hydrocarbons (%)			12.7	12.7	17.3	2.5

SKREO= *Skimmia anquetilia* rainy essential oil, SKWEO= *Skimmia anquetilia* winter essential oil, t = trace >, 0.1. MH= Monoterpene Hydrocarbon, OM= Oxygenated Monoterpene, SH= Sesquiterpene Hydrocarbon, OS= Oxygenated Sesquiterpene, ME= Monoterpene esters, Ses H= Sesterterpene Hydrocarbon, SE= Sesquiterpene Ester.

(13.9%), geijerene (11.7%), α-thujene (11.1%), α-terpineol (6.1%), geranyl acetate (5.1%), α-terpinyl acetate (3.1%), neryl acetate (2.4%) and α-pinene (2.2%). The study of the EO composition of SKREO and SKWEO showed variation in the quantity of percent contribution for the major and minor constituents in both seasons. The oil collected in the rainy season (SKREO) revealed the presence of α-humulene (0.4%) and (2*E*, 6*E*)- farnesol (0.2%), which were found to be missing in SKWEO. Similarly, components like aromadendrene (0.3%) and dictamnol (0.4%) were present in SKWEO but missing in SKREO. The present research compares the seasonal chemical components profiles of SKREO and SKWEO with that of existing report in a detailed manner in Table 1. The GC-MS chromatogram of SKREO and SKWEO is shown in (Fig 2).

To identify possible relationships between volatile compound concentrations and seasonal collection, cluster analysis was applied to a matrix linking EO composition to seasonal variation. The dendrogram obtained from cluster analysis of EOs collected for two seasons revealed segregation of the identified compounds into four main clusters, endorsing the results of the dendrogram displayed in (Fig 3), which revealed the near distance of volatile compound concentrations of SKREO and SKWEO in different seasons. The dendrogram revealed segregation of the identified compounds into four main clusters endorsing the results of major compounds in first cluster (linalyl acetate, linalool, α-thujene, geijerene) and second cluster (α-terpineol, geranyl acetate) whereas minor compounds in the third cluster (α-phellendrene, geijerene, nerolidol, pregeijerene, (Z)-caryophyllene etc.) and trace compounds in the fourth cluster (myrcene, α-terpinyl acetate, sabinene, nerol, elemol etc) as displayed in (Fig 3). The dendrogram revealed the near distance of volatile compound concentrations of *S. anquetilia* obtained from the present study and previous literature.

On comparing the data with previous study, it was observed that the major constituent linalyl acetate was found to be 55.6 % and 7.3 % respectively[12,13], whereas in SKREO and SKWEO it was 15.8 % and 17.6 % respectively. Compounds like geraniol, elemol, nerolidol, guaia-3,9-dien-11-ol, (2*E*,6*E*)- farnesol, (2E,6E)-farnesyl acetate , aromadendrene, *cis*-hydrindane, dictamnol, 8-*epi*-dictamnol, *β*- germacrenol, *β*- eudesmol acetate, lanceol acetate, (Z)- caryophyllene identified in SKREO and SKWEO were totally missing in the previous study.[17] Compounds such as α-thujene, α-pinene, myrcene, sabinene, terpinolene etc. identified as major constituents in SKREO and SKWEO were present in trace amount in previous study. The compounds like geraniol, elemol, nerolidol identified in SKREO and SKWEO were missing in previous study. Similarly, the compounds like *β*-phellandrene, *epi*-cubebol, α-cadinene, nerolidol, viridiflorol,

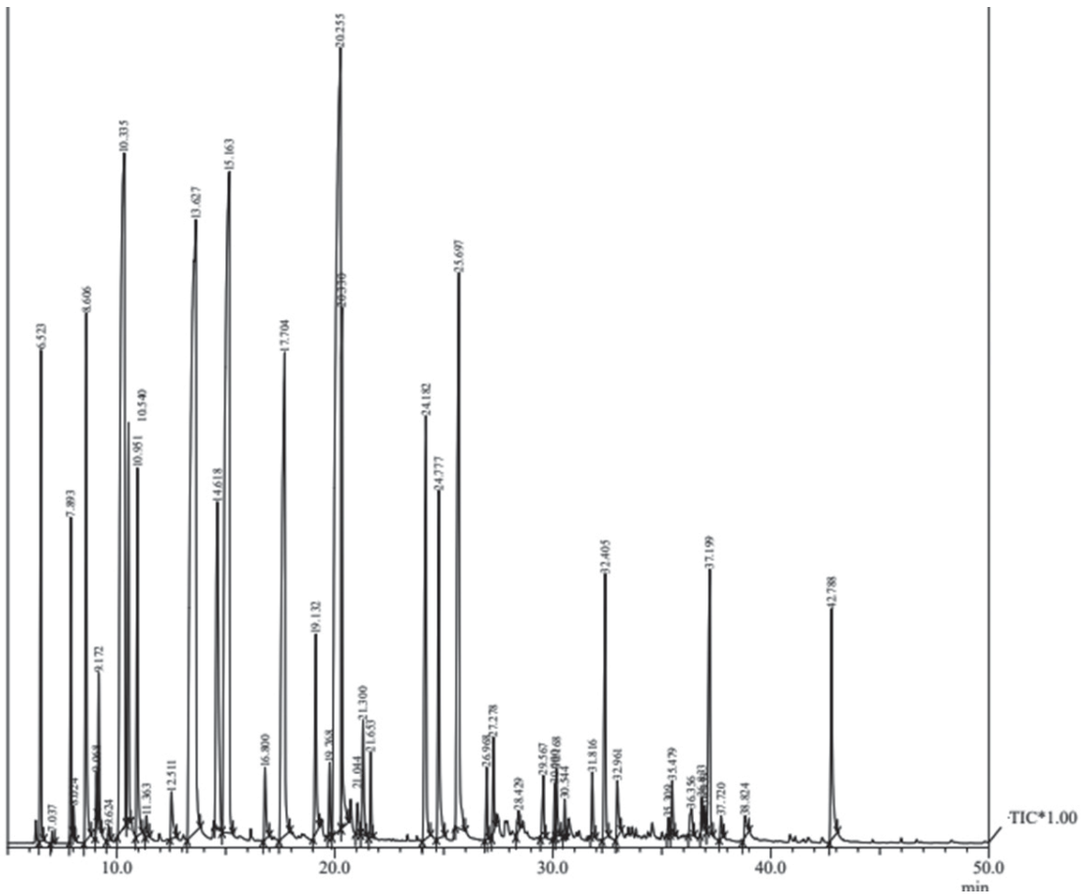

Figure 2. (a) GC-MS chromatogram of SKREO.

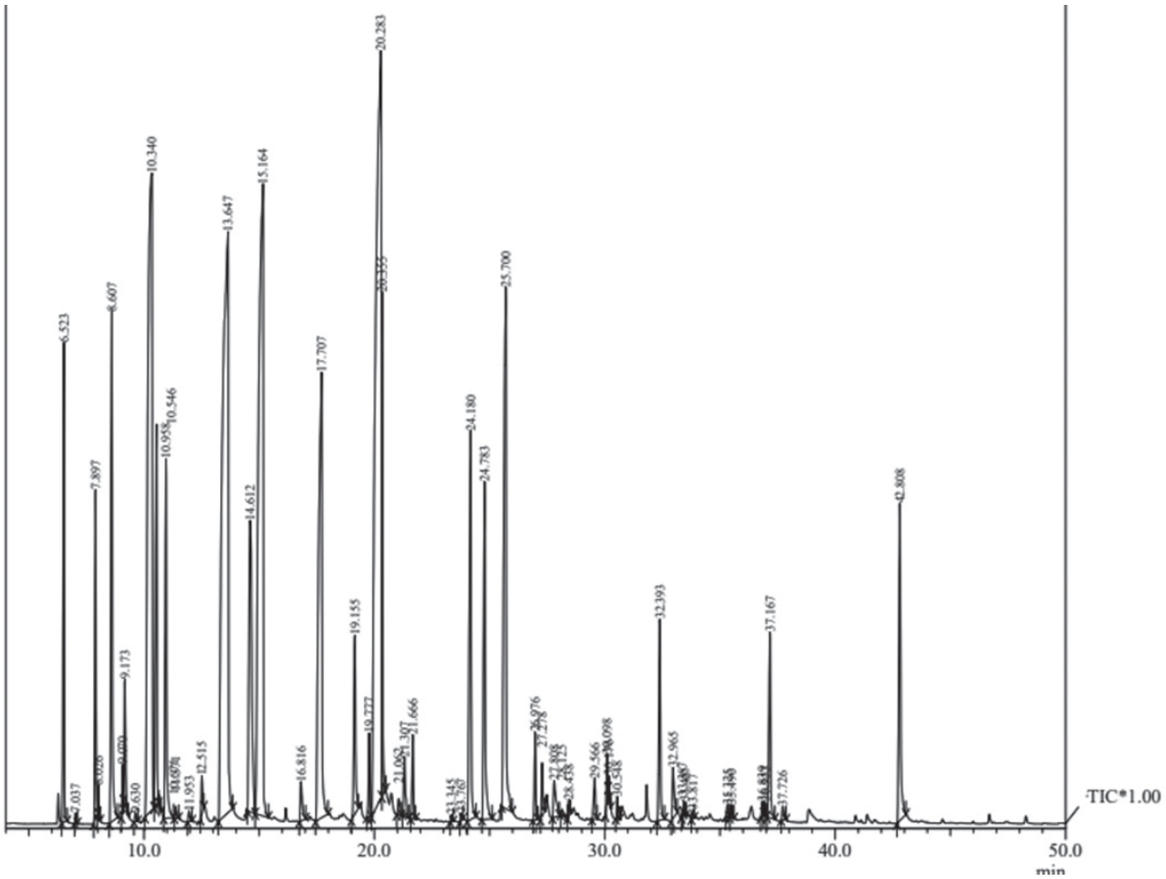

Figure 2. (b) GC-MS chromatogram of SKWEO.

β-eudesmol, selin-11-en-4-α-ol, α-cadinol, bulnesol, α-bisabolol and bicyclovetivenol were identified in prior study but missing in SKREO and SKWEO. The linalyl acetate and linalool have also been reported in aerial parts of *Skimmia laureola* and *Skimmia anquetilia.*[12] Linalool has been reported as a major compound in leaf EO of *Skimmia laureola* from Pakistan, whereas linalyl acetate has been reported as the major constituent in the leaf EO from the central Himalayas of Jammu and Kashmir in India.[26] *β*-phellandrene, *α*-pinene, myrcene, limonene, linalool, linalyl acetate, α-terpineol, neryl acetate, geranyl acetate have been reported in the EO of *S. japonica* ssp. *japonica* and *S. japonica* ssp. *Reevesiana.*[27] Thus, the two main compounds, linalool and linalyl acetate, were found common in all the *Skimmia* species. However, *β*-phellandrene (52.0%), *α*-pinene (13.9%) and limonene (5.95%) have been reported as major compounds in *S. japonica* ssp. *Japonica.*[27] The study and published literature reveal that the linalool and linalyl acetate are the marker compounds in *Skimmia anquetilia*.

3.2 Pesticidal Activities

3.2.1 Antifeedant Activity

The antifeedant activity of SKREO and SKWEO was assessed using the mean leaf area consumed (MLAC) through 'No choice method' in a dose dependent manner against 3rd instar larvae of *S. litura*. Percent antifeeding activity at the high dose level of 8 µL/mL was observed to be 74.06 % in SKREO and 91.67 % in SKWEO. The percent feeding reduced from 58.5 % to 18.12 % in SKREO and from 10.25 % to 1.00 % in SKWEO as the dose value of EOs increased from 2-8 µl/mL. The percent feeding showed an inverse relationship with the percent antifeeding activity. Both SKREO and SKWEO exhibited potent antifeeding activity against 3rd instar larvae of test insect *S. litura*. The detailed antifeeding activity of SKREO and SKWEO has been depicted in Table 2 and 3. The growth and development behavior of EO/extract from *S. anquetilia* has been carried out on *Caryedons erratus* from our laboratory. The antifeedant activity of α-pinene and linalool against agricultural pest has been reported.[28] Geraniol, terpineol and linalool have been reported to possess insect repellent activity.[29] The major compounds such as *trans-β*-ocimene, α-pinene, α-terpineol, linalyl acetate, linalool and dictamnol isolated from *S. laureola* have been reported to be responsible for the insecticidal property.[30] Based on literature search, no reports exist on the antifeedant potential of *S. anquetilia* EO against *S. litura.* As a result, it is being reported for the first time. Based on the aforementioned facts, it can be concluded that anti-feedant activity of SKREO and SKWEO might be possibly due to the presence of major constituents such as linalyl acetate, linalool, α-thujene, geijerene, α-terpineol, α-terpinyl acetate, myrcene, geranyl acetate, geraniol and α-pinene or due to the additive or synergistic effect of major, minor, or trace components of EOs which exhibits

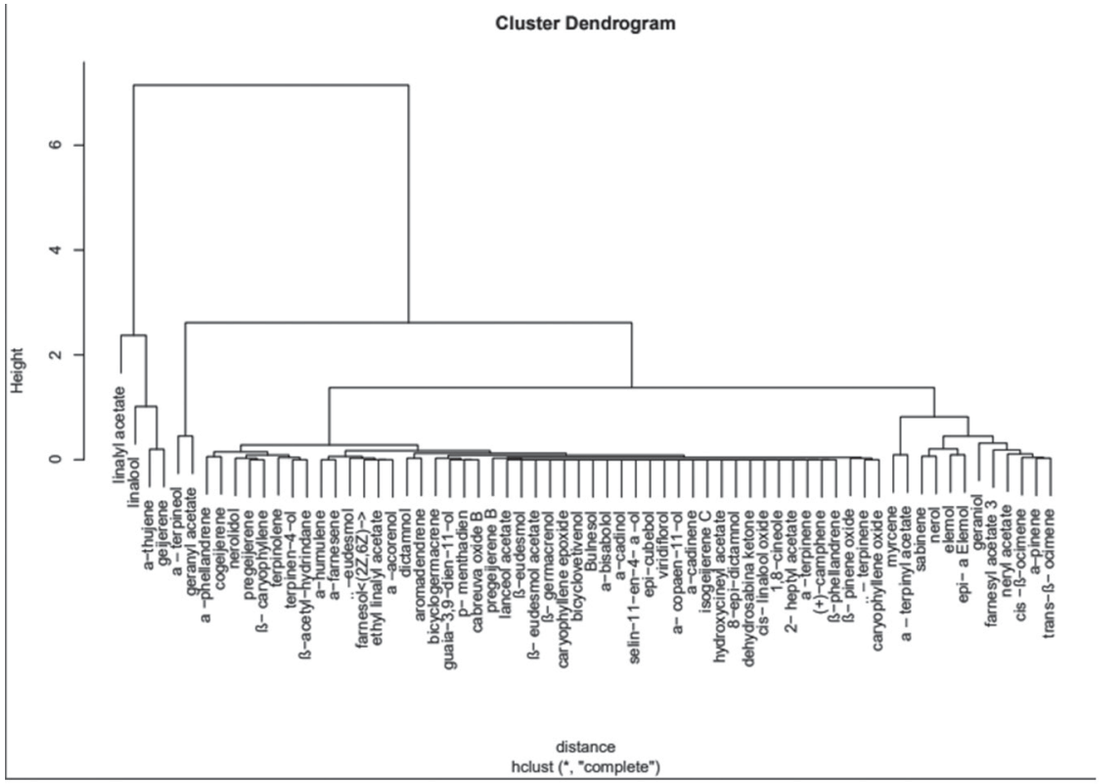

Figure 3. Cluster dendogram of linking essential oil composition to seasonal variation.

Table 2. Antifeedant activity of SKREO [a] against *Spodoptera litura*

Conc. (µL/mL)	Mean Leaf Area Consumed		MLAC [b]	Feeding %	Anti feedant activity	Feeding inhibition	Anti feedant category
	24 Hrs	48 Hrs					
2	8.1	10.62	9.36±1.78	58.5	16.27	8.86	Slight
4	3.43	7.02	5.22±2.53	32.62	53.30	36.34	Moderate
8	2.19	3.61	2.9±1.00	18.12	74.06	58.80	Extreme
Tween 20 (1 %)	9.92	12.45	11.18±1.78	69.87	0	0	
[c] CD1		0.655	**[d] CD2**	0.927	**[e] CD3**		1.311
			[f] CV = 49.85				

[a] **SKREO**= *Skimmia anquetilia* rainy essential oil, [b] **MLAC**= mean leaf area consumed, [c]**CD1**= Critical Difference with respect to (wrt) treatment, [d]**CD2**= Critical Difference wrt concentration, [e]**CD3**= Critical Difference wrt interaction between concentration and treatment, [f] **CV**= Coefficient of Variation

both qualitative and quantitative variations in their make-up.

3.2.2 Nematicidal Activity

3.2.2.1 J_2 Mortality

SKREO and SKWEO exhibited significant mortality against second stage larvae (J_2) of root knot nematode, *M. incognita* after 24, 48 and 72 h. The immobility of SKREO and SKWEO against *M. incognita* was dose and time-dependent when compared to the negative control. As compared to the negative control, SKREO and SKWEO showed dose-dependent and time-dependent immobility against *M. incognita*. The optimal dose level of SKREO was observed to be 5 µl/mL, inhibiting larval mobility by 48.66 %, followed by SKWEO, which inhibited larval mobility by 47.41 %. The mean mortality at 5 µl/mL were observed to be in the order: SKREO (48.66±9.97%) > SKWEO (47.41±7.13%) > control (7.19±4.27%). The percentage mortality of M.

Table 3. Antifeedant activity of SKWEO[a] against *Spodoptera litura*

Conc. (µL/mL)	Mean Leaf Area Consumed		MLAC[b]	Feeding %	Anti feedant activity	Feeding inhibition	Anti feedant category
	24 Hrs	48 Hrs					
2	1.94	1.34	1.64±0.42	10.25	17.07	7.87	Slight
4	0.99	0.91	0.95±0.05	5.94	50.52	33.80	Moderate
8	0.10	0.22	0.16±0.08	1.00	91.67	84.62	Extreme
Tween 20 (1 %)	2.22	1.62	1.64±0.42	12.00	0	0	
[c]CD1		0.640	**[d]CD2**	0.906	**[e]CD3**	1.281	
			[f]CV= 55.785				

[a]**SKWEO**= *Skimmia anquetilia* Winter Essential Oil, [b]**MLAC**= Mean Leaf Area Consumed, [c]**CD1**= Critical Difference wrt treatment, [d]**CD2**= Critical Difference wrt concentration, [e]**CD3**= Critical Difference wrt interaction between concentration and treatment, [f]**CV**= Coefficient of Variation

incognita 2nd instar larvae was observed experimentally in detail, and the results are shown in Table 4.

3.2.2.2 Egg Hatchability

Egg hatching was shown to be significantly inhibited by SKREO and SKWEO in a concentration and time-dependent manner. The emergence rate of eggs was observed to be inversely related to the dosage amount of oil samples and linearly proportional to the exposure time period. SKWEO showed higher inhibitory effect on eggs hatching of *M. incognita* as compared to SKREO. The mean percent rate of egg hatching at a dose level of 1 µL/mL in SKREO (15.98%) and in SKWEO (22.85%) whereas the rate of egg hatching at 5 µL/mL in SKREO (6.43%) and SKWEO (11.27%) revealed that the concentrations of SKREO and SKWEO are function of inhibition in egg hatching. The observation layout of percent egg hatching of *M. incognita* is being depicted in Table 5.

It has been reported that linalool, linalyl acetate, geraniol, *β*-pinene, α-terpineol, α-pinene, (Z)-caryophyllene, terpinen-4-ol, *β*-myrcene, sabinene, α-humulene, α-terpinene, α-terpinyl-acetate were found to be the major components of plant EOs which exhibited anti-hatching and anti-larvae ffect against *M. incognita*. EOs possessing high concentrations of monoterpenoids i.e. borneol, citral, carveol, geraniol, and *α*-terpineol have been reported as effective natural nematicides for the control of *M. Incognita.*[31-33] Additionally, it has been shown that some monoterpenoid components, such as linalool and eugenol, exhibit nematicidal activity with minimal toxicity.[34] The chemical constituents identified in SKREO and SKWEO are in agreement with the aforementioned constituents. It is thus inferred that the nematicidal activity in SKREO and SKWEO towards immobility of 2[nd] instar larvae of *M. incognita* and egg hatching might be possibly due to the presence of aforementioned constituents or addition/synergetic effect of major, minor or trace constituents of EO.

4. CONCLUSION

From reinvestigation of SKREO and SKWEO, it may be concluded that both the oils can be used as antifeedant agents and nematicidals, which shows their potency in various fields of pharmaceuticals, agrochemicals and cosmetics. Linalool and linalyl acetate, from various reports, have been observed as strong bioactive compound and possess different biological activities. In the current investigation, linalyl acetate is the major compound with 15.8 % - 17.6 % composition in SKREO and SKWEO, respectively.

The EO constituents like linalyl acetate, linalool, geijerene, thujene, geranyl acetate, α-terpineol, geraniol, α-pinene, α-terpinyl acetate, *trans-β*-ocimene, *cis-β*-ocimene, sabinene have been reported to have mild to high nematicidal and antifeedant action. In the present study, SKREO and SKWEO have been observed to possess diversified chemical composition with varying chemical makeup along with the above-mentioned compounds.

Therefore, it can be concluded that EOs of *S. anquetilia* can be used as an active agent in controlling important pests of field crops. *S. anquetilia* EO therefore attracts a lot of attention as natural insecticides and can be seen as one of the promising alternatives to conventional insecticides. Hence, these EOs could be used as natural antifeedant and nematicidal instead of the synthetic ones.

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REFERENCES

1. Inoue, M.; Hayashi, S. & Craker, L.E. Role of

Table 4. Nematicidal activity of SKREO and SKWEO against 2nd instar larvae of *M. incognita*

Sample	Dose (µL/mL)	Percent mortality of 2nd instar larvae of *M. incognita*			
		24Hrs	48Hrs	72Hrs	Mean
SKREO [a]	1	27.57±4.43	43.42±9.99	51.92±9.57	40.97±12.35
	3	32.03±7.34	42.14±8.86	54.61±8.22	42.92±11.31
	5	38.00±13.20	50.23±7.69	57.76±9.18	48.66±9.97
SKWEO [b]	1	15.99±9.91	28.67±13.94	39.21±10.85	27.75±11.62
	3	22.89±4.53	38.79±8.40	46.80±7.15	36.16±12.17
	5	39.78±5.53	48.54±5.46	53.91±8.10	47.41±7.13
Control	Tween 20 (1%)	1.57±0.49	9.44±4.34	10.57±1.53	7.19±4.271
			[c]CD (5%)		SEm±
	Hours (H)		6.36		2.18
	Concentration (C)		7.34		2.51
	Interaction (H×C)		12.72		4.36
			[d]CV= 25.43		

[a] **SKREO** = *Skimmia anquetilia* Rainy Essential Oil, [b] **SKWEO**= *Skimmia anquetilia* Winter Essential Oil, [c]**CD**= Critical Difference at 5 %, [d] **CV**= Coefficient of Variation

Table 5. Nematicidal activity of SKREO and SKWEO against egg hatching of *M. incognita* from egg masses

Sample	Dose (µL/mL)	Percent egg hatching inhibition of 2nd instar larvae of *M. incognita*			
		24 Hrs	48 Hrs	72 Hrs	Mean
SKREO[a]	1	8.09±1.48	18.74±1.37	20.96±1.72	15.98±6.87
	3	4.42±0.53	14.73±1.08	14.71±3.49	11.28±5.94
	5	2.71±0.41	10.12±1.99	6.47±1.96	6.43±3.70
SKWEO[b]	1	18.31±2.68	23.78±2.33	26.47±2.60	22.85±4.15
	3	9.01±2.04	18.11±2.74	19.53±2.25	15.55±5.70
	5	6.71±2.17	11.13±1.48	15.97±1.13	11.27±4.63
Control	Tween 20 (1 %)	14.00±1.96	36.46	46.42	32.29±14.33
			CD (5%) [c]		SEm±
	Hours (H)		1.82		0.62
	Concentration (C)		2.10		0.72
	Interaction (H×C)		3.65		1.25
			CV[d]= 12.37		

[a] **SKREO** = *Skimmia anquetilia* Rainy Essential Oil, [b] **SKWEO**= *Skimmia anquetilia* Winter Essential Oil, [c]**CD**= Critical Difference at 5 %, [d] **CV**= Coefficient of Variation

medicinal and aromatic plants: Past, present, and future. *Phcog.-Med. Plants*., 2019, 1-13. doi:10.5772/intechopen.82497.

2. Kumar, S.; Gustav, J.; Dobos, M.D. & Rampp, M.D. The Significance of Ayurvedic Medicinal Plants. *J. Evdi. based Compl. Alt. Med.,* 2017, **22**(3), 494-501. doi: 10.1177/2156587216671392.
3. Lubbe, A. & Verpoorte, R. Cultivation of medicinal and aromatic plants for specialty industrial materials. *J. Ind. Crop Prod.*, 2011, **34**(1), 785-801. doi: 10.1016/j.indcrop.2011.01.019.
4. Verma, P.; Singh, B.; Kaur, A. & Kumar, V. *In-vitro* anti-inflammatory and anti-arthritic activities of ethyl acetate extract of *Skimmia anquetilia* leaves. *J. Med. Herb Ethnomedicine.,* 2020, **6**, 42-44. doi:10.25081/jmhe.2020.v6.6238.
5. POWO 2021. Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. Published on the Internet http://www.plantsoftheworldonline.org/ Retrieved 15.07.2021.
6. Nair, K.N. & Nayar, M.P. Rutaceae In: Hajira, P.K., Nair, V.J. & Daniel, P. (eds.) Flora of India, Botanical survey of India, Calcutta. 1997, **4**, 259-408. doi: 10.1007/s12298-009-0025-7.
7. Uniyal, B.P.; Sharma, J.R.; Choudhery, U. & Singh, D.K. Flowering plants of Uttrakhand (A checklist). Bishen singh Mahendar pal Singh, Dehra Dun, 2007, 404 .
8. Pusalkar, P.K. & Srivastava, S.K. Flora of Uttarakhand, Gymnosperms and Angiosperms (*Ranunculaceae - Moringaceae)*. Botanical Survey of India, Kolkata., 2018, **1**, 1099.
9. Prakash, O.; Gondwal, M. & Pant, A.K. Essential oils composition and antioxidant activity of water extract from seeds and fruit pulp of *Skimmia anquetilia* NP Taylor & Airy Shaw. *Indian J. Nat. Prod. Resour.,* 2011, **2**(4), 435-441.
10. Wani, T.S.; Kumar, N.; Khan, J.; Shah, S.N. & Chandra, S. *In-vitro* cytotoxic activity of *Skimmia anquetillia* Taylor and Airy Shaw essential oils on various human cancer cell lines. *Int. J. Res. Pharm. Chem*., 2016, **6**(1), 89-94.
11. Hussein, R.A. & El-Anssary, A.A., Plants secondary metabolites: the key drivers of the pharmacological actions of medicinal plants. *Herb. Med.*, 2019, **1**, 13. doi; 10.5772/intechopen.76139.
12. Mathela, C.S.; Melkani, A.B. & Pant, A.K. Reinvestigation of *Skimmia laureola* essential oil. *Indian Perfumer*., 1992, **36**(3), 217-222.
13. Gondwal, M.; Prakash, O.; Vivekanand.; Pant, K.A.; Padalia, C.R. & Mathela, C.S. Essential oil composition and antioxidant activity of leaves and flowers of Skimmia anquetilia N.P. Taylor & Airy Shaw. J. Essent. Oil Res., 2012, **1**(24), 83-90. doi:10.1080/10412905.2012.64603.
14. Shah, W.A.; Qurishi, M.A. & Thappa, R.K. Seasonal variation in the essential oil composition of *S. laureola*. *Indian Perfumer*., 2003, **47**, 265-268.
15. Lambert, K. & Bekal, S. Introduction to plant-parasitic nematodes. *Plant Health Instr*., 2002 **10**, 1094-1218.
16. Westphal, A. Sustainable approaches to the management of plant-parasitic nematodes and disease complexes. *J. Nematol.,* 2011, **43**, 122-125. doi: 10.1016/j.fcr.2009.05.009.
17. Pavela, R.; Vrchotova, N.; ERA, B. 2008. Growth inhibitory effect of extracts from *Reynoutria* sp. plants against *Spodoptera littoralis* larvae. *Agrociencia*., 2011, **42**, 573-584.
18. Niranjankumar, B.V. & Regupathy, A. Status of insecticide resistance in tobacco caterpillar *Spodoptera litura* (Fabricius) in Tamil Nadu. *Pestic. Res. J*. 2001, **13**, 86-89.
19. Redwani, A.; Lazrek, H.; Bouallam, S.; Markouk, M.; Amarouch, H. & Jana, M. Larvicidal activity of extracts from Qureus lusitania var infectoria galls (Oliv). *J. Ethnopharmacol.*, 2001, **79**, 261-263. doi: 10.1016/S0378-8741(01)00390-7.
20. Malarvannan, S.; Giridharan, R.; Sekar,S.; Prabavathy , V. R. & Nair, S. Bioefficacy of crude and fractions of *Argemone mexicana* against tobacco caterpillar, *Spodoptera litura* Fab. (Noctuiidae: Lepidoptera). *J. Biopestic*., 2008, **1**(1), 55-62.
21. Al-Banna, L.; Darwish, R.M. & Aburjai, T. Effect of plant extracts and essential oils on root-knot nematodes. *Phytopathol. Mediterr*., 2003, **42**, 123-128. doi: 10.1400/14541.
22. Papachristos, D.P.; Karamanoli, K.I.; Stamopoulos, D.C. & Menkissoglu-Spiroudi, U. The relationship between the chemical composition of three essential oils and their insecticidal activity against *Acanthoscelides obtectus* (Say). *Pest Manag. Sci.,* 2004, **60**, 514–520. doi: 10.1002/ps.798.
23. Adams, R.P. Identification of essential oil components by gas chromatography/mass spectrometry, 4th Edition. Allured business media publishing corporation, carol stream, Illinois, USA, 2007, 804 p.
24. Zandi-Sohani, N.; Hojjati, M.; Angel, A. & Barrachina, C. Bioactivity of *Lantana camara* L. essential oil against *Callosobruchusmaculatus* (Fabricius). *Chil. J. Agric. Res*., 2012, **72**, 502.
25. Eisenback, J.D. Diagnostic characters useful in theidentification of four most common species of root-knot nematodes (*Meloidogyne* spp.). In: Sasser, J.N. and Carter, C.C. (eds.). An advanced treatise on *Meloidogyne*. Volume 1. Biological and control. Raleigh, NC, USA, North Carolina State University Graphics. 1985, 95-112 p.
26. Bhalla, P.; Tripathi, Y.C. & Varshney, V.K. Phytochemistry and biological activity of *Skimmia laureola* leaf essential oil-A review. *J. Essent. Oil Res*.**,** 2021, **33**(4), 315-329. doi: 10.1080/10412905.2021.1895337.
27. Fock, A.; Kubeczka, K.H. & Schultze, W. Comparative

studies on the composition of theessential oils of two subspecies of *S. japonica* Thumb. Proceedings 23rd International Symposium on Essential oils, Ayr, Scotland. 1992, 3-20.
28. Usha Rani, P.; Madhusudhanamurthy, J. & Sreedhar, B. Dynamic adsorption of α-pinene and linalool on silica nanoparticles for enhanced antifeedant activity against agricultural pests. *J. Pest Sci.,* 2013, **87**(1), 191-200.
doi: 10.1007/s10340-013-0538-2.
29. Kamatou, G.P. & Viljoen, A.M. Linalool–A review of a biologically active compound of commercial importance. *Nat. Prod. Commun*., 2008, **3**(7), 1183-1192.
doi:10.1177%2F1934578X0800300727 .
30. Mehmood, F.; Qasim, M.; Khan, Z.U.D.; Iqbal, N.; Mehmood, S.; Lateef, M. & Shahzadi, P. In vitro evaluation of anthelmintic activity of essential oils from different parts of *Skimmia laureola (DC.)* Zucc. Ex Walp., ver. Nair. *Pak. J. Bot*., 2011, **43**(6), 2915-2918.
31. Echeverrigaray, S.; Zacaria, J. & Beltrao, R. Nematicidal activity of monoterpenoids against the root-knot nematode *Meloidogyne incognita*. *Phytopathol.*, 2010, **100**(2), 199-203.
doi: 10.1094/PHYTO-100-2-0199.
32. Daddabbo, T.; Argentieri, M.P.; Laquale, S.; Candido, V. & Avato, P. Relationship between chemical composition and nematicidal activity of different essential oils. *Plants.*, 2020, **9**(11), 1546.
doi: 10.3390/plants9111546.
33. Ntalli, N.G.; Ozalexandridou, E.X.; Kasiotis, K.M.; Samara, M. & Golfinopoulos, S.K. Nematicidal activity and phytochemistry of Greek Lamiaceae species. *Agron.*, 2020, **10**(8), 1119.
doi: 10.3390/agronomy10081119.
34. Sangwan, N.K.; Verma, B.S.; Verma, K.K. & Dhindsa, K.S. Nematicidal activity of some essential plant oils. *Pestic. Sci.,* 1990, **28**(3), 331-335.
doi: 10.1002/ps.2780280311.

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