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Research Article

INFLUENCE OF WILLOW BARK EXTRACTS AND APPLICATION TIMES ON THE PRODUCTION OF ROSELLE

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ABSTRACT

Roselle is an herbaceous perennial and short-day plant widely recognized for its medicinal properties. It is primarily cultivated for its fiber and, more notably, for calyx production. Given its importance, an experiment was conducted to examine the effect of willow bark extracts and their application timings on the growth and yield of roselle. The study was carried out at the Ornamental Horticultural Nursery, The University of Agriculture, Peshawar, Pakistan, using a randomized complete block design with a split-plot arrangement. The experiment consisted of two factors: Factor A, the time of application (20, 40, and 60 days after transplantation), assigned to the main plot; and Factor B, concentrations of willow bark extract (0%, 2%, 4%, 6%, and 8%), assigned to the subplot. In total, 15 treatments were evaluated, each treatment replicated three times. The results revealed that plants treated with 8% willow bark extract exhibited the best performance, recording the least days to flowering (139.26), the highest chlorophyll content (45.60), maximum leaf area (143.88 cm^2), the greatest number of branches (21.11), the highest number of calyces per plant (64.55), calyx yield (4096.11 kg ha^{-1}), and seed yield (2302.56 kg ha^{-1}). The effects of 8% extract were statistically similar to those of 6% extract in most cases. Furthermore, spraying willow bark extract at 60 days after transplantation resulted in a significant increase in the number of calyces per plant (62.73) and seed yield (2285.87 kg ha^{-1}).

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INTRODUCTION

Roselle (*Hibiscus sabdariffa*) is a perennial shrub belonging to the family Malvaceae. Native to Africa, its fleshy calyces are widely consumed in beverages and teas. The plant thrives in tropical and subtropical climates but is highly sensitive to frost. Although it can tolerate partial shade, it performs optimally in open field environments. The flowers are yellowish in color, accompanied by delicate, red calyces (Ansari et al., 2013). The calyces of roselle are rich in organic compounds such as flavonoids, gallic acid, and phenolic acids, which contribute to its nutritional and medicinal value (Higginbotham et al., 2014). Its calyces are in high demand, both fresh and dried, due to their applications in the food and pharmaceutical industries (Cid-Ortega and Guerrero-Beltrán, 2015).

Roselle is widely consumed for its medicinal properties. Various drinks and beverages made from roselle are known to effectively address health conditions such as cancer, high blood pressure, and high cholesterol (Patel, 2014). Moreover, it is used in remedies for diseases like leukemia and liver disorders, primarily due to the presence of bioactive compounds such as sitosterol and tocopherol (Mohamed et al., 2012). Roselle drinks are rich in antioxidants, which enhance the ability of the body to neutralize reactive oxygen species. Regular consumption of roselle after meals has also been shown to significantly aid in weight management (Singh et al., 2017).

Various enzymes in roselle exhibit notable anti-hypertensive and anti-inflammatory properties. Its consumption has been shown to significantly lower blood pressure by inhibiting the angiotensin-converting enzyme (Herrera-Arellano et al., 2007). Furthermore, the intake of roselle calyces can regulate blood insulin levels by enhancing the insulin sensitivity of the body (Mozaffari-Khosravi et al., 2009). Roselle also possesses antibacterial, diaphoretic, sedative, anti-hypertensive, and antifungal properties, making it effective in managing kidney and gastrointestinal diseases (Alarcon-Alonso et al., 2012).

The productivity of roselle can be enhanced through the application of biostimulants, such as willow bark extract (WE). Willow bark extract contains salicylic acid (SA), which promotes plant growth and development (Mutlu-Durak et al., 2023). It regulates key physiological processes, including seed germination, flowering, and root growth, while also modulating the plant’s response to biotic and abiotic stresses. SA improves the ability of the plant to thrive under stress by activating defense mechanisms, particularly systemic acquired resistance (SAR), which strengthens the plant’s capacity to resist pests and pathogens (Volt et al., 2009).

Moreover, SA enhances flowering by regulating both the autonomous and photoperiod pathways that control this process (Martinez et al., 2004). WE is also valued as an organic fertilizer and a natural fungicide, providing a sustainable alternative to synthetic fertilizers (Deniau et al., 2019). Its use supports environmentally friendly agriculture by reducing farming costs and improving profitability.

Given the significance of roselle and the potential benefits of WE, a field experiment was conducted to evaluate the influence of willow bark extract on the growth and productivity of roselle. The study also investigated different application timings to better understand their impact on the development and yield of roselle.

MATERIAL AND METHODS

Experimental conditions

The experiment was conducted at the Ornamental Horticulture Nursery of The University of Agriculture, Peshawar, Pakistan. The Peshawar Valley has a subtropical climate characterized by four distinct seasons: autumn, winter, spring, and summer. The region receives an annual rainfall of approximately 400 mm (Rahman et al., 2019). The experimental site is situated at a latitude of 34.02ºN, longitude of 71.48ºE, and an elevation of 360 meters above sea level (Amanullah et al., 2008).

Experiment layout and treatments

The experiment was arranged in a randomized complete block design (RCBD) with a split-plot arrangement, incorporating two factors. Factor A consisted of willow bark extracts applied at five concentrations (0%, 2%, 4%, 6%, and 8%), which were assigned to the subplots, while Factor B involved the timing of application at three intervals (20, 40, and 60 days after transplantation), assigned to the main plots. There were 15 treatment combinations, each replicated three times. The plant-to-plant and row-to-row spacing was maintained at 50 cm. Treatments were randomly assigned to main and subplots using the fishbowl method to ensure unbiased results.

Field management

Field preparation was completed in March using a rotavator. A basal dose of NPK fertilizer was applied one month before sowing. Standard agronomic practices, including weeding, hoeing, irrigation, and additional fertilizer applications, were uniformly maintained across all plots. Roselle plants were harvested in November and dried in the shade to preserve quality.

Studied parameters

The parameters assessed during the experiment included:

Chlorophyll content (SPAD)

Measured using a plant chlorophyll meter on randomly selected leaves, and the average value was recorded.

Plant leaf area (cm^2)

Determined using a leaf area meter on randomly selected leaves, and the average value was calculated.

Number of branches per plant

Counted on five randomly selected plants and the average was recorded.

Days to flowering

The number of days required to achieve 50% flowering was recorded across all treatments, and the average was calculated.

Number of calyces per plant

Counted on randomly selected plants and the average were calculated.

Calyces yield (kg/ha)

Calyces yield was determined by measuring the total calyces yield per plot and converting it to yield per hectare.

Seed yield (kg/ha)

Measured similarly to calyces yield by calculating the total seed yield per plot and converting it to yield per hectare.

Willow bark extract preparation

WE was prepared using young branches of the willow tree (*Salix alba*). The bark was removed from the branches and chopped into small pieces approximately 1 cm in size. To prepare different concentrations, 20 g, 40 g, 60 g, and 80 g of chopped bark were used for 2%, 4%, 6%, and 8% WE, respectively. The bark was placed in a flask, and the total volume was adjusted to 1000 ml. The mixture was heated in a water bath at 90°C for 30 min while being continuously stirred at 400 rpm. Afterward, it was filtered through a cheesecloth. Once cooled to room temperature, the extract was ready for use (Mutlu-Durak and Kutman, 2021).

Data Analysis

Statistical and graphical analyses were performed using STATISTIX 8.1 software. Analysis of variance (ANOVA) was conducted on all data using a factorial layout within the randomized complete block design (RCBD) with split-plot arrangements. Where necessary, means were separated using the LSD test at either the 1% or 5% level of significance (Jan et al., 2009).

RESULTS

WE significantly influenced chlorophyll content, plant leaf area, number of branches, days to flowering, number of calyces per plant, calyces yield, and seed yield of roselle. The maximum plant leaf area (143.88 cm^2), number of branches per plant (21.11), early flowering (139.26 days after transplantation), number of calyces per plant (64.55), calyces yield (4096.11 kg ha^{-1}), and seed yield (2302.56 kg ha^{-1}) were observed when plants were sprayed with 8% WE, which was statistically similar to the effect of 6% WE in most cases. The highest chlorophyll content (45.60 SPAD) was recorded when the plants were sprayed with 6% WE.

In contrast, the minimum chlorophyll content (35.25 SPAD), plant leaf area (135.16 cm^2), number of branches per plant (16.11), delayed flowering (154.67 days), number of calyces per plant (52.13), calyces yield (3209.89 kg ha^{-1}), and seed yield (1535.78 kg ha^{-1}) were observed when plants were sprayed with distilled water (Table 1 and Table 2).

Mean data on application timing revealed that it significantly influenced days to flowering, number of calyces per plant, and seed yield. Early flowering (141.28 days after transplantation) occurred when plants were sprayed with WE 20 days after transplantation, with a statistically similar effect observed when plants were sprayed 60 days after transplantation (Table 1). The maximum number of calyces per plant (62.73) and seed yield (2285.87 kg ha^{-1}) was recorded when plants were sprayed with WE 60 days after transplantation. This effect was statistically similar to that observed 40 days after transplantation (Table 2).

In contrast, delayed flowering (152.14 days) was observed when plants were sprayed 40 days after transplantation (Table 1). The minimum number of calyces per plant (52.31) and seed yield (1662.46 kg ha^{-1}) was recorded when plants were sprayed 20 days after transplantation (Table 2).

The interaction between WE concentration and application timing was significant for chlorophyll content, days to flowering, and seed yield, as shown in Figures 1, 2, and 3.

DISCUSSION

Roselle is a medicinally significant plant known for its remarkable benefits in managing blood pressure, diabetes, and several other ailments. Although it is a new crop in the region, it is grown on a small scale in the country, highlighting the need to enhance its productivity. WE, which contains SA, plays a crucial role in regulating stomatal movements, influencing photosynthesis, and promoting chloroplast activity, which ultimately increases the chlorophyll content in roselle leaves (Hamad et al., 2021). Moreover, WE affect the leaf area of the plant due to its content of auxins and cytokinins, which stimulate cell division and elongation, resulting in an expanded leaf area. This increased leaf area further contributes to enhanced chlorophyll content in roselle (Ali et al., 2022).

Elbohyl et al. (2018) also reported that SA boosts the chlorophyll content in zinnia plants. WE have been shown to promote plant growth and development, providing sufficient nourishment for an increased number of branches per plant (Pandey, 2015). Furthermore, SA in WE acts as a signaling molecule that activates flowering pathways by promoting the expression of genes such as LEAFY (LFY) and APETALA1 (AP1), contributing to early flowering (Fu et al., 2020). Das et al. (2019) also observed that SA significantly influences the flowering of mango plants by affecting these same genes.

Table 1. Effect of willow bark extracts and application timing on chlorophyll content, leaf area (cm^2), number of branches per plant, and days to flowering.

Willow Bark Extract Concentrations (WE)	Chlorophyll Content (SPAD)	Plant Leaf Area (cm^2)	Number of branches $plant^{-1}$	Days to flowering
0 %	35.25C	135.16C	16.11B	154.67A
2%	38.64B	136.52BC	16.67B	151.08A
4%	40.48B	138.11ABC	19.22A	144.93B
6%	45.60A	142.05AB	19.89A	142.12BC
8%	43.82A	143.88A	21.11A	139.26C
LSD	3.3068	6.3240	2.2518	4.3480
Application time (T) (Days after transplantation)				
20	45.50	146.35	19.53	141.28B
40	37.36	136.63	18.27	152.14A
60	39.41	134.45	18.00	145.80AB
LSD	NS	NS	NS	8.3672
Interaction WE × T	* Figure 1	NS	NS	* Figure 2

Means having different letters are significantly different from each other at P≤ 0.01.

Table 2. Effect of willow bark extracts and application timing on the number of calyces per plant, calyx yield (kg/ha), and seed yield (kg/ha).

Willow Bark Extract Concentrations (WE)	Number of calyces $plant^{-1}$	Calyces yield (Kg/ha)	Seed yield (Kg/ha)
0 %	52.13C	3209.89C	1535.78C
2%	54.20C	3408.67BC	1668.00BC
4%	58.11B	3599.11B	1819.11B
6%	59.67B	4003.67A	2152.00A
8%	64.55A	4096.11A	2302.56A
LSD	3.0324	238.55	174.83
Application time (T) (Days after transplantation)			
20	52.31b	3571.73	1662.46B
40	58.14ab	3692.40	1738.13B
60	62.73a	3726.33	2285.87A
LSD	6.4015	NS	486.30
Interaction WE × T	NS	NS	* Figure 3

Means having different letters are significantly different from each other at P≤ 0.01 and P≤ 0.05.

SA is a major component of WE and serves as a plant growth regulator that promotes cell division in plants. SA enhances the number of branches per plant, which in turn contributes to an increased number of calyces per plant (Ali and Al-Atrakchii, 2022). It stimulates both cell division and elongation, thereby influencing the quality and quantity of flowers and fruits per plant (Ullah et al., 2019). Foliar application of WE led to the maximum number of branches (Table 1) and calyces per plant (Table 2), which resulted in higher calyx yield per plant. This application improved the allocation of resources to the calyces, thereby enhancing both calyx and seed yield in roselle.

When WE were applied 20 days after transplantation, it promoted early flowering in the roselle plants. The early supply of SA triggered the early expression of the flowering locus T (FT) gene, which facilitated the transmission of signals from the leaves to the shoot, prompting the transition of roselle from the vegetative to the reproductive phase (Wada et al., 2010). The later application of WE, specifically 60 days after transplantation, further enhanced the number of calyces per plant and seed yield, as more resources were available to the plant during the onset of the reproductive phase, thereby boosting the production of calyces and seeds in roselle.

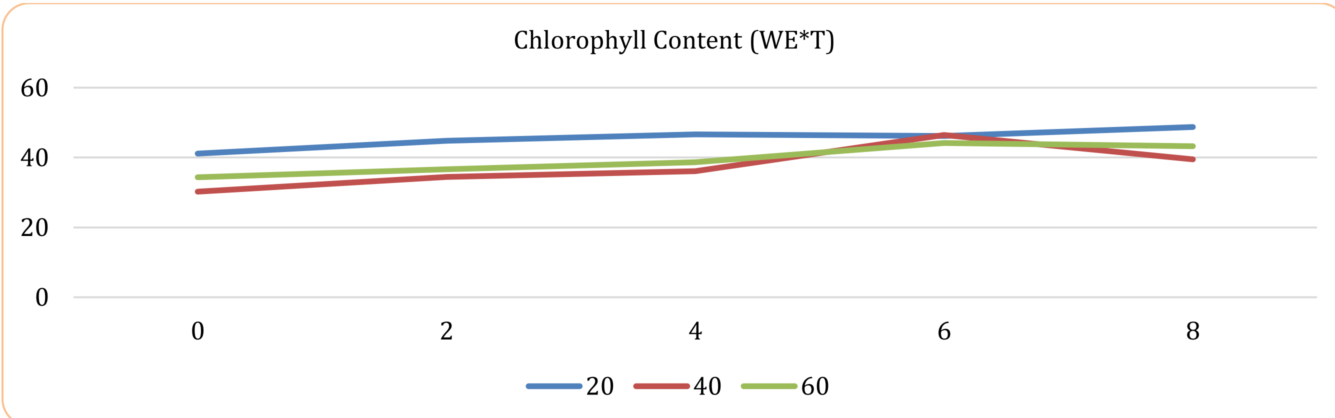

Figure 1. Interaction between willow bark extract and application timing on chlorophyll content (SPAD).

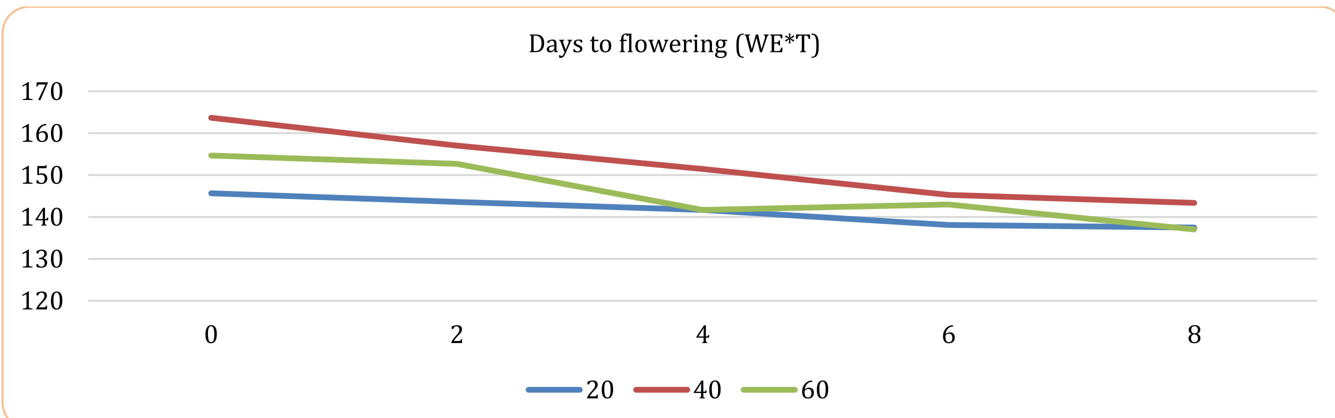

Figure 2. Interaction graph of willow bark extract concentrations and application timing on days to flowering.

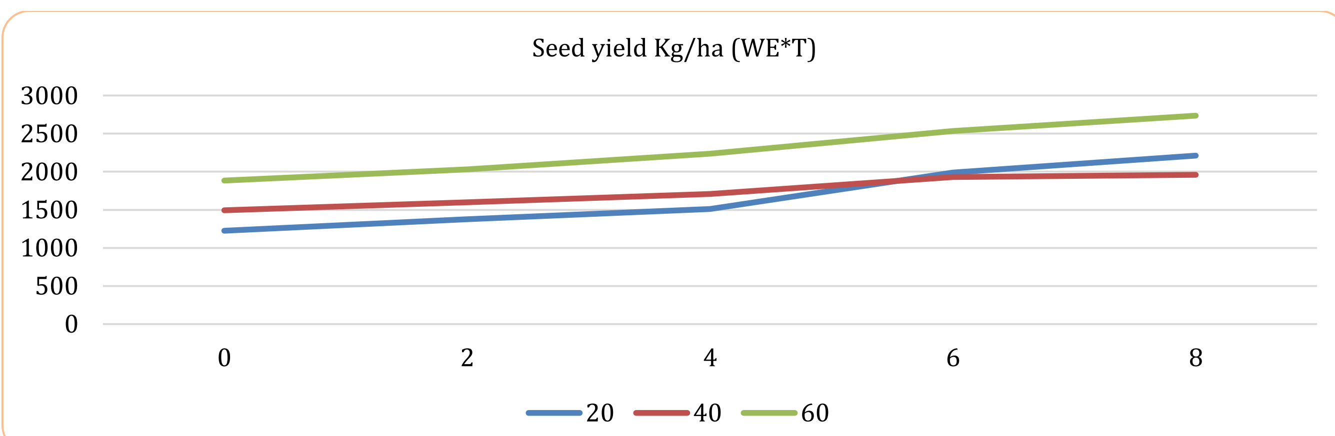

Figure 3. Interaction between willow bark extract and application timing on seed yield (kg/ha).

CONCLUSION AND RECOMMENDATIONS

The application of 8% Willow bark extract (WE) significantly enhanced various growth and yield parameters of the roselle plant, including chlorophyll content (SPAD), leaf area (cm^2), number of branches per plant, days to flowering, number of calyces per plant, calyx yield (kg/ha), and seed yield (kg/ha). The effects of 8% WE were statistically comparable to those of 6% WE in most cases. Furthermore, applying WE 60 days after transplantation resulted in the fewest days to flowering and the highest number of calyces per plant and seed yield (kg/ha). Based on these findings, foliar application of 6% WE 60 days after transplantation is recommended to improve the growth and yield of roselle. Future studies should focus on identifying sustainable practices to further enhance the growth and productivity of roselle in the region, as well as exploring the potential of other bio-stimulants to improve roselle productivity.

AUTHORS' CONTRIBUTIONS

MA designed and supervised the experiment; HA performed the experiment, collected and analyzed the data; SJ, MAK, and MK provided technical assistance in preparing the manuscript; IA provided guidance during the experiment; RZM, IK, and AB reviewed the Manuscript; MK and MA helped in conducting the experiment.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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