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Response of Artemisia plants to benzyl adenine and glycine betaine

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Abstract

The objective of this study was to investigate the effects of foliar application with glycine betaine and benzyl adenine at 0, 100, 200 mgL^{-1} on vegetative growth parameters, yield, biochemical compositions and Artemisinin content of Artemisia plants. Data indicated that the foliar application treatments with glycine betaine at 100 mgL^{-1} and benzyl adenine at 100 and 200 mgL^{-1} were significantly increased all studied growth and yield parameters of Artemisia plant, growth parameters (*i.e.*, plant height, stem diameter, number of branches, fresh and dry weight of stems and leaves, number of inflorescences and fresh and dry weight of inflorescences) in both growing seasons . Furthermore, the foliar application of glycine betaine at 100 mgL^{-1} and benzyl adenine at 100 and 200 mgL^{-1} increased photosynthetic pigments, mineral contents, crude protein and total carbohydrates content in leaves of Artemisia plant. In addition, yield parameters were increased (*i.e.*, Artemisinin content, total alkaloids and total phenolics) when compared with untreated plants (control). Glycine betaine at 100 mgL^{-1} followed by benzyl adenine at 200 mgL^{-1} was the most effective treatment when compared with the control while glycine betaine at 200 mgL^{-1} decreased al above growth parameters . In conclusion, the application of bio stimulating material as a friendly environmental substances increase Artemisinin content with high amount and quality.

Keywords: Artemisia annua, Benzyl adenine, Glycine betaine and Vegetative growth

Introduction

Artemisia annua L., is an annual species in the Artemisia genus of Asteraceae, has a good medicinal value. The whole grass can be used as medicine. It is an ideal pioneer plant resource for natural greening, traditional Chinese medicine, and the greening of barren mountains and has great development and utilization value **(Lu., 2002).** The herb and leaves contain non-volatile sesquiterpenes and are used in the pharmacopoeia. Also, the most important sesquiterpene is Artemisinin and its derivatives, which are used as a treatment for malaria in the Mediterranean region **(Charles *et al.*, 1991); (Woerdenbag *et al.*, 1994); (Gupta *et al.*, 2002); (Ferreira *et al.*, 2005)**. Artemisinin is extracted from *A. annua*, which is helpful for the rapid elimination of Plasmodium falciparum and the elimination of residual parasitism **(Cai et al., 2017)**. It has also been found that a water-soluble polysaccharide isolated from *A. annua* may inhibit the activity of ovarian cancer cells **(Yan et al., 2019)**. *Artemisia annua* is widely distributed throughout the world and widely distributed in China. The Artemisinin content in *A. annua* leaves varies significantly by region, reaching up to 0.86% in some areas of Vietnam, compared to just 0.42% in India **(Woerdenbag et al., 1994).** Additionally, the quality and content of Artemisinin from *A. annua* in different regions are quite different **(Zhang et al., 2017).** Therefore, it is very important to increase the content of Artemisinin and other pharmaceutical components in the production of *A. annua*.

Artemisinin is a sesquiterpene lactone produced in the trichomes of Artemisia annua, and is extensively used in the treatment of malaria. The growth and secondary metabolism of *A. annua* are strongly regulated by environmental conditions, causing unstable supply and quality of raw materials from field grown plants **(Zhang et al 2023)**.

Nowadays, during global warming and environmental changes looking for safe treatments and inducible for enhancing the plants to grow and alleviate these conditions during plant growth including all physiological processes and development, become the big challenge for all the scientists around the world.

Cytokinins play essential roles in the growth and development of plant tissues and organs (**Mujib , 2005** Benzyl adenine (BA), a synthetic cytokinin, is involved in a wide array of biochemical and physiological processes in plants (**Bonilla and Chen , 2015**), it promotes bud and shoot regeneration (**Randall and Ternece 2010**), stimulates plant growth and metabolism (**Argall and Stewart 1984),** helps plants adapt to various environmental stresses (**Ren et al. 2016**), and improves flowering development (**Blanchard and Runkle 2008**), fruit set (**Moatshe et al. 2011**) as well as seed yield and fruit quality (**Greene et al. 2011)** The overproduction of cytokinin in transgenic plants has been shown to enhance photosynthetic rate and water use efficiency (**Rivero et al., 2007**). Application of 6-Benzyladenine (6-BA) can increase cytokinin level in plants, inhibit and scavenge free radicals, prevent ageing of marrow tissue and leaves, extend the duration of photosynthesis and improve factors related to photosynthetic efficiency. These effects contribute to a balanced coordination among yield components, ultimately leading to higher grain yield (**Thomas and Rainer, 2000**). Foliar application of benzyl adenine on *Jasminum sambac* plants led to notable improvements in growth and flowering. Plants treated with BA at 60 ppm exhibited the greatest height and the highest number of branches per plant (8.91 and 8.43, respectively). the heaviest leaves fresh weight/plant followed by using BA at 40 ppm. benzyl adenine at 60 ppm was more effective in increasing No. of flowers/plant as it gave 73.8 and 74.7, followed by using the medium concentration which recorded 66.2 and 68.0 flowers/plant **(Abou El-Ghait-Eman *et al.*, 2020)**.

Glycine betaine is a kind of nitrogen-containing alkyl hydrocarbon compound with a molecular formula of $C_6H_{11}O_2N$ and molecular weight of 117.15 g/mol; It is a membrane-permeable compatible osmolyte and a natural, non-toxic substance widely found through the biological world. Due to these properties, glycine betaine serves multiple important functions in living organisms.. In plants, glycine betaine enhances stress resistance by improving cellular osmotic adjustments, reducing damage to cell membranes from osmotic water loss, stabilizing subcellular structures, and scavenging reactive oxygen species (ROS) **(Carillo et al. 2019).** Additional functions of glycine betaine include preserving the thermodynamic stability of macromolecules and reversing protein misfolding and aggregation without compromising the native functional activities of proteins **(Khan et al. 2010).** It also reduces plasma membrane peroxidation levels, enhances ROS scavenging capacity, and helps maintain cell membrane stability **(Gupta and Huang 2014).** Studies have demonstrated the positive effects of glycine betaine (GB) on the photosynthetic efficiency and yield of Artemisia annua. **Zhang *et al.* (2021)** found that plants treated with 150 mM GB showed a significant increase in chlorophyll content and photosynthetic rate, resulting in greater biomass accumulation and enhanced growth potential under optimal conditions. Similarly, **Ali** et al. **(2024)** reported that application of 150 mM GB under drought stress significantly increased the overall yield of Artemisia annua by 35%, a result attributed to improved biomass accumulation and reproductive performance.

The purpose of this study is to evaluate the effects of applying environmentally friendly growth-promoting substances, such as glycine betaine and benzyl adenine on the growth performance, chemical composition, and bioactive constituent contents of *Artemisia annua* L. plants, with particular emphasis on Artemisinin as the key active ingredient.

Material and Methods

Two field experiments were carried out at the Experimental Farm Station of the Faculty of Agriculture Moshtohor, Benha University, Kalyoubia Gavernorate, Egypt, during the two seasons of 2021/2022 and 2022/2023 to investigate the effects of glycine betaine and benzyl adenine (BA) on growth, yield and some biochemical constituents of *Artemisia annua* plants.

The physical and chemical analysis of the experimental soil are presented in **Table (1)** as an average of the two seasons. Physical and chemical analyses were determined by using standard methods according to **(Black, 1965)**.

Table 1. Physical and chemical properties of the experimental soil during 2022 and 2023 seasons were as follows.

Soil analysis	Seasons	
	2022	2023
	Pre planting	Pre planting
Soluble cations and anions (mmolec L^{-1})		
Ca^{++}	1.3	2.6
Mg^{++}	0.7	1.2
K^{+}	0.4	0.7

Na^{+}	5.1	8.3
Cl^{-}	4.9	10.0
CO_3^{2-}	0.0	0.0
HCO_3^{-}	1.2	2.4
So_4^{2-}	1.4	0.4
Chemical analysis		
E.C* (dSm^{-1})	0.73	1.31
PH	7.92	7.78
Particle size distribution (Mechanical analysis)		
Course sand %	10.5	16.5
Find Sand %	16.1	10.0
Silt %	37.3	36.6
Clay %	36.1	36.9
Soil texture	Clay loam	Clay loam

Plant material and cultivation:

Seeds of *Artemisia* plants were obtained from Experimental Farm Floriculuture, Faculty of Agriculture, Moshtohor, Benha University. The 30-hole nursery trays and the flower pots were sterilized with potassium permanganate solution and hydrogen peroxide to ensure that the containers were clean and sterile. *Artemisia annua* seedlings were raised and then transplanted into flowerpots. Approximately four-fifths of the hole volume in the nursery tray was filled with sterilized peat. Four or Five *A. annua* seeds in each hole were covered with a layer of peat until the hole was filled, and water was sprayed to keep the nursery trays moist. Finally, we put the nursery tray in the screenhouse. Transplanting was carried out when four true leaves of *A. annua* seedlings appeared 30 days after sowing.

Seedlings of *Artemisia* were transplanted on 24th March in the first and second seasons respectively. 1 seedling / hill was transplanted in 4 cm depth on one side of the ridge at 1m apart.

2. Treatments source:

1-Benzyladenine (BA) was exported from Algomhoria Chemical Company, Egypt

2- Glycine betaine was exported from future modern Company, Egypt.

The experiment treatments were as follows:

This experiment included five treatments of glycine betaine and benzyl adenine as follows:

(control , glycine betaine 100 ,200 mgL^{-1} and benzyl adenine 100 ,200 mgL^{-1})

3. Managements Through Plant growth:

Through plant growth and development, plants were sprayed 9 times with different assigned treatments, the first one was at 40 days after transplanting and repeated each 15 days intervals, the spraying solution volume was spraying until the solution runoff from the plant. All other cultural practices were performed as recommended.

4. Sampling and collecting data:

a-Vegetative growth characteristics:

Different morphological characteristics of Artemisia plants at 90, 140 days after transplanting were measured and calculated. Three plants from each treatment were randomly taken from middle rows and then separated into their organs and the following characteristics were recorded: (Plant height (cm)/plant , stem diameter (cm)/ plant , Number of branches/plant , Fresh weight of stems (g) /plant , Fresh weight of leaves (g) /plant , Dry weight of stems (g) /plant, Dry weight of leaves (g) /plant , Fresh weight of flowers (g) /plant , Dry weight of flowers (g) /plant) .The flowers was drayed by on air draying .

b- Biochemical analysis:

Biochemical analyses were carried out on the samples of leaves.

1- Determination of photosynthetic pigments concentrations according to the methods described by **(Wettstein 1957).**
2- Determination of total carbohydrate according to the method described by **(Dubois *et al.*, 1956)**
3- Total nitrogen and crude protein according to the method described by **(Horneck and Miller 1998)** then calculated as mg/g dry weight. Then, the crude protein was calculated according to the equation of **(A.O.A.C. 2005).** Crude protein = Total nitrogen x 3.71
4- Phosphorus according to the method described by **(Sandell 1950)**
5- Potassium according to the method described by **(Horneck and Hanson 1998)**
6- Iron , zinc and Manganese according to the method described by **(Houk et al., 1980)**
7- Determination of Alkaloid Content was determined using the method described by **(Shamsa et al. 2008).**
8- Determination of Total Phenolic Content was determined using the method described by **(Singleton et al. 1999).**

5. Statistical analysis:

Data of growth and yield characteristics were subjected to statistical analysis using a RCPD in which treatments of foliar applications treatments. Using the SPSS 19.0 software package for Windows as duncn letters.

Results and Discussion

1. Vegetative growth parameters of Artemisia plants:

With regard to the effect of foliar application treatments i.e., (Glycine betaine at 100 ,200 and benzyl adenine at 100 , 200 mg l^{-1}), data in (**Tables1, 2 , 3 and 4)** shows clearly that foliar application treatments with the concentrations of benzyl adenine and glycine betaine at 100 mg l^{-1} significantly increased all studied growth parameters (i.e., plant height, stem dimeter , number of branches, , fresh weight of stem and leaves , dry weight of stem and leaves , number of inflorescences, fresh weight of inflorescences and dry weight of inflorescences /plant) while glycine betaine at 200 mg L^{-1} decreased all studied growth parameters at 90 and 140 days after transplanting in both seasons compared with the untreated plants (control). In this respect, foliar spraying with glycine betaine at 100 mg l^{-1} followed by benzyl adenine at 200 mg l^{-1} resulted in the highest mean values of the above mentioned growth parameter traits when compared with control and other treatments application during both seasons.

Generally, the application of benzyl adenine (BA), a synthetic cytokinin, has been widely reported to positively influence plant growth and yield by promoting cell division, morphogenesis, and lateral bud development. In various aromatic and medicinal plants, including *patchouli* and *Chrysanthemum*, BA treatments have resulted in increased branching, leaf retention, and overall plant spread, which enhanced biomass and yield (**Bhaskar et al., 1997; Farooqi et al., 1993).** Exogenous application of glycine betaine significantly enhanced vegetative growth traits in *Artemisia* plants, including shoot length, branching, and biomass accumulation. This growth stimulation is linked to glycine betaine's role in modulating endogenous phytohormone balance and enhancing cell division under non-stress conditions. Recent studies confirm that glycine betaine promotes biomass accumulation in *Artemisia annua* by regulating auxin and gibberellin homeostasis **(Al-Huqail and Soliman, 2023)**. In this respect **(Hasanuzzaman *et al.*, 2020)** While our findings are promising, it is important to note that the magnitude of response may depend on concentration, duration of application, and environmental conditions. Some studies report a biphasic response, where low doses of glycine betaine stimulate metabolite production, while higher doses may have neutral or inhibitory effects.

Table 2. Effect of foliar application with glycine betaine and benzyl adenine on plant height, stem diameter (cm) and number of branches of Artemisia plants at 90 (1st cut) and 140 (2nd cut) days after transplanting during (2021-2022) and (2022-2023) seasons.

Characteristics	Plant height (Cm)		Stem diameter (cm)				Num. of branches / plant			
	1st season	2nd season	1st season		2nd season		1st season		2nd season	
treatments	In the first cut		1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Control	121.88±1.2c	121.88±1.2c	5.94±0.2b	9.33±0.3c	5.55±0.2b	9.50±0.3b	29.66±0.8c	20.66±0.3b	42.11±1.8b	20.11±0.4c
Benzyl adenine 100 mg l^{-1}	128.44±1.2b	128.44±1.2b	5.94±0.2b	10.33±0.3b	5.94±0.2b	10.16±0.3b	38.11±0.8b	21.00±0.4b	43.66±1.8b	22.66±0.4b
Benzyl adenine 200 mg l^{-1}	136.33±1.2a	136.33±1.2a	7.05±0.2a	10.77±0.3ab	7.05±0.2a	11.55±0.3a	42.11±0.8a	23.00±0.4a	52.33±1.8a	24.00±0.4a
Glycine betaine 100 mg l^{-1}	138.00±1.2a	138.00±1.2a	7.50±0.2a	11.16±0.3a	7.50±0.2a	11.88±0.3a	43.22±0.8a	23.33±0.4a	53.55±1.8a	24.66±0.4a
Glycine betaine 200 mg l^{-1}	121.44±1.2c	121.44±1.2c	5.05±0.2c	9.00±0.3c	5.05±0.2c	8.66±0.3c	26.88±0.8d	19.33±0.4c	40.66±1.8b	18.33±0.4d

The data represent the means ± standard deviation (SD) of four replications. In the same column, means followed by the same letter are not statistically different, according to the Duncan Multiple Range Test (DMRT) P≤ 0.05.

Table 3. Effect of foliar application with glycine betaine and benzyl adenine on number of secondary branches, fresh weight of plant and dry weight of plant of Artemisia plants at 90 and 140 days after transplanting during (2021-2022) and (2022-2023) seasons.

Characteristics / treatments	Num. of secondary branches / plant		Fresh weight of plant (g)				Dry weight of plant (g)			
	1 st season	2 nd season	1 st season		2 nd season		1 st season		2 nd season	
	In the second cut		1stcut	2 nd cut	1stcut	2 nd cut	1stcut	2 nd cut	1stcut	2 nd cut
Control	145.22 ±5.2^{d}	161.66 ±8.6^{b}	165.44 ±4.1^{c}	1580.11 ±74.2^{c}	218.33 ±9.4^{c}	2213.55 ±71.0^{c}	45.77 ±1.7^{d}	613.11± 22.5^{d}	51.55 ±1.9^{d}	626.00± 42.0^{c}
Benzyl adenine 100 mg l^{-1}	190.88 ±5.2^{c}	231.33 ±8.6^{a}	204.22 ±4.1^{b}	2306.11 ±74.2^{b}	287.22 ±9.4^{b}	2907.22 ±71.0^{b}	61.33 ±1.7^{c}	842.22± 22.5^{c}	66.66 ±1.9^{c}	870.88± 42.0^{b}
Benzyl adenine 200 mg l^{-1}	214.88 ±5.2^{b}	239.55 ±8.6^{a}	227.88 ±4.1^{a}	2542.77 ±74.2^{a}	303.22 ±9.4^{ab}	3145.55 ±71.0^{a}	73.88 ±1.7^{b}	1059.77 ±22.5^{b}	77.77 ±1.9^{b}	1095.44 ±42.0^{a}
Glycine betaine 100 mg l^{-1}	230.11 ±5.2^{a}	248.33 ±8.6^{a}	238.44 ±4.1^{a}	2697.77 ±74.2^{a}	324.11 ±9.4^{a}	3244.55 ±71.0^{a}	82.55 ±1.7^{a}	1147.77 ±22.5^{a}	84.00 ±1.9^{a}	1178.77 ±42.0^{a}
Glycine betaine 200 mg l^{-1}	133.66 ±5.2^{d}	152.44 ±8.6^{b}	156.55 ±4.1^{c}	1311.44 ±74.2^{d}	199.44 ±9.4^{c}	2109.22 ±71.0^{c}	39.77 ±1.7^{f}	515.33± 22.5^{e}	48.66 ±1.9^{d}	530.77± 42.0^{c}

The data represent the means ± standard deviation (SD) of four replications. In the same column, means followed by the same letter are not statistically different, according to the Duncan Multiple Range Test (DMRT) P≤ 0.05.

Table 4. Effect of foliar application with glycine betaine and benzyl adenine on fresh weight of leaves and dry weight of leaves of Artemisia plants at 90 and 140 days after transplanting during (2021-2022) and (2022-2023) seasons.

Characteristics / treatments	Fresh weight of leaves (g)				Dry weight of leaves (g)			
	1st season		2nd season		1st season		2nd season	
	1stcut	2nd cut	1stcut	2 nd cut	1stcut	2nd cut	1stcut	2nd cut
Control	140.55±4.85^{d}	703.33±23.2^{d}	191.77±17.9^{cd}	907.66±29.57^{c}	53.77±1.07^{d}	308.33±12.7^{c}	48.88±1.54^{d}	311.77±9.34^{c}
Benzyl adenine 100 mg l^{-1}	191.00±4.85^{c}	944.55±23.2^{c}	227.22±17.9^{bc}	1206.22±29.57^{b}	64.22±1.07^{c}	389.00±12.7^{b}	63.22±1.54^{c}	366.00±9.34^{b}
Benzyl adenine 200 mg l^{-1}	211.33±4.85^{b}	1176.0±23.2^{b}	253.77±17.9^{ab}	1427.44±29.57^{a}	70.44±1.07^{b}	414.22±12.7^{ab}	73.66±1.54^{b}	399.22±9.34^{a}
Glycine betaine 100 mg l^{-1}	225.88±4.85^{a}	1252.44±23.2^{a}	297.66±17.9^{a}	1494.55±29.57^{a}	77.88±1.07^{a}	427.66±12.7^{a}	79.00±1.54^{a}	422.22±9.34^{a}
Glycine betaine 200 mg l^{-1}	135.88±4.85^{d}	614.55±23.2^{e}	171.22±17.9^{d}	790.33±29.57^{d}	47.22±1.07^{e}	301.11±12.7^{c}	41.33±1.54^{e}	298.55±9.34^{c}

The data represent the means ± standard deviation (SD) of four replications. In the same column, means followed by the same letter are not statistically different, according to the Duncan Multiple Range Test (DMRT) P≤ 0.05.

Table 5. Effect of foliar application with glycine betaine and benzyl adenine their interaction on number of inflorescences, fresh weight of inflorescences and dry weight of inflorescences of Artemisia plants at 90 and 140 days after transplanting during (2021-2022) and (2022-2023) seasons.

Characteristics treatments	Num.of inflorescences / plant	Fresh weight of flowers (g)	Dry weight of flowers (g)
Control	10.33±0.36^{C}	281.11±7.65^{c}	48.00±0.82^{d}
Benzyl adenine 100 mg l^{-1}	12.66±0.36^{b}	364.66±7.65^{b}	57.66±0.82^{c}
Benzyl adenine 200 mg l^{-1}	14.66±0.36^{a}	382.00±7.65^{ab}	63.33±0.82^{b}
Glycine betaine 100 mg l^{-1}	15.00±0.36^{a}	395.11±7.65^{a}	67.33±0.82^{a}
Glycine betaine 200 mg l^{-1}	8.66±0.36^{d}	254.00±7.65^{d}	43.00±0.82^{e}

The data represent the means standard deviation (SD) off four replications. In the same column, means followed by the same letter are not statistically different, according to the Duncan Multiple Range Test (DMRT) atp 0.05.

2. Chemical compositions of Artemisia leaves:

In Tables (6,7) data report that Artemisia chemical analysis *i.e*, (Photosynthetic pigments ,N, P, K, Fe, Zn, Mn, Total carbohydrates and crude protein) data shows that foliar application treatments with the concentrations of benzyl adenine and glycine betaine at 100 mg l^{-1} increased each of (Photosynthetic pigments (chlorophyll a, b and carotenoids) N, P, K, Fe, Zn, Mn Total carbohydrates and crude protein) in leaves of Artemisia plants while glycine betaine at 200 mg l^{-1} decreased all above chemical analysis in leaves of Artemisia plants at 140 days after transplanting in second season. Also, it could be noticed that maximum increase of pigments, N, P, K, Total carbohydrates and crude protein in leaves existed in case of benzyl adenine at 200 mg l^{-1} followed by Glycine betaine at 100 mg l^{-1} treatments When compared with the control , while Glycine betaine at 100 mg l^{-1} gave the highest concentrations of (Fe, Zn and Mn) followed by benzyl adenine at 200 mg l^{-1} When compared with the control .

Our results agree with many previous studies which found that BA spraying at a concentration of 50, 100, and 150 mg/L improved the chlorophyll contents in *Strelitzia spp* and the best condition was obtained at 50 mg/L treatment. It seems that an increase in photosynthetic pigment contents by BA is due to the direct effect of BA on the photosynthetic reactions and/or the increase in size and number of chloroplasts in leaves as results of cell division (**Abou Rayya *et al.*, 2015)**. **(Nayak et al. 2020).** For instance, in a study on *Zingiber officinale* (ginger), foliar application of BA at 75 ppm combined with 100% recommended NPK fertilizer significantly increased leaf NPK content, indicating improved nutrient uptake efficiency. In general, BA may facilitate increased uptake or utilization of these micronutrients. The effect of benzyl adenine (BA) on micronutrient uptake in *Artemisia* plants is an emerging area of interest, with evidence suggesting that BA may enhance the absorption and mobilization of key elements such as iron (Fe), zinc (Zn), and boron (B) , (**Shinede et al., 2021)**. Although direct studies on *Artemisia* are limited, related research provides compelling evidence. For instance, foliar application of 40 ppm BA in cotton significantly increased total carbohydrate content in leaves, likely due to improved chlorophyll levels and photosynthetic efficiency. These physiological enhancements are relevant to *Artemisia*, where carbohydrate availability supports both vegetative growth and the biosynthesis of secondary metabolites like artemisinin **(El-Shazly 2021)**. Moreover, BA is known to stimulate the expression of genes related to amino acid metabolism and protein assembly. This is supported by findings in other plant systems, such as cotton and sunflower, where BA application led to increased total nitrogen and protein content in leaves **(El-Shazly, 2021; Ewais et al., 2015)**. These physiological responses are likely transferable to *Artemisia*, given the conserved nature of cytokinin-regulated metabolic pathways.

Glycine betaine treatment increased chlorophyll *a*, chlorophyll *b*, and carotenoid contents, reflecting its capacity to stabilize photosystem II and support chlorophyll biosynthesis even in optimal environments , upregulation of chlorophyll biosynthesis genes (*HEMA1*, *CAO*) in *Artemisia annua* following glycine betaine application **(Li et al. 2022)**. Similarly, Plants treated with glycine betaine exhibited elevated levels of nitrogen, phosphorus, and potassium, likely due to improved root membrane functionality and upregulation of nutrient transporter genes, glycine betaine enhances expression of *NRT*, *PHT*, and *HAK* transporter families in *Artemisia*, leading to greater NPK accumulation (**Zhang et al. 2020)**. In general glycine betaine improves root vitality and ion uptake efficiency in non-stressed plants **(Ahmad et al. 2022)**, while **(Gao et al. 2021)** linked its application to increased activity of ATPases involved in nutrient translocation across root membranes.In addition Significant increases in Fe, Zn, and Mn concentrations were observed, suggesting that glycine betaine enhances micronutrient mobility and chelation efficiency in plant tissues without stress induction, also glycine betaine upregulates metal transporter genes (*IRT1*, *ZIP*, *NRAMP*) in *Artemisia annua*, facilitating Fe and Zn uptake **(Wang et al. 2023)**. Additionally, enhanced Mn translocation to leaves via improved xylem loading **(Liu et al. 2022)** . Generally glycine betaine increases organic acid exudation from roots, improving micronutrient solubilization in the rhizosphere under normal growth conditions **(Soliman and Al-Huqail 2024)**. Generally, glycine betaine promoted the accumulation of soluble and total carbohydrates, reflecting improved photosynthetic carbon fixation and sucrose metabolism in source leaves**,** glycine betaine enhances sucrose phosphate synthase (SPS) activity and starch synthesis in *Artemisia* **.(Kurepin et al. 2020)**. Furthermore, **(Liu et al. 2021)** linked its application to increased leaf starch granules and soluble sugar pools, while **(Zhao et al. 2022)** showed improved carbon partitioning to storage organs via upregulation of sugar transporter genes (*SUT*, *SWEET*) under optimal growth. Total protein content increased significantly, suggesting that glycine betaine supports nitrogen assimilation via enhanced nitrate reductase activity and ribosomal protein synthesis. elevated nitrate reductase and glutamine synthetase activities in glycine betaine-treated *Artemisia*, leading to higher protein synthesis **(Ahmad et al. 2023)**. Similarly, **(Gao et al. 2022)** observed increased expression of ribosomal protein genes and improved translational efficiency, while **(Soliman et al. 2021)** confirmed that glycine betaine enhances amino acid pool availability, directly supporting protein biosynthesis in non-stressed plants.

Table 6. Effect of foliar application with glycine betaine and benzyl adenine on photosynthetic pigments concentration (mg/g F.W.) in leaves of Artemisia plants at 140 days after transplanting during (2021-2022) and (2022-2023) seasons.

Characteristics / treatments	Chl (a) (mg/gF.W.)		Chl (b) (mg/ F.W.)		Chl (a+b) (mg/gF.W.)		Carotene (mg/gF.W.)	
	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season
Control	0.90	0.98	0.65	0.63	1.55	1.61	0.77	0.87
Benzyl adenine 100 mg l^{-1}	1.08	1.06	0.68	0.70	1.76	1.76	0.73	0.88
Benzyl adenine 200 mg l^{-1}	1.16	1.20	0.83	0.85	1.99	2.05	0.93	0.90
Glycine betaine 100 mg l^{-1}	1.13	1.15	0.80	0.78	1.93	1.93	0.80	0.89
Glycine betaine 200 mg l^{-1}	0.81	0.83	0.52	0.57	1.33	1.40	0.67	0.64

Table 7. Effect foliar application with glycine betaine and benzyl adenine on some minerals, crude protein and total carbohydrates in leaves of Artemisia plants at 140 days after transplanting during (2022-2023) seasons.

Characteristics / treatments	N%	P%	K%	Fe mg L^{-1}	Zn mg L^{-1}	Mn mg L^{-1}	Carbohydrate %	Crude protein%
Control	2.58	0.34	3.11	0.26	0.22	0.32	37.17	0.41
Benzyl adenine 100 mg l^{-1}	3.08	0.40	3.21	0.33	0.25	0.48	37.96	0.49
Benzyl adenine 200 mg l^{-1}	3.41	0.46	3.95	0.45	0.31	0.57	39.23	0.55
Glycine betaine 100 mg l^{-1}	3.25	0.43	3.74	0.49	0.32	0.61	38.37	0.52
Glycine betaine 200 mg l^{-1}	2.36	0.32	3.08	0.21	0.22	0.26	36.65	0.38

3. Yeild

The effect of foliar application treatments i.e., (Glycine betaine at 100 ,200 and benzyl adenine at 100 , 200 mg l^{-1}), data also in (**Tables 9 and figure 1)** show clearly that different treatments significantly increased Artemisinin content (mg/g DW), Total alkaloids (mg/g) and ,total phenolics (mg GAE/g) concentrations , while glycine betaine at 100 mg l^{-1} gave the highest concentrations followed by benzyl adenine at 200 mg l^{-1}. The other treatments also showed a significant response as it increased Artemisinin content (mg/g DW), total alkaloids (mg/g) and, total pPhenolics (mg GAE/g) concentrations When compared with the control .

1. **Artemisinin content**

A very recent study by **(Liu et al., 2024)** explored the impact of varied nitrogen supply on the growth, Artemisinin content, and overall yield of *Artemisia annua* through changes in endogenous phytohormones and gene expression. Their findings underscore that while BA directly influences cell division and biomass, its ultimate effect on Artemisinin yield is intricately tied to the plant's overall physiological state, heavily influenced by nutrient availability. This suggests a crucial discussion point: simply applying exogenous BA might not maximize yield if other foundational factors, such as optimal nitrogen levels or the plant's endogenous hormonal balance, are not adequately managed. Future research, therefore, needs to explore the synergistic effects of BA application in conjunction with precise nutrient management strategies to achieve the highest possible Artemisinin yield in *Artemisia annua* cultivation. In this respect, glycine betaine application markedly increased Artemisinin accumulation in *Artemisia annua* leaves, potentially by upregulating key genes in the MEP pathway (e.g., *DXR*, *ADS*) and providing methyl donors for sesquiterpene biosynthesis glycine betaine enhances Artemisinin yield by 38% through transcriptional activation of *ADS* and *CYP71AV1* **(Nair et al. 2022).** Similarly, **(Akhtar et al. 2020)** linked glycine betaine to increased flux through the MEP pathway via elevated pyruvate and glyceraldehyde-3-phosphate pools.

2. **Total alkaloid**

In addition the application of benzyl adenine at 60 mg/l on lupine plant *(Lupinus termis L.*). significantly affected biochemical constituents (total phenols, %, Alkaloid) **(Ayad and Gamal El-Din 2011).** Also total alkaloid content rose in glycine betaine treated plants, indicating its role in activating biosynthetic enzymes such as strictosidine synthase under normal growth conditions. upregulation of alkaloid pathway genes (*STR*, *TDC*) in *Artemisia* following glycine betaine treatment **(El-Sayed et al. 2021)**. Moreover, glycine betaine increases precursor availability (e.g., tryptophan) for alkaloid synthesis via primary metabolism modulation **(Chen et al. 2023).**

3. **total phenolic**

In line with this result **(Kecis et al., 2023)**; on mint (*Mentha rotundifolia* L), the application Benzyl adenine significantly influenced the accumulation of phenolic compounds in the plant organs. Treatment with Benzyl adenine also induced remarkable increases in the amounts of individual phenolic compounds, with the greatest increases observed for rosmarinic acids in the aerial parts treated with 20 or 10 mg/mL of Benzyl adenine. Salvianolic acid also showed the most significant increase in the plants treated at 10 mg/mL (from 18.232 to 41.317 µg/g of extract). In addition a significant boost in total phenolic compounds was recorded, likely due to glycine betaine–mediated enhancement of phenylalanine ammonia-lyase (PAL) activity and expression of phenylpropanoid pathway genes , glycine betaine increases PAL and *4CL* gene expression in *Artemisia annua*, leading to higher phenolic yields **(Verma and Mishra 2021) .**

Table 8. Effect of foliar application with glycine betaine and benzyl adenine on artemisinin content (mg/g DW), total alkaloids (mg/g) and total phenolics (mg /g) of Artemisia plants at 140 days after transplanting during (2022-2023) seasons.

Characteristics / treatments	Artemisinin content (mg/g DW)	Total Alkaloids (mg/g)	Total Phenolics (mg /g)
Control	38.09	12.59	23.48
Benzyl adenine 100 mg l^{-1}	43.00	14.19	30.39
Benzyl adenine 200 mg l^{-1}	44.27	14.63	31.70
Glycine betaine 100 mg l^{-1}	46.90	15.30	33.44
Glycine betaine 200 mg l^{-1}	41.46	13.57	28.60

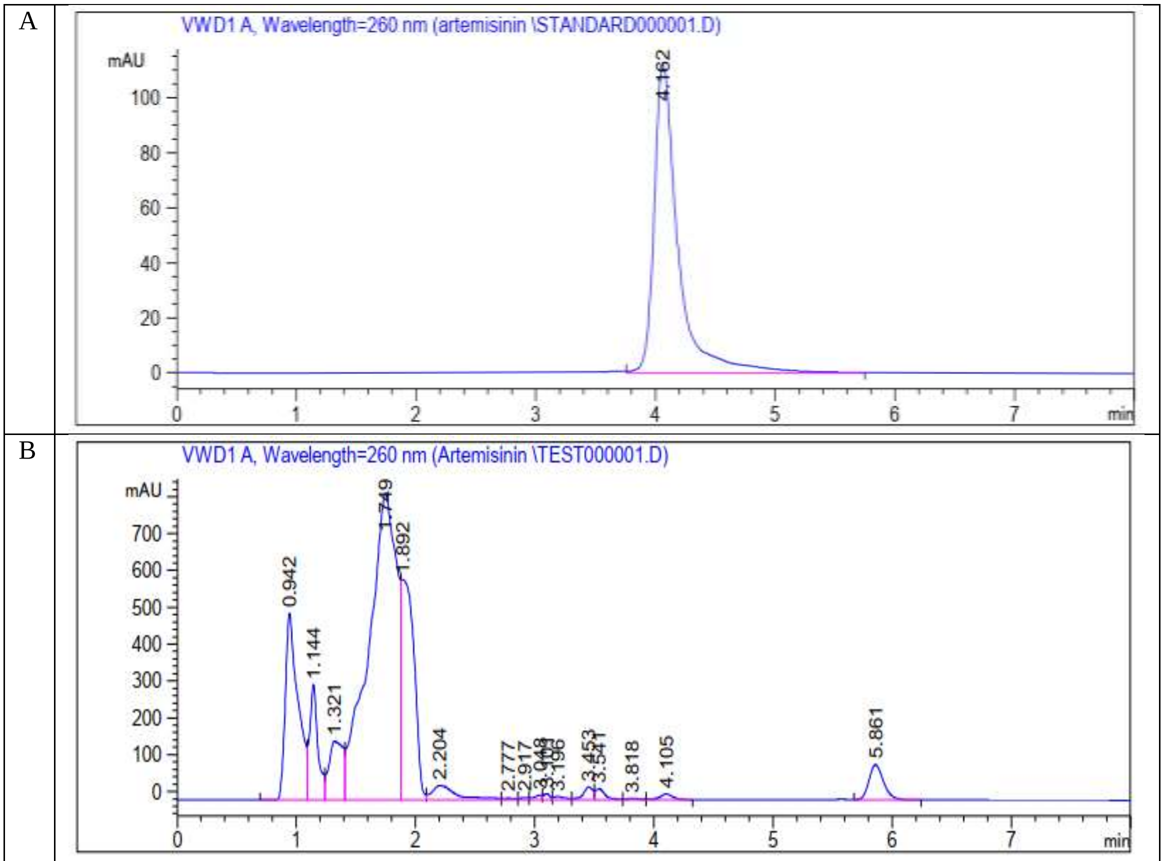

Figure (1): Chromatographic charts of artemisinin standard (A), and samples extracted (B) with UV detector at 260 nm

Conclusion

To get a medicine from medicinal plants become a huge challenge as increases infections and deseases. It could be concluded that these results may be attributed to the role of applied glycine betaine and benzyl adenine to improve the productivity of Artemisia plants by increasing vegetative growth and production chlorophyll contents in leaves, mineral content ,total carbohydrate , crude protein and Artemisinin content .It could be concluded that foliar application with glycine betaine at 100 mg/L, benzyl adenine at 200 mg/L increasing and improvement of growth and productivity of Artemisia plants .

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