Research Article

Pre-sowing Treatment of Alfalfa Seeds with Bio-Extracts for Early Spring Growth Performance

Bushra Qadir[1], Muhammad Asim[2] and Mohammad Akmal[1*]

[1]Department of Agronomy, the University of Agriculture Peshawar, Khyber Pakhtunkhwa, Pakistan; [2]Agric. Research System, Govt. Khyber Pakhtunkhwa, Pakistan.

Abstract | Pakistan faces an acute deficiency of forages for livestock both in terms of dry matter (DM) and total digestible nutrient (TDN). Green forage availability fluctuates in months of the year due to prevailing climate. Alfalfa, with higher potential, can be adjusted in the cropping system for supplying green forage around the year. Nonetheless, its initial growth for stand establishment is a major issue. Treatments of the experiments were four alfalfa varieties (i.e. UCD, Dura, Vernal and Cuf 101) whose seed were treated with overnight in bio-extracts solution made of perennial tree leaves. The study compares early growth performance of alfalfa varieties for forage biomass production. The seed was soaked overnight (14 h) in extracts made from dry powdered leaves of Bakayn (*Azadir achtaindica*), Ber (*Ziziphus mauritiana*) and water (H_2O) and planted in pots on the next morning. The experiment was conducted in a completely randomized design at the University of Agriculture Peshawar during winter 2013-14. Periodic growth sampling was taken by harvesting three pots of treatments starting in early spring and thereafter during tHEgrowth to the maximum five samplings to study roots and shoot growth. Averaged across varieties and extracts, results revealed that plant length (roots with shoot) was significantly ($p<0.05$) changed with a minimum at the thermal unit (TU) 677 to a maximum at TU 3257. Averaged across TU and extracts, the variety Cuf-101 showed the maximum plant length, root length and shoot length. Averaged across TU and varieties, seeds treated with extracts of Bakayn showed higher length than Ber extract and water (control) treatment. Plant length was significantly affected by the interactive effects of treatments (variety x extracts and TU). Similarly, averaged across varieties x extracts, branch number per plant were also found significant ($p<0.05$) which increased at TU from 677 to 1939 slow and 2532 to 3257 at higher rate. Highest branch number was observed for variety Cuf-101. Root volume also increased at TU 2532 and 3257 with maximum for TU 3257. Cuf-101 and Dura varieties were high in ratio of shoot to root (RSR) when compared with variety Vernal and/or UCD. Ratio of root length to root volume (RRLV) was high at TU 1428 and low at TU 677. Variety UCD and Dura, as well as Vernal and Cuf-101 showed similar RRLV. The study suggested that Cuf-101 was relatively better variety for stand establishment in Peshawar's climate. Nonetheless, seed treated with extracts was not as effective as expected focusing growth and development in early spring for alfalfa.

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***Correspondence** | Mohammad Akmal, Department of Agronomy, the University of Agriculture Peshawar, Khyber Pakhtunkhwa, Pakistan; **Email:** akmal_m@hotmail.com
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Introduction

Forage cultivation is important to sustain animal production. It is the cheapest source of feed for livestock in the world. Pakistan is an acute deficit in fodder both as dry matter (DM) and total digestible nutrients (TDN). Most of the fodder in the country is annual, which reflects with high production cost. It is, therefore, not compatible with other seasonal crops in the cropping system. Therefore, a 2% decrease in area under fodder crops cultivation is reported in every next 10 years. Perennial fodder has a great potential to successfully grow in a cropping system with higher net returns. Lucerne, also called alfalfa (*Medicago sativa* L.), suits to the climate of Pakistan with the highest productivity of dry matter production and forage quality. As per available information, history of forage cultivation can be traced back 1300 BC when alfalfa cultivated in Turkey. Widespread uses of forage crops, nevertheless, appeared much later around the beginning of Christian era, when alfalfa was cultivated in several areas of the world, mainly in Mediterranean region of Europe (Kennedy and Mackie, 1995; Summers and Putnam, 2012). Little research work was done from 5th to 12th centuries for improvement of the forage production. Nevertheless, from 13th to 19th centuries a significant advancement observed (Lillak et al., 2005). Forages globally spread with the expansion of European Agriculture to the rest of the world. Use of forage crops development with an expansion of industrialized agriculture involves the use of new techniques and inputs that helps globally in the vertical improvement of forage production (Skerman et al., 1988). Livestock plays a significant role in the small-scale farming systems all over the world providing atraction to cultivated fields with manure for crops and improved quality of food for human consumption. Importance of livestock is an integral part of Pakistan's Agriculture with its share in the Agriculture GDP around 55%. Inadequate livestock nutrition is an issue and a major factor adversely affecting the livestock development in countries like Pakistan (Tesar, 1993). Forage production is the only inexpensive source of animal feed available all over the world and in Pakistan. It is versatile while there is acute deficiency reported since 2000 by increasing number of animals per unit area in relation to the green forage availability. Recently, Pakistan is deficient in total forage availability by 45%, which in terms of available protein is much higher than that figure. Alfalfa is one of the magic crops in cultivation that presently contributes 10% of the total forage production. However, the scope does exist with recent climate change for widespread cultivation of alfalfa in Pakistan (Akmal et al., 2011) due to mild summer and relatively more rainfall from February to August that is termed as the peak forage production period for alfalfa (Lundberg et al., 2004). Alfalfa is famous as "the queen of forages". It is an herbaceous, long-lived perennial legume. It is known as one of the most palatable, nutritious and high yielding multi-cut forage of the world (Summers and Putnam, 2012). Related species are found as wild plants throughout Central Asia and in Siberia but the modern alfalfa presumes to be originated in Northern and Eastern coasts of Mediterranean Sea (Michaud et al., 1988; Summers and Putnam, 2012). Alfalfa also called Lucerne is believed to be the oldest cultivated forage in the United States. It was in cultivation as early as 490 BC by Roman for horses and cattle. It is the leading clover of a world forage production system. It grows well with an increase in daily mean temperature. With the excellent growth in summer season, alfalfa can be fed fresh without supplements and can also be preserved as hay or silages. Conserved fodder is used as a supplement in winter where the green forage production is dormant by limited temperature and mild solar radiations (Akmal et al., 2011). Fermented alfalfa contributes for feed supply and helps to overcome forage shortage in winter (Seguin et al., 2002). Good alfalfa crop is relatively hard to achieve in first establishment year. There are many reasons; one of them is germination and desired optimum density, which suffers from early rain showers after the crop cultivation (Wang et al., 2009). When alfalfa emerged from seed, its initial establishment growth is very slow and weeds growth is faster than alfalfa and hence the crops is suppressed by weeds dominancy. These effect may cause a significant yield loss (Munns, 2002). Seed treatment before sowing has shown preventive measures for a bumper crop growth with desired stand establishment (Peng et al., 2008). Natural flora is rich with plants having a high nutritive value that could be utilized as a seed treatment source for increasing the productivity.

The present study aims to evaluate the crop growth and biomass production in first establishment year of four alfalfa varieties for first-year growth performance as perennial forage production source. We, therefore, compare crop growth and total biomass production of alfalfa varieties in the first-year of the establishment for future biomass production.

Materials and Methods

Experimental design and treatments

The experiment was conducted in winter 2013-14 in the Department of Agronomy, the University of Agriculture Peshawar, Pakistan. Study was focused on roots and shoots development of alfalfa varieties for early vegetative growth and plant development. This was a two factors experiment i.e. (a) four alfalfa varieties and (b) pre-sowing seed treatments with bio-extracts of perennial plants (i.e. Bakayn, Ber and Water) for about 24 h and the treated seeds were sown on next day. Independent set of pots were harvested for plant biomass and the total periodic sampling were used as factor in data analysis. The experiment was conducted in pots (D= 24, H = 50 cm), lying in field condition in open air. Leaves of perennial trees i.e. Bakayn (*Azadirachta indica*) and Ber (*Ziziphus mauritiana*) were collected in mid vegetative growth period from 5-6 years old trees available at the Campus. The leaves were cleaned and oven dried at 60^{O}C for about 45 h. Dried leaves were ground on grinder and passed through 1 mm screen. Ground leaves (30 g) was mixed with de-ionized water (700 ml) and periodically well shaken for about 70 h, filtered from double layers' cheese-cloths and stored in the refrigerator (Chon and Nelson, 2001). Seeds of four varieties of alfalfa (UCD, Dura, Vernal and Cuf-101) were soaked in three different solutions (Bakayn, Ber extracts and water) to compare the growth of alfalfa plants. Seeds were treated in known quality of solutions for about 14 h and planted in pots in a completely randomized design (CRD) in three replications. Before sowing, all pots were filled with mixture of equal quantity of farmyard manure and silt (v/v). Semidried farmyard manure (FYM) was collected from University Dairy Farm and sundried for 10 days. Silt was collected from water canal close to the Farm. Both silt and FYM were thoroughly mixed. Pots were filled with known quantity of substrate (i.e. mixtures of FYM and silt). Each pot was initially sown with 10 treated seeds, which were thinned-out at emergence by leaving three seedlings pot^{-1}. The counted 50 seeds of each variety were soaked in different extracts of Ber, Bakayn and water as treatments on filter papers (Wattman No. 2) in Petri-dishes. On next day, treated seeds were planted manually in pots by placing 10 seeds per pot of treatments that were latter on thin-out after emergence (15 days after sowing). First data were recorded 78 days after sowing (DAS) also expressed in thermal units (TU = 677^{O}C). The subsequent data were recorded periodically 15-20 days interval for the rest of the growth to maximum 5 samplings.

For thermal units (TU), average temperature data were collected from the nearest metrological station (Pakistan Forest Institute, Peshawar). Both the maximum and minimum temperature for a day was averaged and accumulated for the subsequent growth period from sowing to the respective sampling stages. Minimum temperature for a day if below 5^{O}C was not considered in the cumulative TU. The TU scale was used to avoid changes in cuts that were different for the growth period for each day of the following months after sowing.

Measurements and observations

Data were recorded periodically by harvesting three pots of a treatment on a date. All plants were carefully collected in a paper bags and washed under the tap water to remove soil from roots. Shoot and roots were bifurcated with a sharp blade and collected in separate bags. The roots and shoot of each plant were measured with a measuring tape. Data recorded for the length of a plant parts i.e. root and shoot. Three plants averages represent shoot and root length (cm) for a plant within a pot. Each independent pot represents a repeat of the treatment. Total plant length was expressed as sum of the shoot and roots. Root volume (ml) was recorded by immersing all roots in a known quantity of water and the displacement of water in a cylinder was noted as roots volume. Root and shoot weight (mg) was recorded after drying in an air circulating dryer at 70^{O}C for 46 h. Ratio of shoot to root length (RSR), ratio of root length to volume (RRLV) and dry weight to length ratio of each plant were also determined by their respective readings. Branch number of plant was determined by manually counting the primary branches on the main stem at the time of each sampling. Thermal units (TU) were estimated from the mean daily temperature averages (the maximum plus minimum) for the period from sowing to each harvest exercised during the crop growth period.

Statistical analysis

Data were statistically analyzed with SAS program for all periodic samplings taken in account as factor in addition to the given treatments varieties and pre-sowing seed with bio-extracts using appropriate statistical model for the completely randomize design (CRD). Means, where found significant, were separated with least significant difference (LSD) test ($p<0.05$).

Results and Discussion

Data regarding plant length (cm) of alfalfa varieties treated with biological extracts before sowing are shown in Table 1. Averaged across varieties and bio-extracts, length was significantly increased when plant advanced in growth i.e. days after sowing (DAS) expressed in thermal units (TU °C) with minimum at TU 677 to maximum at 3257. Likewise, averaged across TU and bio-extracts, variety Cuf-101 showed highest length, followed by Vernal and Dura with minimum for UCD. Similarly, averaged across TU and varieties, seed treated with Bakayn extracts showed highest length, followed by Ber and water (control). Interaction variety x bio-extracts and TU x variety were significant for plant length (Figure 1a) with a linear response for Cuf-101 and non-linear for rest of the varieties. Interaction varieties x TU showed increments against cumulative thermal units with lowest at 677, followed by 1428, 1939 and highest at 3257 (Figure 1b). Variety Cuf-101 showed relatively better length than rest of the varieties. Dura showed almost no change in length at TU 2532 and 3257. UCD remained lowest in length for all samplings. Averaged across varieties and bio-extracts, the shoot length was significant ($p<0.05$) starting from lowest at TU 677 and highest at 3257. While averaged across TU and bio-extracts, Cuf-101 showed highest shoot length, followed by vernal and Dura with lowest for the UCD. While averaged across TU and varieties, Bakayn was non-significant with Ber and likewise, Ber with control (i.e. water) for shoot length. ANOVA results revealed significant ($p<0.05$) effects for interaction (variety x bio-extract), (TU x variety) and (TU x variety x bio-extract) for shoot length. Cuf-101 showed a linear response for shoot length while rest of the varieties did not show same trends (Figure 1c). Cuf-101 showed the highest shoot length when seed treated with Ber or Bakayn extracts. Variety UCD showed almost similar trend as observed for Dura but with lower values for all treatments. Shoot length of varieties Vernal and Cuf-101 were decreased at Ber and increased at Bakayn with unexpected increased in Cuf-101 at Bakayn extracts. Interactive effects of varieties x TU showed increases in growth of shoot length with lowest at TU 677, followed by 1428, 1939, 2532 and highest at 3257 (Figure 1d). Variety Cuf-101 showed relatively better shoot length than rest of the varieties.UCD remained lowest in shoot length in all sampling (TU) when compared with rest of the varieties. Interaction (TU x bio-extracts x varieties) was significant for shoot length with linear increases against thermal units but with different rates for the bio-extracts with visible differences in varieties at TU 2000 and 3000 for bio-extracts than control (Figure 2a).

Table 1: *Disparity characters of the alfalfa (Medicago sitiva L.) plant treated with bio extracts in early establishment phase of growth.*

DAS TU (°C)	Plant	Shoot	Root	Shoot	Root
	Length (cm)			Dry weight (g)	
Average across (varieties x bio-extracts)					
677	10.79 e	1.76 e	9.03 e	0.39 d	0.21 b
1428	19.65 d	7.27 d	12.38 d	2.46 c	0.49 b
1939	37.46 c	21.42 c	16.04 c	3.52 b	1.06 b
2532	73.63b	50.82 b	22.81 b	4.01 b	4.13 a
3257	92.36 a	68.06 a	24.30 a	6.86 a	3.94 a
Average across (sampling x bio-extracts)					
Varieties (V)					
UCD	41.91 c	25.74 c	16.17 b	3.12 b	2.05 a
Dura	46.94 b	30.53 b	16.41 b	3.86 a	1.74 a
Vernal	44.77 b	27.52 c	17.25 ab	3.55 ab	1.72 a
Cuf – 101	53.49 a	35.67 a	17.82 a	3.27 ab	2.37 a
Average across (sampling x varieties)					
Treatments (T)					
Water	44.42 b	28.70 b	15.71 b	3.34 a	1.46 b
Ber	46.70 b	29.87 ab	16.83 b	3.27 a	2.12 ab
Bakayen	49.22 a	31.02 a	18.20 a	3.73 a	2.32 a
Interactions (significance level)					
Interaction (V x T)					
Variety x Bio-extracts	**	**	Ns	*	ns
Sampling x Variety	**	**	**	*	ns
Sampling x Bio-extracts	ns	ns	**	Ns	ns
Sampling x Variety x Bio-extracts	ns	*	Ns	*	ns

DAS: *Days after sowing;* **TU:** *Thermal Units; Mean followed by a common letter within a category are non-significant ($p<0.05$) using least significant (LSD) test.*

Average across varieties and bio-extracts, root length also significantly ($p<0.05$) increased against accumulative thermal units (TU °C) with lower at TU 677 and higher at TU 3257. Likewise averaged across TU and bio-extracts, Cuf-101 showed maximum root length with a non-significant change from Vernal, followed by rest of the varieties. While averaged across TU and varieties, Bakayn leaf extract showed

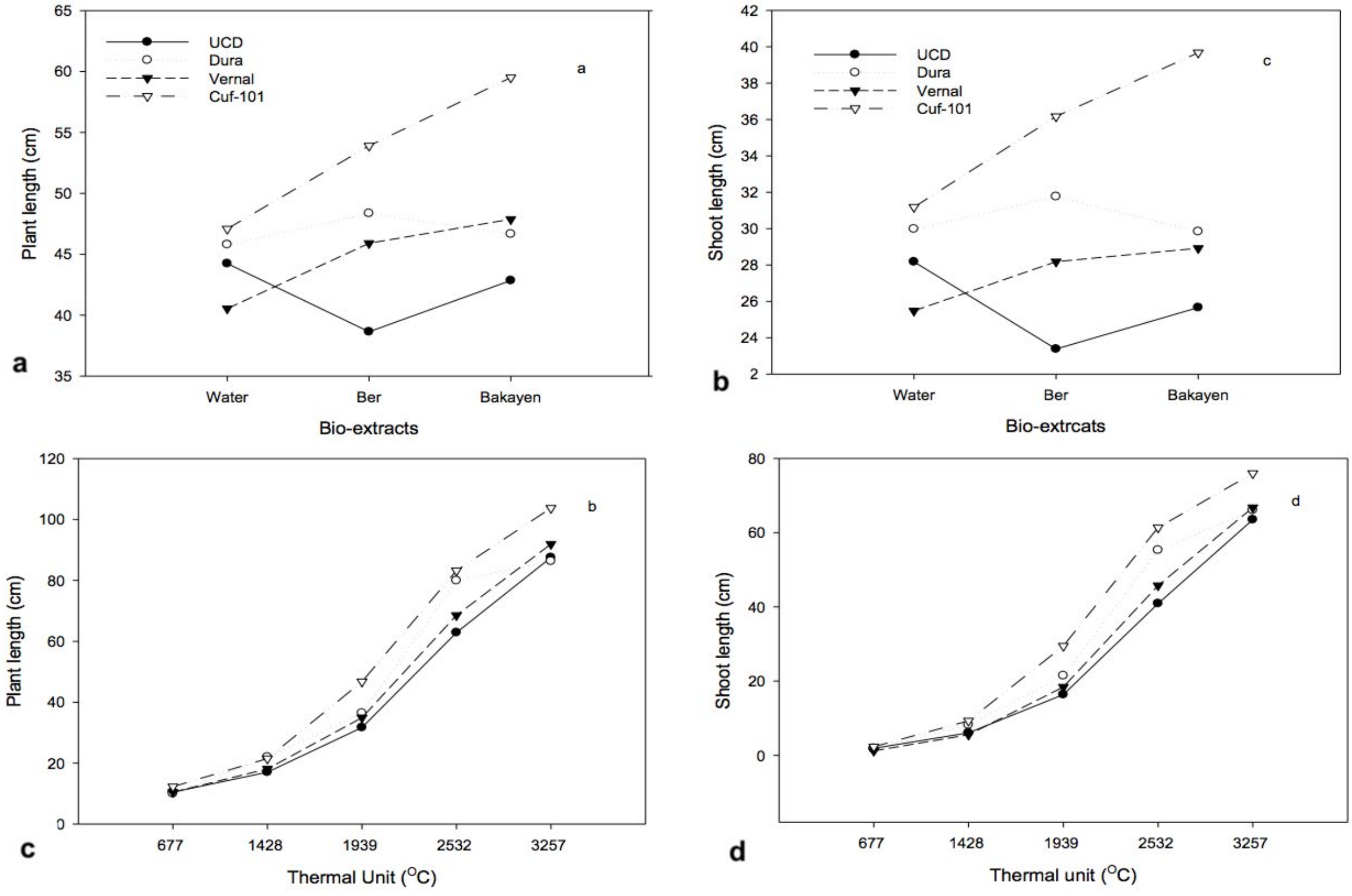

Figure 1: *Interaction (a) extract x variety, (b) TU x variety for plant length, (c) extract x variety, (d) TU x variety for shootlength in early vegetative stage of growth.*

the highest root length, followed by Ber and control (water). Interaction (TU x variety) and (TU x bio-extracts) were significant ($p<0.05$) for root length. Varieties showed an increase in root length when plant proceeds in growth against TU with lowest for TU 677 and highest for 3257 (Figure 3a). Variety Cuf-101 showed relatively better root length than rest of the varieties. Interactive effect of TU and bio-extract for root length was the maximum for Ber and Bakaynand minimum for water (Figure 3b). Bakayn extract showed a bit increased in root length from TU 2532 to 3257. Plant length (roots + shoot) increased with increase in thermal units which is natural while plant grows and contributed towards stem than leaf which brought significant changes in length of the plant as well as shoot that was the main economic yield of alfalfa (Peng et al., 2008). Alfalfa is tap rooted and have strong root system which continue to grow in early establishment phase and hence observed similar in this study while plant grow well during all samplings (Makkar, 2003) that contributed for length as well as for weight of both roots and shoot (Bolinder et al., 2002). Among varieties, Cuf-101 showed better yield performance and growth than rest of the varieties which might be due to climate and variety interaction (Putnam, 2003) or differences in the dormancy levels of the varieties that has performed differently in similar environment and growing media (Lucas et al., 2000). Among treatments, Bakayn leaf extracts showed better plant health with higher length and weight that might have contributed towards favorable effects on germination and growth then Ber or water treatments (Robinson et al., 1992).

Shoot and root dry weight

Data regarding shoot dry weight (g) of alfalfa varieties treated with bio-extracts are shown in Table 1. While averaged across varieties and extracts, mean shoot dry weight was lowest at TU 677 and highest at TU 3257. There was no significant effect ($p<0.05$) on shoot dry weight at TU 1936 and 2532. However, it increased, thereafter, at TU 3257. Likewise, when averaged across TU and bio-extracts, Dura showed highest shoot dry weight, followed by vernal and Cuf-101 with a non-significant difference. Lowest shoot dry weight was observed for UCD. While averaged across TU and varieties, seeds treated with Bakayn and Ber extracts did not differ in shoot dry weight. Shoot dry weight for interaction (variety x bio-extracts), (TU x variety) and (TU x variety x bio-extract) were significant. Cuf-101 and Vernal decreased shoot dry weight with Ber extract than control (Figure 3c). However, as compared to Ber, Bakayn extract increased shoot dry weight. Bakayn extract when compared with water showed higher dry matter. Dura and UCD increased shoot dry weight at Ber extract and decreased at Bakayn. Interactive effect of varieties x TU showed positive changes in dry weight when plant advanced in growth with lowest at TU 677, followed by 1428, 1939 and highest at TU 3257 (Figure 3d). Vernal showed better shoot dry weight than the rest of varieties for all samplings exercised. Variety UCD remained lowest in shoots dry weight when compared with rest of the varieties. Interaction TU x variety x bio-extracts showed increments in shoot dry weights as thermal unit increased with time after sowing but in different fashions for different bio-extracts (Figure 2b). Root dry weight (g) is shown in Table 1. By averaged across varieties and extracts, root dry weight was significant with no effect for TU 677 and 1939 and with significant effects thereafter at TU 2532 with unchanged at TU 3257. By averaged across TU x bio-extracts, all varieties showed non-significant effects on root dry weight. While averaged across TU x varieties, seed treated with either water or Ber extract did not change for root dry weight. Likewise, seed treated with Ber or Bakayn also did not show any significant change in root dry matter.

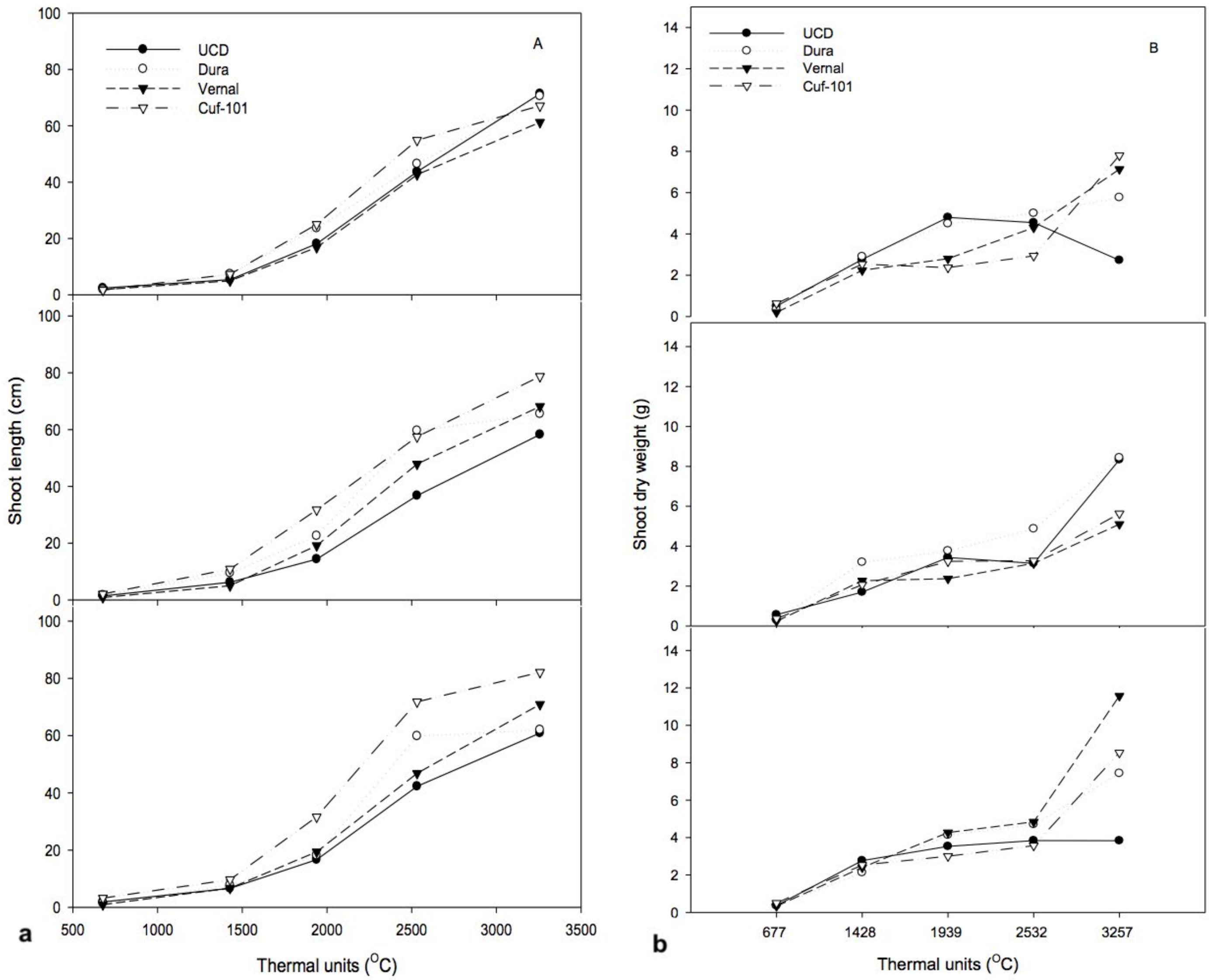

Figure 2: *Interaction (a) TU x extract x variety for shoot length (cm) and (b) shoot dry weight (g) of alfalfa plant in early vegetative stage of growth.*

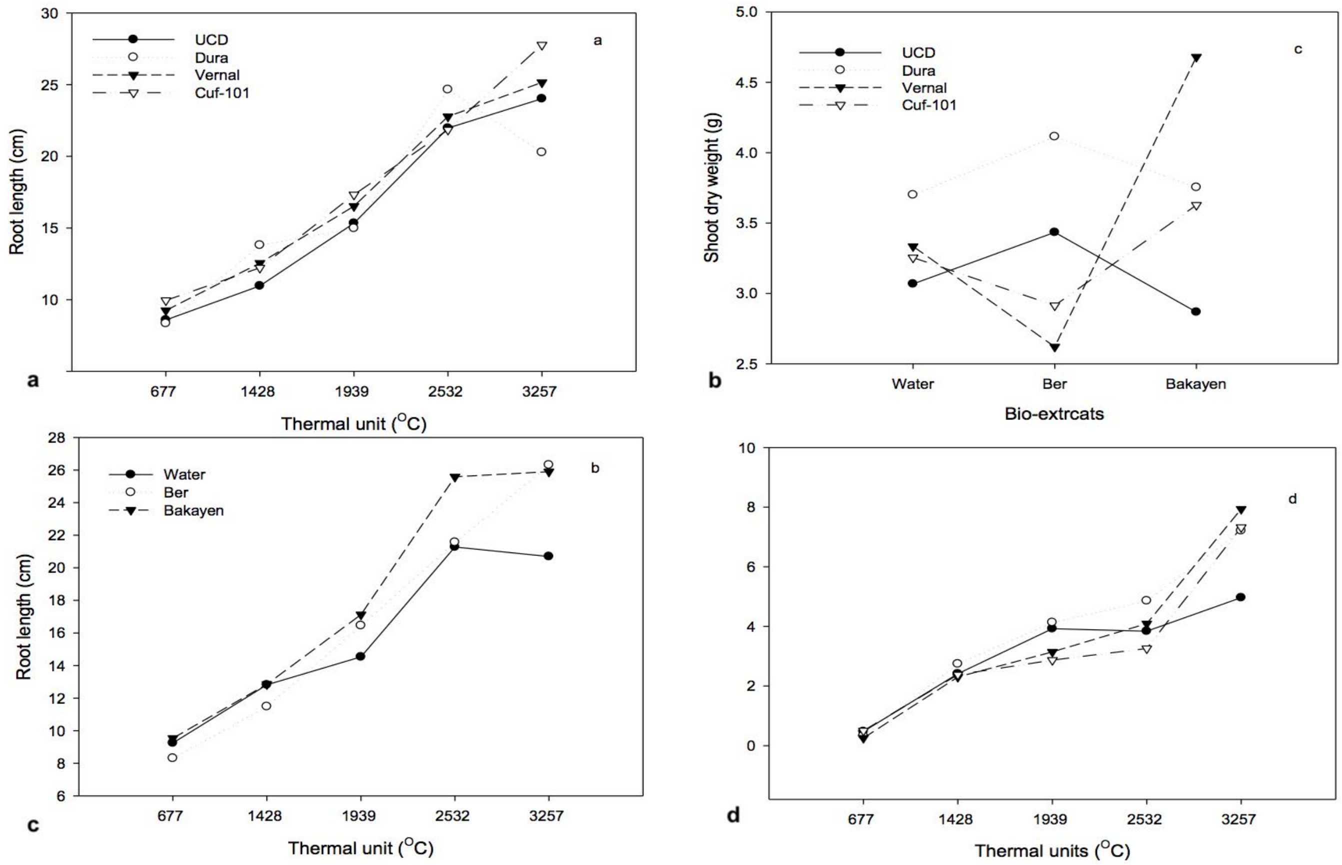

Figure 3: *Interaction (a) TU x variety, (b) TU x extract of root length, (c) extract x variety, (d) TU x variety of shoot dry weight (g) of alfalfa plant in early vegetative stage of growth.*

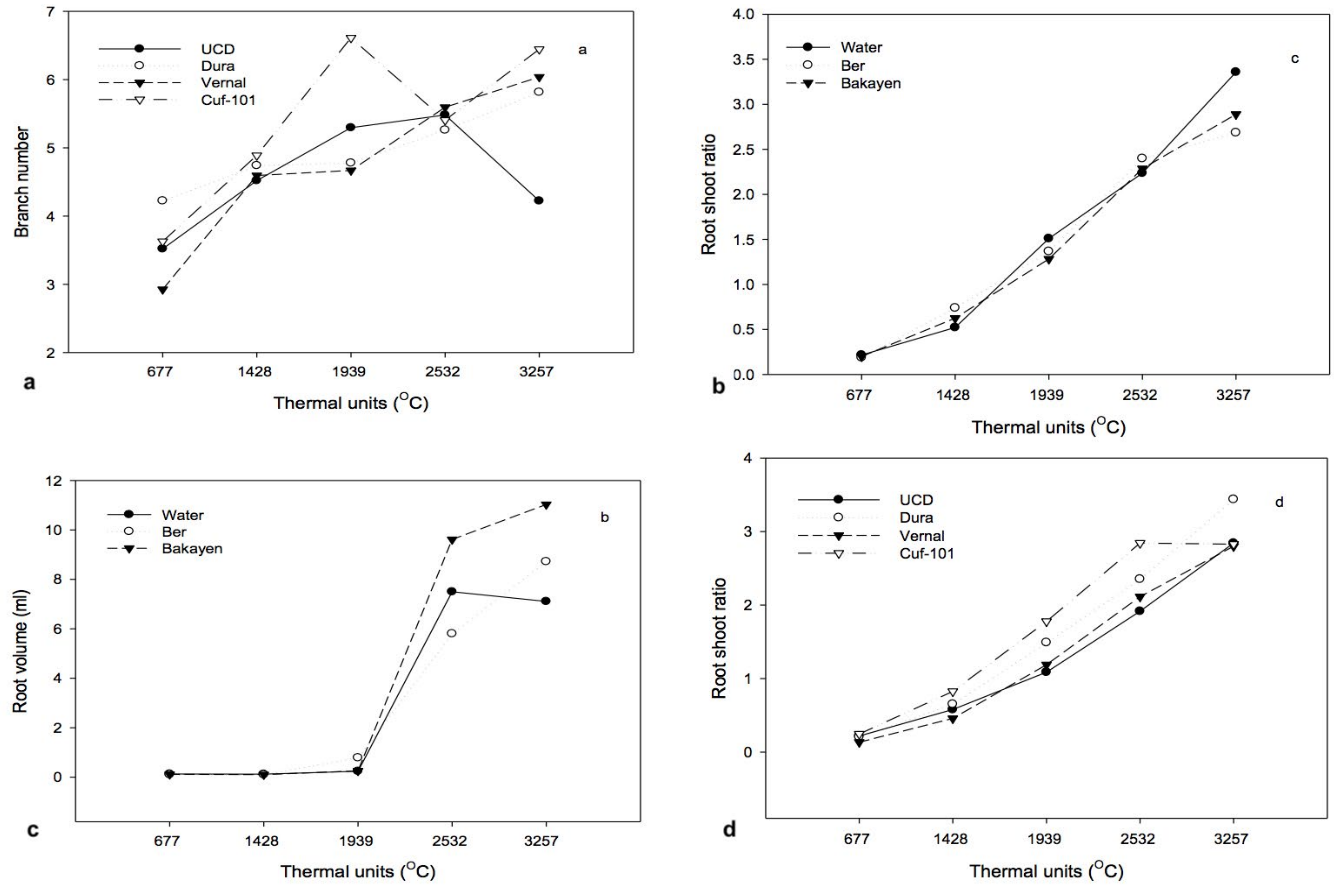

Figure 4: *Interaction (a) TU x extract for branch number (b) TU x extract for root volume, (c) TU x extract and (d) TU x variety for root to shoot ratio (RSR) of alfalfa plant in early stage of growth.*

Branch number and root volume

Data regarding branch number of alfalfa varieties treated with treatments (water, Ber and Bakayn) extracts at different stage of growth are shown in Table 2. Averaged across varieties x bio-extracts, branch number per plant were increased ($p<0.05$) when plant advanced in growth from TU 677 to 1939. However, no further changes in branch number recorded with age at TU 2532 to 3257. While averaged across TU x bio-extracts, varieties showed a significant change in branch number per plant. Cuf-101 showed highest branch number than rest of the varieties. Varieties Vernal, Dura and UCD did not differ in branch number per plant. While averaged across, TU x varieties, bio-extracts showed non-significant response on branch number per plant. All varieties showed an increase in branch number when plant proceeds in age against cumulative thermal units with lowest for TU 677, followed by TU 1428, TU 1939 and highest for the TU 3257. At TU 1939, Cuf-101 showed higher branch number than rest of the varieties, which decreased at TU 2532 with maximum at TU 3257. UCD showed lowest branch number at TU 3257 (Figure 4a). Rest of all the possible interaction was non-significant. Averaged across varieties x bio-extracts, root volume was non-significant when plant advanced in growth (TU 677 to 1939). Root volume increased significantly at TU 2532 and 3257 with highest for TU 3257. While averaged across TU x bio-extracts, varieties showed non-significant effect on root volume. While averaged across TU and varieties, Bakayn showed highest root volume, followed by Ber. Control (water) and Ber extracts did not differ in root volume. Interaction (TU x bio-extracts) showed non-significant effect on root volume from TU 677 to 1939, with highest at TU 3257 for seeds treated with Bakayn extract and lowest for control i.e. water (Figure 4b). Higher branch number per plant is good biomass character of forages. As expected, with increasing accumulative thermal units by time and age branch number increased (Figure 4b) which contributed in dry matter of shoot (Amanullah et al., 2013). Cuf-101 relatively showed better response for branch number and hence results in longer plants with higher biomass (Paula and Pausas, 2011). Extract of Bakayn leaves showed better growth of plants than rest of the treatments, which can be due to activation of enzymes of seed during emergence and thereafter plant vegetative development (Bolinder et al., 2002).

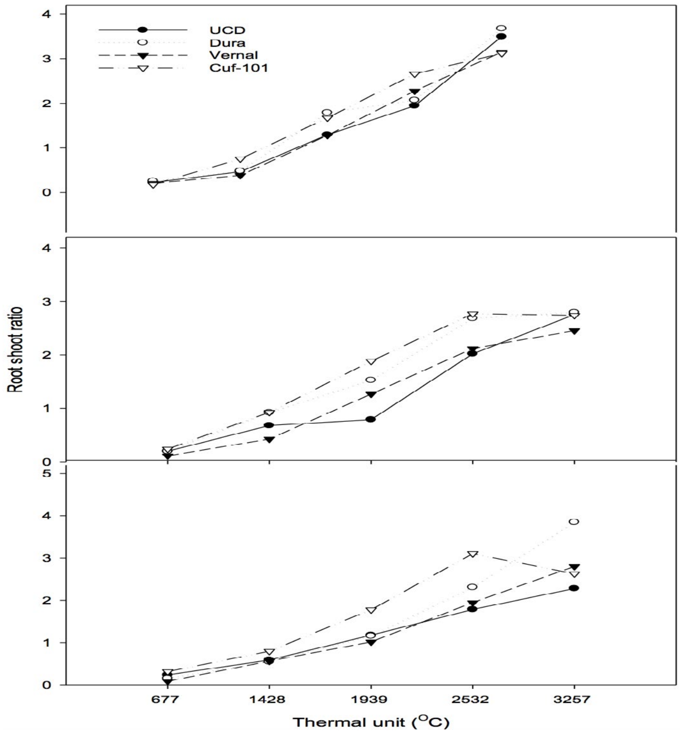

Figure 5: *Interaction (a) TU x extract x variety for root to shoot ratio (RSR) of alfalfa in early vegetative stage of growth.*

Shoot to root ratio (RSR) and root length to volume (RRLV) ratio

Data regarding RSR is shown in Table 2. Averaged across varieties and extracts, RSR were significant from TU 677 to TU 3257 for all varieties. Averaged across TU and extracts, Cuf-101 and Dura showed higher RSR followed by Vernal and UCD that did not differ to other. While averaged across TU and varieties, water showed highest RSR, followed by Ber extract with no-change from control. Ber and Bakayn extracts also did not differ in RSR. Interaction (TU x varieties), (TU x extracts) and (TU x variety x bio-extracts) were differed for RSR. Interaction TU x varieties showed linear increase in RSR with lowest at TU 677, followed by 1428 and highest at TU 3257 for all varieties (Figure 4d). Variety Cuf-101 showed high RSR compared to rest of the varieties with slight reduction at 3257 while rest of the varieties showed a constant increase in RSR. Interactive effects of TU and extracts were significant ($p<0.05$) for RSR with highest for control followed by Bakayn and Ber with upwards trends with age accumulating TU (Figure 4c). Lowest RSR was recorded for Ber than Bakayn or water at any stage of growth. Interaction (TU x extracts x varieties) was also significant for RSR with linear increments for varieties against thermal unit but with different rates for different treatment (Figure 5). Among extracts, water and Ber showed visible differences in RSR within varieties at TU 2532 and 3257 when compared with Bakayn extract. Data regarding root length to volume (RRLV) for water, Ber and Bakayn are shown in Table 2. Averaged across varieties x extracts, RRLV were significant with highest for plant at TU 1428 which respectively decreased at TU 2532. While averaged across TU x extracts, varieties showed significant responses on RRLV. UCD and Dura did not differ in RRLV. Vernal and Cuf-101 also did not differ from each other in RRLV. While averaged across TU and varieties, water did not differ from Ber and likewise did by treatments Ber with Bakayn. All possible interaction was non-significant for RRLV. As expected with plant age, root volume and RSR increased (Fageria et al., 2006). Root volume showed significant change when plant advanced in growth from TU 2532 by more branches and volume that expands with stage of the crop development (Amanullah et al., 2013).

Table 2: *Disparity characters of alfalfa (Medicago sitiva L.) plant treated with bio extracts in early establishment phase of growth.*

TU (°C)	Branches	Root volume	RSR	RRLV
	(No)	(ml)	(cm/ml)	
Averaged across (varieties x bio-extracts)				
677	3.57 c	0.12 c	0.20 e	80.71 b
1428	4.69 b	0.11 c	0.63 d	117.03 a
1939	5.34 a	0.43 c	1.39 c	71.64 c
2532	5.44 a	7.64 b	2.31 b	4.28 d
3257	5.63 a	8.95 a	2.98 a	3.52 d
Average across (sampling x bio-extracts)				
Varieties				
UCD	4.61 b	3.07 a	1.33 b	51.82 b
Dura	4.96 b	3.26 a	1.63 a	55.31 b
Vernal	4.76 b	3.55 a	1.34 b	57.92 a
Cuf –101	5.40 a	3.92 a	1.70 a	56.69 a
Average across (sampling x varieties)				
Treatments				
Water	4.92 a	3.02 b	1.57 a	52.69 b
Ber	4.84 a	3.10 b	1.48 ab	55.28 ab
Bakayen	5.04 a	4.22 a	1.46 b	58.34 a
Interactions (significance level)				
Variety x Bio-extracts	Ns	ns	ns	ns
Sampling x Variety	**	ns	**	ns
Sampling x Bio-extracts	Ns	*	**	ns
Sampling x Variety x Bio-extracts	Ns	ns	*	ns

RSR: *Root to Shoot Ratio;* **RRLV:** *Ratio of root length to volume;* **TU:** *thermal units; Mean followed by a common letter within a category are non-significant (p<0.05) using LSD test.*

Conclusions and Recommendations

It can be concluded from the results that alfalfa seeds treated for 12 h before sowing with Bakayn extracts showed taller plants with longer healthy shoots and roots that are good for crop establishment in early development phase after growth. Among the varieties, Cuf-101 was relatively better with healthy plants for the local environments. Alfalfa plant growth was relatively slow in early 100 days but significantly improved with temperature change thereafter in summer.

Author's contribution

Bushra Qadir: Conducted research and data compilation.
M. Asim: Helped in drafting the manuscript.
Mohammad Akmal: Designed the experiment and provided overall guidance for finalising the manuscript.

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