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Alfalfa (*Medicago sativa)* Mulch on Growth of Troyer Citrange (*Citrus sinensis x Poncirus trifoliata*) Seedlings

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Abstract: ***A greenhouse and plastic house experiments was conducted at Adet Agricultural Research Center and Mertule Mariam Agricultural Technical and Vocation Educational and Training College, in Amhara National Regional State, Northwest of Ethiopia. The objective of the experiments was to determine the effect of alfalfa mulch on growth of Troyer Citrange seedlings. To see the mulch effect 100g dry saw dust was mixed with 160,120, 80, and 40g of dry alfalfa. Randomized completer block design was used with 7 replications. Significant difference in seedling length, number of leaves, seedling diameter, dry weight and leaf area were observed. However, Leaf area ratio was not significantly affected by alfalfa mulch. The highest seedling height, leaf numbers, seedling diameter, dry weight and leaf area were recorded by 120 g alfalfa mulch. The result of the study have revealed that seedling length, number of leaves, seedling diameter, dry weight and leaf area of Troyer Citrange can be increased by manipulating amount of alfalfa mix in mulch.***

Keywords: Lucerne, triacontanol, plant extract, Citrus

1. Introduction

Transplanting citrus rootstocks directly in to the field for budding operation is generally risky and not recommended. Accordingly, many nurseries start raising seedlings and carry out budding on nursery [1]. But, in the nursery citrus seedlings are slower in growth [1] [2]. Well grown seedlings in areas of continuous growth may reach transplanting size to nursery within six months, although a year is generally required under most conditions [1] [2] [3]. Under optimum conditions, trees large enough for orchard planting can be grown from seed in fourteen months [4]. Although some slow growing species may take two years or more to reach adequate size [4]. As a result, citrus seedlings need all required activities of nursery for long period of time. It is clear that the more the seedlings stay in the nursery the more difficult it would be to fulfill required nursery activities such as protecting the seedlings from environmental hazards and diseases. Therefore, it is important to create condition of rapid growth of seedlings in citrus nurseries.

Rapid growth of seedlings can be achieved with plant growth regulators [5] [6]. However, these synthetic growth regulators are expensive and not available in many developing countries like Ethiopia. Alternatively, organic plant extracts such as alfalfa extract are known to have growth stimulating effects. A growth promoter crystalline substance isolated from the alfalfa was identified as triacontanol [7] [8] [9]. Triacontanol is found as an epicuticular wax in many plant species [10]. It is a 30-carbon, saturated, straight chain, primary alcohol with a chemical formula of C30H64O [10] [11] [12]. Growth stimulation of alfalfa is brought about by mulching with alfalfa or watering plants with an extract made by soaking alfalfa in water [9] [13]. In order to get the promoting effect of alfalfa in potted plants, which are just starting their growth cycle, finely chopped alfalfa with some mulch material can be [9]. Therefore, the objective of the present study was to determine the effect of alfalfa mulch on growth of Troyer Citrange seedlings.

2. Materials and Methods

2.1. Experimental Site

The experiment was conducted at Adet Agricultural research center (ARC) and Mertule Mariam Agricultural Technical and Vocation Educational and Training (ATVET) College, which are situated in Northwest of Ethiopia in Amhara National Regional State. The Adet ARC is located at 37°29'E longitude and 11°16'N latitude and at an altitude of 2216 meters while the college is located at 10°50' latitude and 38°16' longitude with elevation of 2850 above see level. In AARC the seedlings were grown in glasshouse with an average night temperature of 11.87°C and in Mertule Mariam ATVET College the seedlings were grown in plastic house with a night temperature of 11°C. In both sites, day temperature was adjusted by natural air circulation not to exceed 30°C.

2.2. Treatments and Experimental Design

Treatments were assigned randomly using a random number table and the arrangement of pots was determined based on the light distribution of the glass house and the plastic house. Treatments included four dose of dry alfalfa (40 g, 80 g, 120 g, and 160 g) mixed with 100 g of sawdust and saw dust mulched control. A total of 7 treatments were tested in a Randomized Complete Block design with seven replications of potted seedlings.

2.3. Raising seedlings

Fresh seeds of Troyer Citrange rootstock were collected from Upper Awash Horticultural Enterprise, Mertijeju Farm. After one day, seeds were extracted and washed with tap water and surface dried and sown in trays containing 1:1 soil and sand. After 60 days, the seedlings were transplanted into individual pots (diameter of 16 cm and height 24 cm) containing soil: sand: compost in a ratio of 2:1:1. The seedlings were grown in glasshouse at Adet and plastic house at Mertule Mariam sites for 15 days to acclimatize with the growth condition before applying the treatments. The each seedling were tagged and used for growth related data collection.

2.4. Alfalfa preparation and application

The seed of alfalfa cultivar # 6984 was obtained from International Live Stocks Research Institution and grown at Mertule Mariam ATVET College. The alfalfa was cut at about 10% flowering stage. Both the stems and the leaves were dried uniformly and separately until they attained constant weight and they were stored in air ventilated room for later use. For treatment application equal weight of dry leaves (blades and petioles) and stems were mixed with saw dust until the desirable treatment weight achieved.

2.5. Mulch preparation and application

Dry saw dust was collected and mixed (100 g) thoroughly with the different rates of dried alfalfa treatments and put on each pots based on the experimental design and lay out for experiment 2. Non alfalfa-mixed 100 g saw dust mulch was applied for control seedlings.

2.6. 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 termination of the study. Starting from the date of treatment application leaf counting was made every 15 days for a period of 90 days. Diameters of the seedlings were measured at 4cm height from soil surface using caliper, starting from date of treatment application every 15 days for 90 days. At the time of lifting the seedlings, 15 days from the final treatment application leaves were separated and leaf area were measured using a leaf area meter and the total leaf area of each seedling was recorded as total leaf area in cm2. To measure dry weight 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 oven dried to a constant weight. The dried tissues were weighed using sensitive balance and expressed as total seedling dry weight.

The Leaf-area ratio (LAR) was calculated as follows:

$$LAR = \frac{LA}{DW} \qquad (1)$$

Where, LA = final total leaf area of seedling
DW = final total dry weight of the seedling

2.7. Statistical Analysis

Analysis of variance was done using Minitab (Minitab Inc. Minitab for windows release 12.12) for Randomized Complete Block Design as to determine differences between treatment means. Multiple comparisons of the treatment means was done by Duncan's Multiple Range Test.

3. Result and Discussion

3.1 Seedling height

The alfalfa mixed saw dust mulch significantly ($p<0.05$) affected the seedling height. The tallest seedlings of Troyer Citrange were recorded by the 120g alfalfa-mixed saw dust mulch, which was 78% increase over the control. The second best responses were shown by 160g mulch and GA3 which increased seedling height by 31% and 29%, respectively, over the untreated control. In a related work, [14] observed seedling height of rhododendron increased by 55.1% over the control by application of chopped alfalfa mixed with aged hardwood bark. Similarly, Giridhar et al [15] also showed significant increase in height of sweet corn and swallow root plants, respectively

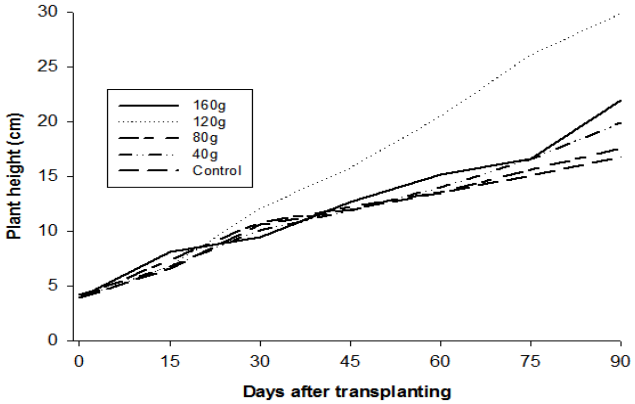

Figure 1: The effect of alfalfa mulch on seedling height

Previous results also showed a significant increase in height of different crops due to the application of mulch [16] - [18] []. This increment in height might be due to the active compound triacontanol or due to the positive effects of the mulch that regulate soil temperature and conserve the moisture. It was also indicated that plants respond to triacontanol application best when conditions for growth, like temperature and moisture, were optimal [19]. Triacontanol effect together with efficient utilization of available water probably has resulted in the increase of height of Troyer Citrange seedlings under the mulch treatments.

3.2 Number of leaves

The alfalfa mixed saw dust mulch was significantly ($p<0.05$) affected the leaf number. The highest leaf number was recorded from the mulch treatment that contained 120g alfalfa mixture starting from 45 days after treatment application. Ninety days later, the 120g alfalfa mulch resulted in a 22% increase of seedling leaf number over plants in the control treatment. The second highest response was shown by 160g alfalfa mulch. The leaf number growth trend was not

significantly different with an increase of alfalfa mulch up to 120g, which then declined at the rate of 160g. Hinerman [14] also showed 27% increase in leaf number of rhododendron due to alfalfa mixed mulch. Previously reports also showed significant increases in leaf number of different plants due to the application of alfalfa mulch or extract up to certain doses [16], [18], [20]. The effects appeared most likely due to the active compound from alfalfa and the mulch effect that might have increased chlorophyll content and net assimilation rate [13], [14], [15], [21] which in turn could have increased emergence of a new shoots, like leaves from the bud.

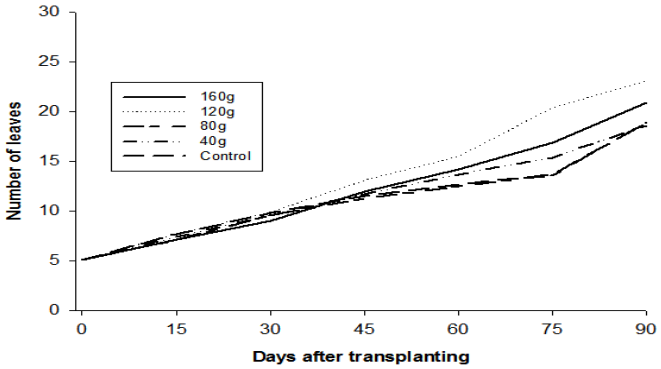

Figure 2: Effect of alfalfa mulch on number of leaves

3.3 Seedling diameter

In the second experiment, alfalfa-mixed saw dust mulch significantly ($p<0.001$) affected diameter of Troyer Citrange seedling starting from 60 days after treatment application. Like the seedling height and leaf number, the largest seedling diameter was recorded when 120g alfalfa was mixed with the mulch, which was 19% increase over the control. The second high response to the alfalfa mulch was shown at 160g rate which increased seedling diameter by 11% over the control. The response is in accordance with the report of Hinerman [14] who observed 27.6% increases in the diameter of rhododendron plant by 125g chopped alfalfa mulch. These effects appear to be due to the active compound found in alfalfa that could enhance cell division and enlargement, thus increasing growth of the seedlings in diameter.

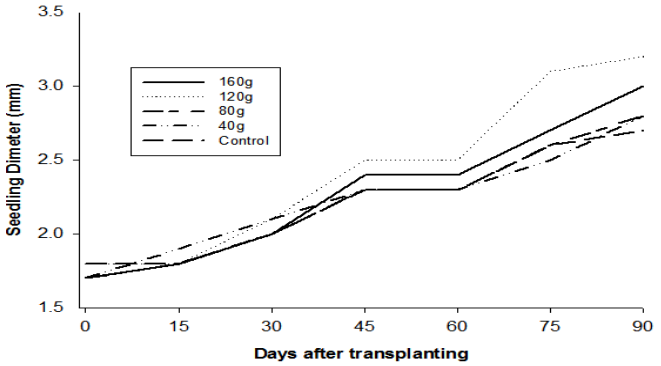

Figure 3: The effect of alfalfa mulch on seedling diameter

Citrus trees are generally known for their rhythmical growth nature, which starting from two leaf stage have continuous vertical growth; when they reach 30cm size, vertical growth of the seedlings stop for about 4 days regardless of the environmental factors [22], which might be the main reason for low response in diameter growth of the seedling to the treatments applied at earlier stages.

3.4 Dry weight, leaf area and leaf area ratio

The alfalfa mixed saw dust mulch significantly ($p<0.01$) affected dry weight and leaf area of the seedlings. However, leaf area ratio was not significantly affected by the applied treatment which was similar result with Bettenbendery [23] (Table 1). The highest seedling dry weight was recorded by 120g alfalfa mixed saw dust mulch, which was 48% more over the control. The second responses were shown by the 160g treatment. Like the other growth parameters, the dry weight of Troyer Citrange seedlings was decreased with an increasing amount of the alfalfa mix up to 120g which then declined with 160g alfalfa treatment. Similarly, the dose response curve for large range in triacontanol concentration (0.01mg/l - 10 mg/l) tended to be quadratic for cucumber, carrot and soya bean [19]. Laughlin [24] also reported similar growth trend for different concentrations of triacontanol in corn. This effect might be caused by production of more number of roots that are capable of absorbing nutrients and moisture efficiently which give rise to better leaf growth and consecutively improve dry matter production with the support of the alfalfa extracted active compound. The effect may be come from not only the alfalfa extract but from the mulch. Significant effect of mulch on dry weight of different crops was reported [16] [18].

Table 1: Dry weight and leaf area of Troyer Citrange seedling

Treatment	*Dry weight (g)*	*Leaf Area (cm2)*
160g	17.5^{b}	221.7^{b}
120g	21.1^{a}	244.6^{a}
80g	14.6^{cd}	197.5^{c}
40g	13.4^{d}	178.9^{d}
Control	13.6^{d}	165.1^{d}

Means within a column followed by the same letter(s) are not significantly different according to DMRT ($p \leq 0.05$)

3.5 Sections headings *Correlation Analysis:*

Simple correlation analysis of means showed a strong correlation of seedling height (0.961 and 0.878), leaf number (0.912 and 0.821) total leaf area (0.894 and 0.947) with the total dry matter of the *Troyer citrange* seedlings indicating the vegetative growth and dry matter accumulation enhancing effects of alfalfa mulch treatments.

Table 2: Correlation of growth parameters

Parameters	*Seedling Height (SH)*	*Number of Leaves (NL)*	*Seedling Diameter (SD)*	*Dry Weight (DW)*
NL	0.869^{**}			
SD	0.962^{***}	0.848^{**}		
DW	0.878^{**}	0.821^{*}	0.966^{***}	
LA	0.927^{**}	0.831^{*}	0.976^{***}	0.947^{***}

*=significant at P<0.05; **= significant at P < 0.01; ***= significant at P<0.001

4. Conclusion

For this study the maximum responses of *Troyer Citrange* for alfalfa mix lay between 120g and 160g. However, it is not logical to recommend greenhouse and plastic house pot experiments for field conditions. However, the result strongly implies that ± 120 g dried alfalfa mulch mix per plant could be manipulated for improvement growth of *Troyer Citrange* seedlings in field condition.

5. Acknowledgement

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