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RESEARCH PAPER

Improvement of essential oil yield and quality of Ajwain's through foliar micronutrient application

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Abstract: Ajwain is an important seed spice crop, yielding an essential oil, which is a secondary metabolite and its accumulation is influenced by multiple factors. Micronutrients play a crucial role and influence the quality of essential oil. To assess the influence of micronutrients impact on essential oil yield, quality and composition an experiment was conducted at College of Horticulture, Bengaluru during *rabi* 2022-23. The experiment was laid out in Randomized complete block design comprising of 15 treatments and their combinations, which were administered as foliar sprays. Quality of the oil was analyzed through GC-MS. Foliar spray of 0.5% $MnSO_4$ at 45 and 60 days after sowing recorded maximum thymol (65.54%); 0.5% $CuSO_4$ at 45 DAS recoded highest essential oil content (2.84%); maximum essential oil yield was recorded in foliar spray of all micronutrients (29.39 kg ha^{-1}). Foliar spray of micronutrients significantly impacted the accumulation of essential oil and thymol, which is the key component of ajwain oil.

Key words: Ajwain, Essential oil, Micronutrients, Thymol

Introduction

Spices plays a very important role in the economy of our country as some of them are exported to earn foreign exchange. Ajwain (*Trachyspermum ammi* L.) belonging to the Apiaceae family is a grassy, annual plant with a white flower and small brownish seeds. Ajwain or Bishop's weed is an annual herbaceous plant, the seeds of which are used for flavouring foods and preservatives (Sathyanarayana *et al.,* 2017). The essential oil from seeds is used in perfumery, essence and medicinal preparations (Nath *et al*., 2008). Ajwain is widely grown in arid and semi-arid regions where soils contain high level of salt. Ajwain is a profusely branched annual herb, 60-90 cm tall, straight stem with inflorescence and compound umbel with umbellets, each containing flowers (Sathyanarayana *et al*., 2017). Ajwain commonly grown as a medicinal plants in Iran, India, Egypt and Europe (Ranjeetha *et al*., 2022). In India it is cultivated in Madhya Pradesh, Uttar Pradesh, Gujarat, Rajasthan, Maharashtra, Bihar and West Bengal. area under the ajwain crop in India is 0.36 lakh ha. With a production of 0.23 lakh MT and productivity of 0.64 MT ha^{-1} (Anonymous., 2022) . The Ajwain fruits yields 2 to 4 per cent brownish essential oil, with thymol as the major constituent (35% to 60%). p-cymene, γ-terpinene, limonene and β-pinene (Zarshenas *et al*., 2014).

Ajwain is popular from ancient times for its use in folk medicines. The seeds contain an essential oil with 60% thymol, which is a strong germicide, antispasmodic and fungicidal properties. Ajwain with its characteristic aromatic smell and pungent taste is widely used as a spice in curries. Its seeds are also used in small quantities for flavouring numerous foods, as preservatives, in medicine and perfumes. Thymol isolated from the oil is a powerful antiseptic and used as an ingredient in a number of skin ointments/powders, deodorants, mouthwashes, toothpastes and gargles (Sathyanarayana and Hadole 2017). Micronutrients tremendously influence the synthesis of essential oils. The availability and amount of these micronutrients affects the biosynthesis of secondary metabolites, thus influencing the essential oil yield, quality and content. Secondary metabolites synthesized by plants are chiefly categorized into: 1) phenol containing compounds like phenyl-propanoids and flavonoids, 2) nitrogen compounds such as alkaloids, cyanogenic glycosides, and glucosinolates and 3) terpenes or isoprenoids (Rietjens *et al*., 2005; Fang *et al*., 2011). Secondary metabolites are produced in some specific tissues and organs of plants, majorly in response of biotic and abiotic stresses. The amount and quality of metabolites formed by plants depends hugely on the intensity and duration of stress and micronutrients are acknowledged to be very crucial in plant secondary metabolite formation during periods of stress. The role of a few micronutrients in secondary metabolite formation has been discussed as follows:

Copper it is used in the form of $CuSO_4$ as an elicitor that can modify the metabolic pathways of plants when added in low concentrations, and can also help in increasing the production of secondary metabolites (Giri and Zaheer, 2016). Manganese plays a critical role in biosynthesis of phenol and lignin (Alejandro *et al*., 2020). It also serves as a cofactor of enzymes involved in isoprenoid biosynthesis (Kollner *et al*., 2008). Boron also plays an important role in secondary metabolism of plants. For instance, it has been observed that the content of essential oil and its active constituents (menthol and menthyl acetate in case of *Mentha arvensis* L. and citral and geraniol in case of *C. flexuous*) were enhanced under the boron treatment at 2.5 mg kg^{-1} (Choudhary *et al*., 2020). Iron plays a major role in secondary metabolite formation of plants. For instance, the highest amount of the major compounds in the essential oil.

Yadegari and Shakerian (2014) observed that Fe was effective in stimulating the accumulation of linalool, nerol, thymol and carvacrol compounds of essential oils of lemon balm (*Melissa officinalis* L.). Zn can considerably influence the expression of the genes involved in synthesis of phenolics (Song *et al*., 2015). Application of Zinc has been shown to directly influence the content of γ-terpinene, p-cymene and β-pinene in essential oil of *Cuminum cyminum* (Rezaeieh *et al*., 2016). Molybdenum had a clear effect on increasing essential oil, oil yield, chlorophylls, carbohydrates and menerals content in some plants. These results were stated by Aly *et al*. (1994) on N, P, K, Zn, B and Mo contents in *Nigella sativa*.

Micronutrients, such as zinc, copper, iron, boron, manganese and molybdenum serve as cofactors or activators for many enzymes involved in metabolic pathways. These enzymes facilitate the conversion of precursor molecules into secondary metabolites (Cakmak and Rengel 2012).

Thymol, is a phenolic compound a major component of ajwain oil, is used to treat gastrointestinal disorders, anorexia and bronchial problems. The oil also contains p-cymene, ã-terpinene, limonene and β-pinene (Zarshenas *et al*., 2014). Besides, p-cymene and γ-terpinene can override the thymol content in some cases (Omer *et al*., 2014; and Moein *et al*., 2015). Balanced nutrition for plants is very important, as generally, farmers used to apply huge amounts of macro elements or fertilizers, with minor attention to the ratio and roles of micronutrients (Aghayie *et al*., 2019). Though the amount of micronutrients required is quite small, still, they play a prominent role in the metabolic and cellular functions performed by plants. Besides, the deficiency, availability and amount of micronutrients affects biosynthesis and concentration of secondary metabolites, thus influencing the essential oil yield, quality and content. Therefore critical aspects need to be considered for micronutrient management. Many crops respond to foliar spray of micronutrients in terms of growth, yield and quality specially involved in synthesis of several secondary metabolites. It is widely reported that foliar application of micronutrients at active growth stages will improve quality in various crops (Kalidasu, 2008).

Understanding the influence of micronutrients on seed quality is essential. Micronutrients have been known to significantly influence the yield and concentration of marker compounds in essential oils produced by ajwain but, for a more comprehensive understanding, the exact mechanism of modifications occurring in plant metabolism is required to be elucidated. Moreover, some micronutrients (especially Fe and Zn) have been well studied for their role in production of secondary metabolites in ajwain but few micronutrients such as Cu, Mn, B and Mo still need to be explored for their mechanism of secondary metabolite production. The methods and timing of micronutrient application is also a subject area that needs to be explored; however, research on this aspect, particularly in ajwain crops in India, is limited. Therefore, this study aims to investigate the effects of micronutrients on the essential oil yield, content and quality of ajwain oil.

Material and methods

A field experiment was conducted at the Department of Plantation, Spices, Medicinal and Aromatic crops, College of Horticulture, UHS campus, GKVK, Bengaluru, Karnataka, India, during *rabi* season of November 2022 to March 2023. Experimental site is located at an elevation of 930 meters above MSL with 12°582 North latitude and 77°352 East longitude which comes under Eastern Dry Zone of Karnataka.

Ajwain seeds (AA-93) were collected from the National research centre on seed spices (NRCSS) Ajmer. The experiment was laid out in Randomized complete block design comprising of 15 treatments (T_1- 100:50:50 kg NPK ha^{-1} (control), T_2- T_1 + $ZnSO_4$ 0.5% at 45 DAS, T_3- T_1 + $ZnSO_4$ 0.5% at 45 and 60 DAS, T_4- T_1 + $FeSO_4$ 0.5% at 45 DAS, T_5- T_1 + $FeSO_4$ 0.5% at 45 and 60 DAS, T_6- T_1 + $CuSO_4$ 0.5% at 45 DAS , T_7- T_1 + $CuSO_4$ 0.5% at 45 and 60 DAS, T_8- T_1 + $MnSO_4$ 0.5% at 45 DAS, T_9- T_1 + $MnSO_4$ 0.5% at 45 and 60 DAS, T_{10}- T_1 + Borax 0.2% at 45 DAS, T_{11}- T_1 + Borax 0.2% at 45 and 60 DAS, T_{12}- T_1 + Ammonium molybdate 0.1% at 45 DAS, T_{13}- T_1 + Ammonium molybdate 0.1% at 45 and 60 DAS, T_{14}- T_1 + 0.5% $ZnSO_4$, $FeSO_4$, $CuSO_4$, $MnSO_4$ + 0.2% Borax + 0.1% Ammonium molybdate at 45 DAS, T_{15}- T_1 + 0.5% $ZnSO_4$, $FeSO_4$, $CuSO_4$, $MnSO_4$ + 0.2% Borax + 0.1% Ammonium molybdate at 45 and 60 DAS) which were replicated twice. The seeds were pre-treated with *Azospirillum* and *Trichoderma* @ 10gm kg^{-1} seed and sown directly in the field at 50 cm x 20 cm spacing by placing four seeds per hill with a shallow depth of 1-1.5 cm. The seeds required 10 days to begin germination and 15 days to completely germinate. To maintain the ideal plant population, gap filling was done at 15 DAS where the seeds failed to germinate. The crop was harvested when it was fully mature, when the majority of the umbels had turned brown (5 months). Plants were cut close to ground level, then they were dried under the sun to achieve 8-10% moisture, seeds were threshed, winnowed and stored.

Extraction of essential oil

One hundred gram of dried seeds were placed in a 2 litre round bottom boiling flask, along with 1000 ml of water. After that, the flask was fitted to the essential oil extraction apparatus (Clevenger's Apparatus). The distillation was carried out on a heated mantle that was thermostatically regulated. The time after which there was no rise in the volume of oil was regarded as the standard time for finishing a batch of samples or the end point of distillation. Taking this into account, the samples were distilled for 3 hours at 80^0 C. The distillate was allowed to cool to ambient temperature and settle until the layer was clear. The excess moisture was removed by centrifuging and stored in glass vials away from direct sunlight. The oil content was estimated after measuring the weight as follows.

$$\text{Essential oil (\% w/w)} = \frac{\text{weight of oil (g)}}{\text{Weight of the sample (g)}} \times 100$$

GC-MS of ajwain essential oil

The GC-MS analysis of ajwain essential oil was performed using Shimadzu QP2020 series gas chromatograph equipped

Table 1. Essential oil constituents of ajwain as influenced by foliar application of micronutrients

Treatments	Essential oil % (W/W)	Essential oil yield kg/ha	Thymol (%)	p-cymene (%)	γ-terpinene (%)	Limonene (%)	β-pinene (%)
Treatment 1	2.03	11.98^{f}	60.45^{abc}	12.88	13.81	4.11^{ab}	2.04^{abcd}
Treatment 2	2.51	24.32^{abcd}	55.40^{d}	14.82	15.15	4.25^{ab}	2.29^{ab}
Treatment 3	2.06	15.82^{ef}	52.30^{d}	14.81	19.73	3.12^{bcd}	2.61^{a}
Treatment 4	2.33	18.30^{de}	50.54^{d}	16.36	17.56	5.01^{a}	1.36^{de}
Treatment 5	2.69	16.61^{ef}	56.72^{bcd}	13.19	18.19	2.73^{bcde}	1.55^{cde}
Treatment 6	2.80	19.75^{bcde}	53.34^{cd}	14.70	17.67	5.02^{a}	1.75^{bcde}
Treatment 7	2.84	14.28^{ef}	56.65^{bcd}	13.29	19.59	2.76^{bcde}	1.58^{cde}
Treatment 8	2.79	20.16^{bcde}	56.42^{bcd}	13.05	18.40	3.05^{bcde}	2.00^{abcd}
Treatment 9	2.50	24.85^{ab}	65.54^{a}	11.57	14.56	1.54^{ef}	1.18^{e}
Treatment 10	2.67	20.04^{bcde}	53.25^{cd}	12.51	14.38	2.05^{def}	1.10^{e}
Treatment 11	2.53	24.68^{abc}	62.16 ^{ab}	14.68	19.71	0.83^{f}	2.10^{abc}
Treatment 12	2.46	18.05^{ef}	54.17^{cd}	11.63	15.25	4.65^{a}	1.11^{e}
Treatment 13	2.75	18.59^{cde}	57.49^{bcd}	14.19	16.34	3.82^{abc}	2.66^{a}
Treatment 14	2.50	29.39^{a}	51.30^{d}	15.22	17.80	2.58^{cde}	1.62^{bcde}
Treatment 15	2.35	29.17^{a}	52.71^{d}	15.29	18.77	1.57^{ef}	1.70^{bcde}
S. Em ±	0.17	2.04	2.45	1.35	1.80	0.50	0.22
C.D @ 5%	NS	6.18	7.42	NS	NS	1.52	0.68

T1: 100:50:50 kg NPK ha^{-1}
T2: T_1 + 0.5% $ZnSO_4$ foliar spray at 45 DAS
T3: T_1 + 0.5% $ZnSO_4$ foliar spray at 45 and 60 DAS
T4: T_1 + 0.5% $FeSO_4$ foliar spray at 45 DAS
T5: T_1 + 0.5% $FeSO_4$ foliar spray at 45 and 60 DAS
T6: T_1 + 0.5% $CuSO_4$ foliar spray at 45 DAS
T7: T_1 + 0.5% $CuSO_4$ foliar spray at 45 and 60 DAS
T8: T_1 + 0.5% $MnSO_4$ foliar spray at 45 DAS
T9: T_1 + 0.5% $MnSO_4$ foliar spray at 45 and 60 DAS
T10: T_1 + 0.2% Borax foliar spray at 45 DAS
T11: T_1 + 0.2% Borax foliar spray at 45 and 60 DAS
T12: T_1 + 0.1% Ammonium molybdate foliar spray at 45 DAS
T13: T_1 + 0.1% Ammonium molybdate foliar spray at 45 and 60 DAS
T14: T_1+ 0.5% $ZnSO_4$, $FeSO_4$, $CuSO_4$, $MnSO_4$+ 0.2% Borax + 0.1% Ammonium molybdate foliar spray at 45 DAS
T15: T_1 + 0.5% $ZnSO_4$, $FeSO_4$, $CuSO_4$, $MnSO_4$ + 0.2% Borax + 0.1% Ammonium molybdate foliar spray at 45 and 60 DAS
Note: Mean with the same letter in each column are not significant different, as indicated by the DMRT test (P< 0.05).

with an SH-Rxi-5Sil column measuring 30 m × 0.25 mm × 0.25 ìm Dia. Components of the oil were detected using Flame Ionization Detector (FID); helium gas was used as a carrier gas at a constant flow rate of 1.20 mm min^{-1} under consistent pressure, and the injection volume was set at 1.0 ìL and a split ratio of 1:50 was employed. The temperature program for the oven was as follows: initially, it was held at 50 ^{0}C for 2 minutes, then ramped up to 220 ^{0}C at a rate of 10 ^{0}C per minute, and finally maintained at 310 ^{0}C for 5 minutes with a ramp rate of 15 ^{0}C per minute. All samples in replicates, were subjected to this analysis and the results were documented. The mass spectrum of the sample was identified by comparing it with a mass spectral library using computer analysis.

Results and discussion

Essential oil content

Foliar application of micronutrients though did not bring about statistically significant differences with respect to essential oil content, the difference between the best treatment *i.e.*, 0.5% $CuSo_4$ at 45 and 60 DAS, which yielded an essential oil content of 2.84% and the control plants without any micronutrient sprays recorded the lowest essential oil content of 2.04% was noteworthy.

Essential oil yield

Micronutrient applications brought out profound impact on the essential oil yield of ajwain as outlined in the Table 1. Among Foliar spray of all micronutrients at 45 DAS, The treatment (T_{14}) recorded the highest essential oil yield of 29.39 kg ha^{-1} which was *on par* with foliar application of all micronutrients twice at 45 and 60 DAS T_{15} (29.17 kg ha^{-1}) and foliar application of $MnSO_4$ at 45 and 60 DAS T_9 (24.85 kg ha^{-1}); while the lowest essential oil yield was recorded in T_1 and T_7 (11.98 kg ha^{-1} and 14.28 kg ha^{-1} respectively). There was an increase in essential oil yield by the effect of micronutrients which may be due to increase in seed yield and also their effect on enzymes activity, metabolism improvement and also due to increase in dry matter yield in treatment which consist of all micronutrients. This results on the effects of micronutrients on coriander plants agreed with the results obtained by Khalid (2015) and Mehrab (2014) reported that trace elements such as Fe, Zn and Mn increased the vegetative growth characters and essential oil yield of different plants such as anise, coriander, sweet fennel and lemon balm.

Thymol content (%)

There was an increase in thymol content in different treatments. Highest thymol content was found in foliar

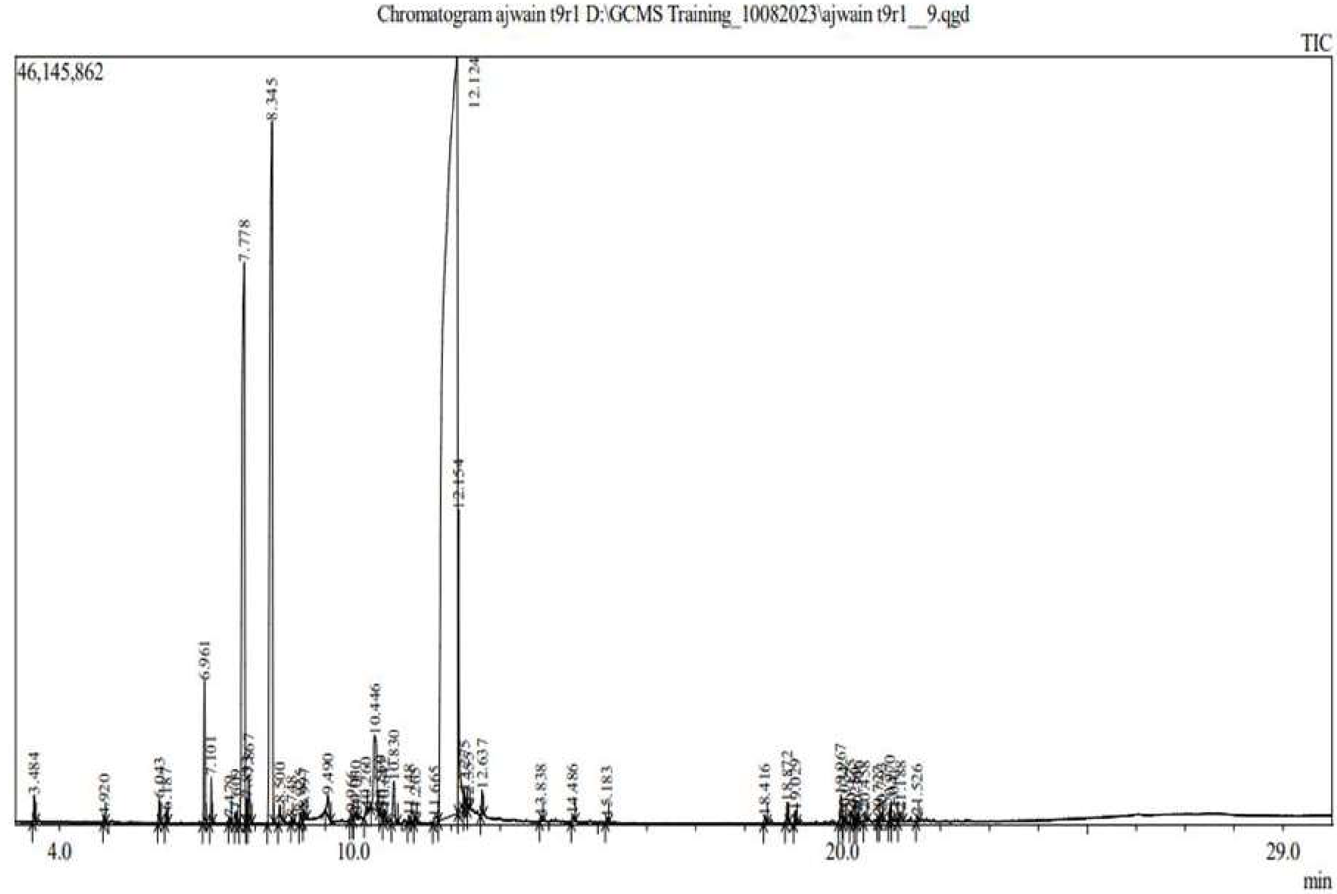

Fig.1. Influence of Foliar application of $MnSO_4$ at 45 and 60 DAS on Essential oil

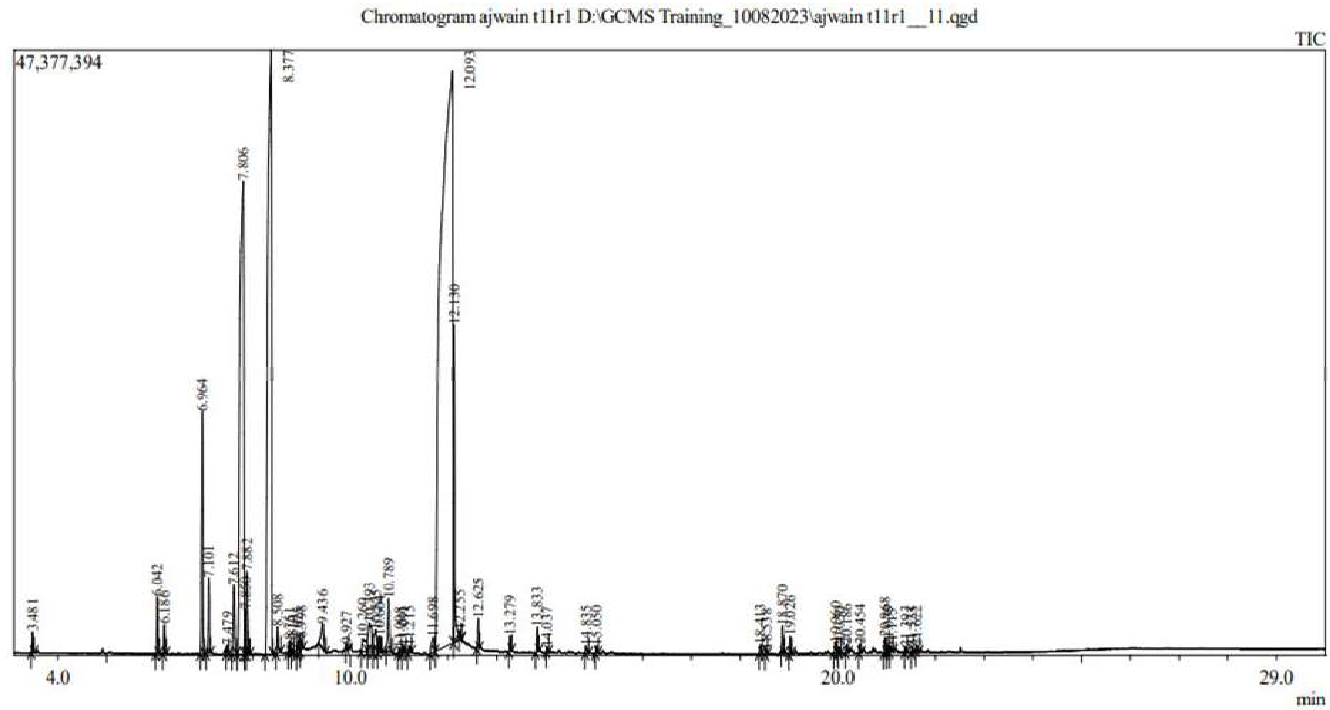

Fig.2. Influence of Foliar application of borax at 45 and 60 DAS on Essential oil

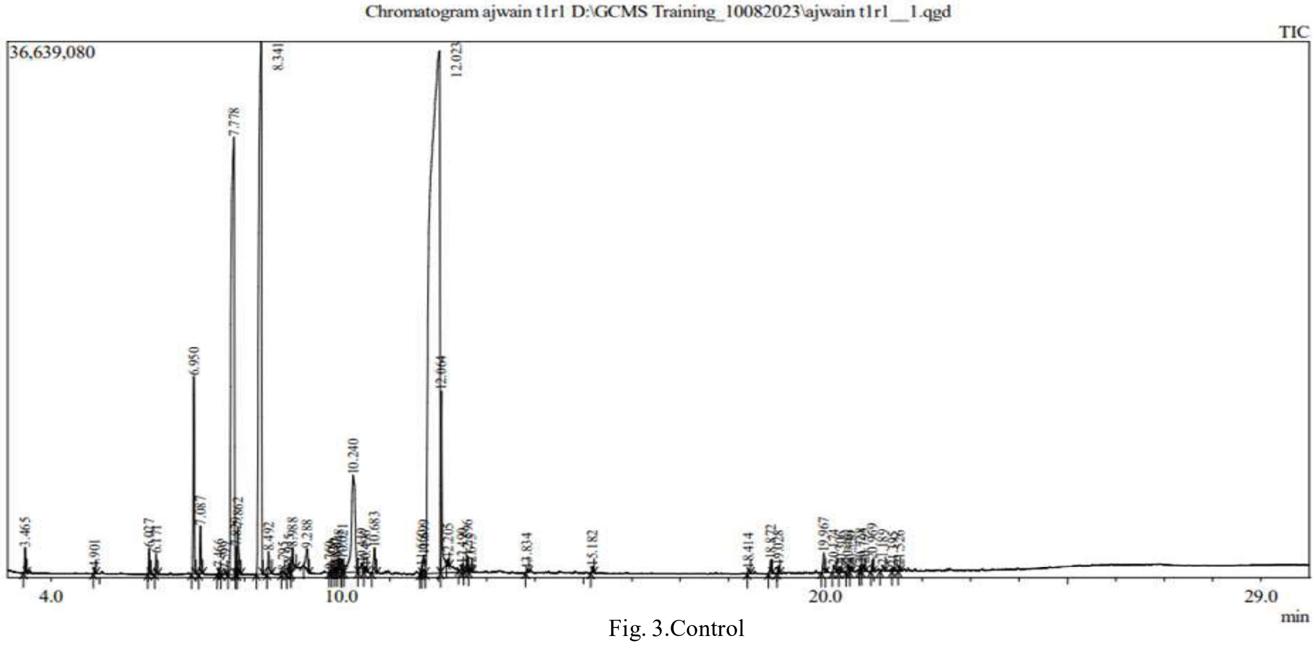

Fig. 3.Control

application of $MnSO_4$ 0.5% at 45 and 60 DAS (T_9) (65.54%) shown in Table 1, Fig 1 and 3 which was *on par* with foliar application of borax 0.2% at 45 and 60 DAS (T_{12}) (62.16%) fallowed by control (T_1). Where as least thymol content was recorded in foliar application of $FeSO_4$ 0.5% at 45 DAS (T_4) (50.54%). This is because thymol belongs to the class of phenolic compounds, where manganese and boron plays a role in the synthesis of phenolic compounds, and its availability may influence the production of thymol in ajwain. Manganese and boron affects the biosynthesis of the essential phenolic compounds which are a large group of secondary plant metabolites. Similar effects were reported by Mohamed *et al.,* (2021) in ajwain with the application of boron at 200 mg l^{-1} gave the maximum values of á-thujene as (50.24%) and application of the $MnSO_4$ at 50 ppm was effective in enhancing thymol and terpinene (El-Wahab *et al.,* 2008).

p-cymene (%)

There is no significant variation registered in cymene content among different treatments (Table 1). Cymene content in different treatments varied from 11.57 to 16.36 per cent. The treatment T_4 exhibited maximum cymene content (16.36%); whereas it's content was lowest in T_9 (11.57%) .

γ-terpinene (%)

No significant difference was registered in terpenene content among different treatments (Table 1). It varied from 13.81 to 19.73%. The treatment T_3 exhibited maximum terpenene content (19.73%); while terpenene content was lowest in T_1 (13.81%).

Limonene content (%)

Limonene content was significantly more in foliar spray of $CuSO_4$ at 45 DAS (T_9) (Table 1, Fig 3) which was *on par* with T_4, T_1, T_{12}, T_{13} and T_2 least was recorded in foliar spray of $MnSO_4$ at 45 and 60 DAS (T_7) (1.54%). This is because the synthesis of limonene often involves the isomerization of a precursor compound called terpinene. Copper catalysts can facilitate this isomerization reaction, helping to convert terpinene into limonene more efficiently. similar results were recorded by (Lizarazo *et al.,* 2021) in caraway where, highest limonene was achieved by the application of Cu in first season with an average of 28.7 mg g^{-1} .

β-pinene content (%)

More β-pinene was observed in foliar application of ammonium molybdate at 45 and 60 DAS (T_{13}) (2.66%) which was *on par* with T_3, T_1, T_2, T_8 and $T_{11,}$ while least was observed in foliar spray of 0. 2% borax at 45 DAS (T_{10}) (1.10%) (Table 1, Fig 4). It is because these micronutrients helps in isomerization of other terpenoids, such as myrcene or carene. Ammonium molybdate, also acts as catalysts in isomerization reactions. They may facilitate the rearrangement of double bonds or other structural changes in the precursor molecules to yield the desired pinene isomers. Similar results were obtained by Hasan *et al.,* (2011) in Clary Sage Seed

Conclusion

From the comprehensive analysis in the study it is conclude that there was significant impacts on essential oil yield, content and quality of ajwain based on various micronutrient applications. Notably, foliar spray of $CuSO_4$ at 0.5% at 45 and 60 DAS and, foliar spray of all micronutrients at once recorded high essential oil yield, foliar spray of $MnSO_4$ at 0.5%, and borax spray at 0.5% twice at 45 and 60 DAS increased thymol content, $CuSO_4$ at 45 DAS increased limonene content and foliar spray of ammonium molybdate at 45 and 60 DAS increased β-pinene content.

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