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Nitrogen, Phosphorous, and Potassium Application Rate on the Young Seedling Growth of *Salvadora persica*

Fawzy F. Lasheen ¹, Ahmed H. Negm ², Soheir E. Hassan ¹, Ehab Azab ^{3,*}, Adil A. Gobouri ⁴ and Mohammed Hewidy ^{1,*}

¹ Horticulture Department, Faculty of Agriculture, Ain Shams University, P.O. Box 68, Hadayek Shubra, Cairo 11241, Egypt; fawzy_lasheen@agr.asu.edu.eg (F.F.L.); so.hassn2013@agr.asu.edu.eg (S.E.H.)

² Arid Land Agricultural Graduate Studies and Research Institute (ALARI), Ain Shams University, Cairo 11241, Egypt; negm.a.h@agr.asu.edu.eg

³ Department of Nutrition and Food Science, College of Science, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia

⁴ Department of Chemistry, College of Science, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia; a.gobouri@tu.edu.sa

* Correspondence: e.azab@tu.edu.sa (E.A.); mohamed.hewidy@agr.asu.edu.eg (M.H.)

Abstract: *Salvadora persica* is the most used medicinal shrub in the Arab world. This experiment was conducted to evaluate seedling performance at different rates of NPK. Seedlings were treated with N:P:K at 4:2:2 (N:P:K 2:2:2 g + 2 g of nitrogen supplemented as ammonium sulfate), 6:3:3 (N:P:K 3:3:3 g + 3 g of nitrogen supplemented as ammonium sulfate), 2:1:1S (N:P:K 1:1:1 g + 1 g of nitrogen supplemented as ammonium sulfate), 2:1:1U (N:P:K 1:1:1 g + 1 g of nitrogen supplemented as urea), 4:1:1S (N:P:K 1:1:1 g + 3 g of nitrogen supplemented as ammonium sulfate), 4:1:1U (N:P:K 1:1:1 g + 3 g of nitrogen supplemented as urea), 6:1:1S (N:P:K 1:1:1 g + 5 g of nitrogen supplemented as ammonium sulfate), 6:1:1U (N:P:K 1:1:1 g + 5 g of nitrogen supplemented as urea) in addition to the control. The results indicated that NPK application significantly affected the vegetative and root growth parameters and the chemical contents. Among the various treatments of NPK, the application of 6:3:3 rates showed the highest values for aboveground parts, root parameters, and greenness level. Therefore, the present study demonstrates the optimum NPK levels that can enhance seedling growth throughout the nursery period.

Keywords: *Salvadora persica*; sewak; NPK; seedling; fertilization rate



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1. Introduction

The toothbrush tree (*Salvadora persica* L.), a member of the Salvadoraceae family, is a special medicinal plant. It is also known as Arak and Miswak in Arabic [1]. *S. persica*, a facultative halophyte, seems to be a potentially valuable oilseed plant for salt- and alkaline-affected soil [2]. These plants accumulate a high salt level in their cells and tissues and deal with the harmfulness of excessive salt by evolved succulence [3]. They are commonly dispersed in the dry areas of Egypt and frequently in salty soil. It is a small upright evergreen tree or shrub, with a trunk that rarely extends more than 30 inches, with a maximum of 3 m.

Plant reproduction occurs through seeds, layering, and commonly by root suckers. It can be coppiced near to the ground. Seeds are spread by birds and often germinate below other nurse bushes, such as *Capparis decidua* or *Tamarix* spp. The growth rate is somewhat slow for the first two to three years after establishing [4].

Sewak is commonly used in Islamic medicine and used by the ancient Arabs to make their teeth white and shiny. The newly flushed leaves are tender and can be eaten in salads and used as a herbal remedy for coughs, asthma, scurvy, rheumatism, and piles [5]. It has been shown to contain many beneficial compounds, i.e., salvadorine, trimethylamine,

mustard oil, sulfur, vitamin C, saponins, resins and traces of tannins, sterols, and flavonoids. The WHO advises and supports the routine of chewing *S. persica* sticks as an effective oral hygiene practice in regions where it is used traditionally. Many researchers have demonstrated the antiperiodontopathic, anticaries, antifungal, and antibacterial effects of the contents *S. persica* sticks [6,7].

The use of numerous medicinal plants is generally limited to native persons. It is of the greatest significance to preserve this information for later generations; otherwise, it will vanish forever with the loss of native healers and people with ancient health care knowledge.

Around the globe, nutritional supply is key for all plants' growth, and they must find the main three elements of fertilization, N, P, and K, but nourishment cannot be efficiently applied in a soil–plant system at proportions exceeding 50% [8]. In the growing cycle of each crop, fragmented nutrition supply is an efficient method to decrease fertilizer loss and to increase its usage [8–10].

The use of a nitrogen gypsum mixture (gypsum + 9 g of N) during the establishment and early growth stages of *S. persica* seedlings showed the best outcomes, where it produced an increase of 41% for height and 35% for crown diameter than the control [11]. In another study, all plant characteristics of *Salvadora oleoides* were enhanced with the application of 100 kg N/ha [12]. The growth of olive trees (cv. Frantoio) was increased significantly when the nitrogen application rate was increased from 250 to 1000 g/tree [13]. The optimum N and K application rates were 750 and 500 g/tree, respectively [13].

NPK fertilizer application produced significant increases in the seedling height, diameter, root length, and fresh and dry weights of the stems, leaves, and roots of *Cordia myxa* seedlings [14]. Still, using a 3:2:1 ratio of NPK followed by 6:2:1 achieved better results for plant height, stem diameter, fresh weight of leaves, stems, and roots compared to the control (without NPK) *Cupressus sempervirens* seedlings [15].

Shehata [16] found that the effect of three NPK levels on *Poinciana regia* seedlings significantly increased stem length, stem diameter, and stem fresh and dry weight using low, medium, or high fertilization rates. To the best of our knowledge, no study has been performed on the effect of NPK application rates on the toothbrush tree's seedling growth. Thus, an experiment was conducted to find the suitable rate of nutrient application at the seedling stage to produce the best plants to transfer to the open field.

2. Materials and Methods

2.1. Plant Material

Seeds of *Salvadora persica* L. were purchased from a commercial nursery in the northern Egypt. They were planted in a nursery in plastic trays. Seedlings were transplanted after 45 days, when the seedlings reached 5 cm in height and had 7 leaves, to the experimental conditions.

2.2. Media Preparation

For this experiment, seeds were planted in a media consisting of 3 kg of vermiculite, 2 kg of peatmoss, 20 mg of chelated Fe, and 1 g/L of Rizolex as a fungicide. Trays were filled with mixed media and irrigated, then covered with a plastic sheet. The cover was then removed after 3–5 days; after 10 days, the media were sprayed with 2 kg/200 L of water NPK (19:19:19), then sprayed with NPK every 15–20 days. Seeds were planted in this media and transplanted later to plastic pots (25 × 18 cm) filled with 1:1:1, peat/soil/sand, by volume. The pH of this substrate was 7.6.

2.3. Fertilization Treatments

The experiment started in March and was terminated by the end of October in an open greenhouse in two successive seasons, 2018 and 2019. The average maximum temperature was 28 °C and the average minimum temperature was 15 °C during the experimental period.

NPK fertilizer treatments were used as units per plant divided across 12 weeks as follows:

- Control treatment (without fertilizers) (control);
- N:P:K 4:2:2 (T1) (N:P:K 2:2:2 g + 2 g of nitrogen supplemented as ammonium sulfate);
- N:P:K 6:3:3 (T2) (N:P:K 3:3:3 g + 3 g of nitrogen supplemented as ammonium sulfate);
- N:P:K 2:1:1S (T3) (N:P:K 1:1:1 g + 1 g of nitrogen supplemented as ammonium sulfate);
- N:P:K 2:1:1U (T4) (N:P:K 1:1:1 g + 1 g of nitrogen supplemented as urea);
- N:P:K 4:1:1S (T5) (N:P:K 1:1:1 g + 3 g of nitrogen supplemented as ammonium sulfate);
- N:P:K 4:1:1U (T6) (N:P:K 1:1:1 g + 3 g of nitrogen supplemented as urea);
- N:P:K 6:1:1S (T7) (N:P:K 1:1:1 g + 5 g of nitrogen supplemented as ammonium sulfate);
- N:P:K 6:1:1U (T8) (N:P:K 1:1:1 g + 5 g of nitrogen supplemented as urea).

2.4. Data Records

2.4.1. Vegetative Parameters

Data were collected at the end of the experiment in both seasons. Five plants were collected from each replicate to inspect the effect of the treatments on the vegetative parameters. For each plant, we collected data on shoot height (cm), the number of shoots, the number of leaves, the fresh weight of the green parts (g/plant), and the dry weight of the green parts (g/plant). After the removal of the plants, the roots were cleaned from the soil with tap water and dried with paper tissues, and the length of the roots (cm), the fresh weight of the roots (g/plant), and the dry weight of the roots (g/plant) were measured.

2.4.2. Chemical Analysis

The plants collected were dried at 70 °C for 72 h. For mineral analysis, dried samples (shoots) were digested in sulfuric acid (H₂SO₄) and hydrogen peroxide (H₂O₂). The total nitrogen content was measured using the micro Kjeldahl method as reported by Pregl [17]. The total phosphorus content was determined using a spectrophotometer as reported by Truog and Meyer [18]. The total potassium content was assessed using a flame photometer, as described by Brown and Lilleland [19]. The greenness level of the leaves was determined using a SPAD 502 Plus Chlorophyll Meter, as described by Lavres et al. [20].

2.5. Statistical Analysis

We used a complete randomized design for the experiment. Each treatment was performed with five replicates. The results are shown as the mean ± standard deviation. Significant differences were established with one-way analysis of variance as described by Snedecor and Cochran [21]. The experimental data were analyzed using SPSS 22. Differences among means were compared using Duncan's new multiple range tests, with $p \leq 0.05$ being considered statistically significant [22].

3. Results

3.1. Effect of Fertilization Treatments on the Aboveground Parameters

Figure 1 shows the effect of the different fertilization rates on the shoot number, the shoot height, and the number of leaves. Figure 2 shows both the fresh and dry weights of the aboveground parts. The N:P:K nutrient supply had a significant effect on young sewak seedlings' aboveground parts. The number of shoots was significantly increased with the highest level of N in the application rates T2 (6:3:3) and T8 (6:1:1U) when compared to the other treatments in both seasons (Figure 1a). In contrast, the lowest number of shoots was observed when plants were not treated (control) or treated with a low dose of T3 (2:1:1S) and T4 (2:1:1U). Seedling heights were three-times higher using the T8 application rate (6:1:1U) than the control (Figure 1b). Combined increases in P and K did not increase the height compared to the control. The lowest plant heights were observed with the T3 application (2:1:1S) rate. The number of leaves was relatively increased when using the T8 (6:1:1U) application rate than the other treatments and the control in both seasons (Figure 1c). The application rates of T6 (4:1:1U) and T8 (6:1:1U) in both seasons showed a significant increase in the fresh and dry weight of the aboveground parts. The fresh and dry weights of the aboveground parts were increased ten-times more than the control,

showing the importance of nutrient supply at the early stages of seedling growth compared to natural situations in arid zones (Figure 2). The use of the T1 (4:2:2) and T2 (6:3:3) application rates with increased P and K doses showed a result closer to the T6 (4:1:1U) and T8 (6:1:1U) application rates. The control had the lowest fresh and dry weight values for the aboveground parts.

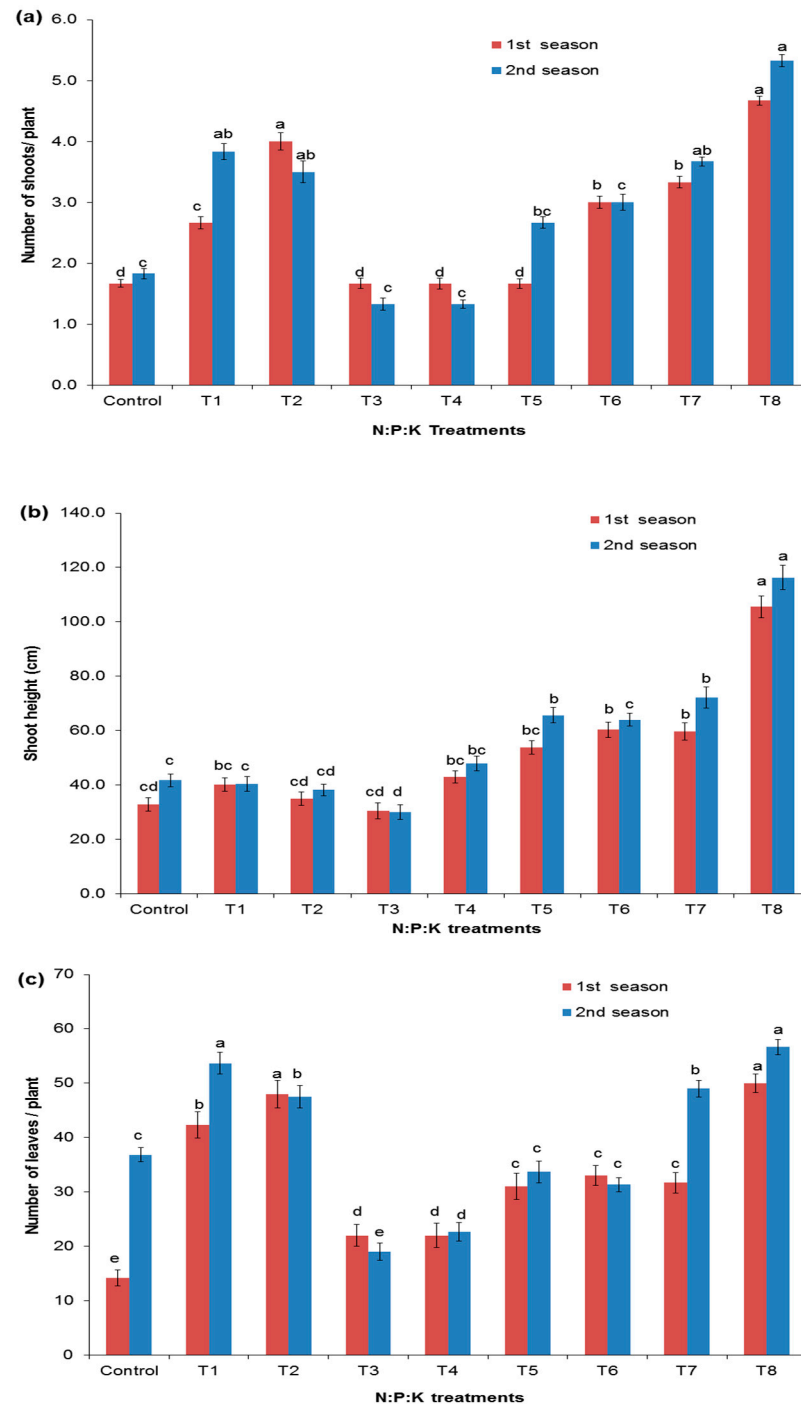


Figure 1. Effect of NPK treatments on the number of shoots (a), the shoot heights (b), and the number of leaves (c) of *Salvadora persica*. Dissimilar letters designate the significance among means ($p < 0.05$) using Duncan's multiple range test (means \pm standard errors ($n = 5$)) for the different fertilization treatments.

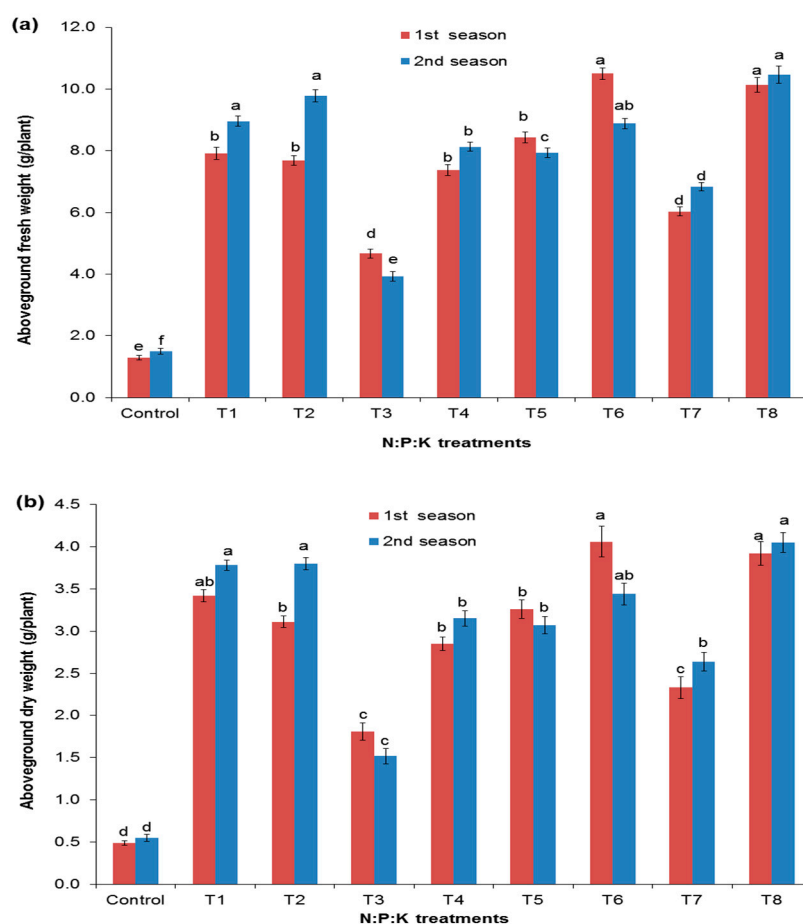


Figure 2. Effect of NPK treatments on the fresh (a) and dry weight (b) of the aboveground parts of *Salvadora persica*. Dissimilar letters designate the significance among means ($p < 0.05$) using Duncan's multiple range test (means \pm standard errors ($n = 5$)) for the different fertilization treatments.

3.2. Effect of Fertilization Treatments on the Root Parameters

Figure 3 shows that the root length and the root fresh and dry weights were significantly affected by NPK application. Both the root fresh and dry weights demonstrated higher values from the T1 (4:2:2) and T2 (6:3:3) application rates in both seasons (Figure 3a,b). Meanwhile, the control had the lowest root fresh weight and dry weights. The root lengths were significantly increased with the T1 (4:2:2) and T2 (6:3:3), T5 (4:1:1S), and T7 (6:1:1S) application rates in both seasons, respectively (Figure 3c). The control had the lowest root lengths in both seasons.

3.3. Chemical Contents of the Aboveground Parts

Figure 4 shows that the leaf greenness was significantly affected by NPK doses. The NPK application rates of T1 (4:2:2) and T2 (6:3:3) in the first season (47.3 and 45.0) and in the second season (48.0 and 45.9), respectively, had the highest greenness values. In contrast, the control had the lowest greenness values (27.7 and 28.0 for the first and second seasons, respectively).

Table 1 shows that the N% in both seasons was affected by NPK application. The T1 (4:2:2) application rate achieved a higher N% (2.07%) than other application rates in the first season. In the second season, the non-treated control achieved a higher N% (2.83%) compared to other NPK application rates. There was no significant difference in the P% among treatments. The NPK application rates affected the K%; the T1 (4:2:2), T4 (2:1:1U), T5 (4:1:1S), and T8 (6:1:1U) application rates had a significant increase in K% compared to

the control in the first season, whereas K% in the second season did not show significant differences among NPK application rates and controls.

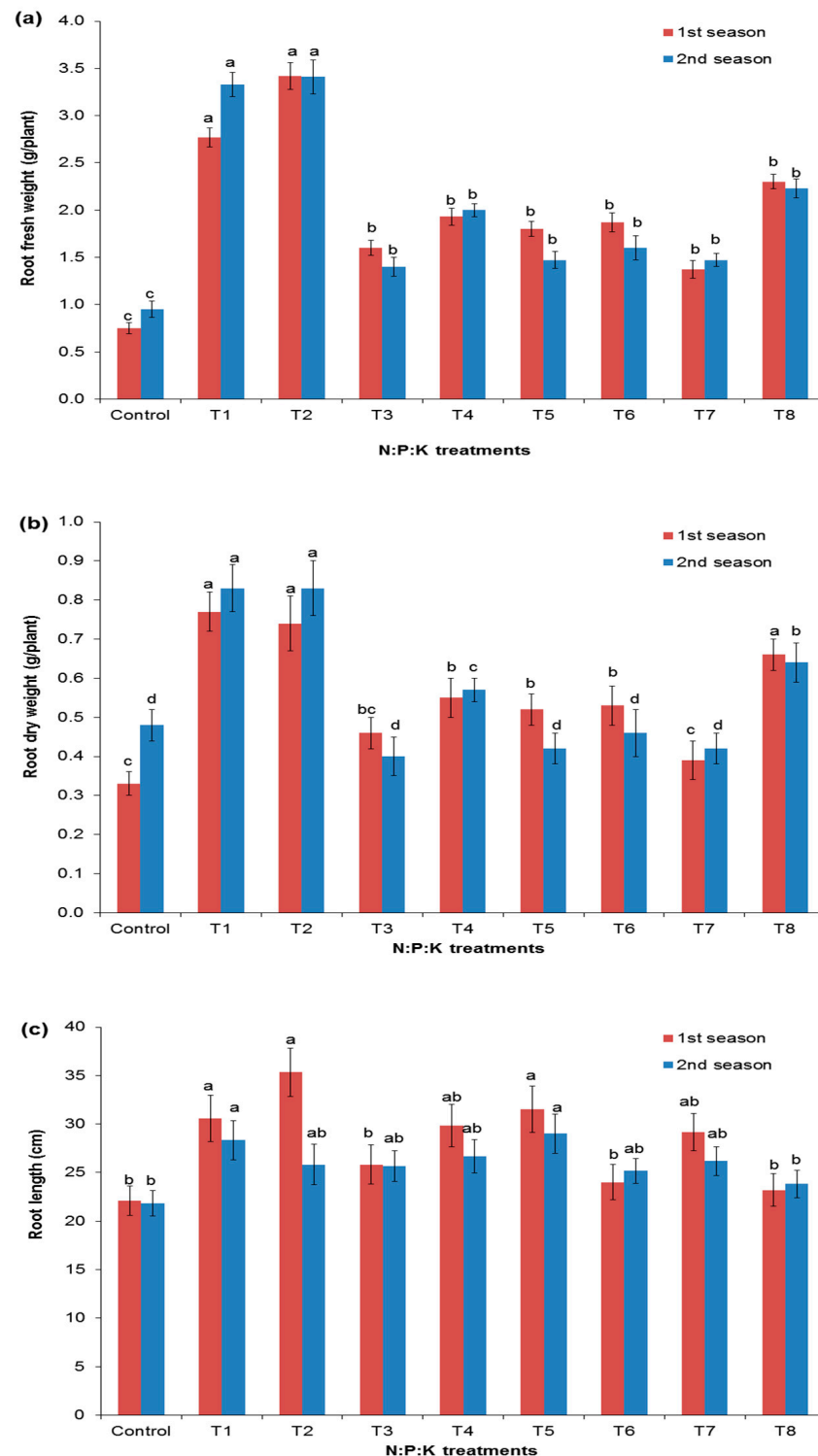


Figure 3. Effect of NPK doses on the root fresh weights (a), the root dry weights (b), and the root lengths (c) of *Salvadora persica*. Dissimilar letters designate the significance among means ($p < 0.05$) using Duncan's multiple range test (means \pm standard errors ($n = 5$)) for the different fertilization treatments.

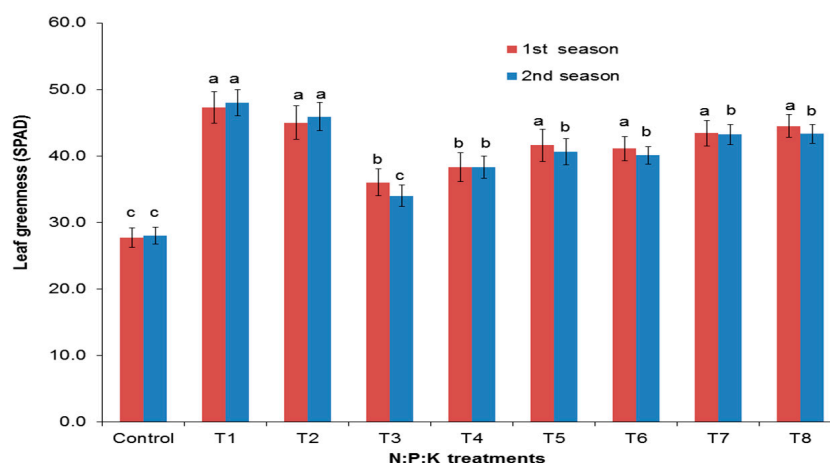


Figure 4. Effect of NPK doses on *Salvadora persica* leaf greenness. Dissimilar letters designate the significance among means ($p < 0.05$) using Duncan's multiple range test (means \pm standard errors ($n = 5$)) for the different fertilization treatments.

Table 1. Effect of NPK doses on the vegetative parameters' percentage of nitrogen, phosphorous, and potassium contents in *Salvadora persica*.

N:P:K (g)	Nitrogen (%)		Phosphorus (%)		Potassium (%)	
	First Season	Second Season	First Season	Second Season	First Season	Second Season
Control	1.62 c	2.83 a	0.25 a	0.36 a	1.2 c	2.2 ab
T1	2.07 a	1.81 c	0.30 a	0.27 a	2.1 a	2.5 a
T2	1.76 b	1.77 c	0.25 a	0.27 a	1.8 b	2.1 b
T3	1.61 c	1.61 d	0.25 a	0.36 a	1.2 c	2.2 ab
T4	1.82 b	1.82 c	0.28 a	0.33 a	2.5 a	2.2 ab
T5	1.95 b	1.95 b	0.30 a	0.27 a	2.1 a	2.5 a
T6	1.90 b	1.90 b	0.25 a	0.27 a	1.8 b	2.1 b
T7	1.80 b	1.80 c	0.25 a	0.36 a	1.2 c	2.0 ab
T8	1.87 b	1.87 c	0.26 a	0.33 a	1.5 a	2.0 ab

Dissimilar letters in the same column designate the significance among means using Duncan's multiple range test ($p < 0.05$).

4. Discussion

The development level of *S. persica* is very slow throughout the first two to three years of growth, requiring a significant intervention for its growth during the early stages. The plant reaches maturity after seven to nine years. In contrast, trees raised from root suckers bear fruit earlier, in approximately five to six years [23].

Ponder [24] found that NPK significantly increased the plant height and stem diameter in fertilized seedlings of *Eucalyptus saligna* and *Juglans nigra* plants, and NPK fertilizers produced significantly taller seedlings of *Eucalyptus tereticornis* than the controls. Krohn [25] and Sundralingam [26] demonstrated that the plant height of *Tectona grandis* responded significantly to NPK treatment compared to the controls. El-Khateeb showed that plant height was significantly increased in the seedlings of *Eucalyptus torquate* and *E. angulosa* at an NPK rate of 25:10:15 [27].

Hassan and Dey [28] demonstrated a significant increase in the aboveground parameters of *Tectona grandis* when NPK was used at 25 g/plant; Alberto [29] showed similar results in *Casuarina equisetifolia*. Additionally, Habba [30] achieved similar results using a 15:15:10 NPK application rate and *Melia azedarach* seedlings, and Krishnamurthy and Vijayan [31] using 15 g/seedling of nitrogen and *Eucalyptus tereticornis* seedlings. At a large scale, Nasr et al. [32] found that the seedlings of *Atriplex* species and *Leucaena leucocephala*

treated with 30:15:15 kg/4200 m² had higher plant growth rates [14]. Plants respond according to their needs. *Cordia myxa* seedlings had better growth with 6:2:1 NKP application rates, whereas, for *Cupressus sempervirens* seedlings, an application rate of 3:2:1 produced a better performance [15]. Improvements in plant characteristics have also been seen with the application of various fertilization rates in *Poinciana regia* [16], *Casuarina*, *Eucalyptus* [33], *Melie azedarach*, *Acacia cyanophylla*, *Casuarina glauca*, *Eucalyptus camaldulensis*, and *Taxodium distichum* [34] seedlings.

In the current research, N application had an encouraging effect on root development, which is in agreement with previous results showing that N has a significant influence on root development, biomass, turn over [35], root development and expansion [36], and greater root-order expansion and branching [37]. The use of N fertilizer can likewise affect the efficiency of water use by manipulating root progress and spreading [38], and an ideal N supply level improves root length and diameter [39], whereas higher or lower N levels have been found to decrease root growth and biomass [40]. Furthermore, N application meaningfully enhanced the diameter of *Larix gmelinii* root tips [41] and the development, root length, and root diameter of *Pongamia pinnata* seedlings [42] and *Acer mono* seedling [38].

Negreiros et al. [43] found a difference in plants' responses to an increase in soil fertility, where the development factors of *Collaea* and *Calliandra* increased with increasing soil richness, while no differences were observed for *Mimosa* and *Chamaecrista*. These species are adapted to low fertility, similarly to in *S. persica*. NPK applications at a higher rate increased the root system's development, in agreement with Razaq et al. [39]; N and P application can increase seedling strength and growth during the nursery period.

Fernández-Escobar et al. [44] found that the leaf N concentration of olive trees was higher when N fertilization was applied to soil (0–1 kg/tree). Low doses of N should be applied through localized fertilization throughout the year. In agreement with the previous findings, Ahmed and Aly [45] showed that all fertilization treatments significantly increased the height, stem diameter, fresh and dry weights of the leaves, stems, and roots of *Acacia saligna* seedlings. Diwivedi et al. [46], on *Jasminum grandiflorum*, found that different N levels of each source significantly increased various vegetative growth traits, such as plant height, stem diameter, leaf area, and the fresh and dry weights of leaves. *Azadirachta indica* seedling characteristics showed an upward trend with a gradual increase in NPK fertilization rate [47]. The application of N stimulates chlorophyll production by increasing the amount of stromal and thylakoid proteins in leaves [48] and increasing the number of chloroplasts during leaf growth [49]. The greenness of leaves depends on P concentration, since it stabilizes the plant as the environmental conditions change [50]; the biochemical features [51] and biosynthesis of pigment molecules depend on ideal P levels [52].

5. Conclusions

The current work shows that NPK fertilization rates significantly influence the growth and root morphology of *S. persica* seedlings. The control seedlings showed inferior plant growth, root characteristics, and greenness levels. The optimal seedling values were obtained using the T2 6:3:3 NPK application rate. Therefore, the results of the present study suggest that optimal NPK levels can ensure the production of vigorous and healthy *S. persica* seedlings. Several gaps and research priorities in medicinal shrub nutrition have been identified, which need to be addressed in the future.

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