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Phytochemical Analysis of *Acmella calva* (DC) R. K. Jansen in Relation to The Mycorrhizal Inoculation

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Abstract:

The present study was undertaken to evaluate the effect of arbuscular mycorrhizal fungal (AMF) inoculation on phytochemical analysis in Acmella calva (DC) R. K. Jansen, an important medicinal plant of the family Asteraceae. Plants were grown under pot culture conditions with AM fungal inoculations in combination (F. mosseae + Gi. margariata) and non-mycorrhizal treatments. Leaves, stems and roots of control and AMF-treated plants were shade-dried, powdered and extracted using Soxhlet separately in petroleum ether, ethanol and distilled water. The extracts were subjected to standard qualitative phytochemical tests to detect alkaloids, glycosides, flavonoids, tannins, saponins, anthraquinones and reducing sugars following established methods. Compared to control plants, AMF-treated plants showed enhanced presence and intensity of alkaloids, glycosides, flavonoids, tannins, saponins, anthraquinones and reducing sugars across different plant parts and solvents. The results indicate that AMF positively influences secondary metabolite biosynthesis and improves the chemical quality of plants. The findings highlight the beneficial role of AMF in improving nutrient acquisition and suggest that mycorrhizal inoculation can be effectively used as a sustainable and eco-friendly approach for enhancing nutrient use efficiency and cultivation of A. calva.

Introduction:

Plants synthesize a wide range of secondary metabolites such as alkaloids, glycosides, flavonoids, tannins, saponins, anthraquinones and reducing sugars, which are chemically distinct compounds derived from primary metabolic pathways. These phytochemicals possess characteristic functional groups such as hydroxyl, carbonyl, amine and glycosidic linkages that determine their polarity, solubility and reactivity in different solvents. Qualitative phytochemical screening using solvents of increasing polarity (petroleum ether, aqueous and ethanol) is a classical chemical approach to identify the nature and distribution of these compounds in various plant tissues (Harborne, 1998; Evans, 2009).

Acmella calva (DC) R. K. Jansen, belonging to the family Asteraceae, is an important medicinal herb widely distributed in tropical and subtropical regions (Jansen, 1981). The plant is traditionally used for its analgesic, anti-inflammatory, antimicrobial, and antioxidant properties, largely attributed to its rich phytochemical profile (Dubey et al., 2013). Recent phytochemical studies on *Acmella* species have reported that the presence of terpenoids, alkamides, phenolics, and essential oils, which showed their medicinal value (Paulraj et al., 2013).

Arbuscular mycorrhizal fungi (AMF) are ubiquitous soil microorganisms forming mutualistic associations with the roots of most terrestrial plants. AMF enhance plant nutrient uptake, particularly phosphorus, nitrogen, and micronutrients, and influence plant growth, stress tolerance, and metabolic pathways (Smith and Read, 2008). The present research suggested that the AMF symbiosis can significantly alter the biosynthesis and accumulation of secondary metabolites in medicinal plants by modulating to their nutrient availability, hormonal balance, and enzymatic activity (Kapoor et al., 2004; Baslam et al., 2011).

Understanding the influence of AM fungi inoculation on phytochemicals can help to optimise cultivation practices for *A. calva*, that will lead to higher yields of bioactive compounds with enhanced therapeutic efficacy. This would enhance the use of *A. calva* in different fields like pharmaceutical, nutraceutical, and herbal medicine industries, where consistency and quality of phytochemicals are critical. Moreover, AMF-based strategies can contribute to soil health, biodiversity conservation, and long-term productivity of medicinal plant systems (Smith et al., 2010). Hence, the present research work was undertaken.

Materials and Methods:

Pant collection and soil samples:

The healthy plants of *A. calva* (Asteraceae) were collected from the Kolhapur (MS.) India. The rhizosphere and non-rhizosphere soil of plant up to the depth of 15 cm were collected in Ziplock polythene bags and brought immediately to the lab.

Isolation of AMF Spores:

The wet sieving and decanting technique (Gerdemann and Nicolson, 1963) was used for AMF spore isolation. 100g of rhizosphere soil sample of *A. calva* was taken for the isolation. The soil solution was prepared the supernatant was decanted through stacked sieves arranged in descending order. After repeated washing with water, trapped spores in the lowermost sieve were then transferred to Whatman filter paper No. 1. The trapped spores were then picked by hypodermic needle from filter paper under the stereo-binocular microscope, then they were observed for identification and used further.

Inoculum Development:

A single spore culture technique was performed for native inoculum development. Isolated, healthy, and identified AM fungal spores (*Funneliformis mosseae,* and *Gigaspora margarita*) were taken for their mass multiplication for the production of infective inoculum. Before mass multiplication, a starter inoculum was developed by the soil funnel culture technique. Sterilised soil and sand were mixed in a 1:1 proportion. The surface-sterilised spores and maize seeds were placed in a funnel assembly for 45 days. Ater successful infection, this starter inoculum was used for mass multiplication on *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.

AMF Inoculation and Experimental Set-Up:

The experiment was set up as T-1: Control, T-2: Inoculation of AMF (*F. mosseae* + *Gi. margariata*). The 35 cm diameter Polyethylene pots were 5 cm less filled than the regular filling for the AMF treatments except for the control. Then, as per the above-mentioned treatments AMF inoculum 200 gm per each pot were spread in the pots, on that a soil layer was added to obtain regular filling, then surface sterilized seeds of *A. calva* were added to each pot. All these experiments were performed in triplicate.

Qualitative Phytochemical Screening:

Qualitative phytochemical screening of leaves, stems, and roots using petroleum ether, ethanol, and distilled water Soxhlet extracts was performed using standard chemical tests to detect the presence of major secondary metabolites.

Alkaloids were detected using Mayer's, Wagner's and Dragendorff's tests, based on precipitation reactions of nitrogenous bases. Glycosides were identified using Keller–Killiani and Legal's tests, indicating sugar-linked aglycones. Flavonoids were detected using Shinoda and alkaline reagent tests, based on color changes due to phenolic structures. Reducing sugars were detected using Benedict's and Fehling's tests, based on oxidation–reduction reactions. Tannins were identified using ferric chloride and lead acetate tests, indicating polyphenolic compounds. Anthraquinones were detected using Borntrager's test, confirming quinone structures. Saponins were detected using the froth test, indicating surface-active glycosides. The results were recorded qualitatively as absent (–), weak (+), moderate (++), or strong (+++) based on the intensity of the reaction (Harborne, 1998; Sofowora, 2008).

Results and Discussion:

The present study investigated the effects AMF inoculum of *F. mosseae* and *Gi. margarita* on the different phytochemicals in *A. calva* after 90 days of inoculation. The results of phytochemical analysis in different treatments of the test plants are presented in Table no. 1.

From a chemical perspective, the qualitative phytochemical profile shows that AMF treatment significantly influenced the biosynthesis, extraction efficiency, and solvent-dependent distribution of secondary metabolites in different plant organs.

In control plants, the chemical constituents were present in limited concentrations, as indicated by weak (+) to moderate (++) reactions. The low response in petroleum ether extracts suggests a comparatively smaller fraction of non-polar compounds under untreated conditions. Alkaloids and anthraquinones, which are nitrogen-containing and aromatic compounds, respectively, were detected mainly in stem and root tissues, indicating localized biosynthesis and storage. Glycosides showed moderate reactions, implying partial conjugation of aglycones with sugar moieties. Flavonoids and reducing sugars exhibited weak or negative reactions, reflecting low phenolic biosynthesis and limited carbohydrate availability. Tannins and saponins, both high-molecular-weight polar compounds, were present at moderate levels, particularly in aqueous and ethanol extracts.

In AMF-treated plants, a clear chemical enhancement of secondary metabolite synthesis was observed. Strong (+++) reactions in aqueous and ethanol extracts indicate increased accumulation of polar and semi-polar compounds, such as flavonoids, glycosides, tannins, saponins and reducing sugars. Chemically, this suggests activation of phenylpropanoid and shikimate pathways, leading to increased synthesis of phenolic compounds and their derivatives. The enhanced presence of flavonoids and tannins confirms an increase in polyphenolic structures rich in hydroxyl groups, which show higher solubility in polar solvents.

Alkaloids showed stronger reactions in treated plants, particularly in ethanol extracts, indicating increased nitrogen assimilation and incorporation into heterocyclic alkaloidal structures. The improved detection of reducing sugars reflects enhanced carbohydrate metabolism and availability of free aldehyde or ketone groups capable of participating in redox reactions. Anthraquinones exhibited increased intensity, suggesting stimulation of aromatic polyketide pathways under AMF influence. Saponins showed consistently strong reactions across all plant parts, indicating increased synthesis of triterpenoid or steroidal glycosides with both hydrophilic and hydrophobic domains.

The mycorrhizal plants recorded an increased uptake of N and P compared to uninoculated control plants. According to Zou et al. (2021), AMF stimulate the production of secondary metabolites like phenol, flavonoid, and carotenoid in plants, as these compounds have antioxidant properties. AMF hyphae could penetrate deep into the soil and acquire nutrients from a large soil volume that would improve the nutrient status of plants (Vandamme et al. 2013). According to Singh et al. (2024), AMF have been well documented to improve plant nutrient uptake, particularly P. Copetta et al. (2021) demonstrated AMF inoculation significantly uplifted height, shoot, and root dry weight by improving specifically P uptake in immobile soil and acquiring macro- and micro-nutrients in host plants that may have led to increased phytochemical content. The findings also revealed that inoculating with *F. mosseae* and *Gi. margarita,* in combination, resulted in a notable improvement in phytochemicals to un-inoculated control.

Conclusion:

It is concluded that inoculation with *F. mosseae,* and *Gi. margarita* showed increasing phytochemicals in *A. calva.* The increased phytochemicals could useful for understanding the mechanism of plant fitness across mineral uptake and increase in plant yield. The increase in concentration phytochemicals and macronutrients in the *A. calva* inoculated with AMF may be due to a large surface area for absorption formed by extra-radical linkage that carries nutrients to the intra-radical mycelium, eventually taken up by host plants with the involvement of P transporters. The present study demonstrated that the plant biomass and phytochemicals in *A. calva* were positively influenced by AMF. Therefore, using AMF can be considered an option for obtaining a higher yield in plant biomass and phytochemicals.

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Table no. 1- Phytochemical analysis of different plant parts of *Acmella calva* after AMF inoculation

Sr. No.	**Tests**	**Sample**	**Petroleum ether**			**Aqueous**			**Ethanol**		
			leaf	**stem**	**root**	**leaf**	**stem**	**root**	**leaf**	**stem**	**root**
1	**Alkaloids**	**Control**	-	++	+	-	++	+	-	-	-
		AMF	+	+++	++	-	++	++	-	+	+
2	**Glycosides**	**Control**	++	-	++	++	++	++	++	-	+
		AMF	+++	+++	+++	+++	+++	+++	+++	+++	+++
3	**Flavonoids**	**Control**	-	-	++	++	+	+	-	-	++
		AMF	-	-	-	-	-	-	-	-	-
4	**Reducing sugar**	**Control**	+	-	-	++	++	++	++	-	-
		AMF	++	++	++	++	++	++	++	++	++
5	**Tannins**	**Control**	+++	++	-	++	+++	+	++	++	-
		AMF	+++	+++	+++	+++	+++	+++	+++	+++	+++
6	**Anthraquinone**	**Control**	++	++	-	++	++	++	++	+++	-
		AMF	+++	+++	+++	+++	+++	+++	+++	+++	+++
7	**Saponins**	**Control**	+++	+++	++	++	++	++	+++	++	++
		AMF	+++	+++	+++	+++	+++	+++	+++	+++	+++

absent (–), weak (+), moderate (++), strong (+++)