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Asian Journal of Plant Science and Research, 2014, 4(6): 47-51

ISSN : 2249-7412
CODEN (USA): AJPSKY

Effect of Alfalfa (*Medicago sativa*) organic extract on the growth of pencil Cedar (*Juniperus procera*) seedlings

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ABSTRACT

A natural growth stimulant, triacontanol can be extracted from commonly grown alfalfa, which might be used as a growth promoter for seedlings. The objective of this study was to determine the effect of alfalfa crude extract on growth of J. procera seedlings. 15 g of equal mixed alfalfa leaves and shoots were soaked in one litter of tap water for 24 hrs, 36 hrs, 48 hrs, 60 hrs and 72 hrs. A 200 ml extract was applied on factorial arranged J. procera seedlings at 5 days, 10 days, 15 days and 20 days interval in Randomized Complete Block Design with 6 replications. J. procera seedling height and dry weight was significantly affected by soaking time and application interval. However, both the height and dry weight were not significantly affected by the interaction effects of soaking time and application interval of the alfalfa water extract. The results of the study have revealed that application of alfalfa water extract could be used as a growth promoter to increase seedling length and dry weight of the seedlings.

Key words: Alfalfa, Growth regulator, Triacontanol, Pencil Cedar, Juniperus

INTRODUCTION

It is generally true that Ethiopia indigenous trees are highly threatened. On the existing plantation activities our people are sticking to exotic species than indigenous species because of the fact that exotic species are fast growing. However, unlike exotic species, indigenous tree species are environmentally friendly, with so many other special benefits like high timber quality. One of the indigenous tree *Juniperus procera* is known to be a valuable timber tree in Ethiopia. It is used as poles, posts, firewood, medicine, ornamental, shade windbreak etc and is termite resistant species [1].

The achievement and activities of forest development are highly dependent on the availability of high quality and quantity of seedlings. However, it is difficult to fulfill these as most indigenous tree seedling are need a long time in the nursery. It is obvious that the more the seedlings are stay in the nursery the more will be the difficulty to fulfill the required nursery activities such as protecting the seedlings from hazard conditions like water deficit and disease problems. In addition, slow growth of seedlings increase the cost of nursery operations. As a result, creating a condition for rapid growth of seedlings is important especially for those slow growing indigenous tree seedlings of Ethiopia.

Commercially available growth-regulators can be mentioned as an option to increase the growth rate of plants [2]. However, these synthetic growth regulators are expensive and not available as required in developing countries like Ethiopia. Alternatively, plant extracts such as alfalfa crude extracts are known to have similar growth stimulating effects. A natural growth stimulant can be extracted from commonly grown alfalfa. This achieved by mulching with alfalfa hay or watering plants with extract made by soaking alfalfa in water [2] [3] [4].

Alfalfa is the most important, drought resistance, perennial forage legume. It can grow at wide range of agro ecologic conditions [5]. Its pellet extract can be used as growth stimulant [2]. Several crops are known to accumulate dry weight rapidly following applications of small amounts of alfalfa under greenhouse and growth chamber conditions [6]. Coarsely chopped alfalfa hay used as band application increased growth and yield of cucumbers, lettuce, tomatoes, and wheat [6]. The chemical that causes the stimulation of this growth has been shown to be the alcohol triacontanol [2] and Triacontanol (TRIA) was first isolated from shoots of alfalfa [7].

In some cases one concentration or amount of a growth regulator stimulates growth and a different concentration or amount restricts growth rate [8]. Both TRIA isolated from alfalfa and synthetic TRIA were active at extremely low concentrations [9]. Too much seems to reduce the stimulating effect [2]. As a result, it is important to determine the appropriate concentration for the plant in concern. Therefore, the objective of this research was to determine the effect of alfalfa organic water extract on the growth of J. procera seedlings.

MATERIALS AND METHODS

Experimental site

The experiment was conducted at Mertule Mariam Agricultural Technical and Vocational Education and Training (MMATVET) College. The college is situated in Amhara National Regional State, Northwest of Ethiopia. The college is located at 10O50' latitude and 38O16' longitude with elevation of 2850 meter above sea level. The seedlings were grown in plastic house with an average night temperature of 10.7OC (Table 1). The day temperature was adjusted by natural air circulation to be 25±2 OC.

Table 1: The average night temperature

Months	Average minimum temperature
May	11.7
June	10.8
July	9.8
August	10.4

Treatment and experimental design

The seed of alfalfa cultivar # 6984 was obtained from International Livestock Research Institution and grown at the college crop field. The alfalfa was cut at about 10% flowering stage. Both the stems and the leaves were dried separately and uniformly until they attained constant weight and they were stored in air ventilated room at ambient temperature (25±1OC) for later use. For treatment application equal weight of dry leaves (blades and petioles) and stems were mixed until the desirable treatment weight achieved. In the other hand, seedlings of pencil cedar were raised in seedling bed and transplanted into equal volume seedling pots. The pots were filled by equal amount of top soil. Based on height, similar seedlings were assigned in to the same experimental block. Treatments were assigned randomly after the seedlings adapted the plastic house condition.

Treatments comprised of factorial combinations of 5 levels of soaking time (24, 36, 48, 60 and 72 h) of alfalfa extract, 4 levels of spray intervals of the extract (5 , 10 , 15 and 20 days) and four extract untreated controls.. The treatments were applied to each experimental unit for 90 days. Equally mixed 15g equally mixed leaf and stem of alfalfa were soaked in 1lt tap water at room temperature, the residue was removed and 200 ml alfalfa extracts was used to spray each seedling. The controls were sprayed with water at each application interval.

Data collection

Seedling height was the height in cm from the ground level to the top of the shoot measured every 15 days starting from treatment application date until 90 days. The seedlings were uprooted with caution 105 days after treatment application and soil was carefully removed and washed. The seedlings (shoot and root) were chopped and dried to a constant weight and dried tissues were weighed using sensitive balance and expressed as total seedling dry weight in g.

Statistical design and analysis

The data were processed with Microsoft Excel 2007. The obtained data was analyzed using the analysis of variance procedure through Minitab (Minitab Inc. Minitab for windows release 12.12) computer program. In addition, Sigma Plot 10.0 was used to drawn graphs.The Means of treatments were distinguished using Duncan's Multiple Range Test at $p \leq 0.05$ [10]

RESULT AND DISCUSSION

Seedling height

The seedling height of Juniperus procera was significantly affected by soaking time ($p<0.01$) starting from 45 days up to 90 days after treatment application. Application interval significant effect was shown at the beginning of growth stage and during the final stages. However, the height was not significantly ($p<0.05$) affected by the interaction effects of soaking time and application interval of the alfalfa water extract starting from 15 days up to 90 days after treatment application (Table 2).

Table 2: Mean square for seedling height and dry weight of *Juniperus procera* subjected to alfalfa water extract

Source of Variation	df	Mean square						
		Seedling height (cm)						Dry Weight (g)
		15	30	45	60	75	90	
A	3	0.54^{ns}	5.37^{**}	10.43^{***}	10.29^{***}	27.49^{***}	46.07^{***}	3.64^{***}
B	5	4.34^{*}	8.75^{***}	3.44^{ns}	4.41^{ns}	12.45^{*}	20.11^{***}	33.92^{ns}
AXB	15	0.83^{ns}	1.51^{ns}	2.01^{ns}	2.21^{ns}	2.63^{ns}	4.19^{ns}	7.57^{ns}
Error	115	1.11	1.29	1.61	1.89	2.37	2.70	2.21

*A=soaking time; B=Application Interval; ns=Non-significant; *=significant at P<0.05; **= significant at P < 0.01; ***= significant at P<0.001*

The tallest seedlings were recorded in treatment combination of 36ST x 10AI, which was about 35% increase over the untreated control. The second highest seedling height was recorded by combinations of 24 ST by 15 or 20 AI, which were increase the seedling height similar to the highest record. The least seedling height was recorded in the untreated seedlings throughout the three months growth period (Fig 1 and Fig 2).

Figure 1: Effect of soaking time on *Juniperus procera* seedling average height

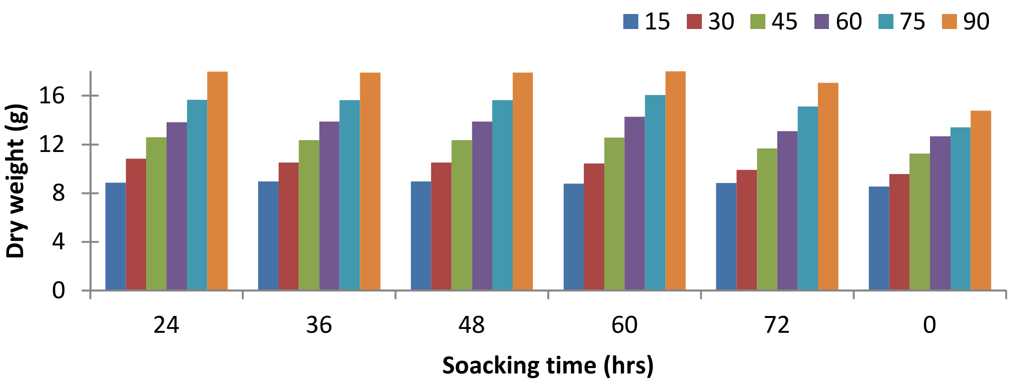
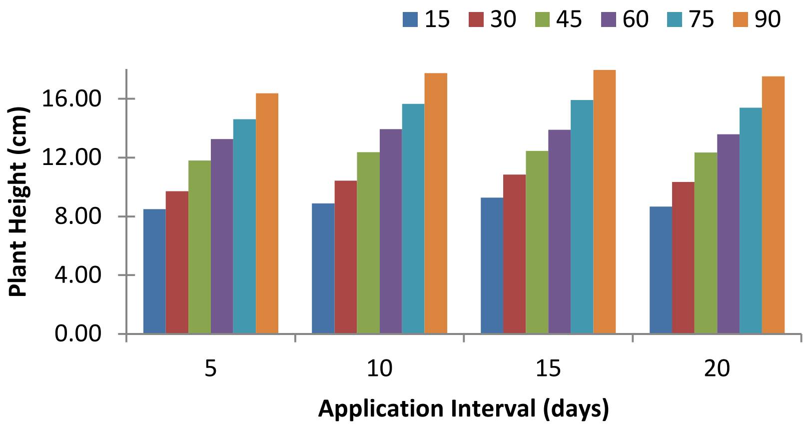


Figure 2: Effect of application interval on *Juniperus procera* seedling height



In related work Hinerman [11] showed that height of rhododendron was significantly affected, where 55.1% increase in height over the control was obtained by application of chopped alfalfa mixed with aged hardwood bark. Other research findings also showed a significant effect of concentration and interval of treatment in height of swallow root and Troyer Citrange [3] [12].

The responses of the seedlings from 15 to 45 days after treatment application were increasing compared to the control. The maximum percentage increase in height was shown during 30 and 45 days after treatment application. The height of the seedling appeared to have decreasing trend with spray interval from 5 to 20 days, while almost constant effect was observed with increasing of soaking time from 24 to 72hrs (Figure 1 and 2). This could be due to early stages effect of the extracted triacontanol. These different responses might be caused by the concentration variability created by soaking and the time of the application [2] [3]. Ries et. al. [13] result also leads to a conclusion not to use a multiple applications, but to apply a single application during the optimum responsive time might also be true for seedling length response of Juniperus procera.

It seems that the extraction of the active compound triacontanol from alfalfa was almost insignificant after 24 hrs of soaking that could be the optimum extraction potential, which is in line with the maximum extraction possibility of the active compound from alfalfa suggested by Ries et al. [20].

These effects most likely appeared due to the active compound extracted from the alfalfa, triacontanol that might have a roll in cell elongation, high chlorophyll content and net assimilation rate [6] [10] [13] . Giridhar et. al. [15] also showed similar response trend for different concentration of triacontanol in the growth of swallow root. An ability of efficient utilization of available water probably results the overall increasing in seedling height of the Juniperus procera [15].

Dry Weight

Dry weight of Juniperus procera was significantly affected by soaking time ($p<0.001$). However, dry weight not significantly ($p<0.05$) affected by the application interval of the alfalfa water extract 90 days after treatment application (Table 1). The tallest seedlings were recorded in treatment combination of 24ST x 15AI, which was about 56% increase over the untreated control. The least seedling height was recorded in the untreated seedlings throughout the three months growth period. Triacontanol increased the dry weight of chrysanthemum by 17% [16]. Similarly, significant results were shown in DW of cucumber, carrot, soy bean, rice and tobacco seedling [12] [17] [18]. The DW of the seedling appeared to have decreasing trend with spray interval from 5 to 20 days, while almost constant effect was observed with increasing of soaking time from 24 to 72hrs (Figure 3 and 4).

Figure 3: Dry weight *Juniperus procera* subjected to alfalfa extract soaking duration

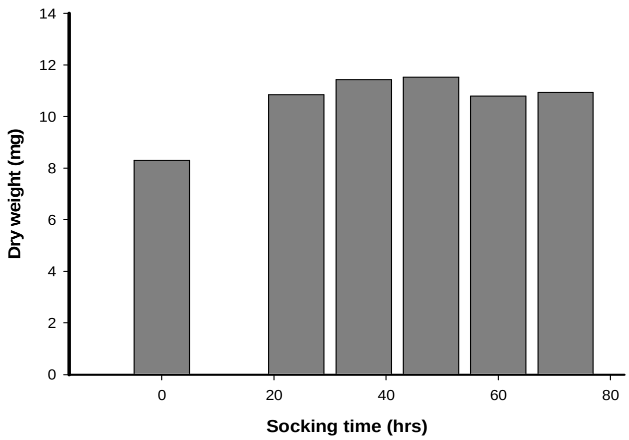
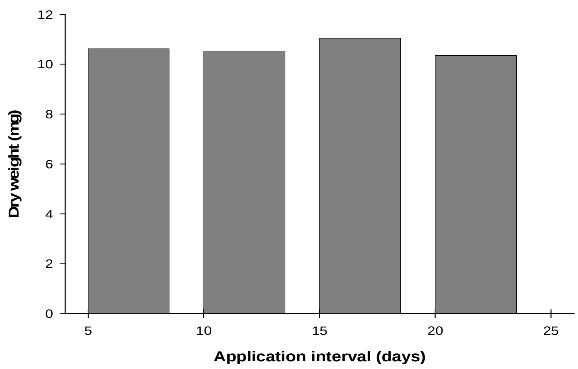


Figure 4: Dry weight *Juniperus procera* subjected to alfalfa extract application interval



The same to seedling height the extraction of the active compound triacontanol from alfalfa was almost insignificant after 24 hrs of soaking that could be the optimum extraction potential, which is in line with the maximum extraction possibility of the active compound from alfalfa suggested by [19]. Increase in dry weight partially can be explained in relation to incorporation of water into products of starch and protein hydrolysis in response to the applied extract. In addition, triacontanol in the extract may cause simultaneous increase in soluble protein, reducing sugars and free amino acids [7]. These changes were possibly due to triacontanol causing an increase in total dry weight and/or percent compositions.

CONCLUSION

In general, though the result of the present study revealed significant effects of water extract of alfalfa, it is not logical to recommend pot experiment results for field conditions. However, the results of the study showed the possibility of using of alfalfa water extract and alfalfa mulch mix as a growth promoter to increase seedling length and dry weight of *J. procera* seedlings.

ACKNOWLEDGMENT
The financial support from Ethiopia Ministry of Science and Technology is gratefully acknowledged. The authors are also greatly appreciate and thankful to Mertule Mariam ATVET College for material and land support, International Livestock Research Institute (ILRI) for providing alfalfa seed and Dilla University for facility to develop the manuscript.

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