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ENHANCEMENT OF QUANTITY OF ESSENTIAL OIL COMPONENTS IN CYMBOPOGAN CITRATUS USING HOMOEOPATHIC ULTRADILUTIONS OF ISOTHERAPEUTICS AND SILICEA

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INTRODUCTION:

In India, 7500 species of higher plants are known for its medicinal uses [1]. The therapeutic properties of various plants mainly depend upon its phytoconstituents. According to the World Health Organization, a variety of drugs are obtained from different medicinal plants and about 80% of the world's developing population depends on traditional medicine for their primary health care needs [2]. Numerous therapeutic agents have been isolated only from the natural sources.

Cymbopogan citratus

Cymbopogan citratus belongs to the family of Poaeceae. It is also known commonly as lemon grass, West Indian lemon grass, oil grass, Melissa grass [3]. It is native to South Asia, South East Asia and Australia. In India it is wildly distributed in the states of Tamil Nadu, Karnataka, Kerala and Maharashtra. It is a tall, perennial sedge throwing up dense fasciclles of leaves from a short rhizome. The culm is stout, erect up to 1.8 m high. Leaves are long, glaucous, green, linear tapering upwards and along the margin. The oil is distilled from the leaves and flowering tops of lemon grass plant and is commonly known as lemon grass oil. [4]

Taxonomical classification of *Cymbopogan citratus:*

Kingdom: Plantae

Division: Spermatophyte

Subdivision: Angiospermae

Class: Monocotyledonae

Order: Cyperales

Family: Poaceae

Genus: *Cymbopogan*

Species: *citratus*

The plant Cymbopogan citratus is mentioned in the Fig.1

Fig.1. *Cymbopogan citratus*

Medicinal uses:

Lemon grass oil is medicinally used as anti-inflammatory, antibacterial, antispasmodic, anticonvulsant,antiemetic,antirheumatic,antiseptic,antioxidant,antimutagenic, antimycobacterial, analgesic, antidiarrhoeal, antinociceptive, antiprotozoan , hypoglycemic, hypolipidemic, hypotensive, free radical scavengers and also has activity in inhibition of *Staphylcoccus aureus,* neurobehavioural effect. It has potential to control ageing process [5].

Culinary uses:

Lemon grass is used since ancient times by the human population by adding it to their food to enhance the flavour and aroma. It is a key ingredient in cuisines of countries like Thailand, Vietnam, Cambodia, Malaysia and Indonesia and in some areas of India and China. Lemon grass is usually consumed in the form of tea [6]. Lemon grass tea has medicinal values as mentioned above and also has a role in improving skin health, hair growth and in weight loss.

It also has an immense role in boosting mental health as it is used to combat depression, bad moods, anxiety and stress.

Other uses:

Lemon grass is also used as mosquito repellent, mosquito larvicidal. Another major and its important commercial value is in the Fragrance and perfumery industry, cosmetics industry .It is used in different systems of medicines. Lemon grass has much economic importance. This oil is a commercially important commodity as it has astounding extensive uses. In India the record of medicinal use of lemon grass dates back to more than 2000 years though its distillation started only in 1890's [5].

Phytochemical composition:

The name lemon grass is attributed to the Lemon like odour of the essential oil, which is due to the high citral content . *C.citratus* oil contains major terpenes such as citral-a or geranial, citral-b or neral, geraniol, borneol, geranylacetate, linalool and many other small components [7]. Citral, the major component of *C.citratus* oil is responsible for the above mentioned extensive and immensely astounding uses of *Cymbopogan citratus.*

Agrohomoeopathy:

The use of homoeopathic method in agriculture was introduced as agrohomoeopathy which allows us to influence biological process of plant to either accelerate or delay the growth. Utilizing homoeopathic dynamizations on plant, guarentees as the mode of preparation assures that plant will no way be contaminated. Important benefits of agrohomoeopathy include economic feasibility and preservation of natural ecology. The homoeopathic preparation can influence the growth, secondary metabolites production and essential oil yield and phytochemical profile when applied during growth of the plants. The homoeopathic dynamizations follow the principles of disintegration of matter and radiation without nuclear rupture through the mechanical action on smaller particles and addition of inert substances with dynamic activity, following the laws of electromagnetic waves: frequency, length and amplitude [8].

REVIEW OF LITERATURE:

Daniel Melo de Castro *et al.,* (2002) conducted a study on *Cymbopogan citratus* plant treated with homoeopathic medicine sulphur, Isotherapeutics and Humic acid in the potencies of 3C,12C,30C,200C,1000C.He evaluated the parameters like fresh weight, dry weight , leaf area, number of tillers, concentration of citral , concentration of it's cis and trans isomer, quantification of the essential oil obtained after extraction and concluded that Isotherapeutics 12C has an overall impact in increasing the quality of the plant as it had significant increase in the physical parameters and reasonable increase in the concentration of citral , cis-citral and trans-citral.[9]

Rovier verdi *et al.,*(2016) conducted a study on *Ocimum basilicum* L. plants treated with Silicea 7C, 12C, 30C , Equisetum 7C, 12C, 30C and distilled water and evaluated the essential oil quantity and quality and concluded that Silicea 30C has an immense impact in increasing the essential oil yield >141%.[10]

Capra *et al.,* (2014) evaluated the effect of Silicea 6C, 12C, 30C, 7x and Equisetum 7x in altering the fresh and dry biomass, quercitin content in the plant *Baccharis trimera* L. and concluded that Silicea 6C has increased the quercitin content by 30% and Silicea 7x has increased the quercitin content by 47% , Equisetum 7x increasing the quercitin content by 45% proving the efficiency of Silicea in altering the quantity of phytochemical components[11]

Duarte *et al.,* (2007) evaluated the quantity of essential oil in *Eucalyptus citriodora* and *Eucalyptus globulus* treated with homoeopathic medicine Phosphorus 3C, 6C, 12C, 30C, 100C, 200C, 1000C, 5000C and concluded that Phosphorus 12C is effective in improving the growth of the plant and increasing the yield of essential oil.[12]

Catarine. M. Nishijima *et al.,* (2014) conducted a study on citral using adult male Swiss mice for its effect of prophylactic and antinociceptive effects in experimental models of acute and chronic pain with the aim of evaluating the effects of orally administered citral in experimental models of acute and chronic nociception , inflammation and gastric ulcers causes by non -steroidal anti inflammatory drugs and concluded that citral administered by intra

gastric pathway causes neurogenic(0-5min) and inflammatory (15-30min) inhibition significantly[13]

MN Boukhatem *et al.,* (2014) in his study Lemon grass essential oil a potent anti-inflammatory and anti fungal drugs revealed that the two major components in lemon grass essential oil is neral and geranial using gas chromatography mass spectrometer method and the concluded that the lemon grass essential oil contains antifungal activity against *Candida albicans*, *C. tropicalis*, and *Aspergillus niger* with different inhibition zone diameters. For evaluating the anti inflammatory property Lemon grass essential oil was administered orally and compared with oral diclofenac as control by which he concluded as Lemon grass essential oil has similar effect as that of the control and it significantly reduced the carrageenan induced paw edema. Anti inflammatory effect of lemon grass essential oil is dose dependent and he has also experimented the effect of lemon grass essential oil as a topical application by demonstrating in a mouse model of croton oil induced ear edema and proved that 5-10ml/ear significantly reduced the ear edema.[14]

Karkala Manvitha *et al.,*(2013) made a review article on pharmacological activities of *Cymbopogan citratus* and established that the citral the main component of lemon grass oil is capable of antibacterial activity to which flavonoids and tannins found in the extract also contribute, antifungal activity is studied against the Candida species and was proved effective, antiprotozoan activity was demonstrated effective against *Crithidia, Blastocrithidia ,*phenolic acids shows anti oxidant profile, the ethanolic extract of lemongrass was found to possess anti-mutagenic properties towards chemical-Induced mutation in *Salmonella typhimurium* strains TA98 and TA100,antimalarial activity was demonstrated on micr infected with plasmodium berghei.[15]

AIMS AND OBJECTIVES:

AIM:

The aim of this research study is to investigate and analyze the essential oil components and growth in *Cymbopogan citratus* plants treated using homoeopathic ultradilutions of Cymbopogan citratus 6C, Cymbopogan citratus 12C and Cymbopogan citratus 30C, Silicea 6C, Silicea 12C and Silicea 30C.

OBJECTIVES:

- To determine the fresh and dry weight of the plant.
- To extract essential oil of lemon grass plant.
- To analyze the essential oil contents obtained using GC-MS analysis.

MATERIALS AND METHODS:

Obtaining the plants:

About 7 *Cymbopogan citratus* plants including 1 control plant was bought in healthy condition from a nursery and was planted in a fertile loamy soil with adequate sunlight on 08/04/2023. The plants were grown for about 60 days for the purpose of acclimatization. Irrigation and weeding was done whenever necessary.

Fig.2. *Cymbopogan citratus* at the time of planting

Obtaining the homoeopathic medicines:

For the purpose of conducting the study isotherapeutic homoeopathic medicine Cymbopogan citratus 6C, Cymbopogan citratus 12C, Cymbopogan citratus 30C and Silicea 6C, Silicea 12C, Silicea 30C was purchased from pharmacy.

Preparation of medicines for application:

The homoeopathic medicines were diluted in plastic containers with a volume of 1L. Inorder to prevent contamination, each medicine was diluted in a separate container. 0.7ml of each medicine was diluted in 1L of water and the solution is homogenized by simple shaking of the container.

Application of medicines to the plants:

The application of medicines were started after the plants reached the age of 60 days. Two different medicines *ie.*Cymbopogan citratus and Silicea, each with 3 different potencies (6C, 12C and 30C) was applied. Control plant was not treated with any medicine. 100ml of

diluted medicine was applied to each plant from its respective container. The diluted medicines were applied at its growth point, therefore wetting the plant and the soil. Application of medicines were carried out every two days always between 7 and 9 hours. Such application of medicine was continued for 90 days.

. Fig.3. Application of medicine to the base of the plant

Collection of plant samples:

The harvest of aerial part of the plants were carried out on 08/09/2023 after 90 days of application of medicines. The plants were cut close to the ground and placed in brown paper bags each labelled separately.

Fig.4. Harvesting of the plant

Fig.5.Harvested plant

Determination of number of tillers and fresh weight:

After the harvest of plants, the number of tillers in each plant was determined. Immediately after the harvest each plant samples were weighed separately inorder to obtain fresh weight of the plant.

Fig.6.Tilles after the harvest

Fig.7. Weighing the fresh weight of the plant

Determination of dry weight of the plant:

The plants were placed in hot air oven with forced air circulation at low temperature of 28$^{\circ C}$ Inorder to reduce heat losses of the volatile compounds in the essential oil during drying

Fig.8. weighing the dry weight of the plant

Extraction of the lemon grass essential oil:

5g of dried plant sample is weighed using physical balance and the extraction was performed using Soxhlet apparatus. Hexane was used as a solvent for the purpose of extraction.

Fig.8. Hexane used for extracting the essential oil

Preparation of the sample for Gc-ms:

The oil extracted from 7 samples is then transferred separately to a glass vial and is labelled. The samples were properly packed and sent for Gc-ms analysis.

OBSERVATIONS AND RESULTS :

After 90 days of application of medicine the following parameters were determined,

Fresh weight of the plant:

Table.1. Fresh weight of the plants

S.No	Sample	Fresh weight (in kg)
1	Cymbopogan citratus 6C	1.952kg
2	Cymbopogan citratus 12C	0.572kg
3	Cymbopogan citratus 30C	1.274kg
4	Silicea 6C	1.574kg
5	Silicea 12C	0.708 kg
6	Silicea 30 C	0.578kg
7	Control plant	1.406kg

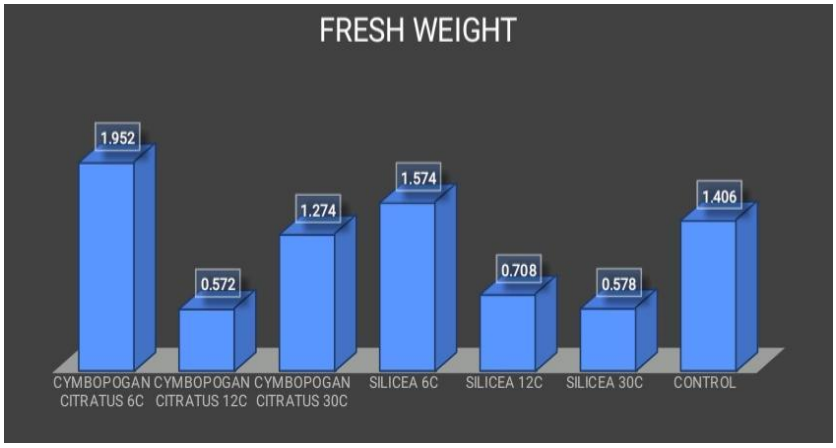

Fig.9. Fresh weight of the plants

Dry weight of the plant:

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Table.2. Dry weight of the plant

S.no	Sample	Dry weight (in kg)
1	Cymbopogan citratus 6C	0.692kg
2	Cymbopogan citratus 12C	0.218kg
3	Cymbopogan citratus 30C	0.382kg
4	Silicea 6C	0.658kg
5	Silicea 12C	0.504kg
6	Silicea 30C	0.170kg
7	Control plant	0.530kg

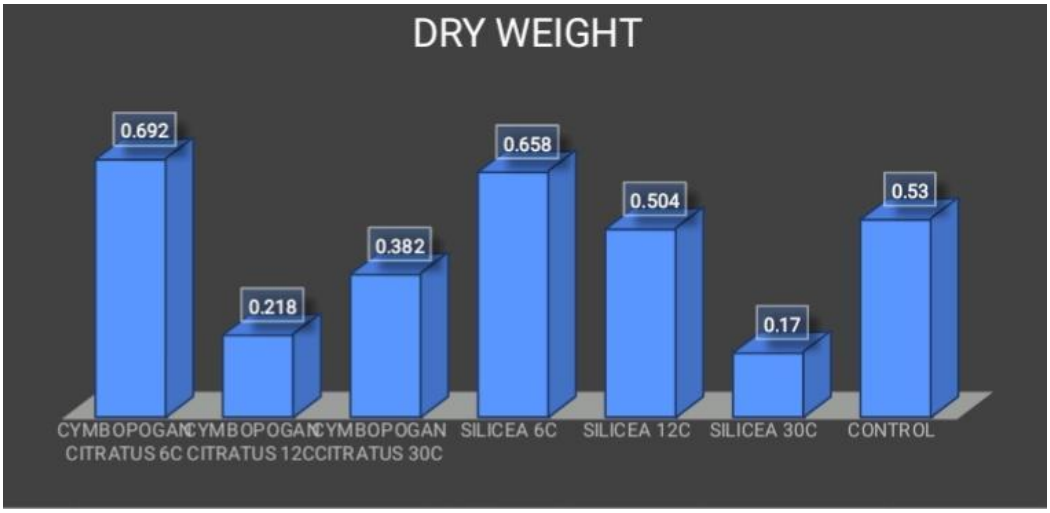

Fig.10. Dry weight of the plant

Numbers of tillers:

Table.3.Number of tillers

S.no	Sample	Number of tillers
1	Cymbopogan citratus 6C	48
2	Cymbopogan citratus 12C	22
3	Cymbopogan citratus 30 C	35
4	Silicea 6C	42
5	Silicea 12C	30
6	Silicea 30C	23
7	Control plant	40

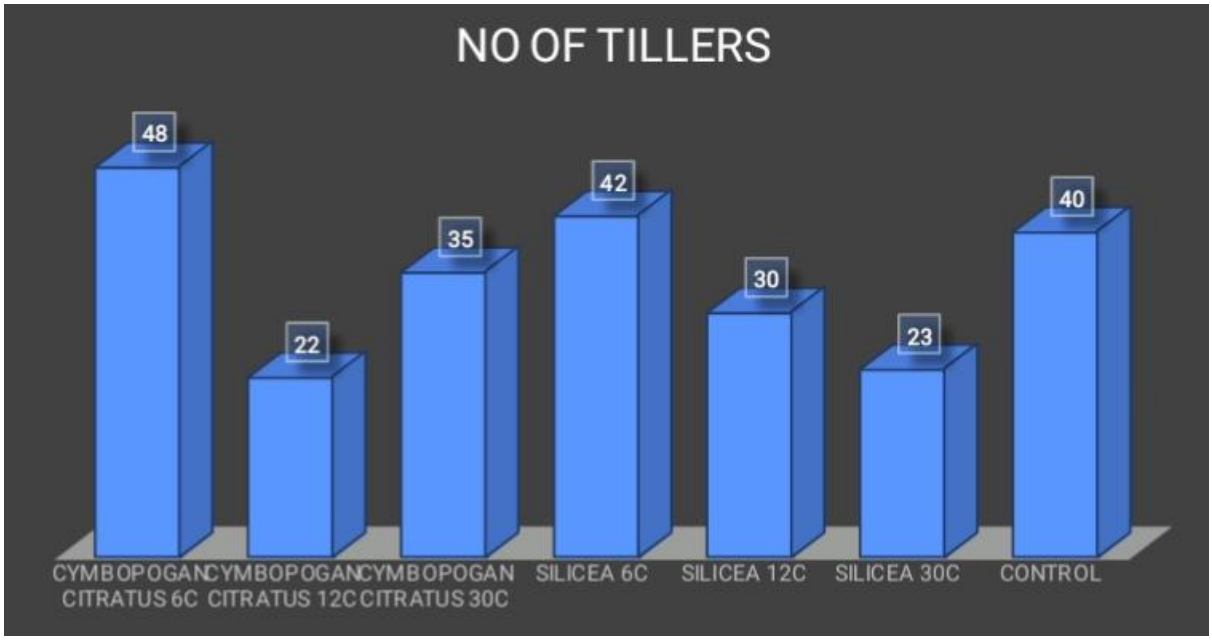

Fig.11.Number of tillers in each plant sample

Concentration of citral:

Table.4. Concentration of citral in %

S.no	Sample	Concentration of citral in %
1	Cymbopogan citratus 6C	Absent
2	Cymbopogan citratus 12C	23.73%
3	Cymbopogan citratus 30C	19.04%
4	Silicea 6C	17.99%
5	Silicea 12C	32.02%
6	Silicea 30 C	22.55%
7	Control plant	

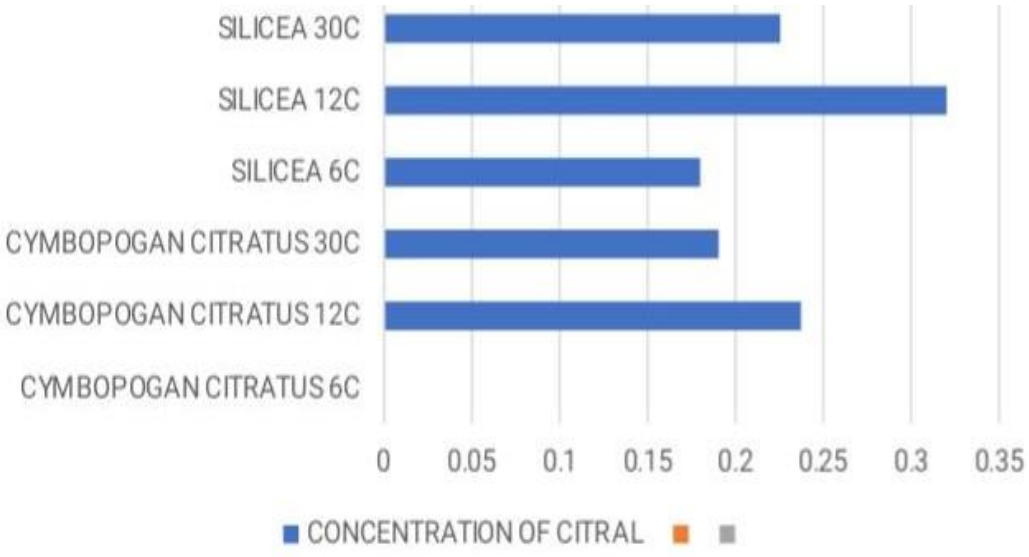

Fig.12. Concentration of citral in each sample

DISCUSSION:

The above mentioned results clearly depict that the growth of the plant quantitatively is increased in the plant treated with Cymbopogan citratus 6C. Silicea 12 C has acted upon the phytoconstituents and has increased the concentration of citral to the highest among the grown samples thus enhancing the growth of the plant qualitatively. On comparing the plants treated with potencies 6C,12C,30C of both Cymbopogan citratus and Silicea, plants treated with 6C potency has an increased growth in terms of fresh weight, dry weight and number of tillers among the plants treated with same medicine and 12 C potency has enhanced the quality of the plant with increased concentration of citral among the plants treated with same medicine. The plants treated with Cymbopogan citratus 6C has the highest value of fresh weight of about 1.952kg, dry weight of about 0.692 kg and with 48 tillers but with no concentration of citral. The plants treated with Silicea 12 C has fresh weight of about 0.708 kg, dry weight of about 0.504 kg and with 30 tillers but has the highest concentration of citral 32.03%.

CONCLUSION:

From the above made observations and obtained results, it clearly depicts that the use of homoeopathic medicine has an action in increasing the weight and overall growth of the plant and also it acts deeper altering the concentration of a essential oil components. Thus agrohomoeopathy can immensely increase the quality of the living mankind . As mentioned earlier, the plant *Cymbopogan citratus* has various uses such as culinary uses, in the preparation of medicines in various systems, in preparation of fragrances and perfumes, mosquito repellents etc. For the purpose of preparation of medicines and in the Fragrances and perfumery industries plants treated with Silicea 12C will be highly beneficial as the above mentioned purposes demand plants with increased quality that is plants with high concentration of citral. For the purpose of increasing the yield of the plant quantitatively Cymbopogan citratus 6C can be used which will benefit in case of its usage in culinary and foods.

SUMMARY:

The study is conducted with the objective of increasing the growth of the plant both quantitatively in terms of weight and qualitatively in terms of concentration of citral, thus improving the quality of life of mankind. About 7 plants of *Cymbopogan citratus* each with a tiller is planted and acclimatized for 60 days. Later for 90 days the applications of the medicines were carried out and then harvested. After harvesting, the number of tillers, fresh weight and dry weight of the sample is determined. The dried sample is powdered and was subjected for extraction of essential oil using hexane as the solvent with the help of soxhlet apparatus. The oil this extracted is carefully labelled and sent for Gc-ms analysis. The above made observations and results clearly depict that for the purpose of increasing the growth of the plant in terms of weight and tillers, treating the plants with Cymbopogan citratus 6C will be effective and for increasing the concentration of citral, treating the plants with Silicea 12 C will be effective.

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