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Cultivation and Evaluation of Antifungal Activity of Citronella and Basil Oil Against Various Fungal Infections

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ABSTRACT:

Combinations of different essential oils are often an option for therapeutic use for human health. Several researchers have examined in detail the modes of action of essential oils and most of their components and combinations. Approximately 90 types of essential oils with more than thousand combinations can be identified as being suggested for dermatological use. This research explores the antifungal properties of essential oils i.e. citronell and basil oil as natural treatment against pathogens responsible for dermatological infections i.e. Tinea corporis and Tinea capitis. The purpose of this research is to describe the properties of essential oils, principally as antifungal agents, and their role in the form of accords of essential oils or combinations.

The essential oil collected from Cymbopogon flexuosus (Lemongrass), Ocimum basilicum (Basil) and Cymbopogon winterianus (Citronella) exhibited strong inhibition against all the selected fungi tested in this study. The therapeutic use of essential oils may also provide a solution for the rapid development of fungal resistance that is problematic with the currently available common antifungal therapeutics.

Keywords: Essential oils, antifungal effect, Trichophyton tonsurans Microsporum canis.

Introduction

Cultivation methods of Citronella

I. Propagation:

The citronella plants were propagated through slips. Direct sowing of seeds or nursery of seeds in the field is not reasonable. Slips were prepared from matured mother plans and then transplanted to the field. Seeds of citronella were not used to avoid the genetic variations and oil yield differences.

II. Soil condition

Citronella can be grow well under varying soil conditions, but the loamy to sandy mold soils with abundant organic matter is the most suitable. The plants were well germinated and grow in

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the soil under the pH range of 6.0 to 7.5. Clayey, water logged soils are not suitable for citronella crop.

III. Climate

The crop of citronella germinates well in rainy season with warm and humid climate. The climate of selected region Kannauj where we cultivated the citronella crop was well warmed and full of humidity condition with rainy season. The climate has humidity and 20-300C temperature which is favorable for plant growth and high oil yielding.

IV. Planting time

The crop of citronella can be grown from the July to August in North Indian reasons. So the citronella was planted in first week of July.

V. Slips preparation

Slips were prepared from matured mother plants of citronella. Each slip was having 2-3 inch stem length with roots. Prepared slips were fresh, thick, green and sappy.

VI. Land preparation and Transplanting

Before the transplanting of citronella the field was 3 times well plowing and applied 15 tons per hectare farmyard manure in the field soil. NPK was also added in the ratio of 180:80:50. Bavistin solution 0.02% was applied in slips just before transplanting for prevention of fungal infection. After that the slips of citronella were transplanted in final prepare field with the certain gap of 60×60 cm between plants which is ideal for citronella cultivation because these gapping of slips resulting healthy and well growth of plant. Irrigation is must after the transplanting so the field was irrigated after the finalize transplanting.

VII. Crop nutrition

The treatment of nitrogen was given to cropped field apart from FYM and other biofertilizer at the time of transplanting. For the requirements, prevention of different infections and diseases and good results of oil yielding, some biopesticides were also applied on citronella crop.

VIII. Weed Control

Weeding process was applied at the time of growing period of crop for two times. After the mature growth in plants there was no requirement of weeding because the plants inhibited the growth of different types of weeds.

IX. Irrigation

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The crop of citronella needs irrigation once or twice a month thereafter if the less amount rain performing. Generally 6 to 8 irrigations are required during complete crop time of citronella.

X. Harvesting:

To avoid any type of contamination at the stage, cautions should be taken for harvesting of citronella. Surface area should be clean where the plants placed after harvesting. The time of harvesting plays an important role in qualitative and quantitative oil production. For good quality and high oil yield, the harvesting is generally complete in bright sunny days.

The crop was harvested in 95 days after transplanting. The herbage was leaved in the shadow place for 10-12 hours to reduce the moisture.

XIII. Processing and isolation of essential oil

Hydro-distillation process was done for obtaining the citronella essential oil. 160 kg per hectare essential oil was obtained. The essential oil was stored in aluminium containers in a cool and dry place.

XI. Yield

An average yield of fresh herbage was 20 to 22 kg. The whole herbage produced the essential oil about 160-170 ml.

Cultivation methods of Sweet Basil

I. Propagation:

The plants of sweet basil were propagated through seeds, but direct sowing of seeds in the field is not reasonable. Seedlings were first raised in the nursery and then transplanted to the field. These plants were highly cross pollinated having best quality oil yield. Hence for planting grower has to collect fresh seeds from the pedigree stock which are in good condition and free from pests.

II. Soil condition

Basil can be cultivated on a wide range of soils, from moderately fertile, well drained loamy to sandy loam soils with a pH ranging from 7.0 to 7.5. While clayey and water logged soils are unsuitable. It is tolerant to higher concentration of copper and zinc, but is susceptible to cobalt and nickel.

III. Climate

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The basil crop comes up well in warm and humid conditioned climate. The climate of selected region Kannauj where we cultivated the sweet basil crop was well warmed and has high humidity condition. The temperature of selected region was 20-300C which is favorable for well growth of plant and high oil production.

IV. Planting time

The crop can be grown well from the middle of February to the end of September and also during Kharif in plains of North Indian reasons. So the sweet basil was planted in mid of April.

V. Nursery preparation

The nursery was prepared with the addition of cow manure in soil of selected field. The biological fungicides and insecticides also used for prevention of bad effects. Nursery was prepared in the shadow place. Soil was well loosed and strewed seeds which were mixed with silica soil in 50:50 ratios. The seeds were placed in 1 cm in depth of field.

VI. Land preparation and Transplanting

The seedlings of six weeks old which were having a height of 10-15 cm were transplanted in the main field. Before transplanting the field was two times well plowing and applied farmyard manure in soil. After that NPK was also added in 30:40:30 ratios. Transplanting should be done preferably in the evening hours to avoid transplantation shock. So we were transplanted the basil plants in evening time with spacing of 50cm between plants line and 40 cm between plants which is ideal for basil cultivation for well growth of plant. Cloudy weather and fine drizzle are considered ideal for transplanting. Irrigate the field at the time of completed transplanting process.

VII. Crop nutrition

Nitrogen was applied apart from FYM and other biofertilizer at the time of transplanting. Some bio pesticides were also applied for requirements to prevent any disease and infection on basil crop.

VIII. Weeding

There were four times weeding processes completed in the total time period of basil crop. First weeding process was done after 15 days of transplanting.

IX. Irrigation

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When this crop is raised as a summer crop, irrigation is required once in a week. But, with the onset of monsoon, irrigation is not required till September. The crop needs irrigation once or twice a month thereafter. In total, 8-10 irrigations are required during complete crop time. Before harvesting, irrigation should be discontinued.

X. Harvesting

Care should be taken at the time period of harvesting of basil to avoid any type of contamination. Clean all the surface areas that come in to contact with the plants during and after harvesting. The time of harvesting plays a major and important role in qualitative and quantitative oil production. Harvesting is usually done in bright sunny days for good oil yield and quality. It is not desirable to harvest the crop if there is a rain during the previous day. At the time of harvesting the plants were in full bloom and the lower leaves started turning yellowish. The crop was harvested after the time period of 70 days after transplanting. Harvesting was done with the help of sickles. The first harvest was taken when the plants were in full bloom. The herbs were leaved in shadow for 4-5 hours to reduce the moisture and also the bulkiness.

XIV. Processing and isolation of essential oil

Essential Oil of sweet basil was obtained by hydro-distillation from the whole herb. Hydro-distillation was carried out by direct heating. The oil was stored in aluminium containers in a cool and dry place.

XI. Yield

An average yield of fresh herbage of sweet basil was 14-15 kg. The whole herb produced 100-120 ml essential oil of sweet basil.

Assessment of selected oils components

All the different components of lemongrass, citronella and basil essential oils were identified through gas chromatography mass spectrometry (GC-MS). There were 50 and 21 chemical components of citronella and basil essential oils were recorded respectively.

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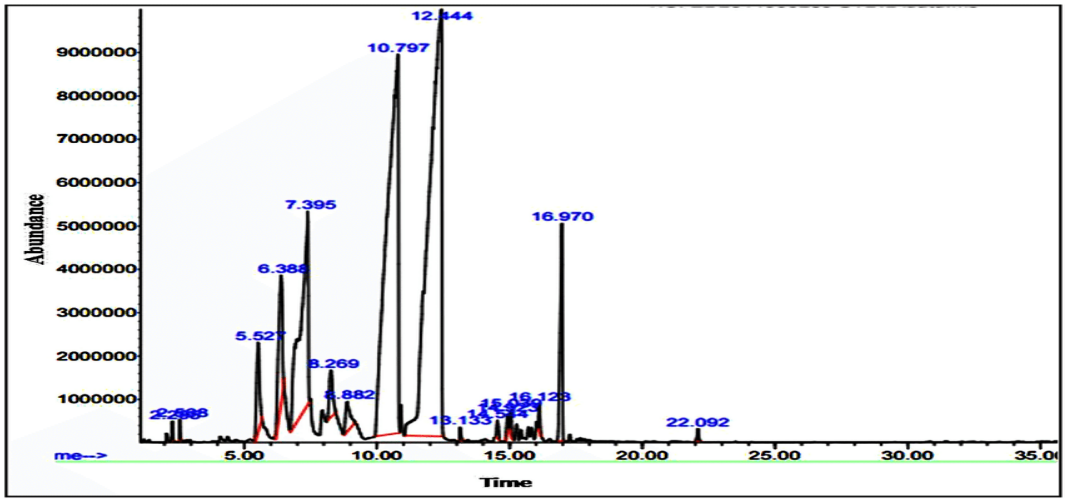

GC-MS Graph of citronella oil

Table 1. Name and percentage of components of citronella essential oil

Sr. No.	Retention Time	Name of the oil components	Percentage of components
1.	5.26	Bergamal	0.06
2.	11.70	Terpineol	1.21
3.	12.63	Linalool	1.62
4.	14.41	Ctronellol isobutanoate	0.11
5.	15.57	Lavandulyl acetate	0.50
6.	16.04	Citronellal	29.15
7.	16.21	Mentha-2,8-dien-1-ol	0.30
8.	16.43	Limonene	0.45
9.	16.78	Decenal	0.16
10.	17.41	Decenal-1-ol	0.06
11.	17.79	Citronellol	7.43
12.	18.19	Neral	6.52
13.	19.20	Geraniol	22.52
14.	19.52	Geranial	5.20
15.	19.78	Neryl acetate	1.86
16.	20.12	Elemene	1.26
17.	20.42	Dodecanal	0.10

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18.	20.55	Caryophyllene	0.12
19.	24.00	Germacrene	1.02
20.	24.22	geranyl acetate	2.63
21.	24.51	Bergamotene	0.11
22.	24.72	Isoeugenol	0.03
23.	25.04	Germacrene	1.09
24.	25.32	Elemol	1.92
25.	25.51	Elemol acetate	1.32
26.	26.02	Limonene	1.27
27.	26.88	Cadinene-γ	0.04
28.	28.04	Methyl linolate	0.02
29.	28.23	Damascene-α	0.52
30.	28.54	Eudesmol -γ	0.30
31.	28.92	Eremoligenol	0.46
32.	29.26	Bisabolene-α	0.01
33.	29.58	Damascene-α	0.02
34.	29.89	Farnesol	0.60
35.	30.09	Methyl isoeugenol	1.30
36.	30.31	Isophorene	1.40
37.	30.64	Myrtanol	0.60
38.	30.89	Linalyl acetate	0.54
39.	31.09	α - pinene	0.16
40.	31.26	Camphene	0.20
41.	31.64	β- pinene	0.21
42.	32.08	Sabinene	0.04
43.	32.32	β- caryophyllene	0.57
44.	32.53	4- Terpineol	1.05
45.	33.20	Cis- ocimene	0.07
46.	33.58	Trans- ocimene	0.70
47.	33.87	p- cymene	0.55
48.	34.56	Terpinolene	1.24

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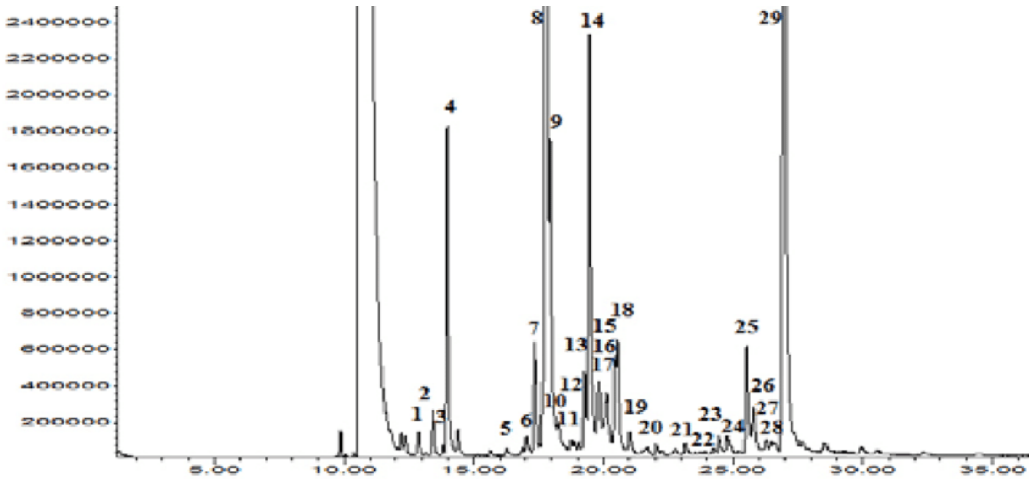
49.	34.95	1- hexanol	0.09
50.	35.09	1- borneol	0.14



GC-MS graph of basil oil

Table 2. Name and percentage of components of basil essential oil

Sr. No.	Retention Time	Name of the oil components	Percentage of components
1.	2.41	1-octen-3-ol	0.07
2.	7.97	6-methyl-5-hepten-2-one	0.19
3.	9.28	1, 8- cineole	0.06
4.	17.92	Linalool	17.61
5.	17.95	Fenchone	0.10
6.	18.31	Sapthulenol	0.61
7.	18.44	terpinen-4-ol	0.47
8.	20.11	eudesmol	0.12
9.	20.56	1, 10-di-epi-cubenol	0.17
10.	21.03	Methyl chavicol	76.36
11.	21.2	trance caryophyllene	0.46
12.	21.49	trans-α- bergamotene	0.62

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13.	21.61	α – humulene	0.10
14.	22.1	germacrene-D	0.15
15.	22.26	Bicyclogermacrene	0.06
16.	22.50	germacrene-A	0.61
17.	23.40	γ cadinene	1.89
18.	25.54	trans-α-bisabolene	0.07
19.	30.05	epi-α-cadinol	0.06
20.	32.71	caryophyllene oxide	0.05
21.	36.40	humulene epoxide II	0.06

Outgrowth of prepared of inoculums

Fungal strain of *Trichophyton tonsurans* 8475 and *Microsporum canis* 3270 was collected from MTCC Chandigarh. *Trichophyton tonsurans* was cultured in Sabourauds agar and *Microsporum canis* was cultured in Emmons modification of Sabourauds agar incubated at 25 C for 7 days (Media and incubation period was prescribed by the MTCC). The outgrowth of both stains was good (Fig. 5.2) in relative media. The inoculums were ready to use for further experimental test.

(A) (B)

Figure: 1. (A) - *Trichophyton tonsurans* and **(B) -** *Microsporum canis.*

Antifungal activity of lemongrass, citronella, basil oils and ketoconazole against *Trichophyton tonsurans* 8475

The antifungal activity of lemongrass, citronella, basil oils and ketoconazole against *Trichophyton tonsurans* 8475 was showed in the zone of inhibition from diameter range of

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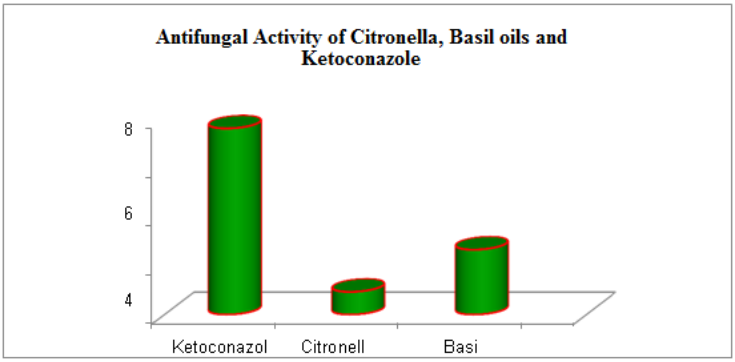
0.93mm to 7.63mm. The values of zone of inhibition displayed in the table 3 and showed graphical form (graph 1) for comparative study.

Table 3. Antifungal activity of essential oils and antifungal drug against *Trichophyton tonsurans* 8475

Sr. No.	Antifungal agents	Zone of inhibition in mm (±SD)
1.	Citronella	0.93 ± 0.15
2.	Basil	2.66 ± 0.20
3.	Ketoconazole	7.63 ± 0.15



Graph 1. Bar graph showing antifungal activity of essential oils and antifungal drug against *Trichophyton tonsurans* 8475

Antifungal activity of Basil and Citronella (BC) Formulations against Trichophyton tonsurans 8475

The antifungal activity of these formulations against *Trichophyton tonsurans* 8475 was identified in the zone of inhibition from the radial diameter range of 1.40mm to 2.53mm. The values of zone of inhibition displayed in the table 4 and graphical form showed in the graph 2.

Table 4. Antifungal activity of Basil and Citronella (BC) formulations against *Trichophyton tonsurans* 8475

Sr. No.	Name of Formulation	Zone of inhibition in mm (±SD)

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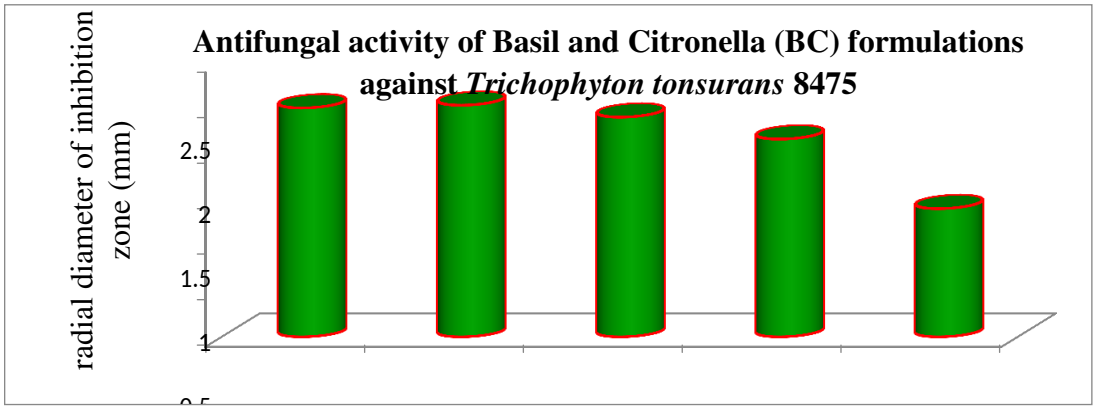
1.	BC1	2.50 ± 0.10
2.	BC2	2.53 ± 0.05
3.	BC3	2.40 ± 0.10
4.	BC4	2.16 ± 0.15
5.	BC5	1.40 ± 0.10



Graph 2. Bar graph showing antifungal activity of basil and citronella formulations against *Trichophyton tonsurans* 8475

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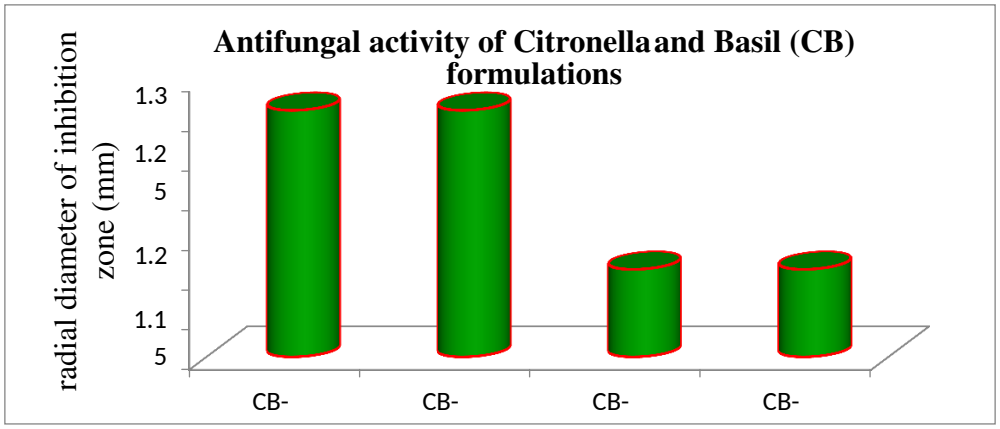
Antifungal activity of Citronella and Basil (CB) Formulations against Trichophyton tonsurans 8475

The antifungal activity of these formulations against *Trichophyton tonsurans* 8475 was identified in the zone of inhibition from the radial diameter range of 1.06mm to 1.26mm. The values of zone of inhibition displayed in the following table 5 and graphical form showed in the graph 3.

Table 5. Antifungal activity of Citronella and Basil (CB) formulations against *Trichophyton tonsurans* 8475

Sr. No.	Name of Formulation	Zone of inhibition in mm (±SD)
1.	CB1	1.26 ± 0.05
2.	CB2	1.26 ± 0.20
3.	CB3	1.06 ± 0.05
4.	CB4	1.06 ± 0.15



Graph 3. Bar graph showing antifungal activity of citronella and basil formulations against *Trichophyton tonsurans* 8475

Antifungal activity of citronella, basil oils and ketoconazole against *Microsporum canis* 3270

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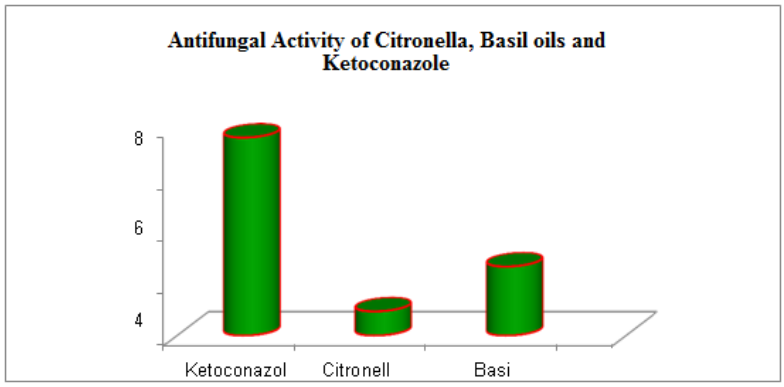
The antifungal activity of lemongrass, citronella, basil oils and ketoconazole against *Microsporum canis* 3270 was showed in the zone of inhibition from radial diameter range of 1.40mm to 7.63mm. The values of zone of inhibition displayed in the table 6 and showed graphical form (graph 4) for comparative study (fig.2).

Table 6. Antifungal activity of Basil and Citronella (BC) formulations against *Microsporum canis* 3270

Sr. No.	Name of Formulation	Zone of inhibition in mm (±SD)
1.	Citronella	1.40 ± 0.36
2.	Basil	2.36 ± 0.05
3.	ketoconazole	7.63 ± 0.15



Graph 4. Bar graph showing antifungal activity of lemongrass, basil and citronella formulations against *Microsporum canis* 3270

Antifungal activity of Basil and Citronella (BC) Formulations against Microsporum canis 3270

The antifungal activity of basil and citronella formulations against *Microsporum canis* 3270 was recorded in the zone of inhibition from the radial diameter range of 1.80mm to 3.10mm. The values of zone of inhibition displayed in the table 7 and showed graphical form in the graph 5.

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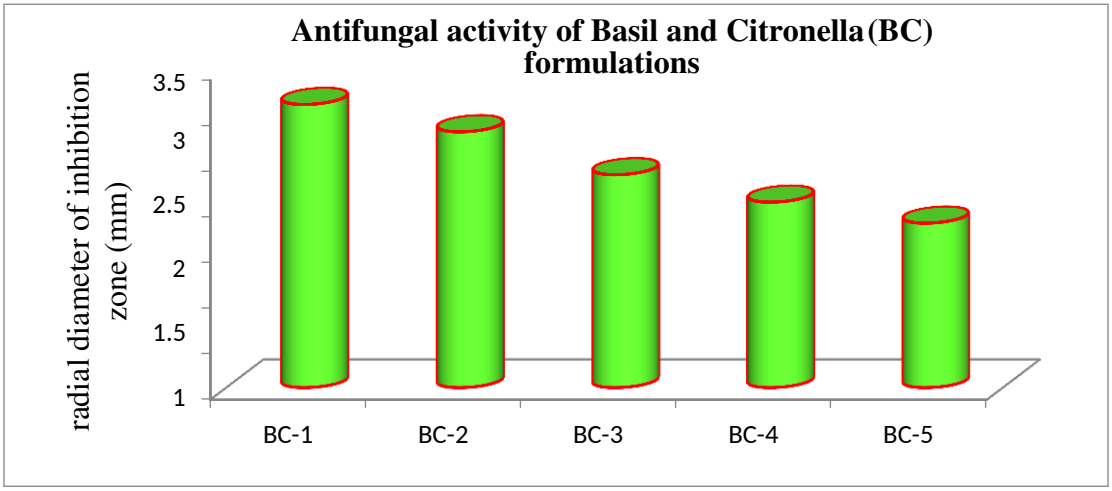
Table 7. Antifungal activity of Basil and Citronella (BC) formulations against *Microsporum canis* 3270

Sr. No.	Name of Formulation	Zone of inhibition in mm (±SD)
1.	BC1	3.10 ± 0.20
2.	BC2	2.80± 0.10
3.	BC3	2.33 ± 0.15
4.	BC4	2.03 ± 0.06
5.	BC5	1.80 ± 0.10



Graph 5. Bar graph showing antifungal activity of basil and citronella formulations against *Microsporum canis* 3270

Antifungal activity of Citronella and Basil (CB) formulations against Microsporum canis 3270

The antifungal activity of these formulations against *Microsporum canis* 3270 was identified in the zone of inhibition from the radial diameter range of 0.73mm to 2.00mm. The values of zone of inhibition displayed in the following table 8 and graphical form showed in the graph 6.

Table 8. Antifungal activity of Citronella and Basil (CB) formulations against

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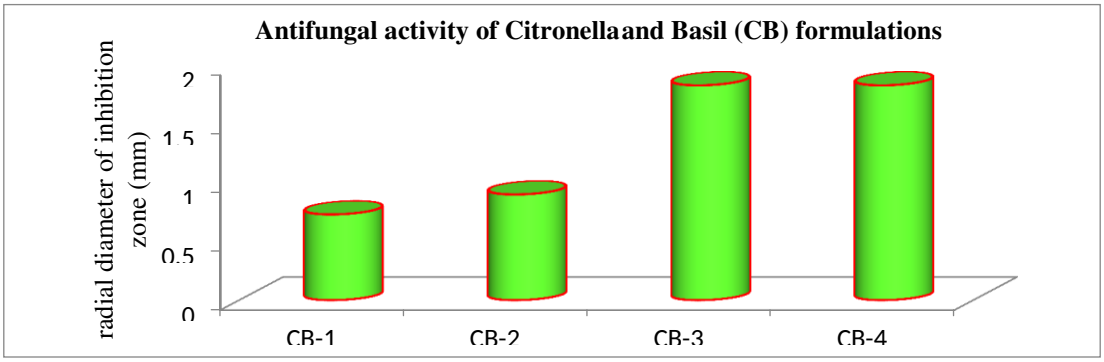
Microsporum canis 3270

Sr. No.	Name of Formulation	Zone of inhibition in mm (±SD)
1.	CB1	0.73 ± 0.05
2.	CB2	0.90 ± 0.10
3.	CB3	1.83 ± 0.11
4.	CB4	2.00± 0.10



Graph 6. Bar graph showing antifungal activity of citronella and basil formulations against *Microsporum canis* 3270

Minimum inhibitory concentration of Basil and Citronella (BC) Formulations against *Trichophyton tonsurans* 8475

The minimum inhibitory concentration of BC formulations against *Trichophyton tonsurans* 8475 was identified from the range of 405µl/ml to 480µl/ml. The values of MIC showed in the table 9 and graphical form showed in the graph 7.

Table 9. Minimum inhibitory concentration of Basil and Citronella (BC) Formulations against *Trichophyton tonsurans* 8475

Sr.No.	Name of Formulation	MIC (µl/ml)
1.	BC1	405
2.	BC2	410
3.	BC3	425

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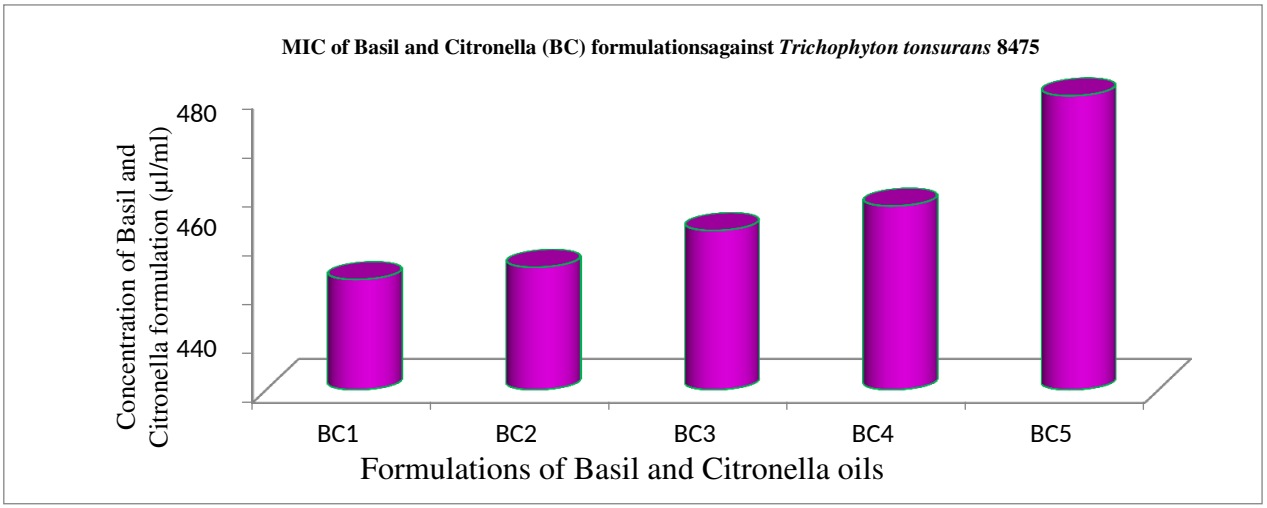
4.	BC4	435
5.	BC5	480



Graph 7. Bar graph showing MIC of Basil and Citronella Formulation against *Trichophyton tonsurans* 8475

Minimum inhibitory concentration of Citronella and Basil (CB) Formulations against *Trichophyton tonsurans* 8475

The minimum inhibitory concentration of these formulations against *Trichophyton tonsurans* 8475 was recorded from the range of 410µl/ml to 475µl/ml. The values of MIC showed in the following table 10 and graphical form showed in the graph 8.

Table 10. Minimum inhibitory concentration of Citronella and Basil (CB) formulations against *Trichophyton tonsurans* 8475

Sr.No.	Name of Formulation	MIC (µl/ml)
1.	CB1	410
2.	CB2	425
3.	CB3	455
4.	CB4	475

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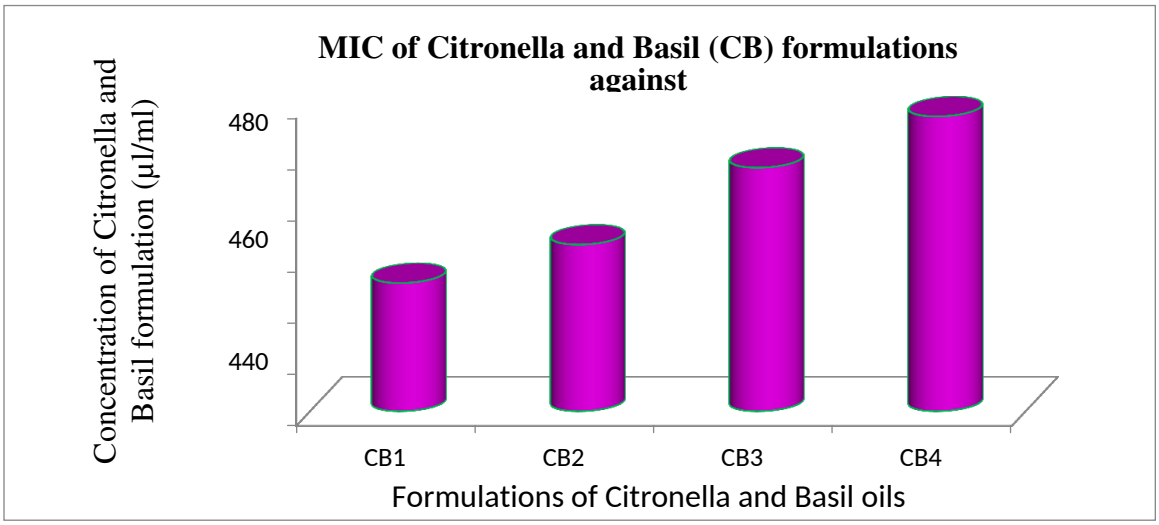

Graph 8. Bar graph showing MIC of Citronella and Basil formulations against *Trichophyton tonsurans* 8475

Minimum inhibitory concentration of Basil and Citronella (BC) Formulations against *Microsporum canis* 3270

The minimum inhibitory concentration of BC formulations against *Microsporum canis* 3270 was recognized from the range 210µl/ml to 318µl/ml. The values of MIC displayed in the following table 11 and graphical form presented in the graph 9.

Table 11. Minimum inhibitory concentration of Basil and Citronella (BC) formulations against *Microsporum canis* 3270

Sr. No.	Name of Formulation	MIC (µl/ml)
1.	BC1	210
2.	BC2	235
3.	BC3	295
4.	BC4	315
5.	BC5	318

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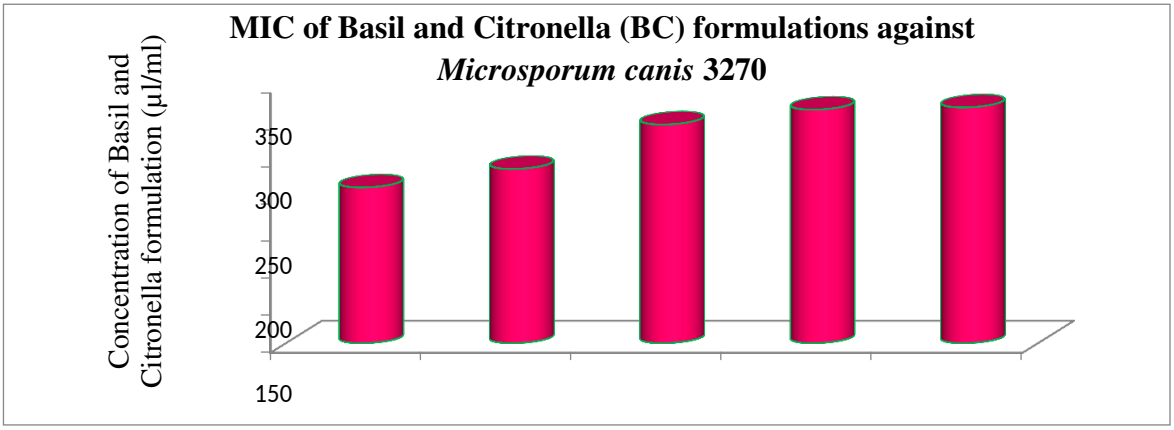

Graph 9. Bar graph showing MIC of Basil and Citronella formulations against *Microsporum canis* 3270

Minimum inhibitory concentration of Citronella and Basil (CB) formulations against *Microsporum canis* 3270

The minimum inhibitory concentration of these formulations against *Microsporum canis* 3270 was recorded from the range 385µl/ml to 550µl/ml. The values of MIC displayed in the following table 12 and graphical form presented in the graph 10.

Table 12. Minimum inhibitory concentration of Citronella and Basil (CB) formulations against *Microsporum canis* 3270

Sr. No.	Name of Formulation	MIC (µl/ml)
1.	CB1	550
2.	CB2	500
3.	CB3	395
4.	CB4	385

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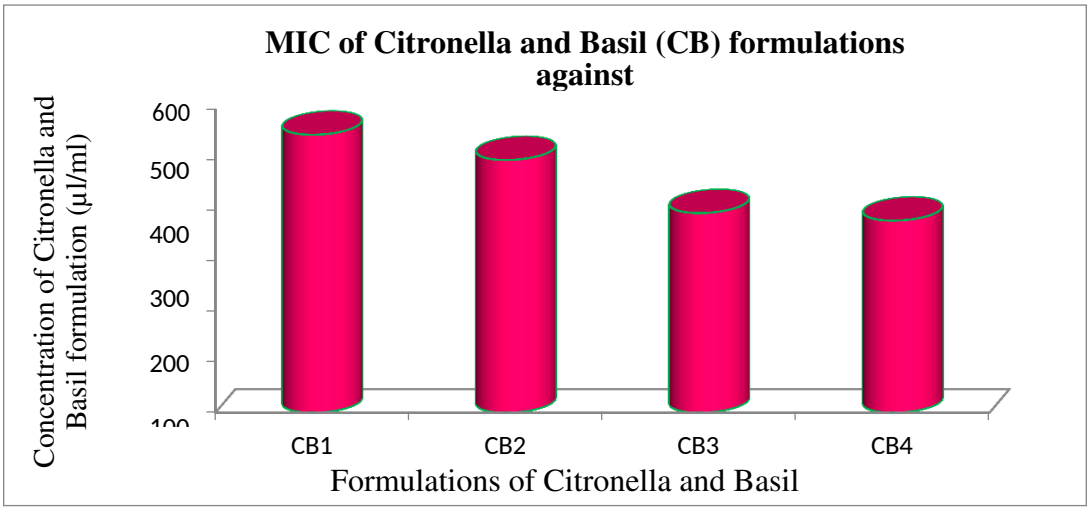

Graph 10. Bar graph showing MIC of Citronella and Basil formulations against *Microsporum canis* 3270

Comparative study of antifungal activities and MICs of all the formulations for dose standardization

According to the present study essential oils and their formulations were capable for inhibited the growth of *Trichophyton tonsurans* 8475 and *Microsporum canis* 3270. The high values of antifungal activities in zone of inhibition and low values of minimum inhibitory concentrations were the best resulting data. That specific resulting values formulation has great efficiency against *Trichophyton tonsurans* 8475 and *Microsporum canis* 3270. Resulting the MICs ranging between 1 µl/ml to 20 µl/ml and zone of inhibition ranging between 7.66 mm to 7.0 mm were sufficient for inhibiting the both of selected fungal growth. The comparative data displayed in the table 13. The selection of those high and low values of essential oil formulations were required for the standardization of doses against selected fungus.

Table 13. MIC values of all the formulations of essential oils.

Formulations	**MIC (µl/ml) against *Trichophyton tonsurans* 8475**	**MIC (µl/ml) against *Microsporum canis* 3270**
Citronella	400	600

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Basil	500	200
Ketoconazole	0.1	0.1
BC-1	405	210
BC-2	410	235
BC-3	425	295
BC-4	435	315
BC-5	480	318
CB-1	410	550
CB-2	425	500
CB-3	455	395
CB-4	475	380

Table 14. Zone of inhibition values of all the formulations of essential oils.

Formulations	**Zone of inhibition in (mm) against *Trichophyton tonsurans* 8475**	**Zone of inhibition in (mm) against *Microsporum canis* 3270**
Citronella	0.93	1.4
Basil	2.66	2.36
Ketoconazole	7.63	7.56
BC-1	2.5	3.1
BC-2	2.53	2.8
BC-3	2.4	2.33
BC-4	2.16	2.03
BC-5	1.4	1.8
CB-1	1.26	0.73
CB-2	1.26	0.9
CB-3	1.06	1.83
CB-4	1.06	1.83

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Conclusion

It is clear that the antifungal effects of essential oils used for skin disorders can be either valuable or unfavorable and requires a thorough further scientific investigation of the phytochemistry, toxicity and other pharmacological activities. It is also suggested that the essential oils are not only screened for antifungal properties against *T. tonsurans* and *M. canis*, but also studies on the isolated compounds be subjected to these pathogens of specific dermatological relevance.

The use of essential oils in the form of blends or combinations of two or more oils considered to be a skill where the oils are carefully selected and combined with the target of overall healing the symptoms of individuals. The objective of blending is to generate a synergistic therapeutic effect where the blends of essential oils are greater than the individual essential oil. Co-ordination can be realized if the compounds in the respective essential oil are able to affect different target sites. After all, the study concluded that the formulations of essential oils, can initiate a synergistic antifungal effect. However, further investigations are needed to determine the synergistic effects of different oils and their compounds, as well as the best possible doses and methods of application in the field.

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