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CONTENTS

Seasonal changes in yield, composition and fumigant action of essential oil of *Murraya koenigii* L. against *Rhyzopertha dominica*(F.) and *Sitophilus oryzae* (L.)

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ABSTRACT: Experiments were conducted to study the variation in the yield and composition of essential oil of *Murraya koenigii* and its effect on fumigant action against *Rhyzopertha dominica* and *Sitophilus oryzae.* The essential oil was extracted from the leaf of test plant in each month of the year and its composition was determined through Gas Chromatography-Mass Spectrometry. The samples of spring (March-April), summer (May-June), rainy (July-September), autumn (October-November) and winter (December- February) were pooled to study the effect on test insects. The yield of essential oil varied from 0.07-0.43% in different month and maximum oil was obtained in November and December. The amount of monoterpenes was higher in autumn season (61.60%) followed by summer (59.00%), spring (55.80%), winter (55.41%) and rainy season (43.37%). On the other hand, sesquiterpenes were higher in spring season (36.46%) followed by winter (30.28%), autumn (28.55%),summer(26.60%) and rainy season(24.73%). Total terpenes were highest in spring (92.25%) followed by autumn (90.15%), winter (85.69%), summer (85.60%) and rainy season (68.10%). The essential oils of all the seasons inhibited 97.8 to 100.0 per cent progeny production at higher concentrations of 0.20 and 0.10% (w/v).At lower concentrations of 0.05, 0.025 and 0.012%, oils extracted in spring season was most effective against *R. dominica* and *S. oryzae,*while lowest efficacy was shown by oil extracted in rainy season. Results demonstrated that the efficacy of oil was dependent on level of constituents which varied with stage of plant and season.

Key words: Essential oil, fumigant toxicity, *Murraya koenigii,* monoterpenes, Rutaceae, *Rhyzopertha dominica,* sesquiterpenes, seasonal variation, *Sitophilus oryzae*

In due course of evolution, plants have developed numerous secondary plant metabolites for their defence against herbivores. However, these phytochemicals have also been found to influence the behaviour, growth and development of insects not co-evolved with it. Many of such chemicals belonging to various groups such as terpenoid, phenol and glucosinolate have been found to possess significant pest control properties against field and storage pests as they affect the feeding, breeding and survival of insects (Golob and Webley, 1980; Jacobson, 1983; Jilani, 1984; Grainge and Ahmed, 1988; Rajendran and Sriranjinia, 2008). Non-volatile secondary plant metabolites may be much useful against insect pests infesting crops under field condition while volatiles phytochemicals may be used for the control of storage pests. In past few decades fumigant toxicity of many essential oils has been studied against major insect pests of stored grain such as *Sitophilus oryzae*(Linnaeus), *Rhyzopertha dominica*(Fabricius), *Tribolium castaneum*(Herbst), *Callosobruchus chinensis* Linnaeus, *Sitotroga cerealella*(Olivier),*Corcyra cephalonica*(Stainton), etc. and some of these have shown promising activity(Singh *et al.*, 1989; Shaaya *et al.*, 1990, 1997; Tunc *et al.,* 2000; Tripathi *et al.,* 2002; Lee *et al.*, 2001a & 2001b;Rajendran and Sriranjinia, 2008; Tewari and Tiwari, 2008 ; Geetanjly and Tiwari, 2015;Gangwar and Tiwari, 2017;Kumar and Tiwari 2017a; 2017b; 2018a; 2018b; Kumar *et al.,* 2020; Sharma and Tiwari, 2021a; 2021b).However, due to various reasons, no commercial formulation has been developed so far from these tested essential oils for the management of storage insect pests. Some studies have indicated that yield and content of the essential oil depend on several extrinsic and intrinsic factors, including soil and climate conditions, harvesting season, and storing condition (Ram *et al.*, 2005;Verma *et al.*, 2009, 2010a;2010b) which may influence the toxicity against insect pests (*Jemaa et al., 2012*).The essential oil of *Murraya koenigii,* an edible plant, was reported to be toxic to *C. chinensis* (Pathak *et al.,*1997; Paranagama *et al.*, 2002),*R. dominica* (Geetanjly *et al.,* 2016; Kumar *et al.,* 2018a; 2018b; Joshi and Tiwari, 2019; Kumar *et al.,* 2019),*S. oryzae*

(Kumar *et al.,* 2018;Kumar *et al.,* 2019) and *T. Castaneum* (Kumar *et al.,* 2018;Kumar *et al.,* 2019a; 2019b). However, seasonal changes in its efficacy against most important insect pests of stored cereals such as *R. dominica* and *S. oryzae*, is still unknown. In the present investigation an attempt was made to study the effect of season on the yield and composition of essential oil of *Murraya koenigii* and its fumigant toxicity against *R. dominica* and *S. oryzae.* The study would be useful in identifying the most appropriate time period for harvesting and extraction of essential oil from this plant species for further use.

MATERIALS AND METHODS

Culture of insects

All the experiments were conducted in Post Harvest Entomology Laboratory of Department of Entomology, G.B. Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar. However, GC-MS analysis of oil was performed at Advance Instrumental Research Facility, Jawaharlal Nehru University, New Delhi. The test insects were cultured in the plastic jars of about 0.50 kg capacity at 27.0$\pm$1.0 ˚C temperature and 70.0$\pm$5.0 per cent relative humidity. For the proper aeration, the lid of the jar was bored to make a hole of 1.8 cm diameter which was covered with 30 mesh copper wire net. The adults of *R. dominica* and *S. oryzae* were reared on the untreated seed of wheat variety PBW-343 which was used after disinfestation in the oven at 60 °C for 12 hrs. After disinfestations the moisture content of the grain was measured and raised to 13.5 per cent by adding water in the grain. The quantity of water required to raise the moisture content was calculated by using following formula (Pixton, 1967).

$$\text{Quantity of water to be added} = \frac{W_1\,(M_2\text{-}M_1)}{100 - M_2}$$

Where,

W_1 = Initial weight of grain

M_1 = Initial moisture content

M_2 = Final moisture content

After mixing the water, the grain was kept in closed polythene bags for a week at room temperature for equilibration of moisture content. The grain was then filled in plastic jar and 50 adults of test insect were released in each jar after which it was kept in control room. First generation adults (0-7 days old) were used for experimental purpose.

Collection of plant materials and extraction of oils

Fresh leaves of *M. koenigii* were collected twice from Medicinal Plants Research and Development Centre (MRDC), Pantnagar at 15 days interval in each month throughout the year in different seasons e.g. Spring (March-April), Summer (May-June), Rainy (July-September), Autumn (October-November), Winter (December- February) in year 2014-15. The semi-dried leaves were subjected to steam distillation in a Clevenger Apparatus . After collection, the leaves were washed thoroughly in water and spread under shade to get the surface dry. A weighed quantity of 2400g fresh leaves was then kept for 3 days at room temperature for semi-drying after which it was used for oil extraction. Essential oil was extracted twice at 15 days interval in each month after which it was transferred directly in extraction burette and then in a measuring cylinder to calculated the volume (ml) of essential oil per 100g of fresh plant material. Anhydrous sodium sulphate was used to remove trace of moisture from essential oil which was stored in air tight container in a refrigerator at 4 °C.

Analysis of essential oil by GC-MS (Gas chromatography- Mass spectrometry)

GC-MS analysis of essential oil was performed in Advanced Instrumental Research Facility (AIRF), Jawahar Lal Nehru University (JNU) New Delhi. GC-MS data was obtained on a Shimadzu GCMS-QP-2010 plus system using AB inno-wax column (60 m x 0.25 mm id, film thickness 0.25 m). Analysis was performed using the following temperature program: oven keep isothermally at 60^{0}C for 2 min, increased from 60^{0}C to 200^{0}C at the rate of 3^{0}C/min for 2 min and from 200^{0}C to 250^{0}C at the rate of

10^{0}C/min for 5 minute. The injection temperature was 260^{0}C. Helium was used as carrier gas with a flow rate of 3.0 ml/min and split ratio 110:0. Scan time and Mass range were 0.20 sec and 40-650 m/z respectively. EI source and mass range were 70 eV and 40-650 amu.

Identification of compounds

The volatile compounds of essential oil were identified by calculating their retention time, relative to n-alkenes and data for authentic compounds available in Willey, NIST and Perfumery libraries and also by matching their mass spectrum fragmentation patterns with corresponding data stored in the mass spectra library of the GC-MS data system and their published mass spectra. The relative percentage amount of each identified compound was obtained from the electronic integration of its FID peak area.

Experiment detail

The experiments were conducted under controlled conditions at 27.0±1.0 ˚C temperature and 70± 5 per cent relative humidity in the plastic vials (10×4cm) having 50g wheat grain (moisture content 13.5 per cent). Ten adults of test insect (0-7 days old) were released in each vial after 24 hrs of which required quantity of oil poured on the absorbing mat was placed inside the grain. Screw cap of vial was tightly closed and made completely airtight by sealing with paraffin wax strip. Each treatment was replicated thrice and untreated grain was used as control. Insects were then allowed to feed and breed for one month after which F1 progeny emerged in each vial was counted thrice at two days interval and its sum was used as number of adults emerged in each treatment. Duncan-Multiple Range Test was used to determine the significance of differences between means. Correlation analysis was performed to study the effect of minimum and maximum temperature on yield of oil while relationship between monoterpene content of oil in different seasons and its fumigant activity was quantified by regression analysis.

RESULTS AND DISCUSSION

Effect of season on the yield of essential oil of *M. koenigii*

The average yield of essential oil of *M. koenigii* and its correlation with standard weather parameters from January to December is summarized in Table 1 from which it is evident that the yield of oil varied from 0.07 to 0.43% in different months. Maximum yield was obtained from the leaves extracted in November and December (winter) during which it varied from 0.41 to 0.43%. During this period maximum temperature ranged from 27.5 to 20.5 °C while minimum temperature was 10.2 to 7.6˚C with relative humidity of 90-94%. As compared to it, some reduction in the oil content was observed during the month of January, February and March when the yield varied from 0.31 to 0.34%. During this period, maximum and minimum temperature ranged from 27-17˚C and 13-8.1˚C, respectively. Significant reduction in essential oil was noticed in the month of April which indicated only 0.14% recovery of oil. Minimum yield of essential oil was recorded from the month of May to August during which it varied from 0.07 to 0.09% only. From April to August maximum temperature ranged from 32.44-37.8 ˚C while minimum temperature varied from 15.9 to 26.1 ˚C. The oil content started to increase from September and October which recorded 0.15-0.17%

Table 1: Yield of essential oil of *M. koenigii* in different months

S.N.	Month	Oil yield % (v/w)	Temperature ˚C	
			Max	Min.
1	January	0.34d*	17.0	8.1
2	February	0.31c	21.1	9.2
3	March	0.34d	27.4	13.0
4	April	0.14b	34.0	15.9
5	May	0.08a	37.3	21.9
6	June	0.09a	37.8	26.1
7	July	0.07a	32.4	25.9
8	August	0.08a	33.4	25.9
9	September	0.15b	32.8	23.5
10	October	0.17b	30.4	17.8
11	November	0.41e	27.5	10.2
12	December	0.43f	20.5	7.6
	Correlation of weather parameters with oil yield		-0.8**	-0.9**

*Means followed by different letters are significantly different

Table 2: Variation in the essential oil composition of *Murrayakoenigii* in different months and seasons

Compounds	RT	Content (%)MOD																	
			Spring			Summer			Rainy		Autumn				Winters				
		MarT1	AprT2	Mean	May T3	JunT4	Mean	JulyT5	AugT6	SepT7	Mean	OctT8	NovT9	Mean	DecT10	JanT11	FebT12	Mean	MOD
Terpenoids																			
Monoterpenes																			
α-Ocimene	8.0	29.0	28.4	28.70±0.42	26.4	27.5	26.95±0.78	-	20.5	-	6.83±11.84	23.4	32.2	27.80±6.22	26.8	-	25.3	17.37±15.06	MS
β-Ocimene	9.8	0.5	t	0.25±0.35	t	t	0.00±0.00	-	0.5	-	0.17±0.29	0.8	t	0.40±0.57	0.5	-	t	0.17±0.29	MS
α-Pinene	6.0	-	-	0.00±0.00	-	-	0.00±0.00	-	-	-	0.00±0.00	-	-	0.00±0.00	-	11.3	-	3.77±6.52	MS
β-Pinene	7.4	10.1	9.8	9.95±0.21	8.6	9.0	8.80±0.28	4.7	6.3	2.3	4.43±2.01	7.8	10.5	9.15±1.91	8.7	2.4	8.3	6.47±3.53	MS
α-Terpinene	8.7	0.5	t	0.25±0.35	t	t	0.00±0.00	-	t	-	0.00±0.00	t	-	0.00±0.00	5.4	0.3	-	1.90±3.03	MS
α-Phellandrene	7.2	3.7	3.4	3.55±0.21	3.2	-	1.60±2.26	-	-	-	0.00±0.00	6.5	2.5	4.50±2.83	t	-	6.7	2.23±3.87	MS
β-Phellandrene	7.2	-	-	0.00±0.00	-	3.1	1.55±2.19	-	-	-	0.00±0.00	-	-	0.00±0.00	-	-	-	0.00±0.00	MS
Myrcene	7.7	1.3	1.2	1.25±0.07	1.0	1.0	1.00±0.00	t	1.3	-	0.43±0.75	1.3	1.0	1.15±0.21	1.3		1.2	1.25±0.07	MS
3-Carene	11.4	-	t	0.00±0.00	-	-	0.00±0.00	-	t	-	0.00±0.00	t	-	0.00±0.00	t	0.5	t	0.17±0.29	MS
Limonene	9.2	6.2	6.7	6.45±0.35	9.9	8.7	9.30±0.85	-	4.9	-	1.63±2.83	5.5	12.5	9.00±4.95	7.6	11.2	5.8	8.2±2.75	MS
γ-Terpinene	10.2	1.1	0.9	1.00±0.14	0.6	0.5	0.55±0.07	-	t	-	0.00±0.00	t	-	0.00±0.00	1.0	-	-	0.33±0.58	MS
Nerol	12.1	t	t	0.00±0.00	t	0.5	0.25±0.35	1.1	0.7	0.9	0.90±0.20	0.7	0.7	0.70±0.00	1.0	1.5	t	0.83±0.76	MS
Sabinene	7.2	-	-	0.00±0.00	-	-	0.00±0.00	1.5	-	1.1	0.87±0.78	-	-	0.00±0.00	-	0.4	-	0.13±0.23	MS
α-Thujene	5.8	-	-	0.00±0.00	-	-	0.00±0.00	t	0.5	t	0.17±0.29	t	-	0.00±0.00	-	-	t	0.0±0.00	MS
Norbornane	6.5	1.4	1.3	1.35±0.07	1.2	1.3	1.25±0.07	06	1.0	t	0.53±0.50	1.1	1.5	1.30±0.28	1.2	2.0	1.2	1.47±0.46	MS
Eucalyptol	9.3		-	0.00±0.00	2.6	2.6	2.60±0.00	-	1.2	31.9	11.03±18.08	0.9	3.5	2.20±1.84	0.8	8.8	0.7	3.43±4.65	MS
Myrtenal	16.1	-	-	0.00±0.00	t	0.7	0.35±0.49	15.2	-	9.8	8.33±7.71	t	t	0.00±0.00	-	1.7	t	0.57±0.98	MS
verbenone	16.7	-	-	0.00±0.00	0.6	0.6	0.60±0.00	t	-	0.9	0.30±0.52	-	0.6	0.30±0.42	-	3.0	t	1.00±1.73	MS
Carvone	18.2	-	-	0.00±0.00	t	0.6	0.30±0.42	0.7	t	2.0	0.90±1.01	—	0.8	0.80±0.00	-	1.7	t	0.57±0.98	MS
α-Fenchol	6.5	2.1	2.2	2.15±0.07	2.5	2.5	2.50±0.00	3.0	2.3	3.3	2.87±0.51	2.5	2.3	2.40±0.14	1.9	5.0	2.5	3.13±1.64	MS
Pinocarveol	21.3	-	-	0.00±0.00	t	t	0.00±0.00	0.6	t	0.9	0.50±0.46	-	0.6	0.30±0.42	-	0.8	-	0.27±0.46	MS
Camphoraldehyde	13.0	-	-	0.00±0.00	t	t	0.00±0.00	-	-	-	0.00±0.00	-	0.6	0.30±0.42	-	1.7	-	0.57±0.98	MS
Limonene oxide	13.5	-	-	0.00±0.00	-	-	0.00±0.00	0.6	t	1.4	0.67±0.70	-	t	0.00±0.00	-	0.6	-	0.20±0.35	MS
Pinocarvone	14.6	-	t	0.00±0.00	t	t	0.00±0.00	t	t	t	0.00±0.00	t	t	0.00±0.00	-	0.7	t	0.23±0.40	MS
α-Terpineol	16.1	-	-	0.00±0.00	-	-	0.00±0.00	1.0	-	1.3	0.77±0.68	-	-	0.00±0.00	-	-	-	0.00±0.00	MS
α-Pinene oxide	12.4	-	-	0.00±0.00	-	-	0.90±0.14	1.0	-	1.4	1.23±0.72	-	-	0.00±0.00	-	-	-	0.00±0.00	MS
Longipinanol	39.0	-	-	0.00±0.00	-	-	0.00±0.00	-	-	0.5	0.17±0.29	-	-	0.00±0.00	-	0.6	-	0.20±0.35	MS
Terpinen-4-ol	15.3	1.2	0.6	0.90±0.42	0.5	0.5	0.50±0.00	t	1.9	t	0.63±1.10	1.9	t	0.95±1.34	0.9	-	1.4	0.77±0.71	MS
Sesquiterpenes																			
Copaene	23.8	0.8	0.8	0.80±0.00	-	0.6	0.30±0.42	-	-	-	0.00±0.00	-	t	0.00±0.00	0.5	-	t	0.17±0.29	MS
Elemene	22.7	1.0	1.2	1.10±0.14	1.0	1.0	1.00±0.00	1.4	1.9	2.3	1.87±0.45	1.4	1.0	1.20±0.28	1.3	0.8	1.9	1.33±0.55	MS
(E)-Caryophyllene	31.0	16.1	15.4	15.75±0.49	10.5	10.0	10.25±0.35	2.3	9.4	-	3.90±4.90	14.8	3.7	9.25±7.85	15.2	-	12.1	9.10±8.03	MS
α-Humulene	27.1	4.7	4.5	4.60±0.14	3.1	2.9	3.00±0.14	1.0	3.0	-	1.33±1.53	4.8	1.0	2.90±2.69	4.5	-	4.2	2.90±2.52	MS
γ-Muurolene	28.0	0.5	0.5	0.50±0.00	t	t	0.00±0.00	t	t	t	0.00±0.00	t	t	0.00±0.00	t	t	t	0.00±0.00	MS
α-Selinene	28.8	2.6	2.4	2.50±0.14	1.5		1.50±0.00	0.8	1.1	t	0.63±0.57	2.5	0.6	1.55±1.34	2.3		1.9	2.10±0.28	MS
β-Selinene	28.4	1.3	1.5	1.4±0.14	1.0	2.7	1.85±1.20	1.3	1.0	1.0	1.10±0.17	1.5	0.7	1.10±0.57	-	0.9		0.45±0.64	MS
β-Bisabolene	29.1	t	t	0.00±0.00	t	t	0.00±0.00	-	-	-	0.00±0.00		-	0.00±0.00	-	-		0.00±0.00	MS
γ-Cadinene	29.5	t	t	0.00±0.00	t	t	0.00±0.00	t	t	-	0.00±0.00	t	t	0.00±0.00	t	-	t	0.00±0.00	MS
α-Copaene	23.8	-	-	0.00±0.00	0.6		0.60±0.00	t	t	-	0.00±0.00	t	-	0.00±0.00	-	-		0.00±0.00	MS
α-Muurolene	28.0	1.1	t	0.55±0.78	-	-	0.00±0.00	t	0.8	-	0.27±0.46	0.6	-	0.30±0.42	t	-	t	0.00±0.00	MS
Selinene	28.8	-	-	0.00±0.00	-	-	0.00±0.00	-	-	-	0.00±0.00	-	-	0.00±0.00	1.0	-	1.4	0.80±0.72	MS
Epi cubenol	34.0	0.7	t	0.35±0.49	0.5	0.5	0.50±0.00				0.00±0.00	-		0.00±0.00	t	-	-	0.00±0.00	MS
α-Cyperone	38.4	-	-	0.00±0.00	t	t	0.00±0.00	-	t	0.5	0.17±0.29	-	t	0.00±0.00	-	0.5	-	0.17±0.29	MS
Caryophyllene oxide	31.0	4.7	6.0	5.35±0.92	0.5	8.0	4.25±5.30	17.9	0.6	18.8	12.43±10.26	10.1	10.1	10.10±0.00	4.1	17.5	11.6	11.07±6.72	MS
Nerolidol <(E)->	31.9	-	1.8	0.90±1.27	1.4	1.3	1.35±0.07	-	-	-	0.00±0.00	0.9	-	0.45±0.64	-	-		0.00±0.00	MS
Spathylenol	34.8	-	-	0.00±0.00	0.7	t	0.35±0.49	-	-	-	0.00±0.00	-	t	0.00±0.00	-	0.7	t	0.23±0.40	MS

Humulene epoxide	33.3	0.9	1.2	1.05±0.21	1.6	1.7	1.65±0.07	3.6	2.0	3.5	3.03±0.90	1.5	1.9	1.70±0.28	0.7	3.2	2.0	1.97±1.25	MS
Nerolidol <(Z)->	31.7	2.0	-	1.00±1.41	-	-	0.00±0.00	-	-	-	0.00±0.00	-	-	0.00±0.00	-	-	-	0.00±0.00	MS
Selin	35.2	-	1.2	0.60±0.85	-	-	0.00±0.00	-	-	-	0.0±0.00	-	-	0.00±0.00	-	-	-	0.00±0.00	MS
Miscellaneous compounds																			
Benzene	9.0	0.5	0.6	0.55±0.07	1.3	1.0	1.15±0.21	t	1.4	-	0.47±0.81	0.4	1.8	1.10±0.99	0.6	1.7	2.0	1.43±0.74	MS
Pinonic acid	19.6	-	-	0.00±0.00	t	t	0.00±0.00	t	t	-	0.00±0.00	-	-	0.00±0.00	-	0.7	t	0.23±0.40	MS
Thujyl acetate	21.0	-	-	0.00±0.00	1.1	1.1	1.10±0.00	1.0	0.8	1.7	1.17±0.47	-	1.5	0.75±1.06	-	3.3	0.5	1.27±1.78	MS
Chrysanthenol	21.8		-	0.00±0.00	t	0.5	0.25±0.35	t	t	0.8	0.27±0.46	-	0.8	0.40±0.57	-	1.4	-	0.47±0.81	MS
Intermedeol	33.6	1.5	t	0.75±1.06	1.0	1.2	1.10±0.14	t	1.6	1.3	0.97±0.85	1.7	t	0.85±1.20	1.2	0.9	1.6	1.23±0.35	MS
Isobornyl	36.4	-	t	0.00±0.00	t	t	0.00±0.00	0.7	t	0.5	0.40±0.36	-	t	0.00±0.00	-	0.7	t	0.23±0.40	MS
Benzoate	32.0	-	-	0.00±0.00	-	-	0.00±0.00	0.8	0.6	0.6	0.67±0.12	-	-	0.00±0.00	-	0.6	-	0.20±0.35	MS
Cyclohexene	22.1	0.6	0.5	0.55±0.07	-	-	0.00±0.00	0.5	11.7	-	4.07±6.62	t	0.5	0.25±0.35	0.5	-		0.25±0.35	MS
α-Campholenic aldehyde	13.0	-	-	0.00±0.00	-	-	0.00±0.00	0.5	t	1.0	0.50±0.50	-	-	0.00±0.00	-	-	-	0.00±0.00	MS

Grouped components	Spring	Summer	Rainy	Autumn	Winter
Monoterpenoids	55.80	59.00	43.37	61.60	55.41
Sesquiterpenoids	36.45	26.60	24.73	28.55	30.28
Total terpenes	92.25	85.6	68.1	90.15	85.69

SD= Standard Deviation(±); t= Trace (<0.05%); MOD= Mode of Identification; MS= Mass Spectra; RT, Retention Time

yield. The result clearly indicates that oil content decreased with increasing temperature; hence they are negatively correlated with each other. The low oil content from April to August may also be correlated with the age of leaf which is new and tender during these months. Presence of mature leaves from September to March may be the main reason for high oil contents as plant metabolites are accumulated more in old leaves. Seasonal variation in the essential oil has also been reported earlier in aromatic plants (Verma *et al.,* 2010 a,b; Padalia *et al.,* 2011). Essential oil yield from fresh leaves of *M. koenigii* varied from 0.15% to 0.18% in chemotype 'A' and 0.12% to 0.14% in chemotype 'B' in different seasons (Verma *et al.,* 2012). Oil yield of *C. distance* varied 0.34 to 0.44% during the year, with highest in winter season (0.44%) followed by spring (0.42%) (Verma *et al.,* 2013). The result showed that oil recovery is very high from November to March due to which this period is most suitable for harvesting of leaves and extraction of essential oil

Effect of season on the chemical composition of essential oil of *M. koenigii*

GC-MS analysis identified fifty-nine constituents in the essential oil of *M. koenigii* which represent 88.68% of total oil composition in various seasons (Table 2). Interestingly, all the seasons were dominated by monoterpenes (43.37-61.60%) followed by sesquiterpenes (24.73-36.45%). Major constituents (%) identified in the essential oil were α-Ocimene (6.83±11.84 to 28.70±0.42), β-Pinene (4.43±2.01 to 9.95±0.21), limonene (6.45±0.35 to 9.0±2.83), α-Phellandrene (1.60±2.26 to 4.50±2.83), norbornane (0.53±0.50 to 1.47±0.46), caryophyllene (3.90±4.90 to 15.75±0.49), caryophyllene oxide (4.25±5.30 to 12.42±10.26), humulene epoxide (1.05±0.21 to 3.03±0.3), elemene (1.00±0.35 to 1.87±0.45), α-selinene (0.63±0.57 to 2.50±0.41), α-humulene (1.33±1.53 to 4.60±0.14) and α-fenchol (2.40±0.14 to 3.13±4.65).

Chemical composition of *M. Koenigii* essential oil changed with month and season of collection (Table 2). Monoterpenes were recorded in higher quantity during autumn season (61.60%), while sesquiterpenes reached their higher value in spring season (36.45%).

Percentage of individual component obtained in different season indicated that content of α-Ocimene (28.70%), β-

Table 3: Fumigant action of essential oil of *Murraya koenigii* extracted in different seasons against *R. dominica*

S. No	Concentration % (v/w)	Spring		Summer		Rainy		Autumn		Winter	
		Number of adults emerged*	Per cent inhibition	Number of adults emerged*	Per cent inhibition	Number of adults emerged*	Per cent inhibition	Number of adults emerged*	Per cent inhibition	Number of adults emerged*	Per cent inhibition
1	0.2	0.00±0.00[a]	100.0	0.00±0.00[a]	100.0	0.00±0.00[a]	100.0	0.00±0.00[a]	100.0	0.00±0.00[a]	100.0
2	0.1	0.00±0.00[a]	100.0	0.00±0.00[a]	100.0	1.33±2.16[a]	98.7	0.00±0.00[a]	100.0	0.00±0.00[a]	100.0
3	0.05	10.08±2.69[b]	90.1	11.42±4.27[b]	89.1	23.17±1.53[b]	78.55	14.25±2.28[b]	85.8	15.50±2.43[b]	85.9
4	0.025	35.83±3.22[c]	64.8	56.75±3.99[c]	45.6	59.17±1.11[c]	45.21	37.50±2.99[c]	62.8	40.33±2.16[c]	63.5
5	0.012	58.92±1.55[d]	42.2	89.50±0.20[d]	14.2	96.39±0.91[d]	10.75	65.75±0.50[d]	34.7	69.33±2.94[d]	37.2
	Control	102.00±2.08[e]	0.0	104.33±3.79[e]	0.00	108.00±1.21[e]	0.00	100.67±1.31[e]	0.00	110.33±1.19[e]	0.00

*Means followed by the different letters in a column are significantly different according to Duncan-Multiple Range Test, ±Standard deviation,

Table 4: Fumigant action of essential oil of *Murraya koenigii* extracted in different seasons against *S. oryzae*

S. No.	Concentration % (v/w)	Spring		Summer		Rainy		Autumn		Winter	
		Number of adults emerged*	Per cent inhibition	Number of adults emerged*	Per cent inhibition	Number of adults emerged*	Per cent inhibition	Number of adults emerged*	Per cent inhibition	Number of adults emerged*	Per cent inhibition
1	0.2	0.00±0.00[a]	100.0	0.00±0.00[a]	100.0	0.00±0.00[a]	100.0	0.00±0.00[a]	100.0	0.00±0.00[a]	100.0
2	0.1	0.00±0.00[a]	100.0	0.00±0.00[a]	100.0	2.67±1.61[a]	98.1	0.00±0.00[a]	100.0	0.00±0.00[a]	100.0
3	0.05	18.17±2.25[b]	91.5	36.92±3.40[b]	79.9	54.22±2.74[b]	61.0	23.84±1.65[b]	83.3	22.72±4.07[b]	84.8
4	0.025	87.83±4.83[b]	58.8	113.08±0.74[c]	38.3	112.56±4.23[c]	20.9	75.42±2.61[c]	47.0	94.17±2.72[c]	37.1
5	0.012	133.00±3.47[b]	37.6	148.50±2.12[d]	19.0	138.11±3.19[d]	3.0	108.34±3.19[d]	23.9	122.72±2.65[d]	18.0
	Control	213.00±3.06[b]	0.0	183.33±3.51[e]	0.0	142.33±2.52[e]	0.0	142.33±2.65[e]	0.0	149.67±2.00[e]	0.0

*Means followed by the different letters in a column are significantly different according to Duncan-Multiple Range Test ± Standard deviation

Pinene (9.95%), caryophyllene (15.75%) and α-selinene (2.50%) were higher in spring season, while β-phellandrene (1.55%), limonene (9.30%), β-selinene, (1.85%), α-copaene (0.60%) and epi-cubenol (0.50%) recorded higher during summer season. The amount of eucalyptol (11.03%), myrtenal (8.33%), limonene oxide (0.67%), α-Pinene epoxide (1.23%), elemene (1.87%), caryophyllene oxide (12.43%), humulene epoxide (3.03%), ethyl acetate (1.17%) and cyclohexane (4.07%) was found to be higher in rainy season. Moreover, α-phellandrene (4.50%) and α-Fenchol (3.13%) were noted higher in autumn and winter season, respectively. Others minor and trace constituents of essential oil were less in quantity, however, they play important role. The flavor and aroma depends on major constituents of essential oil (Nigam and Purohit, 1961; Walde *et al*., 2006; Chowdhary *et al.,* 2008 and Malwal and Sarin, 2010).

Effect of essential oil on development of *R. dominica* and *S. oryzae*

Effect of essential oil of *M. koenigii* extracted in different seasons on development of *R. dominica* is presented in Table 3 and Figure 1, which indicates that oil extracted in different seasons exhibited variation in their efficacy against *R. dominica* at lower concentrations. At higher concentrations (0.1-0.2%), oil extracted in all the seasons completely checked the progeny production of test insect. However, at lower concentrations maximum and minimum efficacy was obtained in oil extracted in spring and rainy season, respectively. The essential oil extracted in spring season suppressed 90.1, 64.8

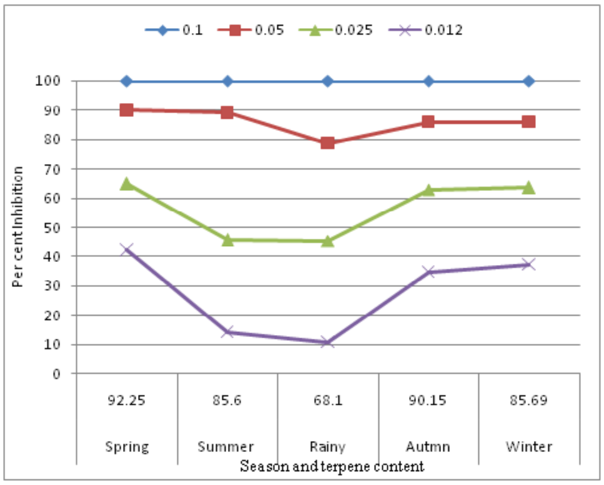

Fig. 1: Relationship between seasonal monoterpene content of oil (%) and its fumigant activity against *R. dominica*at different concentrations

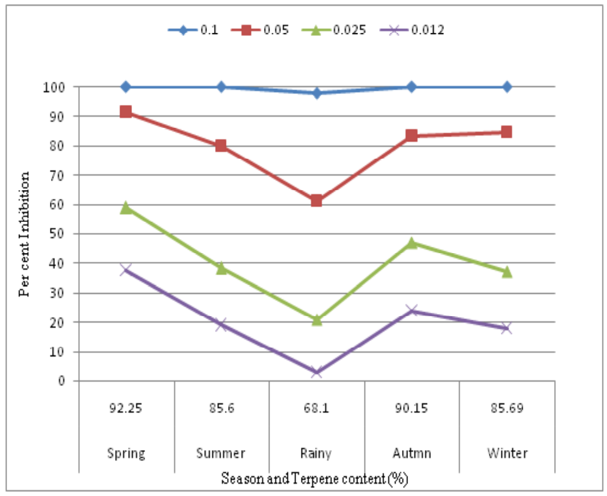

Fig. 3: Relationship between seasonal monoterpene content of oil (%) and its fumigant activity against *S. oryzae*at different concentrations

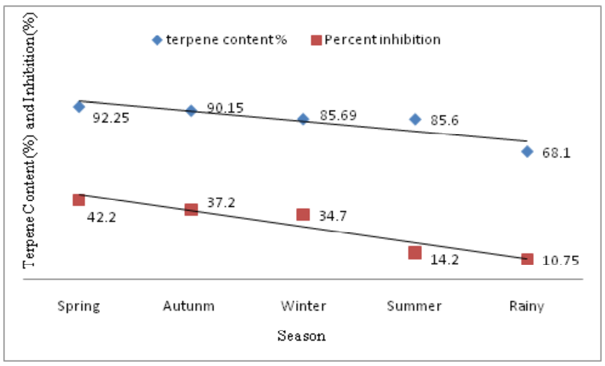

Fig. 2:Relationship between monoterpene content of oil in different seasons and its fumigant activity against *R. dominica* at 0.012%

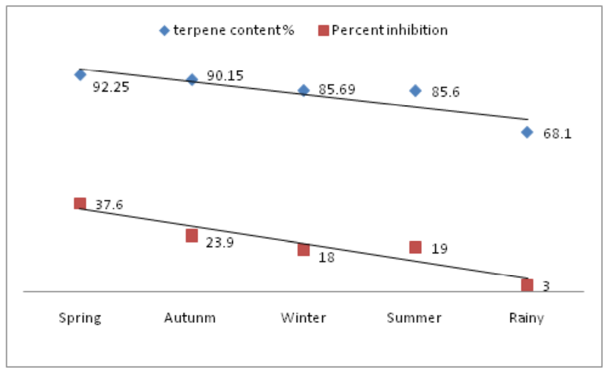

Fig. 4: Relationship between monoterpene content of oil in different seasons and its fumigant activity against *R. dominica* at 0.012%

and 42.2 per cent progeny at 0.05, 0.025 and 0.012 per cent, respectively, which declined to 78.55, 45.21 and 10.75 per cent in rainy season. The efficacy of the oil at lower concentration was positively correlated with terpene content of the oil (Figure 2). Data present in Table 4 and Figure 2 indicates that no seasonal difference was noticed in the efficacy of essential oil of *M. koenigii* against *S. oryzae* at 0.10 to 0.20 per cent at which it completely checked the growth and development of insect, however, the efficacy of the oil varied at lower concentrations and oil extracted in spring showed maximum efficacy which was again lowest in rainy season. The essential oil extracted in spring season suppressed 91.5, 58.8 and 37.6 per cent progeny of *S. oryzae* at 0.05, 0.025 and 0.012 per cent, respectively, which declined to 61.0, 20.9 and 3.0 per cent in rainy season. The efficacy of the oil at lower concentration was positively correlated with terpene content of the oil (Figure 4).

CONCLUSION

On the basis of above mentioned study it is concluded that spring and autumn are the best period for the collection and extraction of essential oil from *M. koenigii*. The study also demonstrated that the essential oil of this plant is highly effective against *R. dominica* and *S. oryzae* due to which it may be explored further for management of both the insect pests.

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