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Efficiency of salicylic acid to enhance the growth attributes of *Bacopa monnieri* (L.) under water deficit conditions

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Abstract
Salicylic acid (SA) has been recognized as a strong tool and act as signaling molecule for sustainably alleviating adverse effects of abiotic stresses particularly water deficit conditions. An experiment was therefore conducted to test whether the application of SA at various concentrations (25, 50, 75, 100, 125 and 150 mg/L) as a foliar spray would protect Brahmi (*Bacopa monnieri* L.) plants when subjected to water deficit stress based on reduce irrigation; (i) Well watered (regular), (ii) Water deficit A (two times within a week) and (iii) water deficit B (only 1 time within a week). Under water deficit SA improved the growth rate of medicinal herb *Bacopa monnieri* thorough increasing the numbers of shoot, shoot length, leaves number, fresh and dry herbage yield as compared to control. It appeared the best ameliorative remedies of SA obtained when *Bacopa monnieri* were sprayed at 75 mg/L under stress exposure, while higher conc. (above 100 mg/l) showed less improvement.

Keywords: *Bacopa monnieri*, growth attributes, salicylic acid, water deficit, medicinal herb

Introduction
Bacopa monnieri, is one of the most potentially therapeutic plants also known as Brahmi belongs to family Scrophulariaceae, its small herb prefers to grow in sandy and damp areas. The plant is predominantly located in the plains of Australia, India, Pakistan, Afghanistan, Nepal, Africa and Asia. Soft herbaceous cuttings are used to propagate the plant (Aguiar and Borowski, 2013) [3]. Ayurveda refers to it as a "Medhya-Rasayana" because of its potential to stimulate intellect properties. This herb attracted the attention of enthusiastic phytochemical researchers for its remarkable pharmacological activities like neuropharmacological, anxiolytic, anticonvulsant, hepatoprotective, anti-bacterial activity. Other activities of *Bacopa monnieri* such as anti-inflammatory, cardiotonic, anticancer, bronchodilatory and vasodilatory make it more precious (Bhandari *et al*., 2020) [7]. Nootropic properties of the plant are primarily attributed to the presence of triterpenoid glycosides saponins called 'bacosides' namely Bacoside A which was earlier considered to be isomers and now characterized to be a mixture of four triglycerides saponins *viz.* bacoside A3, bacopaside II, isomer of jujubogenin and bacopasaponin C. In recent years, "Memory Plus", a product that contains the standardized extract of bacosides from brahmi, has been marketed in India. Subsequently, several formulations containing *Bacopa monnieri* extracts standardized for bacoside content have appeared in the global market **(**Singh, 2013) [27]. Almost the entire commercial requirement is met solely from the wild natural populations. However, the rainy season is preferred in many regions of India for the cultivation of *Bacopa monnieri* due to the high irrigation require throughout the culture period. Although, there was a tremendous demand for raw materials from industry throughout the year, so agriculture systems needed to adopt substantial tactics for production with limited water availability.

In many arid and semi-arid regions around the world, water deficiency is probably the biggest obstacle to crop productivity (Kapoor *et al*., 2020) [14]. Water scarcity has an effect on a wide range of morphological and physiological processes, which hinders overall plant growth, development, and yield. When there is a water shortage, a range of stressful events might combine to induce osmotic and ionic imbalance in plants (Mukarram *et al*., 2021) [18]. Several studies have demonstrated that plant growth regulators (PGR) molecules like salicylic acid, jasmonic acid, calcium, abscisic acid, polyamines, and nitric oxide participate in extensive cross-talk and signaling processes between pathways in plant cells that are associated with the biosynthesis of secondary metabolites, antioxidants and compatible molecules over stress exposure (Isah, 2019) [12].

Salicylic acid (SA), one of these molecules which alters a number of metabolic pathways including synthesis, oxidation and some biological activities including respiration, photosynthesis and nutrients absorption (Borsani *et al*., 2001) [8]. SA can reverse the drought stress effects by increasing photosynthesis rate, chlorophyll, carbohydrates, and proline contents and amend the growth attributes of plants (Su *et al*., 2018) [29]. Many studies have assessed the function of SA as a key signaling molecule in response to both abiotic and biotic stress factors Hence, in the present study, evaluating the efficacy of salicylic acid (SA) to ameliorate growth and growth attribute of the *Bacopa monnieri* (L.) plants under water deficit conditions.

Method and Material

The pot experiment was carried out in the Department of Plant Physiology CBS&H, GBPUA&T Pantnagar (Uttarakhand) during the years 2021 and 2022 (February-May). Geographically experimental site is situated in subtropical region with warm and dry summer, and cool winter. For vegetative propagation, an 8–10 cm cuttings of the plants were employed, and three cuttings were inserted in each pot having area 0.456 square meters with 20 Kg soil capacity. After a month of plant establishment, pots were divided into three groups for given the water deficit treatment i.e., first group received regular irrigation (well watered), second group irrigated two times in a week (water deficit A) and third one received only one time irrigation within a week (Water deficit B). Salicylic acid (SA) was administered as a first foliar treatment with varying concentrations (25, 50, 75, 100, 125, and 150 mg/L) after one week of water deficit exposure, and a second foliar treatment was applied after one month following the first spray. The plant samples were taken after 7 days from each spray to determine the growth characteristics such as number of shoots, shoot length, leaves numbers, fresh and dry herbage yield.

The total number of erect shoots from each pot was counted after each spray. For shoot length analysis the three longest upright shoots from each replication were selected randomly and measured with the help of cm scale and expressed in cm. One of the upright shoot was selected randomly and the leaves were counted. The counted number of leaves multiplied with the total number of shoots (measured above), this help in calculating total number of leaves that later expressed in per pot. The whole plant (including leaves, stem and roots) taken for measure the fresh herbage yield at harvest after both spray. Subsequently harvested samples leave to dry in oven at 55 ºC for dry herbage yield.

Statistical Analysis

Experiment was performed in completely randomized block design **(**CRBD) with three replications for each treatment. ANOVA of collected data was calculated by using statistic software STPR15 at 5% probability level ($p \leq 0.05$).

Results

Number of shoot per pot

It has been observed that *Bacopa monnieri* under water deficit exposure showed significant decline by 18.26, 20.01% in number of shoots per pot i.e., water deficit B (61.09, 72.28) at T15 during both spray times (first and second) without SA, while water deficit A exhibit less harm on shoots numbers per pot in 2021. Similar tend also found in 2022 about water deficit impact on shoot numbers. Apart that foliar spray of salicylic acid effective with medium concentrations (75 and 100 mg/l conc.) for increment in the numbers of shoot per pot by 16.8, 8.23% at first foliar spray and 12.14, 5.81% at second foliar spray in comparison to respective controls, but higher concentration of SA (above 100 mg/l) were not showed significant improvement during 2021 and similar trend followed by 2022 also. However, salicylic acid also helps to mitigate water deficit impact on *Bacopa monneri* through improving number of shoots per pot significantly i.e. Water deficit A (84.60, 98.47) at T11 after both (first and second) foliar spray of SA with 75 mg/l over respective controls (75.23, 90.36) in 2021. Although SA higher concentration (more than 75 mg/l) was showed only slight increase in shoots number per pot over both water deficit conditions (A & B) during both spray time in 2021 and 2022 (figure 1).

Number of leaves per pot

Current investigation indicate that without SA treated water deficit (B) plants at both spray time indicated significant decline in number of leaves per pot by 27.40, 29.22% (2021) and 25.13, 22.39% (2022) in comparison to respective control. Moreover, both foliar spray of SA shown maximum enhancement in leaves numbers per pot by 31.51, 44.26% (with 75 mg/l conc.) followed by 27.23, 20.28% (with 100 mg/l conc.) at T4 and T5 when supplemented to plant individually, while lowest and highest concentrations was slightly effective during 2021. Therefore foliar application of 75 mg/l conc. of SA also indicate the enhancement in leaves number per pot by 22.76, 26.40% significantly during 2021 and 39.21, 36.81% during 2022 recorded after both (first and second) spray respectively over water deficit (A), whereas similar SA application was slightly effective over severe deficit condition B (figure 2). Similar trend was observed in 2022 also about SA influence on leaves number per pot with and without water deficit stress.

Shoot length (cm)

Shoot length considered important growth attributes which influenced easily through abiotic stress particularly from water deficit (B) exposure during present investigation and significantly lower shoot length by 5.26, 11.83% found at both spray time without SA application in T15 (8.60, 8.03 cm) over respective controls (9.07, 9.10 cm) in 2021. Although after SA foliar spray with moderate concentration (75 mg^{-1} L) at both spray time significantly improve the shoot length by 4.89, 2.71% i.e. water deficit A in T11 (9.51, 9.35 cm) and by 6.33, 2.89% in same treatment T11 (9.62, 9.49 cm) than control (9.08, 9.10 cm) in two constitutive years 2021-2022, respectively. Besides that present outcomes also revealed that alone SA application with moderate concentrations can modulate the shoot length by 5.92%, 5.33% and 4.45% followed by T4, T5, T6 (75-125 mg/l) at first spray and 4.87%, 4.06% and 3.26% in same SA concentrations after second spray respectively over controls during 2021. While lowest and highest SA concentrations have not significant effect on Bacopa shoot length in water deficit and normal conditions. Similar influence of SA application was followed by 2022 in shoot length when spray on stress and non-stress plants after both times (figure 3).

Fresh herbage yield (g/pot)

Bacopa monnieri under water deficit exposure showed

significant decline in fresh herbage yield by 15.30, 18.51% i.e., water deficit B (61.80, 70.81 gm per pot) at T15 during both spray times (first and second) without SA, while water deficit A exhibit less harm in comparison to respective controls (72.97, 86.90 gm per pot) during 2021. Similar tend also found in 2022 about water deficit impact on fresh herbage yield. Apart that foliar spray of salicylic acid effective with medium concentrations (75 and 100 mg/l conc.) for increment in the fresh herbage yield gram per pot by 17.42, 16.66% at first foliar spray and 19.09, 9.83% at second foliar spray followed by T4 and T5 Treatments in comparison to respective controls, but higher concentration of SA (above 100 mg/l) were not showed significant improvement during 2021 and similar trend followed by 2022 also. However, salicylic acid showed mitigation effect during water deficit (A & B) exposure on *Bacopa monneri*, significant increase in fresh herbage yield by 11.69% and 10.09% after first spray when applied SA with 75 mg/ l, similar increment also recorded during second spray time with same concentration over respective controls in 2021. Although SA high concentration (above 75 mg/l) was showed only slight increase in fresh herbage yield insignificantly over both water deficit conditions (A& B) during both spray time in 2021 and 2022 presented in figure 4.

Dry herbage yield (g/pot)

Our results showed that without SA treated water deficit (B) plants at both spray time indicated significant drop in dry herbage yield by 27.66, 26.02% (2021) and 26.35, 24.81% (2022) in comparison to respective control. Moreover, both foliar spray of SA shown maximum enhancement in dry herbage yield (g/pot) by 46.24, 24.41% (with 75 and 100 mg/L conc.) at first spray followed by second spray times with same SA concentrations when supplemented to plant individually, aside from lowest (up to 50 mg/l) and highest (above 100 mg/l) concentrations was slightly effective during 2021. Therefore SA also intensify the dry herbage yield (g/pot) by 26.87, 41.47% (with of 75 mg/l conc.) followed by 22.03, 17.00% (with 50 mg/l conc.) significantly during 2021 and 36.71, 32.58% (with 75 mg/l conc.) followed by 23.88, 6.12% (with 100 mg/l conc.) during 2022 recorded after both spray (first and second) respectively over water deficit (A). Furthermore, SA application in similar way influenced the dry herbage yield (g/pot) under severe deficit condition B during 2021 and 2022 (figure 5).

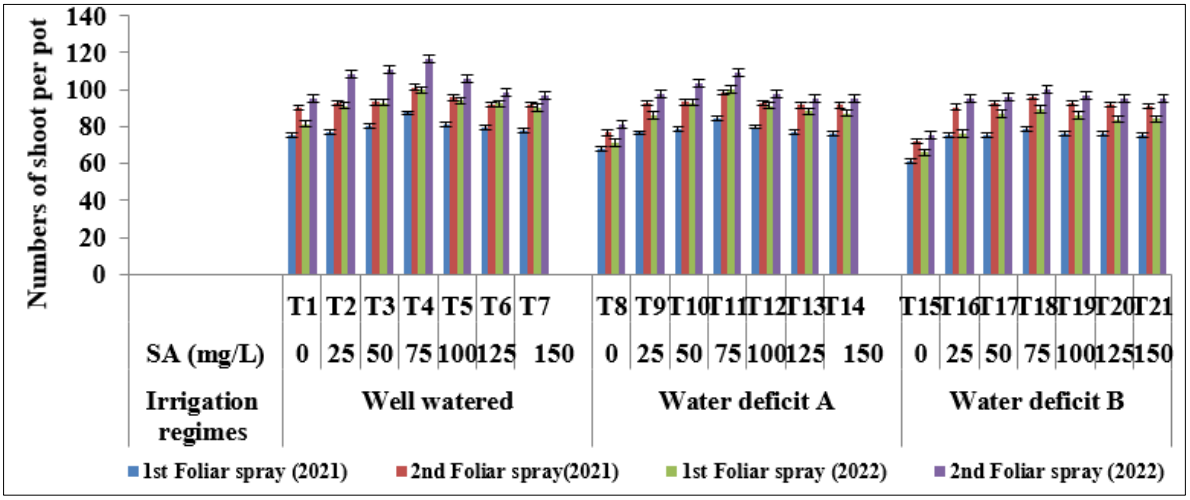

Fig 1: Effect of salicylic acid application on a number of shoots per pot in *Bacopa monnieri* (L.) at first and second foliar spray during 2021 and 2022 under different irrigation regimes. Vertical bars indicate ± standard error of mean (n=3).

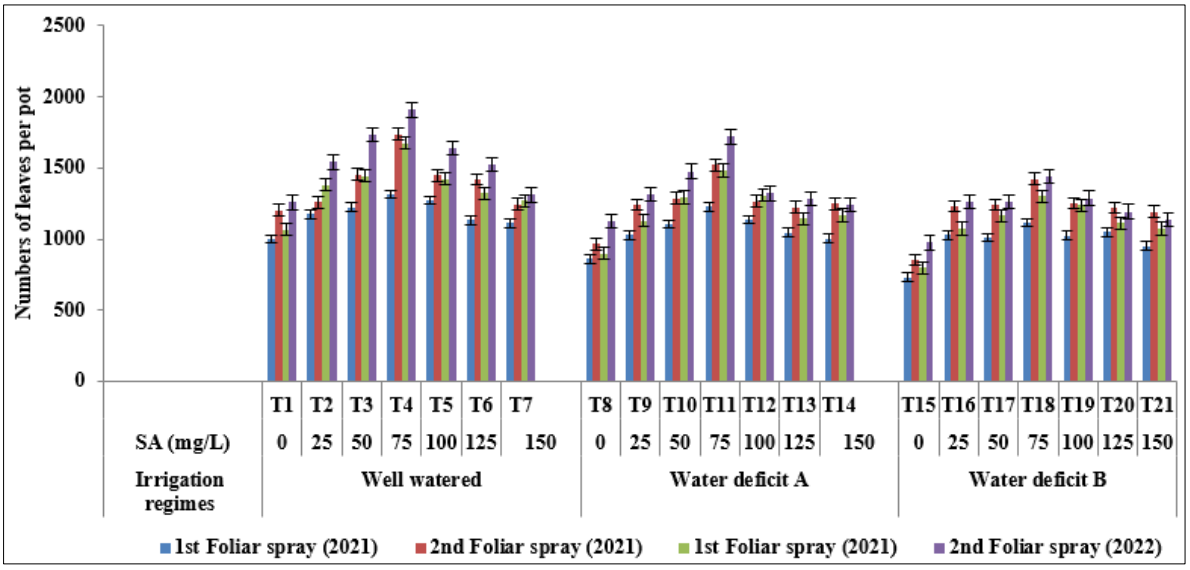

Fig 2: Effect of salicylic acid application on a number of leaves per pot in *Bacopa monnieri* (L.) at first and second foliar spray during 2021 and 2022 under different irrigation regimes. Vertical bars indicate ± standard error of mean (n=3).

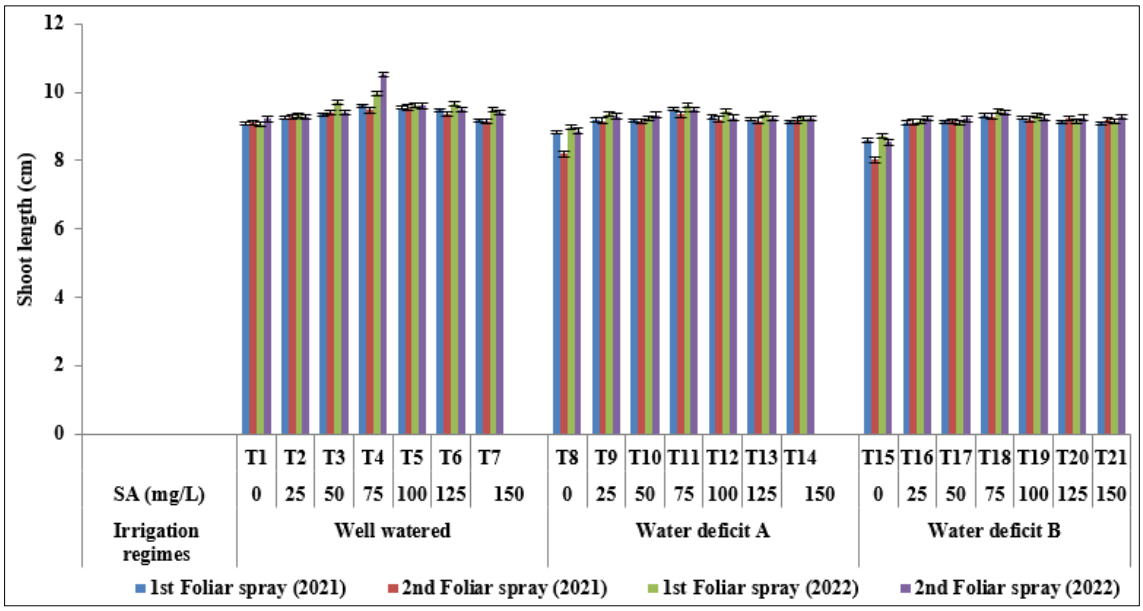

Fig 3: Effect of salicylic acid application on a number of shoots length (cm) in *Bacopa monnieri* (L.) at first and second foliar spray during 2021 and 2022 under different irrigation regimes. Vertical bars indicate ± standard error of mean (n=3).

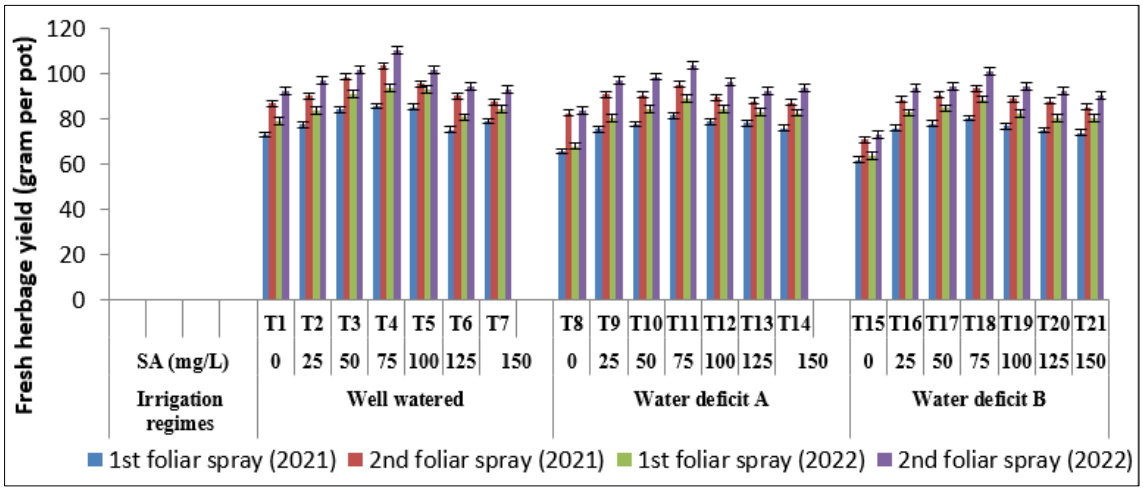

Fig 4: Effect of salicylic acid application on fresh herbage yield (g/pot) of *Bacopa monnieri* (L.) at first and second foliar spray during 2021 and 2022 under different irrigation regimes. Vertical bars indicate ± standard error of mean (n=3).

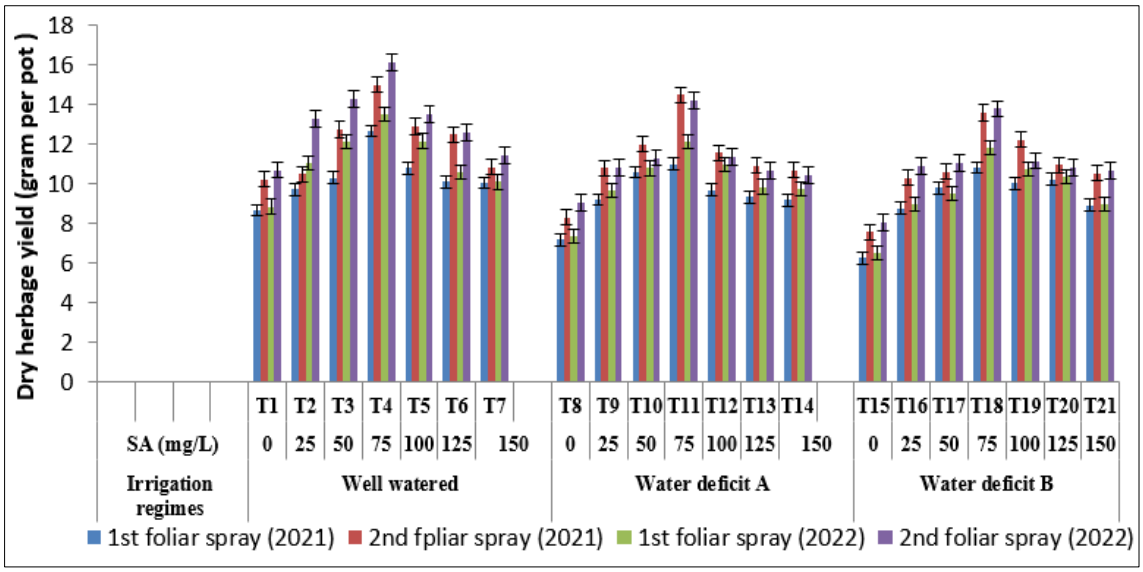

Fig 5: Effect of salicylic acid application on dry herbage yield (g/pot) of *Bacopa monnieri* (L.) at first and second foliar spray during 2021 and 2022 under different irrigation regimes. Vertical bars indicate ± standard error of mean (n=3).

Discussion

Water deficit prominently caused the imbalance in osmotic status of plants which provoke the alteration in plant physiology accompanied by modulation in the metabolism; occurred stomatal closure and reduction in photosynthetic rate ultimately diminished the plant growth (Ludlow and Muchow, 1990) [17]. Besides that a reduction in meristematic activity might be linked with a fall in leaf turgor pressure influenced by water deficit consequently negatively affects cell development and growth in the shoot region of plants (Muller *et al*., 2011) [19]. Earlier research exhibited *Bacopa monnieri* (L.) response towards water deficit situation induced by osmotic imbalance (excess salt exposure). When Bacopa shoots exposed to excess (200 mM) salt contain medium, significant decline in shoots number were noticed after 28 days of inoculation in comparison of control (0 mM). The plant ability to maintain turgor abolished due to water deprive induced by osmotic stress which may affect the metabolic processes, and thus the growth of a plant hamper (Ahir *et al*., 2013) [4]. It was also showed that excess NaCl (100 mM conc.) contained culture medium lead stunted shoot growth of *Bacopa monnieri* might be linked with osmotic imbalance (Dogan, 2020) [9] Additionally another study also explained similar negative impact from water deficit exposure on growth parameters such as leaves number, stem length and ultimately yield of Milk thistle (Farhadi *et al*., 2016) [10]. The negative consequence of reduced irrigation was reported on Bacopa plant when given water after 5 days instead regularly. Results showed that the final yield (dry biomass) of plant declined significantly under prolong reduced water situation (Singh *et al*., 2021) [28]. Impaired photosynthesis is result of negatively modulate leaf gas exchange attributes due to water deficit stress consequently reduces the biomass yield. Researcher identified similar negative impact from water deficit (-1.3 MPa) induce stress on leaves numbers and biomass in *Aristotelia chilensis* (González-Villagra *et al*., 2022) [11]. Our results also showed maximum decline in growth parameters i.e. shoot length, numbers of shoot and herbage yield (fresh and dry) when plant were exposed to severe water deficit (B), while moderate water deficit (A) exhibit lessen deterioration on *Bacopa monnieri*. In recent years, the use of plant growth regulators (PGRs) to strengthen crop tolerance and resilience to drought has been investigated as a potential tactic. Phytohormones are significant signaling molecules that can also counteract the negative effects of drought by modifying the physiological and biochemical responses in plants (Ulfat *et al*., 2017; Jahan and Rautela, 2022) [30, 13]. Number of research supported the PGRs (Plant growth regulators) i.e. salicylic acid (SA) supplementation to recover the growth and yield of plants over water deficit exposure. Because outcomes of research reports reveled that SA exogenous application with appropriate amount influenced the growth by improving photosynthesis as well as the antioxidants and osmolytes synthesis which help to maintain turgor under water deprivation (Raza, *et al*., 2019) [22]. Another studies also showed SA potency to improve growth characteristics of sesame plants through enhance endogenous ethylene level which linked to an increase in cell division in the meristematic regions. Besides that improve antioxidants increased the detoxification of ROS leading improve growth and productivity under water limited conditions (Najafabadi and Ehsanzadeh, 2017) [20]. Similar impact of SA with 3 mM conc. was able to mitigate water deficit induced oxidative stress in *Rosmarinus officinalis* through modulating anti-oxidative mechanism as well as revive the growth (Abbaszadeh, *et al*., 2020) [1]. Many researchers also reported similar efficacy of exogenous SA application to increase growth of plants in terms of height and number of leaves over water deficit in Carthamus tinctorius, *Ammi visnaga* and *Egletes viscosa* (Shaki *et al*., 2018; Osama *et al*., 2019; Batista *et al*., 2019) [25, 21, 6]. These results are in line with SA foliar application used with moderate concentration (75-100 mg/l) amended the growth attributes significantly in stress as well as normal conditions during present investigation. In contrast highest concentration of SA (above 100 mg/l) was insignificant about improvement of growth attributes over stress and normal. It is well known that plants cause stomatal closure under drought stress to prevent water loss, contrary to restrict the CO_2 assimilation and constrain the growth rate due to impaired photosynthesis process. According to recent study SA (with 2 mM concentration) foliar spray improve the photosynthesis rate over water stress (75% field capacity) impact in comparison to control (100 field capacity) plant of *Impatiens walleriana* (Safari *et al*., 2021) [24]. In addition Largia *et al*., (2015) [15] explained the implementation of SA elicitation effect on dry matter of Bacopa in *in-vitro* grown shoots. The SA with 75-100 µM concentration was found most effective to improve the dry mass of 30 days old shoots. In addition Sharma *et al*., (2015) [26] used salicylic acid (SA) as elicitors to study the impact on *Bacopa monnieri* during *the in-vitro* experiment. Following a 9-day exposure period, cultures exposed to 50 µM SA raised the dry weight (8.14 mg/g) over control. Similarly SA (0.5 mM) influence on fresh, dry weight and grain yield recorded in *Foeniculum vulgare* when subjected 35% and 75% soil moisture conditions (Askari and Ehsanzadeh, 2015) [5]. However, SA application under water deficit stress prevented the growth reduction rate. The highest fresh and dry weight was obtained from the *Mentha piperita* plants with 2 mM of SA treatment under mild stress (Abdi and Karami, 2020) [2]. Furthermore, another finding indicate that increase in dry weight of wheat plants under stress in response to SA application due to the induction of antioxidant responses that protect the plant from damage and enhance the protein synthesis (Saboon *et al*., 2015) [23]. Moreover, recent study exhibited that SA (0.1 mM) could be reversed the water stress imposed negative impact via improving gas exchange and redox homeostasis in plants (Lobato *et al*., 2021) [16].

Conclusion

Bacopa monnieri thrives in wet areas or with regular watering as a perennial plant with an annual cycle of growth. During investigation Bacopa plants were grown under water deficit conditions and found declined growth and growth attributes. However, foliar supplementation of Salicylic acid in different concentrations efficiently amends the growth attributes and mitigate the harmful impact of water deficit and can be used as elicitor. Therefore, it is concluded that when SA applied in an optimum amount, can be beneficial for plants to grow even in water deficit area.

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