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Incidental finding of root knot symptoms in *Lavandula angustifolia* Mill: First report from India

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Abstract
Some plants of *Lavandula angustifolia* Mill. appeared to be weaker than the rest which lead to the exploration of prominent knot and gall like structures in their respective root system that are in turn characteristics for root knot nematode infection. Based upon the prominence of infection present in the roots, these infected plants were categorised into three groups having mild infection, high infection and severe infection. A total of thirty two traits were considered for analysis out of which seventeen traits were morphometric and fifteen traits were chemometric in nature. A reduced percentage concentration in a number of essential oil components were observed in the severely infected plants. This is an alarming finding because the quality of essential oil of lavender is invariably dependent on the presence of adequate component concentration of its essential oil which in the present study was reduced due to root knot nematode infection.

Keywords: ANOVA, Correlation, INDIA, *Lavandula angustifolia* Mill, Root knot nematode

Abbreviations: EO = Essential oil, MAP = Medicinal and Aromatic Plants, RKN = Root Knot Nematode

1. Introduction
Host-parasite interaction has always been a preferred area of investigation. This kind of interaction is a global problem, which demands investigations, precautions, preventions and solutions. Root knot disease is predominantly caused by a specific genus of nematode called *Meloidogyne* spp. The host range of this root knot nematode (RKN) is extremely vast (Hussey and Janssen, 2002; Brito *et al*., 2008) [25, 3] inflicting serious and irrecoverable damage to the host. Instances of RKN infection in plants, leading to sumtotal reduction of quantitative and qualitative yield is echoed from every agro-economic sectors of the world.
This notorious nematode has overpowered almost 3000 host defence mechanisms (Hussey and Janssen, 2002) [25], in almost all kinds of crops (wild crops, cultivated crops and weeds) including medicinal and aromatic plants(MAPs). The RKN when present in higher population in MAPs seriously affect them in yield related aspects (Haseeb *et al*., 1996; Haseeb *et al*., 1984; Sivakumar and Vadivelu, 1997; Tanda *et al*., 1989; Mukhopadhyaya *et al*., 1980; Mustika, 1992; Rhoades, 1988; Chinappen *et al*., 1988) [22, 19, 51, 53, 34, 35, 45, 7] and conversely, there are also certain medicinal and aromatic herbs which can contain and control the expansion of this phytoparasite (Haroon and Huettel, 1991; Haseeb and Butool,1990; Mukhopadhyaya *et al*., 1980; Tanda *et al*., 1989; Taylor and Sasser, 1978) [13, 14, 34, 53, 54]. Among the well described species of *Meloidogyne* (Brito *et al*., 2008) [3], *M. arenaria, M. hapla, M. incognita,* and *M. javanica* are considered as the most dominant ones responsible for the plight of agricultural and horticultural crops (Hussey and Janssen, 2002) [25].
Infection of RKN is also prevalent in the members of Lamiaceae e.g. infection of *Meloidogyne incognita* on *Ocimum canum, Lucas aspera, Ocimum americanum* (Gowda *et al*., 1995) [10], *Prunella vulgaris, Lamium amplexicaule, Leonurus cardiaca, Nepeta cataria* (Davidson and Townshend, 1967) [8]; *M. floridensis* on *Leonotis nepetaefolia* (Kaur *et al*., 2007) [28]; *M. javanica* on *Leonurus sibiricus* (Moraes *et al*., 1972) [33]; *M. exigua* on *Stachys arvensis, Leonurus sibiricus* (Lima *et al*., 1985) [30].
Lavender (*Lavandula angustifolia* Mill.) is a well known intensely aromatic herb of Lamiaceae, which is grown for its high quality essential oil (EO) having immense global demand. The best quality of EO is mainly derived from the spikes of the herb (Talal *et al*., 2005) [52]. Europe continues to be the geographic hub of global lavender oil market (clinching about three-fifth of total production) with Bulgaria being the largest producer of EO.

Lavender was introduced to Indian agro-climatic condition in 1957. The Kashmir valley was considered to be a favourable zone for its establishment and much of the credit goes to Sir Col. R. N. Chopra (Singh *et al*., 2007; Verma *et al*., 2010; Shawl *et al*., 2000; Handa *et al*., 1957) [50, 57, 49, 12]. Expansion and improvement programs in relation to lavender followed, but with a very slow pace due to various complications. However, international standards in EO was eventually attained and INDIA began producing and exporting lavender EO. The most recent Indian export estimate of lavender EO for the period of February, 2013 to November, 2016 indicates a total value $3,523,934.

In India, among the many nematode species that infect paddy and wheat, *M. graminicola* is known to be the most devastating species which reduces crop yield by 21% (Prasad *et al*., 1987; Bridge *et al*., 1990) [43, 2]. Yield loss in upland, rainfed and direct seeded plants rose up to 50% under field condition (Lorenzana *et al*., 1998) [31]. *M. graminicola* was also responsible for the reduction in farmers' field yield of rice in Nepal by 40% (Sharma- Poudyal *et al*., 2002, Sharma *et al*., 2001) [48, 47]. A new RKN species, *M. luci* was identified from the roots of *Lavandula spica* L collected in Rio Grande do Sul, Brazil (Carneiro *et al*., 2000; Carneiro *et al*., 2014) [6, 5]. *M. luci* also serves as a parasite to *S. tuberosum* L., *Oxalis corniculata* L., *Cordyline sp*. and *S. lycopersicum* (Maleita *et al*., 2018) [32]. *M. arenaria* is a parasite of horticultural crops in tropical and subtropical regions being responsible for significant yield losses (Hunt & Handoo, 2009) [24]. *Meloidogyne spp* are responsible for an estimated annual loss of $100 billion (Sasser *et al*., 1987) [46]. Among a list of economically important nematode species, *Meloidogyne incognita* and *M. arenaria* are the most damaging ones. *M. incognita* thrives in every temperate and tropical country of the world being the most damaging crop pathogen as reviewed by Trudgill and Blok, 2001[56].

Root knot infection by nematodes in *Lavandula angustifolia* is reported very recently from Greece (Gonçalves *et al*., 2020) [9] and Turkey (Özalp *et al*., 2020) [36] whereas it was reported earlier from Brazil (Pauletti and Echeverrigaray, 2002; Carneiro *et al*., 2000) [42, 6]. Gonçalves *et al*., 2020[9] represented the first report of natural infestation of *Lavandula angustifolia* by *M. hapla* in Greece. However, infection caused by the same species in Greece was earlier reported based on morphological features by Hirschman *et al*., 1966; Koliopanos, 1980; Pyrowolakis, 1980; Vovlas and Antoniou, 1987; Vlachopoulos, 1994 [23, 29, 44, 59, 58]. Özalp *et al*., 2020 [36] first reported RKN infection in *Lavandula angustifolia* by *Meloidogyne arenaria* in Turkey.

Scientific endeavours for RKN infection and its allied aspects (Janssen *et al*., 2016; Jones *et al*., 2015) [26, 27], particularly in aromatic plants in India is rising decorously. Aromatic plants like *Ocimum sanctum* (Haseeb *et al*., 1999) [17], *Hyoscyamus muticus* (Butool *et al*., 1998) [4], *Hyoscyamus niger* (Pandey, 1997; Haseeb *et al*., 2000) [37, 21], *Ocimum basilcum* (Haseeb *et al*., 1988; Tiwari *et al*., 2017) [20, 55], *Pogostemon cablin* (Borah *et al*., 2018; Pandey *et al*., 2009) [1, 38], *Ocimum canum* (Haseeb *et al*., 2000)[18], *Mentha arvensis* (Haseeb and Shukla, 2000; Pandey *et al*., 2010; Pandey *et al*., 2011) [15, 39, 40], *Matricaria chamomilla* (Pandey *et al*., 1999; Gupta *et al*., 2017) [41, 11], *Ocimum kilimandscharicum* (Haseeb *et al*., 1998) [16] etc. have come under investigation predominantly by CSIR-Central Institute of Aromatic Plants (CIMAP), Lucknow.

In the same way an incidental finding for the presence of knot like structures in the roots of some plants of *Lavandula angustifolia* appeared to be a result of RKN infection. In the light of the most recent investigations by Özalp *et al*., 2020 and Gonçalves *et al*., 2020[36,9] regarding RKN infection of *L. angustifolia* and with the help of morphological determinants there is a substantial indication that the root knot appearance in lavender is due to the infection of RKN. Since, no previous record or report of RKN infection of lavender exists from India, the present finding is therefore the first report of RKN infection of *Lavandula angustifolia* from India. Like other lavender growing countries in the world, in India it is also grown as an export oriented crop having a great demand for its EO. The present study will thus open up new research vistas for lavender in India like - plant protection, plant pathology (crop nematology), agro-technology, soil biochemistry, allelopathy and so on.

2. Materials and Methods

2.1 Plant sample and site of experiment.

The lavender plants were available at the at CSIR-Indian Institute of Integrative Medicine (IIIM), Field Station at Chatha, Jammu, India, located at 32°39’40”(N) to 32°40’00”(N) latitude and 74°48’40”(E) to 74°49’10”(E) longitude, with an altitude ≈300m above sea level. The climate remains semiarid to subtropical with minimum and maximum night and day temperatures ranging between 1–11°C to 15–17°C during winter and 25–30°C to 35–40°C during summer.

2.2 Morphological features of root knot symptom and scale.

It was observed that about 12.50% plants of *Lavandula angustifolia* exhibited uncommon appearance when compared to others. Investigation of the root system of these plants uncovered the presence of root-galls or knots of varying sizes on the rootlets. The root hairs were greatly reduced in most cases.

A scale of 0-3 was used to visually segregate the infections in roots with 0 as no infection, 1 as mild symptomatic infection, 2 as high symptomatic infection and 3 as severe symptomatic infection.

2.3 Morphometric traits, data recording and data analysis

Data of seventeen morphometric traits like shoot length (length of plant from the pot soil surface measured in centimetres), root length (length of roots of the plant from the pot soil surface measured in centimetres), shoot-root length ratio, shoot spread (circular spread of the shoot measured in centimeters), root spread (circular spread of the root measured in centimeters), number of primary branches in shoot (total number of branches emanating from the main stem), branch length (length of a branch from the apical tip to its base on the main stem measured in centimetres), stem circumference (circular measurement of the stem just above the pot soil of the potted plant measured in centimetres), number of primary branches in root, fresh weight of plant (total weight of the shoot and root system of the live plant in grams), approximate shoot fresh weight (total weight of the shoot system of the live plant in grams), approximate root fresh weight (total weight of the root system of the live plant in grams), shoot-root fresh weight ratio, severity of infection (scale of 0 to 3, where 0=no infection, 1=mild, 2=high and 3=severe), growth habit (represented by 1 and 2 being erect and angular nature respectively), angle of inclination from soil surface (degrees), colour of leaves (represented by 1 and 2 being greenish and bluish-green respectively) and percentage content of fifteen

major essential oil components like — eucalyptol; endo-Borneol; 3-Carene; camphene; α-Pinene; p-Cymene; o-Cymene; cyclobutane,1,2-dicyclopropyl-; bicyclo[2.2.1]heptan-2-ol,1,7,7-trimethyl-,(1S-endo)-; linalool; D-Limonene; (+)-2-Bornanone; α-Phellandrene; β-Phellandrene; linalyl acetate; extracted from sampled leaves using HS-GCMS for fifteen treatments (thirteen infected plants named as — MSP1, MSP2, MSP3, MSP5, MSP6, HSP7, HSP8, HSP9, HSP10, SSP11, SSP12, SSP14, SSP15 and two checks — CMSP4 and CSSP13) growing in pots in glasshouse condition were recorded.

The experiments were conducted using a completely randomized design. The statistical analysis was performed by one-way analysis of variance (ANOVA) using SPSS statistical software (version 20 for Windows) and MS Excel-2007. Correlation coefficients were calculated amongst all traits and significant positive and negative values were demarcated.

2.4 Head Space-Gas Chromatography-Mass Spectrometry (HS-GCMS) analysis

The qualitative and quantitative estimation of leaf volatiles for glass house grown lavender plants were performed on a Shimadzu Nexis GC-2030 hyphenated with GCMS-TQ8040 instrument and samples were introduced through HS-20 headspace sampler. Fresh leaf material of 500 mg sample was kept in 20 ml head space flat base vial fitted with crimp cap and silicon/PTFE 18 mm 35 SHORE septum. The vial was incubated in head space heater for 5 min at 120ºC with 50 kps pressure. The loop temperature was 110ºC and transfer-line temperature was kept at 120ºC. The sample was introduced into the split/splitless injector in the split mode (1:25) at 280ºC.The column oven temperature was programmed from 90ºC to 120ºC at the rate of 3ºC/min with final hold of 2 min. High purity helium gas was used as a carrier gas (1 ml/min) and a SH-Rxi-5S MS (30 m × 0.25 mm; 0.25 µm film thickness) column was employed for separation. Identification of compounds was based on retention time, elution order, relative retention index using a homologous series of n-alkanes (C8–C25 hydrocarbons) with those of literature. Further identification was made by matching the recorded mass spectra with those stored in the inbuilt mass spectral library. The percentage determination was based on peak area normalization.

3. Results

A total of 12.50% potted lavender plants exhibited knot like appearance in their roots. These affected plants were visually differentiated into 3 categories — mild infection, high infection and severe infection. The overall morphological appearance of these roots were exactly identical to those represented by Özalp *et al*., 2020 and Gonçalves *et al*., 2020 [36, 9] in their individual studies of RKN (*Meloidogyne sp*) infection on *Lavandula angustifolia.*

The one way ANOVA reflects highly significant differences existing between the thirty two traits from the overall fifteen treatments (MSS=64225.14**), the six mild symptomatic treatments with (MSS=16914.17**), the four high symptomatic treatments (MSS=15128.12**) and the five severely symptomatic treatments (MSS=34926.01**) at $\alpha \leq 0.01$ level of significance as represented in the table (1).

Table 1: ANOVA representing significant differences in thirty two traits for 15 treatments of *L. angustifolia* Mill.

1. Cumulative Anova					
	Source of Variation	**SS**	**df**	**MS**	**F**
	Between Groups	1990979.43	31	64225.14**	91.52
	Within Groups	314377.90	448	701.74	
	Total	2305357.30	479		
2. Anova For Mild Infection					
	Source of Variation	*SS*	*df*	*MS*	*F*
	Between Groups	524339.13	31	16914.17**	28.97
	Within Groups	93402.34	160	583.80	
	Total	617741.50	191		
3. Anova for high infection					
	Source of Variation	*SS*	*df*	*MS*	*F*
	Between Groups	468971.60	31	15128.12**	28.27
	Within Groups	51381.40	96	535.22	
	Total	520353.00	127		
4. Anova For Severe Infection					
	Source of Variation	*SS*	*df*	*MS*	*F*
	Between Groups	1082706.40	31	34926.01**	60.11
	Within Groups	74370.094	128	581.02	
	Total	1157076.50	159		

Where, ** = $p < 0.01$.

The mild symptomatic group represented by six treatments exhibited the highest value of eleven traits, the high symptomatic group represented by four treatments exhibited the highest value of six traits while the severe symptomatic group represented by five treatments exhibited the highest value of eleven traits as represented in the table (2).

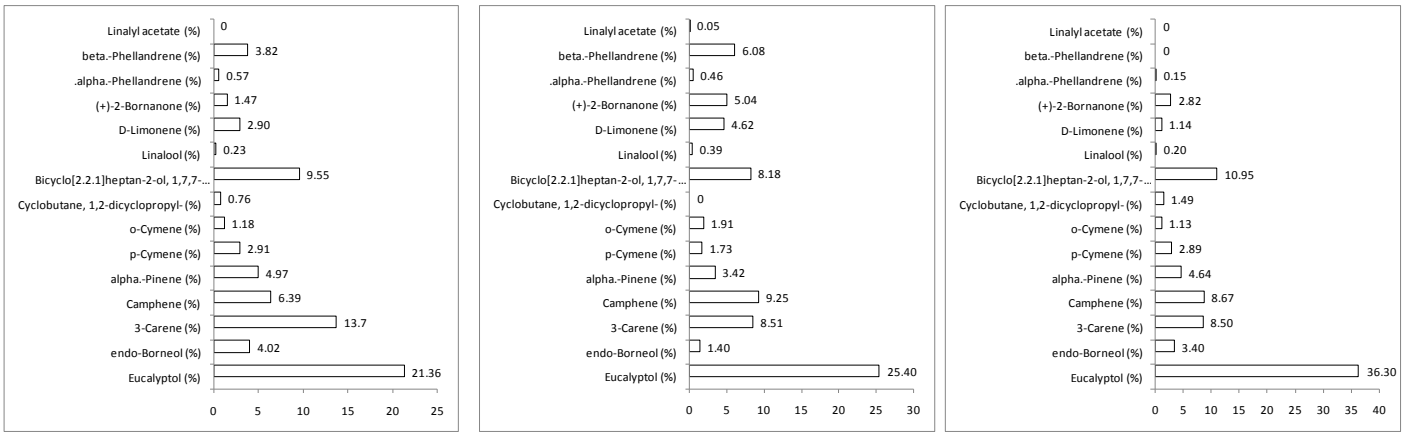
Table 2: Effect of RKN infection on some traits in the three categories of infected treatments of *L. angustifolia* Mill.

Sl. No	Traits	MSG	HSG	SSG
1	Shoot length (cm)	37	-	-
2	Root length (cm)	28	-	-
3	Shoot-Root length ratio	-	-	3.17
4	Shoot spread (cm)	-	-	70.65
5	Root spread (cm)	72.22	-	-

6	No. of primary branches in shoot	16	-	-
7	Branch length (cm)	-	-	25
8	Stem circumference (cm)	-	-	4.473
9	No. of primary branches in root	59	-	-
10	Fresh plant weight (g)	-	-	440
11	Approximate shoot fresh weight (g)	-	-	330.60
12	Approximate root fresh weight (g)	-	-	166.63
13	Shoot-Root weight ratio	-	-	3.17
14	Eucalyptol (%)			59.45
15	endo-Borneol (%)	24.11	-	-
16	3-Carene (%)	18.28	-	-
17	Camphene (%)	-	16.35	-
18	α-Pinene (%)	8.62	-	-
19	p-Cymene (%)	4.72	-	-
20	o-Cymene (%)	5.69	-	-
21	Cyclobutane, 1,2-dicyclopropyl- (%)	-	-	7.45
22	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)- (%)	-	-	22.92
23	Linalool (%)	-	0.48	-
24	D-Limonene (%)	17.42	-	-
25	(+)-2-Bornanone (%)	-	15	-
26	α-Phellandrene (%)	-	1.24	-
27	β-Phellandrene (%)	-	24.32	-
28	Linalyl acetate (%)	-	0.19	-
	Total number of highest observation in respective groups	11	06	11

Where, MSG=Mild Symptomatic Group, HSG= High Symptomatic Group and SSG=Severe Symptomatic Group,

However, the severely symptomatic group exhibited highest degree of infection followed by a uniformly erect growth habit (having an angle of inclination of 90 degrees) and with a greenish colour of leaves. The effect of this infection mainly reduced the p-Cymene, (+)-2-Bornanone, Linalool and D-Limonene component concentration of the EO in most of the hosts to a great extent in comparison to the checks (Figure 1).

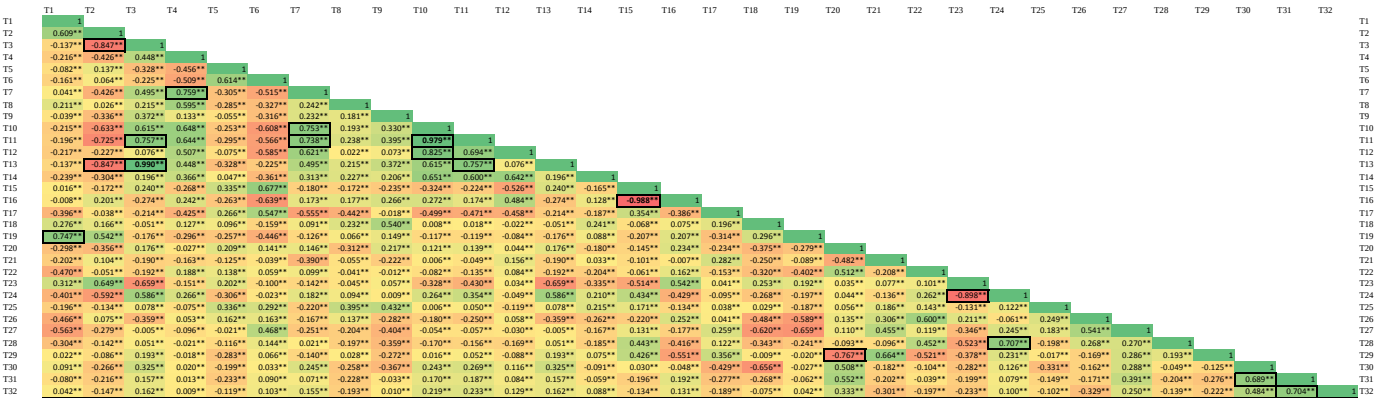


(a) (b) (c)

Fig 1: Mean variation in the percentage component concentration in EO of *Lavandula angustifolia* within the three symptomatic groups — (a) Mild Symptomatic Group, (b) High Symptomatic Group and (c) Severe Symptomatic Group,

The mean comparisons of the EO constituents among the three grades (mild, high and severe) of infection indicated a decline in the concentration of β-phellandrene, α-phellandrene, D-Limonene, linalool, o-cymene, p-cymene, α-pinene, 3-carene and endo-borneol whereas a rise in the concentration was detected in eucalyptol, camphene, cyclobutane,1,2-dicyclopropyl-, bicyclo[2.2.1]heptan-2-ol,1,7,7-trimethyl-,(1S-endo)- and (+)-2-bornanone (Figure 1).

The correlation studies (Figure 2) exhibited a very high positive significant correlation between — shoot-root length ratio and shoot-root fresh weight ratio (0.990**), fresh plant weight and approximate shoot fresh weight (0.979**); a high positive significant correlation between — fresh plant weight and approximate root fresh weight (0.825**); a medium positive significant correlation between — shoot spread and branch length (0.759**), shoot-root length ratio and approximate shoot fresh weight (0.757**), approximate shoot fresh weight and shoot-root weight ratio (0.757**), branch length and approximate shoot fresh weight (0.738**), o-cymene and D-limonene (0.707**), β-phellandrene and linalyl acetate (0.704**) etc. The correlation studies (Figure 2) also exhibited a very high negative significant correlation between — growth habit and angle of inclination from soil surface (-0.988**); a high negative significant correlation between — p-cymene and o-cymene (-0.898**), root length and shoot-root length ratio (-0.847**), root length and shoot-root weight ratio (-0.847**), 3-Carene and (+)-2-Bornanone (-0.767**) etc.



Where, T1=Shoot length (cm), T2=Root length (cm), T3=Shoot-Root length ratio, T4=Shoot spread (cm), T5=Root spread (cm), T6=No. of primary branches in shoot, T7=Branch length (cm), T8=Stem circumference (cm), T9=No. of primary branches in root, T10=Fresh plant weight (g), T11=Approximate shoot fresh weight (g), T12=Approximate root fresh weight (g), T13=Shoot-Root weight ratio, T14=Severity of infection, T15=Growth habit, T16=Angle of inclination from soil surface (degrees), T17=Colour of leaves, T18=Eucalyptol(%), T19=endo-Borneol(%), T20=3-Carene(%), T21=Camphene(%), T22=α-Pinene(%), T23=p-Cymene(%), T24=o-Cymene(%), T25=Cyclobutane, 1,2-dicyclopropyl-(%), T26=Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-(%), T27=Linalool(%), T28=D-Limonene(%), T29=(+)-2-Bornanone(%), T30=α-Phellandrene(%), T31=β-Phellandrene(%), T32=Linalyl acetate(%). **=p<0.01;

Fig 2: Correlation matrix represented by thirty two traits and fifteen treatments of *L. angustifolia*.

4. Discussion

The prevalence of a few species of RKN (*Meloidogyne sp*), causing knot like appearance in the infected roots of *Lavandula angustifolia* has been reported earlier by Özalp *et al*., 2020 and Gonçalves *et al*., 2020[36,9] in their individual studies carried out in Turkey and Greece respectively. The present incidental finding is also in complete consonance to it and is the first report of RKN infection from India causing morphologically distinguishable and characteristically identifiable knots in the roots of *L. angustifolia*.

This present study also brings into focus the effect of RKN infection on seventeen morphometric and fifteen chemometric traits in three categories of infection grouped as mild, high and severely symptomatic. The quality of economically valuable EO of lavender is predominantly governed by the components like linalool, linalyl acetate and limonene. Most of the chemometric traits (EO components) exhibited reduction in their percentage composition through the three symptomatic levels. Stress factor (in this case biological stress caused by RKN infection in *L. angustifolia*) has also played its usual role by initially improving the percentage concentration of EO components from mild to high symptomatic category but ultimately the final percentage concentration of EO components declined from high to severe symptomatic categories. In the light of RKN infection, this adverse manifestation was most evident.

The correlation study estimated the overall positive and negative significant effects of all the thirty two traits involved in this present study. The pair of traits represented by shoot-root length ratio and shoot-root weight ratio was positively highly significant in contrast to negatively highly significant pair of traits represented by growth habit and angle of inclination from soil surface.

5. Conclusion

The morphological investigation of knot like structures present in the roots of MSG (represented by MSP3; Figure 3), HSG (represented by HSP8; Figure 4) and SSG (represented by SSP11; Figure 5) of *L. angustifolia* was due to the infection of RKN whose ill effects were prominently reflected on the morphometric and chemometric traits in comparison to the control CSSP13 (Figure 6) considered in the study. The percentage concentration of the components of the EO exhibited a decline towards the severity of infection. Hence, the qualitative value of the essential oil of *L. angustifolia* was affected negatively.

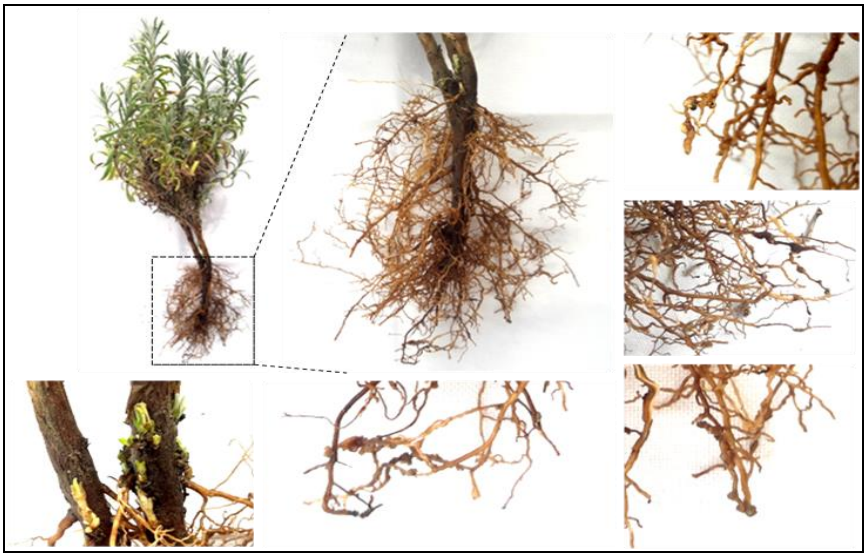


Fig 3: Intensity of RKN infection in MSP3 — a representative of mild symptomatic group of *L. angustifolia* Mill.

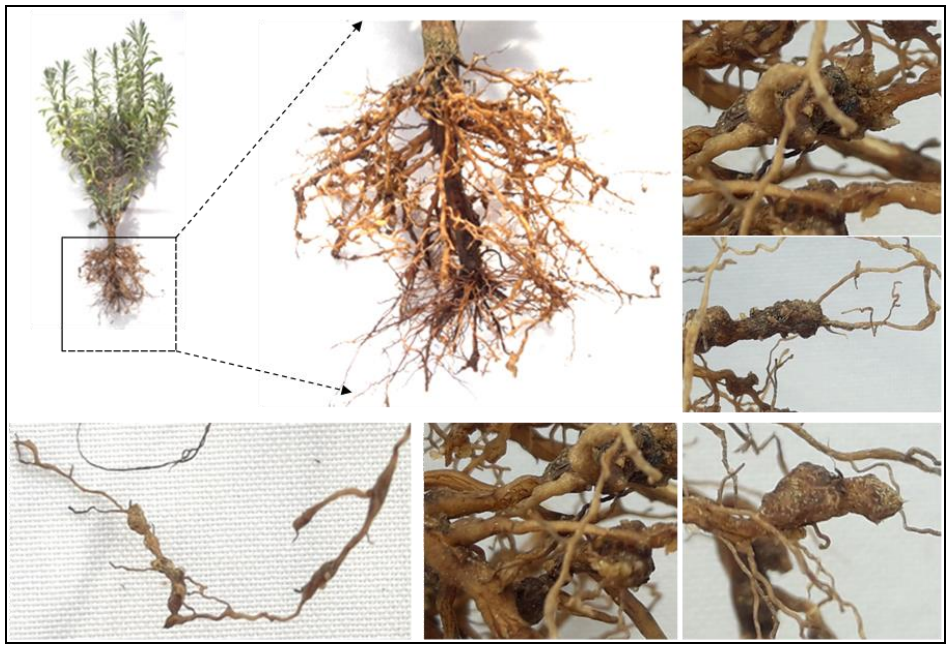


Fig 4: Intensity of RKN infection in HSP8 — a representative of high symptomatic group of *L. angustifolia* Mill.

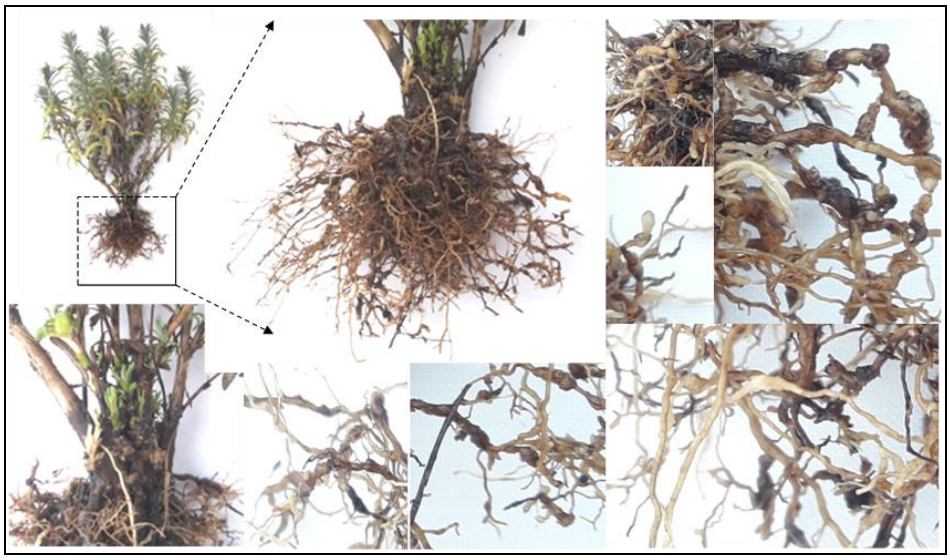


Fig 5: Intensity of RKN infection in SSP11 — a representative of severe symptomatic group of *L. angustifolia* Mill.

Fig 6: Control CSSP13 having no infection in roots.

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7. Conflict statement
No conflict of interest is declared by the author.

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