Effect of multicomponent aqueous plant extracts on the growth and biochemical composition of *Mentha arvensis* L. and *Centella asiatica* L.

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Abstract: The present study aimed to analyze the effect of different concentrations of aqueous extracts of *Tephrosia purpurea, Ipomea carnea, Trianthema portulacastrum,* and *Tithonia diversifolia* on *Centella asiatica* and *Allium cepa, Solanum tuberosum* and *Calotropis gigantia* on *Mentha arvensis* respectively. Extracts of 5%, 10%, 20% & 50% concentrations were prepared and fertigated by pot experiments. Results of this study showed that extracts of *T.purpurea* (50%), *I. carnea* (10% & 20%), *T. portulacastrum* (10%, 20%& 50%) and *T. diversifolia* (5% &10%) enhanced the growth of *C. asiatica* when compared to the control. *Calotropis* extract *(*15%) enhanced the growth of *M. arvensis* and lowest growth was recorded in onion peel extract (5%). *T. diversifolia* extract at lowest concentration (5%&10%) increased the leaf surface area (38.8±0.4cm^2), petiole length (17.6±0.6cm) and fresh (120.6±2.0g) & dry weight (52.4±0.5g). Lower concentrations of *T. purpurea* (5% &10%) and higher concentrations of *I. carnea* (50%) inhibited the growth, number of leaf, leaf surface area (3.8±0.7cm^2), petiole length (2.63±0.3cm) and fresh (10.8±1.7g) & dry weight (3.1±0.7g) in *Centella asiatica* and *Calotropis* extract (15% concentration) on *M. arvensis* increased the number of leaves (57±3), leaf surface area (12.7±0.9**)**, shoot length (39±2 cm^2) and total fresh weight (170±1.5g) when compared to the control. Significant reduction existed in all the parameters at higher concentrations of the extracts used.

Key-Words: Aqueous extracts, growth, biochemical parameters, *Centella asiatica, Mentha arvensis.*
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1. Introduction

In recent years, extracts of plants are being commercialized in crop improvement. They have emerged as promising components of nutrient supply systems being environment-friendly and cost-effective. Its utilization is one of the important soil management practices which substantially increase crop yield and soil fertility over the past century (Vitousek *et al.* 1997*;* Tilman *et al.* 2002). *Allium cepa*, *Solanum tuberosum,* and *Calotropis gigantea* are used as raw materials during compost preparation. *Tephrosia purpurea* is considered a source of green manure in crop fields (Sagar *et al*. 2018). *Ipomoea carnea* is known to activate the microbial activity of soil (Moindi *et al*. 2012; Sharma & Bachheti, 2013) *Trianthema portulacastrum* has been suggested for green manuring due to considerable amounts of nitrogen, potassium, and phosphorus present in the plant. *Tithonia diversifolia* is used as green manure to increase soil fertility in Western Kenya (Jama *et al*. 2000).

Mints (*Mentha* spp.) are aromatic and medicinal herbs, frequently used in traditional and folk medicines globally for its antimicrobial and antioxidant properties. They grow best in confined areas such as containers, as top-dress plants with a thin layer of organic fertilizer every day. The leaves are the principle site for oil biosynthesis. In recent times, water scarcity has caused a drastic reduction in the biomass of the crop and various irrigation and fertigation strategy is followed which allows uniform water supply and nutrient distribution for enhanced growth. In India, *Centella asiatica* L. frequently suffers due to the increasing use of herbicides. Although over-exploitation of the species is now widespread due to high market demand, no serious effort has been made for its planned cultivation. Hence, considering the above facts, the present study, which could

benefit the farmers after conclusive results, was designed to study the effects of different plant extracts on the growth and biochemical composition of *M.arvensis* and *C. asiatica*.

2. Materials and methods

The study samples viz the whole plant of *C. asiatica, M. arvensis, C.gigantea, T. purpurea, I. carnea, T. portulacastrum,* and *T. diversifolia*; peels of *A. cepa* and *S. tuberosum* were collected from their natural habitat and identified by Department of Botany, PSG College of Arts & Science, Coimbatore, Tamilnadu, India. 20gms of materials of the peel of *A.cepa*, peel of *S.tuberosum*, and the stem of *Calotropis* were taken individually and in combination (1:1), soaked overnight using 100 ml of distilled water and ground. The filtered extracts were taken for further treatment of the *M. arvensis*. 20gms of materials (*T. purpurea, I. carnea, T. portulacastrum,* and *T. diversifolia)* were taken individually and in combination (1:1). They were soaked overnight using 100ml of distilled water individually and ground. The filtered extracts were taken for further treatment of *C. asiatica*.

Single-node stem cuttings from *M.arvensis* and *C.asiatica* were planted into pots. The pots were fertigated with filtered extracts (5%, 10%, 15% & 20) of onion peel, potato peel & *Calotropis* on the former and filtered extracts (5%, 10%, 15% & 20) of *T. purpurea, I. carenea, T. portulacastrum* & *T. diversifolia* on the latter respectively. Control was irrigated with tap water. Continuous irrigation for all the pots was given daily basis using tap water. Mature plants were analyzed after 30 days of planting. The leaves and stems were collected separately. The standard methods were adopted for the analysis of the following parameters such as morphological analysis (number of leaves, shoot length, and leaf surface area), total fresh weight, soil analysis (physicochemical properties), isolation of essential oil, and GC-MS analysis of essential oil. All measurements were done in replicates and the mean was calculated using SPSS software.

2.1 Morphological analysis:

The total number of leaves was counted. Petiole and root length were measured in cms. The total leaf area was calculated by measuring the length and width multiplied by a correlation factor (0.66) derived from the method of Yoshida *et al*. (1972). The values of leaf area were expressed in cm^2.

$$\text{Leaf area} = L \times W \times 0.66 \quad (1)$$

2.2 Total fresh and dry weight:

After 90 days of planting the plant samples were harvested and washed thoroughly using tap water. The total fresh weight (leaves, stem and root) was weighed using Shimadzu electronic balance. They were dried in a hot air oven at 80ºc for 24 hours. The dry weight was recorded. The value of fresh and dry weights was expressed in g.

2.3 Soil analysis (Physicochemical properties):

50 grams of air-dried soil (< 2 mm) was weighed and 50 ml of distilled water was added using a graduated cylinder to a 50-ml volumetric flask, mixed well with a glass rod, and allowed to stand for 30 minutes. The suspension was stirred every 10 minutes during the period.

2.3.1 pH:

The pH meter was calibrated before taking the measurement. The combined electrode was immersed (about 3 cm deep), and the reading was taken after 30 seconds with one decimal. The electrode was removed from the suspension and rinsed thoroughly with distilled water in a separate beaker and the excess water was dried with a tissue. The value of pH was noted.

2.3.2 Electrical Conductivity:

The suspension was filtered using suction. A round Whatman No. 42 filter paper was placed in the Buchner funnel. The filter paper was moistened with distilled water and it was ensured that it was tightly attached to the bottom of the funnel with all holes covered. The suction was opened and the suspension was added to the Buchner funnel. The filtration process continued until the soil on the Buchner funnel started cracking. The conductivity meter was calibrated before taking the measurement. The clear filtrate was transferred into a 50-ml bottle and the conductivity cell was immersed in the solution and the readings were taken. The conductivity

cell was removed from the solution, rinsed thoroughly with distilled water, and the excess water dried with a tissue. The value of electrical conductivity was noted.

2.3.3 Organic Matter:

Total Organic Matter was determined by the method reported by Walkley-Black (1934).

2.3.4 Nitrogen:

Total nitrogen was estimated by the Kjeldahl method.

2.3.5 Phosphorus and potassium:

Phosphorus and potassium content was evaluated by the method used by Olsen *et al*. (1954).

2.4 Biochemical analysis:

Total chlorophyll content was estimated by the method of Arnon (1949).

2.5 Leaf nutrients analysis:

Total nitrogen was determined by the Kjeldahl method. Phosphorus, potassium, and micronutrients were determined by the method used by Chapman and Pratt (1961).

2.6 Isolation of essential oil:

Leaves of Japanese mint (*M. arvensis)* were harvested from a well-grown plant of the treated extracts. The plants were washed with distilled water and subjected to steam distillation in a Clevenger-type apparatus. The collection of essential oils were filtered through a 0.45µm filter and kept at 4ºC until further analysis.

2.7 GC-MS analysis of essential oil:

The essential oils were analyzed by GC/MS using a Hewlett-Packard GC system (HP6890 series II) coupled with a mass detector (MSD5973) equipped with an HP-5MS capillary column (5 % phenyl methylsiloxane, 30 m x 0.25 mm id., 0.25µm film thickness) (Agilent Technologies, Palo Alto, CA). For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used over a scan range of 40-400 amu. Helium was used as a gas carrier at a flow rate of 1ml/min. The split ratio was adjusted at 25:1. The column temperature was initially kept at 60ºC for 4 min, then gradually increased up to 240ºC at an increment of 3ºC/min, and finally held isothermal for 10 min. The percentage of components was calculated from total ion chromatograms.

3. Results and discussion

The results of the study are presented in tables (1-15). The steam-distilled yields were about 0.3%. In *C. asiatica* aqueous extract of *I. carnea* (20% concentration) increased the total number of leaves (59±2), aqueous extract of *T. diversifolia* (20% concentration) decreased the total number of leaves (6±1) when compared to the control (Table 10**).** An increase in the total number of leaves (57±3) using *M. arvensis* aqueous extract (15% concentration) and a decrease in the total number of leaves (17±2) using aqueous extract of onion peel (5% concentration) was observed when compared to the control (Table 10**).** Our findings are in with accordance the study on the growth of purple nutsedge which exhibited significant inhibition of shoot and tubers using various mango aqueous extracts (Rafat *et al*. 2010).

Aqueous extract of *T. diversifolia* (5% concentration) increased the petiole length (17.6±0.6 cm), leaf surface area (38.8±0.4 cm^2), and root length (27.1±0.7cm) whereas aqueous extract of *T. purpurea* (20% concentration) decreased the same (3±0.4 cm), (3.8±0.7 cm^2) and (3.3±0.2cm) respectively (Table 2).

Treatment of *Centella asiatica* with leaf and stem extract of *T. portulacastrum* produced more leaves in minimum germination time. The result was contradictory for germination of rice seeds which was significantly affected by soaking the water extracts of *T. portulacastrum* in distilled water. Maximum germination time (MGT) was found when rice seeds were soaked in root and leaf extracts of *T. portulacastrum*. The stimulatory functions of these chemicals were evident in the significant enhancement of the growth parameters (petiole height, fresh weight, dry weight, and leaf area) of *C. asiatica.*

Table 1: Effect of various extracts on the number of leaves in *C.asiatica*

S.no	Name of the Treatment	Days	Number of leaves recorded at different percentage levels			
			5%	10%	20%	50%
1.	*Tephrosia purpurea*	30	5±1	28±2	13±1	11±1
		90	13±2	8±1	5±2	44±1
2.	*Ipomea carnea*	30	7±1	18±1	21±1	17±1
		90	31±2	54±2	59±2	13±1
3.	*Trianthema portulacastrum*	30	10±1	13±1	15±2	20±1
		90	33±1	39±2	48±3	46±2
4.	*Tithonia diversifolia*	30	8±1	13±1	10±2	11±2
		90	49±2	42±2	6±1	21±3
5.	Combination (1:1:1:1)	30	11±2	14±1	12±2	22±2
		90	17±2	43±2	7±2	10±2
	Control (H_2O)	30	9±1			

6.		90	15±2

#Mean ± S.E

Table 2: Effect of various extracts on petiole length in *C.asiatica*

S.no	Name of the treatment	Petiole length (cm) recorded at different percentage levels			
		5%	10%	20%	50%
1.	*Tephrosia purpurea*	15.4±0.3	2.63±0.3	3±0.4	11.6±0.7
2.	*Ipomea carnea*	9.6±0.6	10.1±0.2	13±0.3	7.6±0.7
3.	*Trianthema portulacastrum*	4.6±0.3	8.6±0.1	14.1±0.8	13.2±0.5
4.	*Tithonia diversifolia*	17.6±0.6	8.3±0.4	8±0.4	12.5±0.2
5.	Combination (1:1:1:1)	5.1±0.2	10.8±0.1	3.3±0.2	2.7±0.4
6.	Control (H_2O)	5.5±0.6			

#Mean ± S.E

Table 3: Effect of various extracts on the root length in *C.asiatica*

S.no	Name of the treatment	Root length (cm) recorded at different percentage levels			
		5%	10%	20%	50%

1.	*Tephrosia purpurea*	9.1±0.6	3.3±0.2	7.7±0.4	10.4±0.3
2.	*Ipomea carnea*	17.3±0.5	29.5±0.3	14.7±0.4	6.7±0.5
3.	*Trianthema portulacastrum*	17.9±0.2	20.4±0.4	18.8±0.3	16.3±0.5
4.	*Tithonia diversifolia*	20.9±0.3	27.1±0.7	1.9±0.2	11±0.5
5.	Combination (1:1:1:1)	12.9±0.5	7.9±0.5	3.5±0.2	4.5±0.3
6.	Control (H_2O)	12.6±0.4			

#Mean ± S.E

Table 4: Effect of various extracts on leaf surface area in *C.asiatica*

S.no	Name of the treatment	Leaf surface area (cm^2) recorded at different percentage levels			
		5%	10%	20%	50%
1.	*Tephrosia purpurea*	16±0.2	3.8±0.7	5.8±0.3	18.6±0.2
2.	*Ipomea carnea*	13.7±0.2	13.6±0.1	26.7±0.1	6.4±0.7
3.	*Trianthema portulacastrum*	9.9±0.5	13.6±0.8	23.3±0.4	26.1±0.3

4.	*Tithonia diversifolia*	38.8±0.4	37.6±0.3	7.6±0.1	14.6±0.1
5.	Combination (1:1:1:1)	11.6±0.5	25.4±0.2	3.9±0.4	6.1±0.1
6.	Control (H_2O)	8.8±0.4			

#Mean ± S.E

Table 5: Effect of various extracts on total fresh weight of *C.asiatica*

S.no	Name of the treatment	Total fresh weight recorded at different percentage levels			
		5%	10%	20%	50%
1.	*Tephrosia purpurea*	28.6±1.5	6.7±2.9	27.1±2.9	80.2±1.6
2.	*Ipomea carnea*	77.1±2.0	104.7±2.6	113±3.3	10.8±1.7
3.	*Trianthema portulacastrum*	53.0±1.3	88.0±2.5	83.3±1.5	105.5±2.1
4.	*Tithonia diversifolia*	120.6±2.0	90.4±1.7	8.8±1.9	34.4±2.4
5.	Combination (1:1:1:1)	40.8±2.2	78.4±2.9	7.1±0.6	10.7±2.0
6.	Control (H_2O)	39.7±1.4			

#Mean ± S.E

Table 6: Effect of various extracts on total dry weight of *C.asiatica*

S.No	Name of the treatment	Total dry weight (g) recorded at different percentage levels			
		5%	10%	20%	50%
1.	*Tephrosia purpurea*	7.2±0.7	1.7±1.1	6.5±0.9	32.8±0.8
2.	*Ipomea carnea*	29.4±0.7	45.7±0.9	49.2±1.4	3.1±0.7
3.	*Trianthema portulacastrum*	21.6±0.6	24.3±1	23.9±0.8	46.1±0.6
4.	*Tithonia diversifolia*	52.4±0.5	26.8±0.2	3±0.7	9.2±0.6
5.	Combination (1:1:1:1)	11.7±0.1	20.2±0.7	2.3±0.5	2.6±0.4
6.	Control (H_2O)	11.1±0.3			

#Mean ± S.E

Table 7: Physicochemical properties of the soils fertigated with various extracts of *C.asiatica*

S.no	Name of the treatment	pH	EC dS/m	Org C%	N kg/ha	P kg/ha	K kg/ha
1	Control(H_2O)	6.5	0.47	0.44	147	9.44	188
2	*Tephrosia purpurea* (50%)	6.4	0.39	0.38	142	10.32	196

3	*Ipomea carnea* (50%)	6.5	0.54	0.43	144	10.38	182
4	*Trianthema portulacastrum* (10%)	6.4	0.28	0.42	148	10.72	192
5	*Tithonia diversifolia* (20%)	6.7	0.33	0.40	140	10.40	190
6	Combination (1:1:1:1) (50%)	6.8	0.40	0.40	140	10.60	184

Table 8: Effect of various extracts on total Chlorophyll content in *C.asiatica*

S.no	Name of the treatment	Chlorophyll a	Chlorophyll b	Total chlorophyll
1.	*Tephrosia purpurea* (10%)	0.071±0.0	0.045±0.0	0.117±0.0
2.	*Ipomea carnea* (20%)	0.294±0.0	0.105±0.0	0.399±0.01
3.	*Trianthema portulacastrum* (20%)	0.402±0.0	0.178±0.0	0.580±0.0
4.	*Tithonia diversifolia* (10%)	0.395±0.0	0.174±0.0	0.570±0.0
5.	All (1:1:1:1) (10%)	0.078±0.0	0.046±0.0	0.125±0.0
6.	Control (H_2O)	0.402±0.0	0.136±0.02	0.562±0.01

#Mean ± S.E

Table 9: Leaf nutrients analysis in *C.asiatica*

S. No	Name of the treatment	N	P	K	Ca	Mg	Zn	Cu	Fe	Mn
		%			Ppm					
1.	Control (H_2O)	3.4	0.27	1.73	1341	286	32	16	251	38
2.	*Tephrosia purpurea* (10%)	3.7	0.24	1.88	1732	324	36	21	276	30
3.	*Ipomea carnea* (20%)	2.9	0.22	1.49	1550	338	34	19	228	41
4.	*Trianthema portula castrum* (20%)	3.1	0.28	1.58	1389	327	40	16	259	44
5.	*Tithonia diversifolia* (10%)	3.2	0.25	1.69	1432	315	37	20	244	37

#Mean ± S.E

Table 10: Number of leaves *Mentha arvensis*

S.no	Name of the Treatment	Number of leaves recorded at different percentage levels			
		5%	10%	15%	20%
1.	Onion peel extract	17±2	22±2	28±8	37±2
2.	Potato peel extract	25±2	36±3	32±2	26±2
3.	*Calatropis* entire plant extract	54±3	57±3	90±2	55±3
4.	All (1:1:1)	26±2	35±2	33±3	39±2
5.	Control	35±3			

Table 11: Leaf surface area of *Mentha arvensis*

S.no	Name of the Treatment	Leaf surface area (cm^2) recorded at different percentage levels			
		5%	10%	15%	20%
1.	Onion peel extract	3.6±0.9	6.1±0.7	4.9±0.2	3.6±0.9

2.	Potato peel extract	4.8±0.5	3.6±0.4	2.6±0.5	5.3±0.8
3.	*Calatropis* entire plant extract	4.9±0.4	5.5±0.5	12.7±0.9	8.0±0.8
4.	All (1:1:1)	4.7±0.8	3.2±0.6	2.8±0.7	3.1±0.8
5.	Control	3.0±0.5			

#Mean ± S.E

Table 12: Shoot length of *Mentha arvensis*

S.no	Name of the Treatment	Shoot length (cm) recorded at different percentage levels			
		5%	10%	15%	20%
1.	Onion peel extract	12±0.6	13.8 ±0.8	14.6±0.2	17.1± 0.2
2.	Potato peel extract	16.1±0.7	15.3±1.5	16±2	16.6±1.5
3.	*Calatropis* entire plant extract	14.6±2	23.6±3	39±2	30.6±2
4.	All (1:1:1)	15.3±1.5	19.3±1.5	13.3±1.5	17.3±2.5

5.	Control	23±2

#Mean ± S.E

Table 13: Total fresh weight of *Mentha arvensis*

S.no	Name of the Treatment	Total fresh weight (g) recorded at different percentage levels			
		5%	10%	15%	20%
1.	Onion peel extract	20±2.5	35±3	37±2.8	41±1.2
2.	Potato peel extract	40±2	30±2.1	20±1	37±1.7
3.	*Calatropis* entire plant extract	50±2.6	80±1.5	170±1.5	120±1.5
4.	All (1:1:1)	28±1.9	70±2.2	51±1.8	80±1.6
5.	Control	40±1			

#Mean ± S.E

An increase in total fresh weight (120.6±2.0 g) and total dry weight (52.4±.5 g) in *C. asiatica* with an aqueous extract of *T. diversifolia* (5% concentration) was observed compared to the aqueous extract of *T. purpurea* (20% concentration) (6.7±2.9 g) and (1.7±1.1 g)

(Table 5 & 6). In *M. arvensis* , the aqueous extract of *Calotropis* (15% concentration) increased the total fresh weight (170±1.5g) and shoot length (39±2 cm**)**, whereas the aqueous extract of potato peel (15% concentration) decreased the same (20±1g) and (12±0.6 cm) respectively (Table 12 & 13).

3.1 Soil analysis:

The results are presented in Table 14

Table 14: Soil analysis of *Mentha arvensis*

S.no	Name of the treatment	pH	EC dS/m	Orc C%	N kg/ha	P kg/ha	K kg/ha
1	Control	7.1	0.51	0.38	140	9.65	174
2	Onion peel extract	6.97	0.42	0.38	146	10.36	160
3	Potato peel extract	6.84	0.40	0.41	151	10.52	176
4	*Calotropis* entire plant extract	6.90	0.38	0.42	160	11.21	170

#Mean ± S.E

Figures

Figure 1 Effect of *T. purpurea* aqueous extract on *Centella asiatica*

Figure 2 Effect of *I.carnea* aqueous extract on *Centella asiatica*

Figure 3 Effect of *T. portulacastrum* aqueous extract on *Centella asiatica*

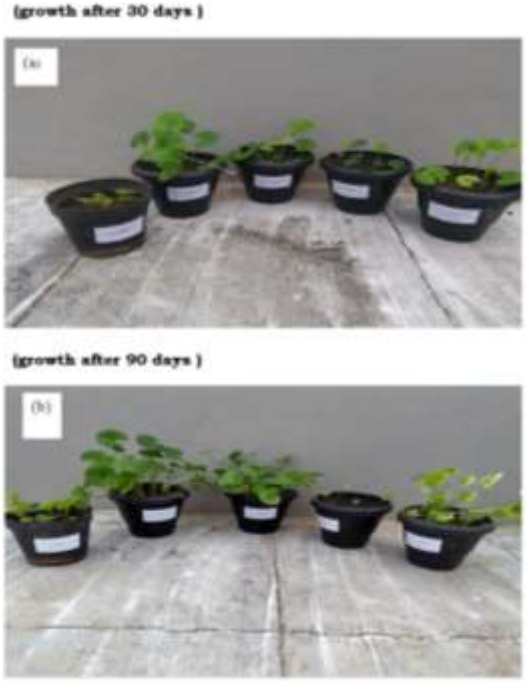


Figure 4 Effect of *T.diversifolia* aqueous extract on *Centella asiatica*

Figure 5 Effect of combination of 2 to 5 (1:1:1:1) aqueous extract on *Centella asiatica*

Figure 6 Treatment of Onion and potato aqueous extract on *Centella asiatica*

Figure 7 Treatment of *C.gigantea* and mixed combination of aqueous extract on *Menta arvensis*

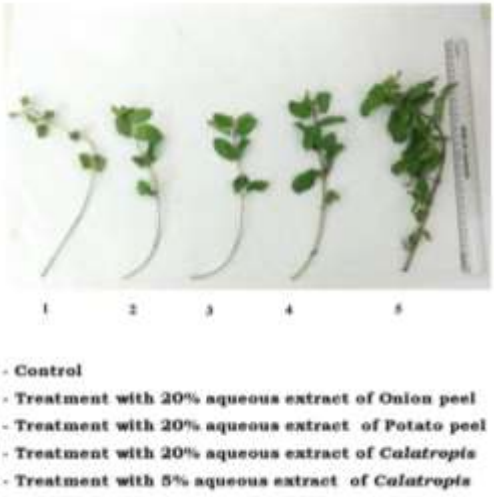


Figure 8 Treatment of Onion and potato peel of aqueous extract on *Menta arvensis*

1 - Control (H_2O)

2 – Treatment with 10% aqueous extract of *Tephrosiapupurea*

3 - Treatment with 20% aqueous extract of *Ipomea carnea*

4 - Treatment with 5% aqueous extract of *Tithonia diversifolia*

Figure 9 Petiole length of *Centella asiatica*

1 – Control (H_2O)

2 - Treatment with *Tephrosia purpurea* aqueous extract (a -10%; b - 50%)

3 - Treatment with *Ipomea carnea* aqueous extract (a -10%; b - 50%)

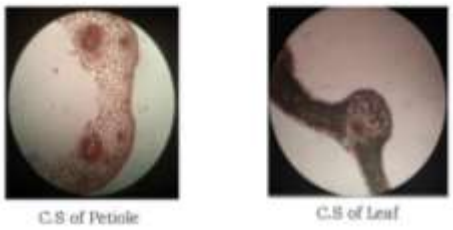
4 - Treatment with *Trianthema portulacastrum* aqueous extract (a -5%; b - 50%)

5 - Treatment with *Tithonia diversifolia* extract (a -10%; b - 20%)

Figure 10 Root length of *Centella asiatica*

1 - Control (H_2O)
2 - Treatment with *Tephrosia purpurea* (10%) aqueous extract
3 - Treatment with *Ipomea carnea (20%)* aqueous extract
4 - Treatment with *Trianthema portulacastrum* (50%) aqueous extract
5 - Treatment with *Tithonia diversifolia* (5%) aqueous extract
6 – Treatment with combination of 2 to 5 (1:1:1:1) (10%) aqueous extract

Anatomical study in *C. asiatica*



C.S of Petiole C.S of Leaf

Figure 11 Leaf surface area, anatomical study of petiole and leaf of *Centella asiatica*

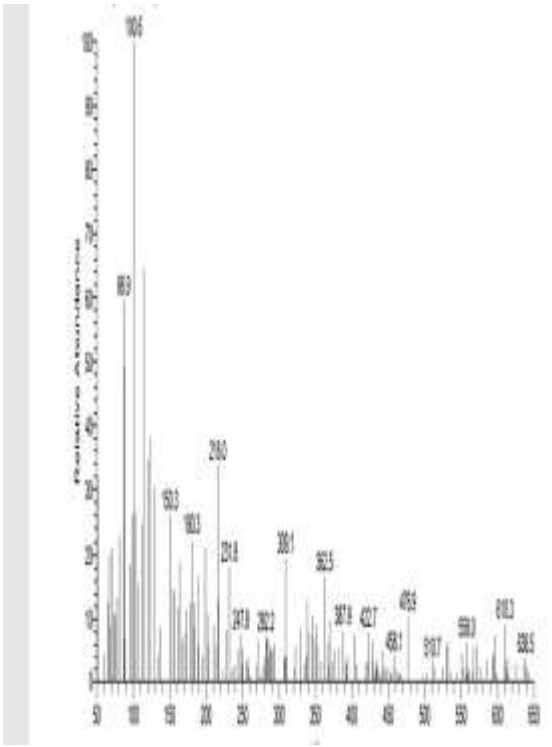


Figure 12 GC-MS analysis of *Mentha arvensis* oil

3.2 GC-MS analysis

In GC-MS analysis 30 bioactive phytochemical components were identified in the hexane extract of *M. arvensis*. Among the identified compound, 5-(4-Hydroxyphenyl) -10,20-bis (3-methoxyphenyl) -15-Propylporphyrin, 5- (deuteriomethyl)- 3-methyl-4- nitroisoxazole were reported as major compounds followed by 5,10-bis(3-aminophenyl)-15,20-diphenylporphyrin,{12,12,17,18,22,23 Hexamethyl2,7 anthraquinono[26,27b] phthalocyanine}zinc,7,8-Dimethoxy-13-carbomethoxy-15-à- (3,4,5-trimethoxybenzoxy)- 13,14 didehydroalloberban, (-)- Asimilobin and {1',2'-bis (Methoxycarbonyl) -1,1,6,7,11,12-hexamethylbenzo [16,17-d] phthalocyanine} zinc.

Table 15: GC-MS analysis of *Mentha arvensis* oil

S. No	RT	Name of the compound	Molecular Formula	Molecular Weight (g/mol)	Peak area
1	4.49	7,8-Dipropyl-2-ethoxycarbonyltetrathia [7]helicene	$C_{31}H_{26}O_2S_4$	558	2.94
2	5.63	2,2-Bis[4-[(4,6-dichloro-1,3,5-triazin-2-yl)oxy]phenyl]-1,1,1,3,3,3-hexafluoropropane	$C_{21}H_8Cl_4F_6N_6O_2$	630	2.33
3	7.55	5,10-bis(3-aminophenyl) -15,20-diphenylporphyrin	$C_{44}H_{32}N_6$	644	2.64

4	7.85	5H-Cyclopropa [3,4]benz[1,2-e]azulen-5-one, 3,9,9a-tris(acetyloxy) -3-[(acetyloxy)methyl]-2-chloro-1,1a,1b,2,3,4,4a,7a, 7b,8,9,9a-dodecahydro- 4a,7b-dihydroxy- 1,1,6,8-tetramethyl-	$C_{28}H_{37}ClO_{11}$	584	3.07
5	9.36	Oleic acid, 3-(octadecyloxy)propyl ester (CAS)	$C_{39}H_{76}O_3$	592	2.38
6	11.52	13-(3,4,5-trimethoxyphenyl)- ,4,8,11-tetraoxadispiro [4.1.4.3]tetradecane	$C_{19}H_{26}O_7$	366	3.54
7	12.94	5-(4-Hydroxyphenyl)- 0,20-bis(3-methoxyphenyl)- 15- Propylporphyrin	$C_{43}H_{36}N_4O_3$	656	5.79
8	15.17	{12,12,17,18,22,23- Hexamethyl-2,7- anthraquinono[26,27-b] phthalocyanine}zinc	$C_{38}H_{30}N_4O_2Zn$	638	4.31
9	16.29	6,6''-Bis(chloromethyl) [4,4':6',4''-terdibenzofuran]	$C_{38}H_{22}C_{l2}O_3$	596	2.56

10	17.51	(RR)/(SS) and (RS)/(SR)-8-(2-chlor-1,2-diphenylethyl) -3,7-dihydro- 1,3,7-trimethyl- 1H-purin-2,6-dion	$C_{22}H_{21}ClN_4O_2$	408	3.32
11	18.08	Pregnan-20-one, 3,11,21-tris [(trimethylsilyl)oxy]-, O-methyloxime, (3à,5á,11á)-	$C_{31}H_{61}NO_4Si_3$	596.087	3.99
12	18.71	4,4'-(o-Xylylenedithio) bis(5-carbomethoxy- 1,3-dithiol-2-one	$C_{18}H_{14}O_6S_6$	518	2.84
13	24.07	8,10-bis(1',1'-Dimethylethyl) -4,6-bis(4'-methylphenyl) -3,7-dithiatricyclo [4.4.0.0(2,8)]deca-4,9-diene	$C_{30}H_{36}S_2$	460	2.61
14	25.41	Norvenlafaxine	$C_{16}H_{25}NO_2$	263.381	3.14

15	26.92	5''-(1,1-Dimethylethyl)-2,2',2'',2''',2''''-pentamethoxy[1,1':3',1'':3'',1''':3''',1''''-quinquephenyl]-3,3''''-dimethanol	$C_{41}H_{44}O_7$	648.796	3.15
16	27.74	5,11,17,23-Tetra-t-butyl-25,26,27,28-tetrahydroxycalix-4-arene	$C_{44}H_{56}O_4$	648.928	3.18
17	29.33	5,10-bis(3-aminophenyl)-15,20-diphenylporphyrin	$C_{44}H_{32}N_6$	644.782	4.43
18	29.79	7,8-Dimethoxy-13-carbomethoxy-15-à-(3,4,5-trimethoxybenzoxy)-13,14-didehydroalloberban	$C_{31}H_{37}NO_9$	567.635	4.25
19	30.63	5-(Ethynyl)non-1-en-8-yn-5-yl acetate	$C_{13}H_{16}O_2$	204.269	2.91
20	31.06	(-)-Asimilobin	$C_{35}H_{62}O_6$	578.875	4.24

21	31.42	Bicyclo[3.1.1]hept-3-en-2-yl 2,2,2-trifluoromethyl Ether	$C_9H_{11}F_3O$	192.181	2.39
22	32.28	Benzeneacetic acid, 3-methoxy-4- [(trimethylsilyl)oxy]-, trimethylsilyl ester (CAS)	$C_{15}H_{26}O_4Si_2$	326.539	3.52
23	2.58	norepinephrine-pentatms	$C_{23}H_{51}NO_3Si_5$	530.09	2.61
24	34.24	5-(deuteriomethyl)-3- methyl-4-nitroisoxazole	$C_5H_3D_3N_2O_3$	142.06	4.65
25	35.29	{1',2'-bis(Methoxycarbonyl)-1,1,6,7,11,12-hexamethyl benzo[16,17-d] phthalocyanine }zinc	$C_{34}H_{32}N_4O_4$ Zn	626.034	4.21
26	36.33	36,37,38-Trimethoxy- 5,10,15-trimethyl- 22,25,30,33-tetraoxa- 1,19-diazapentacyclo [17.8.8.1(3,7).1(8,12).1(13,17)]octatriaconta- 3,5,7(36)nonane	$C_{38}H_{52}N_2O_7$	648.841	2.41

27	36.66	2,7,12,17-tetrabrom-(all-às)cyclotetrathiophen (2,7,12,17-tetrabromcycloocta [1,2-b:4,3-b':5,6 -b":8,7-b"']tetrathiophen	$C_{16}H_4Br_4S_4$	644.064	2.53
28	39.57	2-Cyclohexylamino-cyclohex-2-en-1-one	$C_{12}H_{19}NO$	193.29	3.11
29	40.27	2,2-Bis[4-[(4,6-dichloro -1,3,5-triazin-2-yl)oxy]phenyl]-1,1,1,3,3,3- hexafluoropropane	$C_{21}H_8Cl_4F_6N_6O_2$	632.125	4.28
30	41.12	4-Bromophenyl)bis (2,4-dibromophenyl)amine	$C_{18}H_{10}Br_5N$	639.805	2.65

Treatment of *C. asiatica* with *Tithonia diversifolia* extract at lower concentration increased the number of leaves, petiole length, leaf surface, root length, and fresh & dry weight. A similar observation was noticed in growth parameters (shoot height, fresh weight, dry weight, leaf area, and ratio) of *Zea mays* treated with fresh stem extract of *Tithonia diversifolia* (Oyerinde *et.al.* 2009). The potentiality of *T. diversifolia* as green manure and organic fertilizer for vegetable crops is already known. Our findings are in accordance with the results of the growth of purple nutsedge recorded by different mango aqueous extracts or powder concomitant with the accumulation of phenolic compounds that might indicate a sort of allelopathic stress (Baloch *et al.* 2014, Kiran & Patra, 2003). In the present study, *T. diversifolia* may contain allelochemicals that perform both stimulatory and inhibitory functions. The stimulatory functions of these chemicals were evident in the significant enhancement of the growth parameters (petiole height, fresh weight, dry weight, and leaf area) of *C. asiatica.*

Treatment of *T. purpurea* at lower concentrations decreased the number of leaves, petiole length, leaf surface, root length, and fresh & dry weight in *Centella asiatica*. Higher concentrations of the extract increased the number of leaves and inhibited the root length. The use of legume green manures is complicated, because of potential problems with phytotoxicity of the residues for several weeks following incorporation into the soil (Liang *et al.* 2006) which would have been a reason for the decreased root length in the present study. Soil containing *Euphorbia hirta* powder showed a significant reduction in the

fresh and dry weight of wheat plant (Mojid *et al*. 2012). Treatment of *Centella asiatica* individually with leaf and stem extract of *T. portulacastrum* produced more number of leaves in minimum germination time. The result was contradictory to the germination of rice seeds which was significantly affected by soaking the water extracts of *T. portulacastrum* in distilled water (Mubeen e*t al*. 2011). Maximum germination time (MGT) was found when rice seeds were soaked in root and leaf extracts of *T. portulacastrum*.

In this study highest growth was recorded in a 15% concentration of aqueous extract of *Calotropis*, while the lowest growth was recorded in a 5% aqueous extract of onion peel. This may be due to the release of ammonium-nitrogen through volatilization losses (Solomon *et al*. 2001). Similar reports on the effect of kitchen waste on plant growth and productivity have been studied for tomato in pot experiments by Vanlauwe *et al* (2001). 15% concentration of *Calotropis* aqueous extract increased the total number of leaves, leaf surface area, shoot length and total fresh weight compared to control in *M. arvensis*. This could be attributed to the addition of nitrogen in adequate amounts which results in the enhancement of yield due to a positive effect on plant growth, fresh leaf weight, and root development (Jilani *et al*. 2010).

A 5% concentration of onion peel aqueous extract decreased the total number of leaves, shoot length, and total fresh weight compared to the control. A similar observation was noted in the findings of Resende *et al.,* (1999) in radish. Chlorophyll content (mg/g of fresh weight) was recorded. Treatment of *T. portulacastrum* increased the total chlorophyll content of *C. asiatica* compared to *T. purpurea*. A good and optimum supply of green manure is associated with increased plant growth due to which the plants explore more soil nutrients and moisture. Plant leaf nutrients such as N, P, K, and micronutrients (Ca, Mg, Zn, Cu, Fe & Mn) were rich in the plant extracts when compared to the control. N and K were higher with the treatment of *T. purpurea*, whereas lower with the treatment of *I. carnea*. P was higher with the treatment of *T. portulacastrum*. Micronutrients were most abundant in the treatment with *T. purpurea* on *C. asiatica*. The soil containing aqueous extract of 15% *Calotropis* extract had the highest levels of nitrogen and phosphorus compared to the control. Nitrogenous fertilizers may have a positive impact on soil health (Singh, 2018). Aqueous extract of potato peel (20%) increased potassium when compared to the control. N, P, and K sources are well known to contribute to soil moisture (Garter, 1967; Stevenson & Bates, 1968).

In this study, 0.03ml oil was obtained from *M. arvensis* treated with a 15% concentration of *Calotropis* aqueous extract. *Mentha* retains its nutritional values even after steam distillation and oil extraction and has been reported to have great promise as an organic source of plant nutrients (Chattopadhyay *et al*. 1993, Patra & Singh, 1993, Patra & Anwar, 1997). *Mentha arvensis* essential oil is the main source of natural menthol. The genetic characteristics of the essential oil quality of *Mentha arvensis* are not fully understood (Kumar *et al*., 2000). Only a few genes have been identified and cloned, although several workers are actively involved in the study of the monoterpenoid pathway of *Mentha arvensis* and related mint. Among the identified compound,5- (4-Hydroxyphenyl)-10,20- bis(3-methoxyphenyl) -15 - Propylporphyrin, 5-(deuteriomethyl)-3-methyl-4-nitroisoxazole was found as major compound followed by 5,10-bis(3-aminophenyl)-15,20- diphenylporphyrin, {12,12,17,18,22,23- Hexamethyl-2,7-anthraquinono [26,27-b] phthalocyanine} zinc, 7,8-Dimethoxy-13-carbomethoxy-15-à-(3,4,5-trimethoxybenzoxy)-13,14-didehydroalloberban, (-)- Asimilobin and {1',2'-bis(Methoxycarbonyl)-1,1,6,7,11,12-hexamethylbenzo [16,17-d]phthalocyanine} zinc.

4. Conclusion

In menthol mint, there has been limited work regarding its organic cultivation. Therefore, producing essential oil through organic farming has great importance in the present context. In a mint cropping system, the joint application of organic fertilizer and green manure plays a significant role in sustaining crop productivity and restoring soil fertility (Patra & Anwar, 1997).

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References

[1] Tilman, D., Lehman C., and Thomson, K. 1997. Plant diversity and ecosystem productivity: Theoretical considerations. Proceedings of the *National Academy of Sciences of the United States of America*. **94**:1857–1861.

[2] Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H., Tilman, D.G. 1997. Human alteration of the global nitrogen cycle: Sources and consequences. *Ecological Applications*. **7:**737–750.

[3] Sagar, M., Zaman, A., Tanuj, K. M., and Jnana, B. P. 2018. Green manures in agriculture: A review. *Journal of Pharmacognosy and Phytochemistry*. **7**(5): 1319-1327.

[4] Moindi, J., Onyambu, E., Kiprono, S. J., Titus, S. K. and Onyancha, W. 2012. Studies of Biodegradation of *Ipomoea carnea* Weed from Kavinadu Big Tank in Pudukkottai District (India). *Journal of Natural Sciences Research*. **2**:6.

[5] Sharma, A. and Bachheti, R.K. 2013. A review on *Ipomoea carnea*. *Int J Pharm Bio Sci*., **4**(4): 363 – 377.

[6] Jama, B., Palm, C.A., Buresh, R.J., Niang, A., Gachengo, C., Nziguheba G., and Amadalo, B. 2000. *Tithonia diversifolia* fertility Improvement in Western Kenya: A review. *Agro. For. Syst*., **49**: 201-221.

[7] Yoshida, S, Forno, D.A., Cock, J.H .1972. "Laboratory Manual for Physiological studies of rice". I*nternational Rice Research Institute.*2nd Ed.

[8] Walkley, A. and Black, I. A. 1934. An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci*. **37**: 29-37.

[9] Olsen, S. R. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. *United States Department of Agriculture;* Washington.

[10] Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant physiology*, **24**(1), 1.

[11] Chapman, H. D., and Pratt, P. F. 1961. Methods of analysis for soils, plants and water. *Univ. California*, Berkeley, CA, USA.

[12] El-Rokiek, Kowthar G; Rafat. R. El-Masry; Nadia K. Messiha and Salah A. Ahmed. The Allelopathic Effect of Mango Leaves on the Growth and Propagative Capacity of Purple Nutsedge (*Cyperus rotundus* L.) *Journal of American Science* 2010;**6**(9)

[13] Baloch, P. A., Uddin, R., Nizamani, F., Solangi, A.,& Siddiqui, A. (2014).Effect of nitrogen, phosphorus and potassium fertilizers on growth and yield characteristics of radish (*Raphinus sativus* L.). *Am. Eurasian. J. Agric. Environ. Sci*, **14**(6), 565-569.

[14] Liang, B., Lehmann, J., Solomon, D., Kinyangi, J., Grossman, J., O'neill, B., & Neves, E. G. (2006). Black carbon increases cation exchange capacity in soils. *Soil Science Society of America Journal*, **70**(5), 1719-1730.

[15] Mojid, M. A., Wyseure, G. C. L., & Biswas, S. K. (2012). Requirement of nitrogen, phosphorus and potassium fertilizers for wheat cultivation under irrigation by municipal wastewater. *Journal of soil science and plant nutrition*, **12**(4), 655-665.

[16] Mubeen, K., Nadeem, M. A., Tanveer, A., & Zahir, Z. A. (2011). Allelopathic effect of aqueous extracts of weeds on the germination and seedling growth of rice (*Oryza sativa* L.). *Pakistan Journal of Life and Social Sciences*, ***9***(1), 7-12.

[17] Kiran, U., & Patra, D. D. (2003). Medicinal and aromatic plant materials as nitrification inhibitors for augmenting yield and nitrogen uptake of Japanese mint (*Mentha arvensis* L. Var. Piperascens). *Bioresource Technology*, **86**(3), 267-276.

[18] Mojid, M. A., Wyseure, G. C. L., & Biswas, S. K. (2012). Requirement of nitrogen, phosphorus and potassium fertilizers for wheat cultivation under irrigation by municipal wastewater. *Journal of soil science and plant nutrition*, **12**(4), 655-665.

[19] Vanlauwe, B., Wendt, J., & Diels, J. (2001). Combined application of organic matter and fertilizer. *Sustaining soil fertility in West Africa*, (sustainingsoilf), 247-279.

[20] Jilani, M. S., Burki, T., & Waseem, K. (2010). Effect of nitrogen on growth and yield of radish. *Journal of Agricultural Research*, ***48***(2), 219-225.

[21] Garter, L. (1967). The Zinoviev Letter.

[22] Stevenson, C. K., & Bates, T. E. (1968). Effect of Nitrogen to Phosphorus Atom Ratio of Ammonium Phosphates on Emergence of Wheat (*Triticum vulgare*) 1. *Agronomy Journal*, ***60***(5), 493-495.

[23] Chattopadhyay, A., Subrahmanyam, K., & Singh, D. V. (1993). Recycling of nutrients in Japanese mint-assessment of soil fertility and crop yield. *Fertilizer research*, ***35***(3), 177-181.

[24] Patra, D. D., Ram, M., & Singh, D. V. (1993). Influence of straw mulching on fertilizer nitrogen use efficiency, moisture conservation and herb and essential oil yield in Japanese mint (*Mentha arvensis* L.). *Fertilizer research*, ***34***(2), 135-139.

[25] Patra, D. D., & Anwar, M. (1997). Residue recycling for restoring soil fertility and productivity in Japanese mint (*Mentha arvensis*) mustard (Brassica sp.) system. In *Plant Nutrition for Sustainable Food Production and Environment* (pp. 587-588). Springer, Dordrecht.

[26] Resende. F.V.. Souza, L.S.. Oliveira, P.S.R.. Gualberto. R. 2005. Uso de cobertura morta vegetal no controle da umidade e temperatura do solo, na incidência de plantas invasoras e na produção da cenoura em cultivo de verão, Ciência agrotecnica **29**: 100-105.

Singh, B. (2018). Are nitrogen fertilizers deleterious to soil health?. *Agronomy*, ***8***(4), 48.

[27] Oyerinde, R. O., Otusanya, O. O., & Akpor, O. B. (2009). Allelopathic effect of *Tithonia diversifolia* on the germination, growth and chlorophyll contents of maize (*Zea mays* L.). *Scientific Research and Essay*, ***4***(12), 1553-1558.

[28] Kumar, S., Bahl, J.R., Bansal, R.P., Kukreja, A.K., Garg, S.N., Naqvi, A.A., Luthra, R. and Sharma, S. 2000. Profiles of the essential oils of Indian menthol mint *Mentha arvensis* cultivars at different stages of crop growth in northern plains. *Journal of Medicinal and Aromatic Plants Sciences* **22**: 774-786.