GC-MS analysis of the bioactive compounds of *Acmella calva* (DC) R. K. Jansen influenced by AMF

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ABSTRACT
Medicinal plants are widely recognized as a storage pool of numerous effective drugs, which have pharmaceutical efficacy in diverse diseases based on the presence and abundance of secondary metabolites. *Acmella calva* (DC) R. K. Jansen is a medicinal herb traditionally used as a toothache plant to treat stomatitis, mouth ulcers, and different gum-related problems. The present study aimed to investigate the effect of three distinct species of Arbuscular mycorrhizal fungi (AMF) individually (*Funneliformis mosseae*, *Glomus macrocarpum, and Gigaspora margarita*) and in combination (*F. mosseae* + *Gi. margarita*) on the phytochemical composition in *A. calva.* The findings showed an increased concentration of terpenoids, alkaloids, hydrocarbons and lipids in ethanolic extracts of *A. calva* inoculated with AMF. The GC- MS screening of ethanolic extract of *A. calva* revealed the presence of terpenoids, alkanes, hydrocarbons and lipids as major active compounds with the varying % area found in combined AMF (*F. mosseae* + *Gi. margarita*) treatment, it evidenced by their highest % peak area. Furthermore, the plants inoculated with *F. mosseae* showed nine different bioactive compounds viz. sesquiterpenoids, hydrocarbon, terpenoid, alkane, ester and terpenoids groups. Among them, Phytol reported with highest peak area (40.69%), followed by 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (4.55%), and Nonacosane (4.36%). *A. calva* inoculated with *G. macrocarpum* showed the highest % peak area of Phytol (21.62%), followed by Neophytadiene (12.93%), and 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (7.80%). Inoculation of *Gi. margarita* showed five different compounds with the highest % peak area of Stigmasterol (45.34%), followed by Neophytadiene (22.77%), and Phytol (21.62 %) in comparison with control. From GC-MS analysis of *A. calva*, it was concluded that Mix AMF (*F. mosseae* + *Gi. margarita*) inoculation enhanced the production of secondary metabolites in *A. calva* which may be due to improved mineral nutrition and transfer in the AMF host interphase. The present study exhibited the inoculation of AMF leads to an increase in medicinal potential of *A. calva.*

Introduction

Acmella calva (DC) R. K. Jansen is well known perennial herb that is native to India, with reputed medicinal values (Rafi et al. 2022; Nabi et al. 2016). The plant is commonly used as a traditional remedy for diverse range of aliments including analgesic, hypoglycaemic, antidepressant, antidiarrheal, tooth and gum aches (Rafi et al. 2022; Panday et al. 2009). The therapeutic properties of *A. calva* are attributed to the phytochemical compounds which have physiological effects on human body. Previous investigations on *A. calva* have found a variety of bioactive compounds including carbohydrates, Alkaloids, Phenolic compounds, Flavonoids, Glycoside, Tannins, Steroids, Proteins, amino acids, Gum, and Acidic compounds (Ramproshad et al. 2019; Dhivya and kalaichelvi 2017). Recently, Patole and Khilare (2024) reported several bioactive compounds from *A. calva* such as Caryophyllene, Neophytadiene, and N-Isobutyl-(2E,4Z,8Z,10E)-dodecatetraenamide which revealed their good medicinal capacity. Therefore, enhancing the accumulation of these phytochemicals in *A. calva* may improve their quality for the pharmaceutical sector and boost their traditional and medicinal applications.

The interaction of AMF and host plant enhances plant growth and modifies the level of plant secondary metabolites (Zubek et al. 2015; Dhalaria et al. 2020). Hence the application of AMF to *A. calva* species had been proposed. In present study, it was hypothesized that the application of AMF may enhances the synthesis of phytochemicals in *A. calva*. However, in this contest, no investigation has been done on effect of AMF on phytochemical potential of *A. calva*. Therefore, objective of the present investigation was to evaluate the effect of AMF inoculation on secondary metabolites by GC-MS in *A. calva*. Moreover, phytochemical analysis using GC-MS was done in both AMF inoculated and non inoculated plants of *A. calva*.

Materials and methods

1. Pant collection and soil samples

Healthy plants of *A. calva* (Asteraceae) were collected from the Kolhapur region (MS.) India. The plant is authenticated and voucher specimen (SP001) was deposited at Department of Botany, Balwant College, Vita. The rhizospheric and non-rhizospheric soil of plant up to the depth of 15 cm were collected in Ziplock polythene bags and brought immediately to the lab and used for isolation of AMF spores.

2. Isolation of AMF spores and Inoculum development

The wet sieving and decanting technique (Gerdemann and Nicolson, 1963) was used for AMF spore isolation. Isolated, healthy, and identified AMF spores (*Funneliformis mosseae, Glomus macrocarpum,* and *Gigaspora margarita*) were taken for their mass multiplication and production of infective inoculum. A starter inoculum was developed by the soil funnel culture technique before mass multiplication. Firstly, isolated AMF spores were surface sterilized using 200 ppm streptomycin sulphate for 3-5 min then washed with sterilized distilled water and again sterilized with 2 % chloramines T solution for 3-5 min. Sterilized soil and sand in the proportion of 1:1 was used for preparation of starter inoculum. The surface sterilized spores and maize seeds were placed in funnel assembly. Root infection was checked after 14 days and entire assembly was continued for 45 days. Then starter inoculum was used for mass multiplication using *Pennisetum purpureum* as host. After completion of set the root samples were checked for infectivity. This mixture of sand, soil, and infected root samples were served as native inoculum for the pot culture experiment.

3. AMF inoculation and experimental set-up

In order to explore impact of AMF a pot culture experiments were performed in greenhouse conditions at the Botanical Garden of Balwant College, Vita (MS.) India. The experiment was set up as T-1: Control, T-2: *Funneliformis mosseae,* T-3: *Glomus macrocarpum,* T-4: *Gigaspora margarita,* T-5: Mix AMF (*F. mosseae* + *Gi. margariata*). The 35 cm diameter Polyethylene pots were filled 2 cm less than the regular filling for the AMF treatments except for the control. As treatments mentioned above, AMF inoculum of 200 gm per each pot were spread on the pots, then a layer of 2 cm soil was added to obtain regular filling, then surface sterilized seeds of *A. calva* were added in each pot. The experiments were performed in triplicate.

4. Extraction

By the completion of the experiment after 90 days plants are harvested. The harvested plants of *A. calva* were shade dried and powdered using a mechanical grinder. The grounded powder was used for phytochemical extraction. 10 gm of powdered samples were taken for extraction using ethanol (100ml) as a solvent in Soxhlet assembly to obtain 10% w/v concentration until complete extraction and the extract was used for GC-MS analysis.

5. GC-MS analysis

Phytochemical screening of *A. calva* using Chromatography coupled to Mass Spectrometry (GC-MS) was done according to Cruz et al. (2020). The Soxhleted ethanolic extracts of the test plant were used for the study of bio-constituents using GC-MS (TQ8050). The method adopted for GC–MS analysis was conducted as the instrument built with a capillary column of 30 m length, 0.25 mm diameter, 0.25 µm film thickness, column oven temperature 50 ^{0}C, and injection temperature 250.0 ^{0}C, column flow rate 1.01 mL/min. The chemical constituents were identified by matching the mass spectra and retention periods with the spectra from NBS/NIST and Wiley Libraries. The improved concentration of bioactive molecules after the reproductive stage of the plants had been noted, hence, the GC-MS profiling of *A. calva* investigation was carried out after 90 days of growth period.

Result and discussion

Effect of AMF inoculum treatments on Quantitative phytochemicals by GC-MS analysis

A significant effect of AMF on *A. calva* for its phytoconstituents was detected in the ethanolic extract (Table no. 1 and Fig. no. 1 to 5). Phytol a terpenoid in *A. calva* varied depending on integrated AMF inoculation. The ethanolic extract of *A. calva* inoculated with *F. mosseae* + *Gi. margarita* showed considerably more terpenoids (Caryophyllene oxide; Methane, tricyclohexyl-; Neophytadiene; Phytol) in caparison with control. However, when *A. calva* were inoculated with *Gi. margarita* alone, no significant difference was seen. *F. mosseae* inoculated plants estimated Terpenoids and sesquiterpenoid followed by *G. macrocarpum* treated plants with

terpenoids (Neophytadiene and Phytol) sesquiterpenoid (3,7,11,15-Tetramethyl-2-hexadecen-1-ol), Glycoside (Card-20(22)-enolide, 3,5,14,19-tetrahydroxy-, (3.beta.,5.beta.)-), and hydrocarbon (Triacontane, 1-iodo-) and *Gi. margarita* exhibited with sesquiterpenoid (1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-,[1ar-(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha.)]-; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol), Terpenoid (Neophytadiene; Phytol), and lipid (Stigmasterol), while minimum phytochemical constituents were observed in control with terpenoids (Cyclohexane, 1-(cyclohexylmethyl)-2-methyl-, cis-; Neophytadiene; Phytol) and sesquiterpenoid (3,7,11,15-Tetramethyl-2-hexadecen-1-ol).

Table no. 1: Effect of AMF inoculum treatments on Quantitative phytochemicals by GC-MS analysis of *A. calva*:

Sr. No	Name of the compound	Area %				
		T-1	**T-2**	**T-3**	**T-4**	**T-5**
1	1-Butanamine, N-methyl-N-2-propenyl-	-	-	-	-	1.65
2	1H-Cycloprop[e]azulen-7-ol,decahydro-1,1,7-trimethyl-4-methylene-,[1ar-(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha.)]-	-	3.82	-	6.78	-
3	2,2-Dicyclohexylbutane	-	1.25	-	-	-
4	2-Decanone	-	-	-	-	5.71
5	2-Methyltetracosane	-	2.61	-	-	-
6	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	2.42	4.55	7.80	3.49	14.98
7	Bis(2-ethylhexyl) phthalate	-	-	-	-	4.42
8	Card-20(22)-enolide,3,5,14,19-tetrahydroxy-, (3.beta.,5.beta.)-	-	-	6.77	-	-
9	Caryophyllene oxide	-	-	-	-	9.33
10	Cyclohexane, 1-(cyclohexylmethyl)-2-methyl-, cis-	19.20	-	-	-	-
11	Hexadecanoic acid, ethyl ester	-	-	-	-	5.60
12	Hexanoic acid, anhydride	-	-	-	-	0.44
13	Methane, tricyclohexyl-	-	-	-	-	2.84
14	Neophytadiene	3.01	3.55	12.93	22.77	9.04
15	Nonacosane	-	4.36	-	-	-
16	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	-	-	3.51	-	-
17	Octadecanoic acid, 17-methyl-, methyl ester	-	-	-	-	5.30
18	Oxalic acid, 3,5-difluorophenyl tetradecyl ester	-	2.95	-	-	-
19	Phytol	18.05	40.69	21.62	21.62	74.94
20	Stigmasterol	-	-	-	45.34	-
21	Tetratetracontane	-	1.98	-	-	-
22	Triacontane, 1-iodo-	-	-	9.35	-	-

AMF inoculations with combined AMF treatment showed the greatest terpenoid concentrations in ethanolic extract which was greater than inoculations with other treatments. The lowest phytochemicals were detected in non inoculated plants.

Overall, the observations revealed that the ethanolic extract of *A. calva* inoculated with combined AMF treatment showed substantial increase in all the phytochemicals. The phytochemical analysis of secondary metabolites in *A. calva* confirmed the existence of Terpenoids, alkane, alkaloid, hydrocarbons, lipids, sesquiterpenoids, glycosides, volatile organic compounds, fatty acids, esters, and one non phytochemical compound in ethanolic extract of *A. calva* inoculated with strong presence observed in mixed treatment with 11 different types of phytochemicals. These results are in agreement with Yamuna et al. (2017); Melato et al. (2024). According to them secondary metabolites possesses numerous therapeutic and biological properties in *Gomphrena globosa.* They also reported *G. globosa* and *Ruta graveolens* were anticipated to have a wide range of potential medicinal applications. The quantitative study revealed that the number of phytochemical compounds were improved in *A. calva* when inoculated with AMF particularly mixed form of AMF. The results are corroborated with Dhalaria et al. (2024) in *G. globosa*; Lone et al. (2015) in *Allium cepa*, Melato et al. (2024) in *R. graveolens*. The combined AMF treatment exhibited the most elevated levels of secondary metabolites as compared to isolated AMF treatment. This may be due to competitive interaction between two AMF species.

Fig. no 1: GC-MS chromatographic profile of ethanolic extract of non inoculated *A. calva* (T-1)

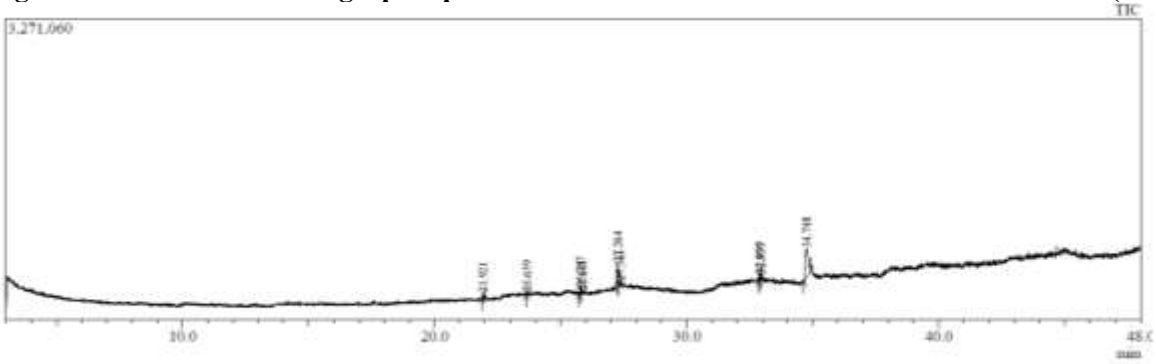


Fig no. 2 GC-MS chromatographic profile of ethanolic extract of *A. calva* inoculated with *F. mosseae* (T-2)

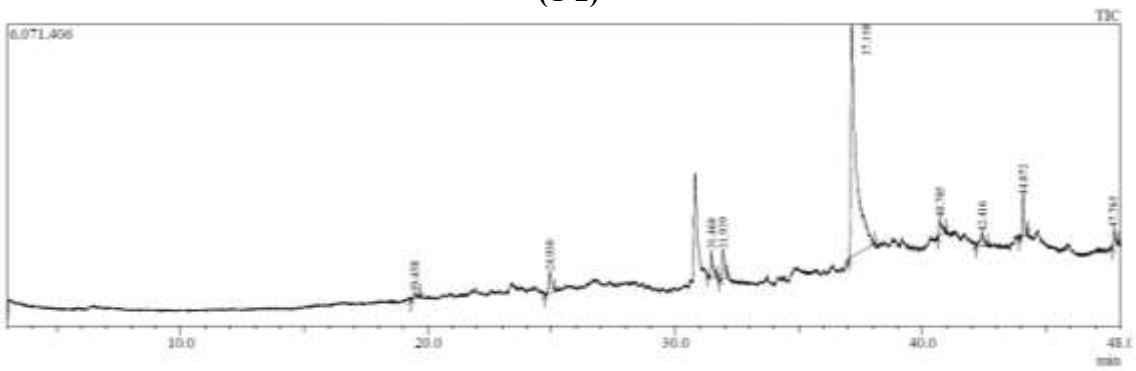


Fig no. 3 GC-MS chromatographic profile of ethanolic extract of *A. calva* inoculated with *G. macrocarpum* (T-3)

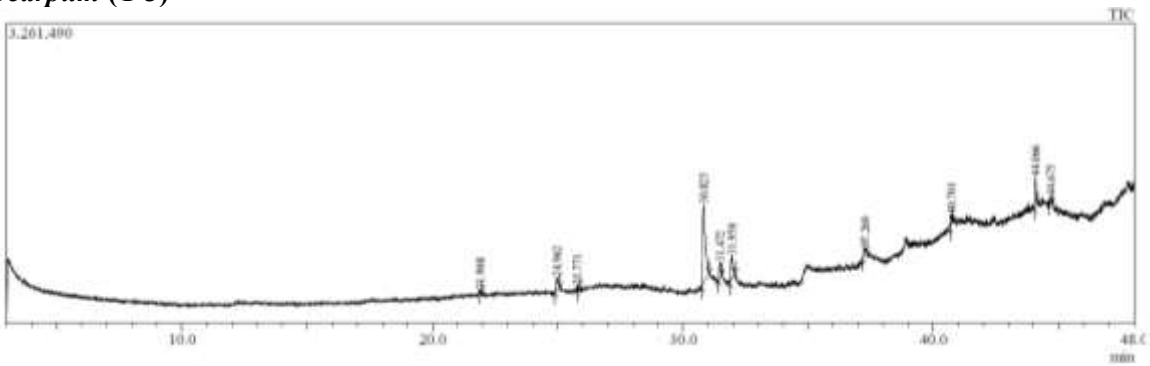


Fig no. 4 GC-MS chromatographic profile of ethanolic extract of *A. calva* inoculated *Gi. margarita* (T-4)

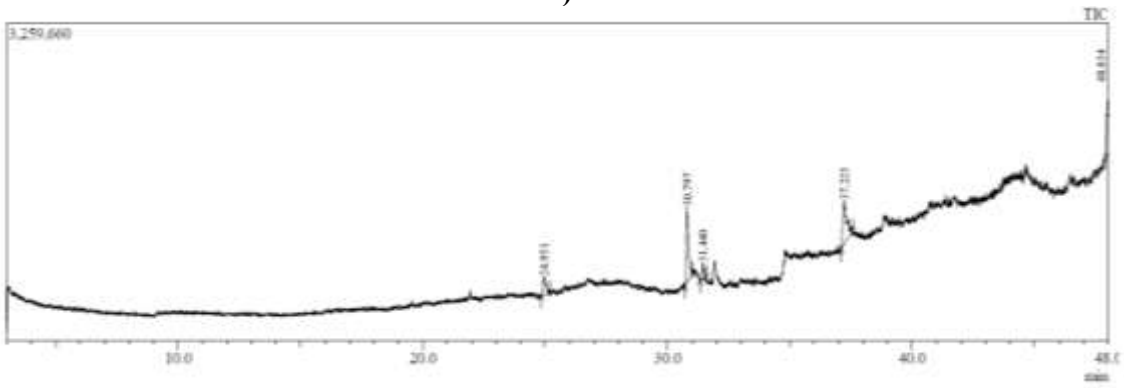
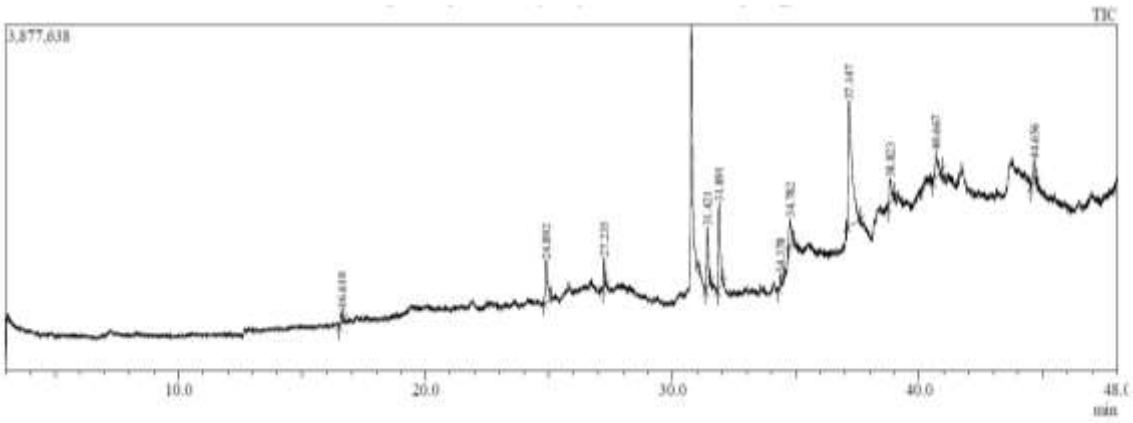


Fig no. 5: GC-MS chromatographic profile of ethanolic extract of *A. calva* inoculated Mix AMF (T-5)



The GC-MS analysis of the ethanolic extracts at peak growth revealed that the presence of AMF enhances the synthesis of various phytochemicals in *A. calva*, while they were less evident in non mycorrhizal samples. Furthermore, the identified compounds had different % peak area in which the highest peak area found in combined AMF treatment. Previous investigations showed that some phytochemicals were biologically active viz. 3,7,11,15-Tetramethyl-2-hexadecen-1-ol and Phytol demonstrated to have Anti-inflammatory, Antimicrobial, Antitumor, and Antithrombotic effect (Ko and Cho 2018). Neophytadiene described to have Anti-inflammatory, Antimicrobial, Cardioprotective, and Antioxidant property (Endris et al. 2024). These biological activities of the phytochemical compounds reported in *A. calva* extract supports the therapeutic potential of the plant. These results are agreement with Dhalaria et al. (2024) And Melato et al. (2024). They have reported that the enhancement in production of secondary metabolites due to co-inoculation of mixed AMF due to improved mineral nutrition.

Thus, AMF application boost the bioactive constituents with the strongest effect on the increase in the concentration bioactive compounds present in the *A. calva*. The synergistic relationship between AMF and *A. calva* plant can also accelerated for the increased production of phytochemical after the inoculation with AMF.

Conclusion

The findings revealed that the inoculation of *F. mosseae* + *Gi. margarita* in combination enhanced the number of phytochemicals in *A. calva*. This enhancement is due to the synergistic relationship between AMF and *A. calva* and may be due to transfer in the AMF host interphase. From the present investigation it has been observed that inoculation of AMF leads to an increase in medicinal potential of *A. calva*. Furthermore, more investigation is also required to identify potential mechanism of action induced by AMF in enhancing the number of phytochemicals.

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