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In-vitro establishment, germination and growth performance of Babel *Acacia* (*Acacia jacquemontii* Benth)

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Abstract
The main objectives of the present study were to determine the growth behavior, germination percentage in petri dishes at different time duration and different field soil mediums, collected from the depths of 0-0.5 m, 0.5-1.0 m and above >1.0 m at different time periods during the growing seasons of 2013 to 2014. Ripened pods of *Acacia* species were collected from the field and seeds were isolated from pods. Then such seeds were stored in sealed paper bags for one a week and were dried under normal room temperature. Growth parameters of the twigs were measured after every 15 days. In nursery conditions, seeds soaked with cold/hot water had higher germination (82-94%) in petri dishes than the non-soaked seeds of the species. Seeds sown in pots had 51-70% germination. Seedlings of the species had higher fresh/dry weights of their shoots than the roots. In contrast, the roots of the *Acacia* seedlings elongated at a higher rate than the shoots.

Keywords: *Acacia*, germination %, root-shoot elongation, root–shoot ratio

1. Introduction

Scientific name: *A. jacquemontii* Benth (Fabaceae), common name "Babel" or "Kikri" in Pakistan. In India, this shrub is called as "Bhu-Banwali", "Gulli Bouli" and "Ratabouli" (Mertia *et al*., 2009) [26]. This plant species mainly occurs in desertic regions of Australia and Africa. In Asia it is widely distributed in Pakistan, India, Afghanistan, Iran and Iraq (Mertia *et al*., 2009) [26]. On sand dunes and interdunal sandy plains, it naturally distributes in patches, but on bare undulating sand dunes its frequency and density is more. It is a potential but lesser known multipurpose shrub of arid and semi-arid regions (Singh *et al*., 2003).

This *Acacia* is an erect, multi-stemmed, and small to large shrub. It can attain a height ranging from 1.5 to 4.5 m in different habitats and soil types. The crown is variable in size, flattened, spreading and erect. The number of stems or branches on the individual plant varies from 4 to 46. Due to multi-stem growth characteristics, the plants attain a good canopy spread within 4 to 5 years. The growth of shrub is very fast in early stage while it slows down after 5 to 6 years. Plant stems are stiff, smooth and brown in color. Thickness of stem varies from 1.0 to 5.9 cm. Twigs are zigzag with grayish brown bark. Young shoots of the stems are slightly short and soft. Spines are arranged in paired, straight, slender and 2.0 to 5.0 cm long. They are white in color and most often smooth. Leaves are bi-pinnate 2.5-5.0 cm long with 2-4 pairs of pinnae. The Leaflets of this species are in 5-10 pairs, sessile, 2.5-3.0 mm long, linear oblong, obtuse, and glabrous. Common petiole is 2.5 to 5.0 cm long with small or indistinct glands between the upper pair of pinnae (Mertia *et al*., 2009) [26].

This shrub produces yellow sweet scented flowers, inflorescence globose heads, 12-16 mm in diameter, peduncles 2-3, slender, axillary, close cluster, bracts 2-3, about the middle of the peduncle. Calyx campanulate, 1.2 to 1.5 mm long; the teeth short, deltoid and corolla 3 mm Long, lobes ovate-oblong, acute. Stamens indefinite, anthers are not gland tipped (Parveen and Qaiser, 1998) [33]. Pollens are 12-16 celled polyads and tectum subpsilate. Length, breadth and exine thickness of pollens are 39.40, 50.26 and 1.79, um, respectively and ovary included in calyx tube or inferior. This species produces sweet scented flowers and attracts birds and insects toward extra floral nectories. The yellow sweet scented flowers of this shrub make the birds and insects main vectors of pollination (Ford and Forde, 1976). Pods of this shrub show considerable variation in shape, size and color.

They are stalked ovate oblong, round at base, flat, straight, transversely veined, glabrous and 4 to 6 seeded. The length of the pod of this shrub varies from 5.2 to 10.0 cm, and 1.0 to 1.7 cm in width while weight of individual pod ranges from 0.2 to 0.6 gram. The pods of this species are pinkish-white in color with prominent pink colored lining. Seeds are brown to dark brown in color, smooth, compressed and 5.5 to 7.5 mm in diameter. The individual seed weight ranges from 0.03 to 0.06 g while the weight of its100 seeds is about 4.9 g (Mertia *et al*., 2009) [26].

The seed setting is mainly controlled by evaporation of this shrub (Khan, 1970). Seed setting is poor if windy days are prolonged. Pods are dehiscent and burst on drying. Fallen seeds are blown by wind to distant places. Some seeds are also buried in the ground with deposition of windblown sand on them. Seed of this species dispersal also takes place by animals, which pass out undamaged seeds through the digestive tract. The phenological behavior of this shrub is mainly influenced by rainfall, temperature and evaporation. Rainfall usually affects leafing while temperature influences flowering and fruiting. The time of flowering varies at different locations. In hyper arid conditions, the flowering on the plants initiate in middle February while the pods mature either at the end of April or early May the year (Bhandari, 1990) [2].

The seeds of this shrub start germinating when favorable conditions are available. The germination of seed is epigeal. The radicle emerges and moves downward. The growth and elongation of roots is faster than of shoot. The primary tap root is long and thick (Mertia and Prasad, 2005) [22]. Young seedlings of the shrub are relatively more susceptible to frost than the mature plants (Joshi *et al*., 1983) [16]. Young plants (seedlings) oftenly grow very fast and can attain a height of 32 to 70 cm in a year after transplanting in the field. In shallow and gravely soils the growth of seedlings are slow and poor (Sharma *et al.,* 1984) [38]. This species develops profuse root system. Main root divides into several sub roots called lateral roots which combine and make a strong root network that binds sand in the rhizosphere. Normally its root penetrate 4 to 6 meter deep in search of water, whereas in sand dunes the roots may penetrate even deeper in search of water (Mertia *et al*., 2009) [26]. This species starts coppicing either after the period of every 5 to 6 years or when its plants attains a height of about 4 meters (Mertia and Prasad, 2005) [22].

2. Material and methods

Ripened pods of *Acacia* species were collected from the field and seeds were isolated from pods. Then such seeds were stored in sealed paper bags for one a week and were dried under normal room temperature. Only mature and uniform sized seeds were used in the following germination experiments.

2.1 Seed germination in petri dishes

Mature and uniform seeds of this species were sown in large petri dishes. Before sowing in the petri dishes, seeds were soaked with cold water (for 12 hours) and hot water (100 ^{0}C for 3 hours). In 3 petri dishes, cold water-soaked seeds were used while in another 3 petri dishes hot water-soaked and 3 petri dishes, untreated seeds were used. Seed germination percentage was recorded on hourly basis. In each petri dish a blotting paper was laid out and 100 seeds were placed at equal spacing on the blotting papers. Later on, seeds in the Petri dishes were covered with another blotting paper. Small quantities of water were added over the blotting papers so that they may be become moist and wet.

2.2 Pots experiments

Mature and uniform seeds of this species were sown in the earthen pots in nursery conditions. In this experiment, 300 pots were used and in each pot 10 non-water soaked seeds were sown. Each pot contained 5 kg of field soil, collected from the depths of 0-0.5 m, 0.5-1.0 m and above >1.0 m. First 100 pots contained field soil collected from the depth of 0-0.5 m while other 100 pots had field soil collected from the depth of 0.5-1.0 m. The reaming 100 pots contained field soil collected from the depth of above 1.0 m. After seed sowing 500 ml water was supplied to each pot on daily basis for the period of one week. Daily seed germination was recorded up to 19 days.

2.3 Root-shoot experiments

In this experiment, pots of seed germination test were used. The pots were divided into 3 categories i.e. pots filled with the soils of 0-0.5 m, 0.5-1.0 m and above 1.0 m depths. In each pot category, there were 63 pots which were selected randomly. At the end of pot germination test, only one seedling per pot was kept and the other seedlings were uprooted manually from the pots of each category. Later on the seedlings of the pots were tested for their root-shoot weights and elongation.

2.4 Root-shoot weights and elongation

Three weeks later when the germination was completed in all the pots, the plant species was studied for its root-shoot elongation. For fresh and dry weights of root-shoots, 3 seedlings, 1 seedling from each of 3 pots of each category were uprooted and washed with tape water so that soil particles should be detached from the roots. In this way, 9 seedlings from 3 categories of the pots were uprooted and later were dried for 3 days in the open air. With the help of knife, roots and shoots were separated at their joint point (collar). During this experiments, 500 ml of water was provided regularly by biweekly basis. The data on the root-shoot fresh/dry weights and root-shoot elongation of *Acacia* seedlings were recorded on bimonthly basis for 14 months (from February 2013 to March 2014). The mean values of root-shoot weights and elongation obtained from seedlings of three category of pots at each bimonthly period were combined has single mean values.

3. Results

3.1 Petri dishes experiments

Mean values of germination percentage of *Acacia* seeds in petri dishes are given in Fig. 4.20. Results indicated that the seeds soaked in cold water had about 33% germination after 8 hours of their sowing in the petri dishes. The seeds soaked with hot water and untreated seeds (control) did not show any germination in these hours. After 16 hours of sowing, seeds treated with cold and hot water indicated about 60% and 66% germination respectively, while there was no seed germination in case of untreated seeds (control). After 24 hours period of sowing, cold water-treated and hot water-treated seeds showed about 78% and 76% germination respectively, while untreated seeds (control) had about 15% germination. After 32 hours of sowing, cold water-treated and hot water-treated seeds showed about 94% and 82% germination respectively. The germination of untreated seeds was about 32%. The above results depicted that seeds of this species had more germination when treated with cold water than the others two treatments.

3.2 Pots experiments
Mean values of seed germination of *Acacia* in the pots are given in Fig. 4.21. Germination results showed that during first week of February 2013, the seed germination in the field soils, collected up to the depths of 0.5 m, 0.5-1.0 m and above 1.0 m were about 29%, 30% and 36% respectively. During the second week of the month, the seed germination in the soils, collected from the depths of 0.5 m, 0.5-1.0 m and above 1.0 m were about 48%, 54% and 65% respectively, while in the 3rd week the seed germination in the soils of these depths were about 51%, 57% and 70% respectively. The findings of germination indicated a regular increase in seed germination in each week at each soil depths. Overall the seed germination in each week was highest in the soil collected from the depth of above 1.0 m. Minimum germination of seeds was found in the shallow soils in each week.

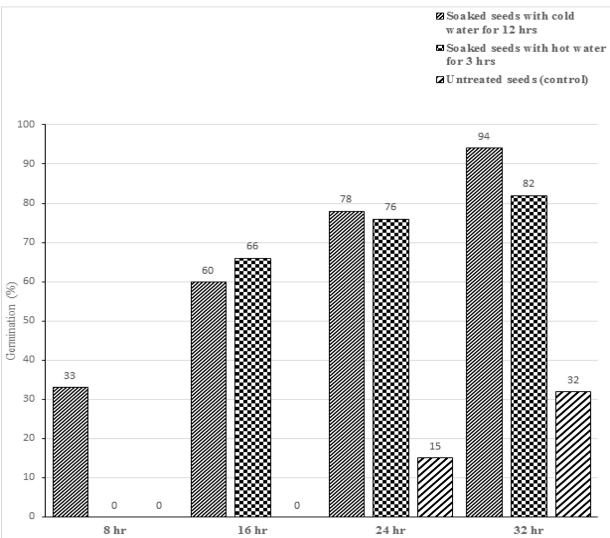

Fig 1: Hourly germination of *A. jacquemontii* seeds in different water treatments in petri dishes in 2013

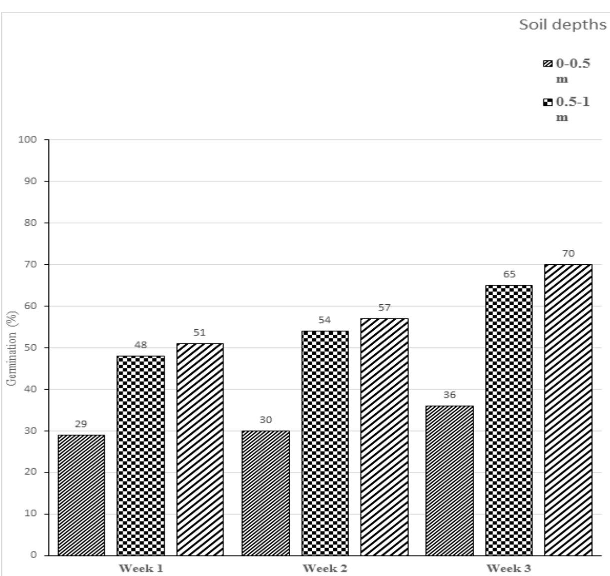

Fig 2: Seeds germination in pots of *A. jacquemontii* at 3 soil depths during February in 2013

3.3 Seedlings growth performance of root-shoot
Large variation in all the growth characteristics was observed during in period of March 2013 to May 2014 the data recording in green house study in Department of Forestry, Range Management and Wildlife University of Agriculture Faisalabad.

The linear relationship between shoot lengths of Babel *Acacia* in different soil depths was shown in Fig. 1. Highest plant growth was obtained in deep type of soil followed by medium soil, whereas in shallow soil, the growth was least. Among overall plant growth, shoot length attained minimum and maximum growth in three type of soils under consideration which increased from a mean values of 8.00 cm to 67.31 cm in deep soil, while in medium soil from a mean values of 7.73 cm to 55.25 cm and in shallow soil, from a mean values of 5.70 cm to 48.94 cm was observed respectively.

Regarding root length plant growth in deep soil expressed maximum increase from a mean values of (164.89 cm) followed by from a mean values of (142.55 cm) in medium soil and in shallow soil from a mean values of (131.15 cm) respectively. Similarly minimum root length was noticed in deep soil from a mean values of (9.99 cm) followed by from a mean values of (9.10 cm) in medium and in shallow from a mean values of (9.54 cm) respectively shown in Fig.2. Similarly maximum from a mean values of (20.99 gm) shoot fresh weight was gained in deep type followed by medium type from a mean values of (18.87 gm) and shallow type from a mean of (15.57 gm) respectively. While minimum shoot fresh weight of deep type was gained from a mean values of (0.71gm) followed by medium type from a mean values of (0.58 gm) and in shallow type from a mean values of (0.41) was observed respectively as shown in the Fig. 3.

Mean values of root fresh weight (maximum) as 16.87 gm, 15.99 gm and 13.88 gm, while minimum as 0.68 gm, 0.54 gm and 0.47 gm was observed in plants growing in deep type, medium type and shallow type soils respectively (Fig. 4).

Among overall plant growth, shoot dry weight attained minimum and maximum growth in three type of soils under the consideration which increased from a mean values of 0.17gm to 6.25 gm in deep type soil, while in medium type from a mean values of 0.11gm to 5.21 gm and in shallow, from a mean values of 0.10 gm to 4.78 gm was gained shown in Fig. 5 respectively.

Regarding root dry weight plant growth in deep soil expressed maximum increase from a mean values of (5.23 gm) followed by from a mean of (3.99 gm) in medium and in shallow from a mean values of (3.10 gm) respectively. Similarly minimum root dry weight was noticed in deep type from a mean values of (0.21 gm) followed by from a mean values of (0.19 gm) in medium and in shallow from a mean values of (0.15 gm) respectively shown in Fig. 6.

The polynomial relationship between root and shoot ratio of Babel *Acacia* in different soils was showed in Fig. 7. Plants attained minimum and maximum root/ shoot ratio in three type of soils increase from a mean values of (1.25 to 2.95) in deep type of soil, while in medium type of soil from a mean values of (1.18 to 2.58) and shallow type of soil from a mean values of (0.75 to 0.90) respectively.

Regarding plant growth in deep soil expressed maximum increased in diameter from a mean values of (5.12 mm) followed by from a mean of 4.91 mm) in medium and in shallow from a mean values of (4.12 mm) respectively. Similarly minimum increased in diameter was noticed in deep type from a mean values of (0.33 mm) followed by from a mean values of (0.31 mm) in medium and in shallow from a mean values of (0.28 mm) respectively shown in Fig. 8.

The polynomial relationship between numbers of branches of Babel *Acacia* in different soils was showed in Fig. 9. Plants attained minimum and maximum numbers of branches in three type of soils increase from a mean values of (0.00 to 4.80) in deep type of soil, while in medium type of soil from a mean values of (0.00 to 4.20) and shallow type of soil from a mean values of (0.00 to 2.80) respectively.

The annual growth of the plant is mainly influenced by the rainfall. The linear relationship between annual rainfall and various growth parameters are presented in Table 10. The rainfall contributes maximum toward growth of all parameters and explains considerable variation in them.

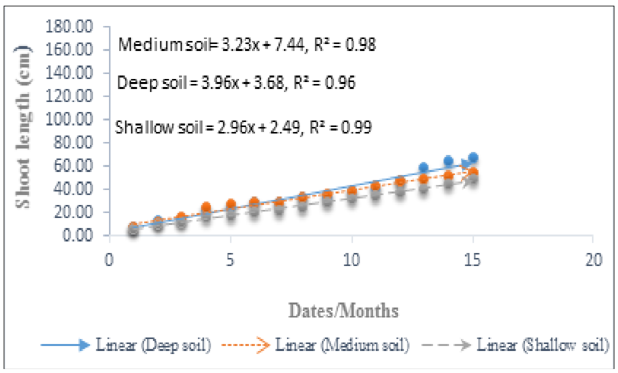

Fig 1: Shoot length of Babel *acacia*

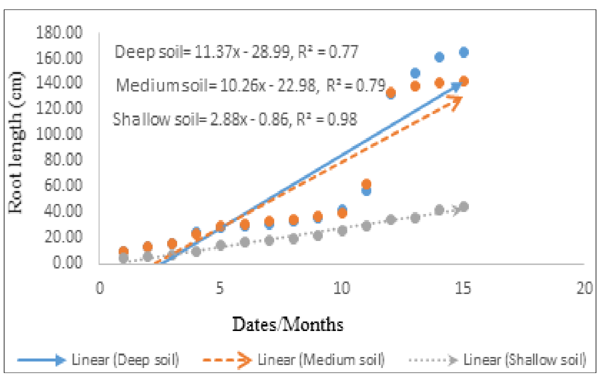

Fig 2: Root length of Babel *Acacia*

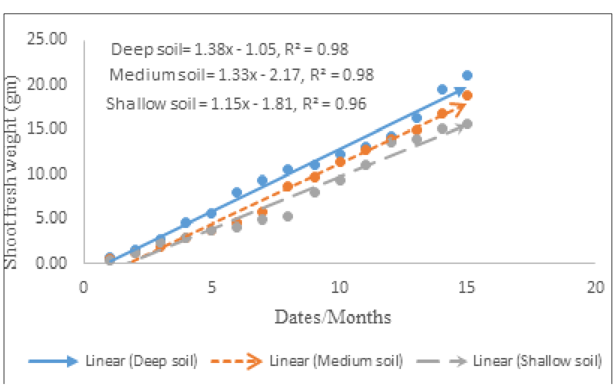

Fig 3

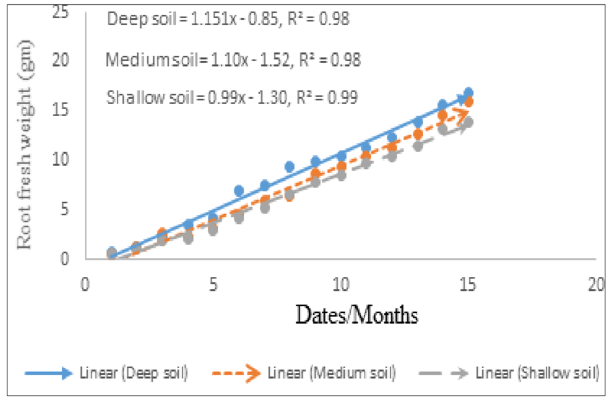

Fig 4: Root fresh weight of Babel *Acacia*

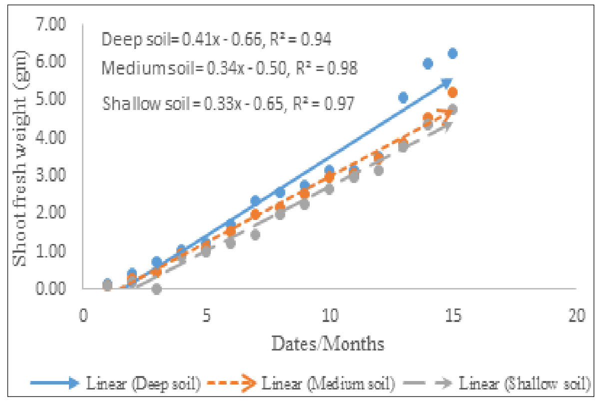

Fig 5: Shoot dry weight of Babel *Acacia*

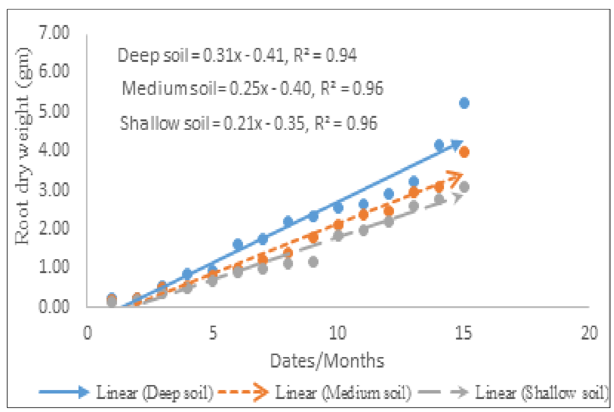

Fig 6: Root dry weight of Babel *Acacia*

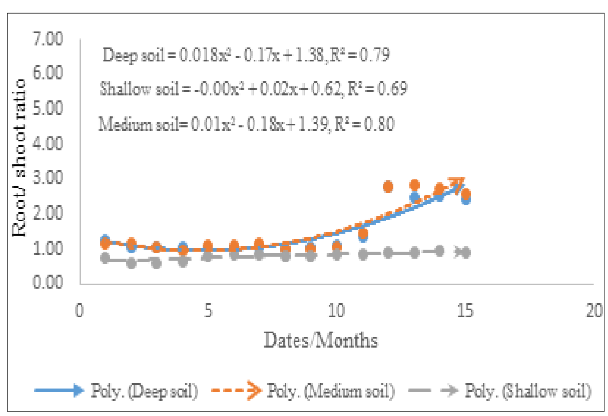

Fig 7: Root/ Shoot ratio of Babel *Acacia*

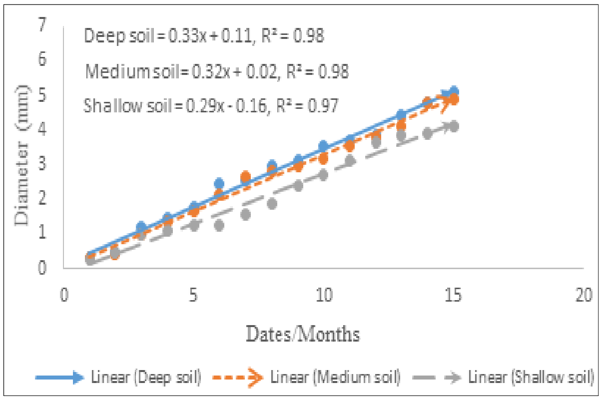

Fig 8: Diameter of Babel *Acacia*

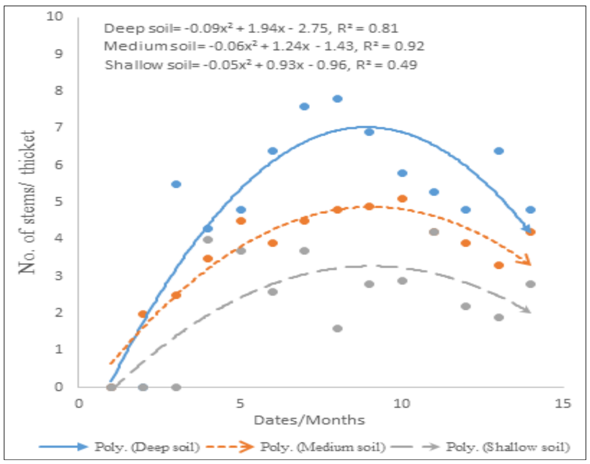

Fig 9: Number of stems/ thicket of Babel *Acacia*

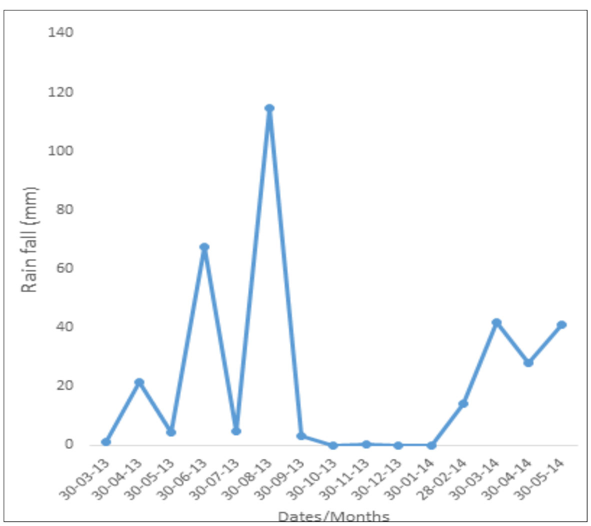

Fig 10: Rain fall data during 2013-2014

4. Discussion

Cold/hot water soaked seeds had greater germination (over 80%) than the non-soaked seeds. Higher germination of the soaked seeds indicated that the soaking was very helpful in softening and breaking the hard seed coats leading to increased germination of the seeds. Mertia and Prasad, (2006) reported that seed dormancy in many *Acacia* species and other woody plants occurs because of the hard seed coat. The hard seed coat may be impermeable for water and gases or it may be mechanically debarring the emergence of the embryo from the seed. According to previous studies conducted by (Edwards, 1973; Clement *et al.*, 1977; Raddad, 2007 and Danthu *et al.*, 1992) [9, 34, 8] most of the *Acacia* species have hard coat, which studied one of the several strategies for survival in the spatial and temporal adaptable environment.

In another study Lorilla, (1992) [24] reported that *Acacia mangium* was not germinate if not treated with cold and hot water. Nasroun and Al-Mana, (1992) [30] similarly, postulated that boiling the seeds of *A. salicina*, *A. saligna*, *A. seyal*, *A. farnesiana* and *A. tortilis* did not increase their germination percentage significantly. This may be due to varying the response of *Acacia* to hot water treatment with species (Bebawi and Mohammed, 1985) [1]. Soaking in tap water for 24 or 48 or 72 in the present study resulted in lower germination percentage comparing with boiling in water. Similar result was obtained previously by Goda, (1987) [12] who found soaking the seeds of *Acacia nilotica* in tap water for 72 h promoted a very slight germination (4%). Danthu *et al.* (1992) [8], therefore, recommended untreated seeds for field sowing and 12-24 h soaking in water for tree nurseries and showed that all *Acacia* species had the greatest germination percentage when boiled in water and left to cool to room temperature apart from *Acacia ehrenbergiana* which did not so, but had higher and similar percentages either when soaked in tap water for different times or untreated.

The higher germination of soaked seeds also depicted that the species had smaller amounts of inhibitions (like abscisic acid etc.) in its seeds. Magnani *et al.* (1993) [28] reported that a number of inhibitors are found in the seeds of *Acacia* species or other woody plants. These inhibitors often decrease the germination of seeds.

Species should higher germination (70%) in pots having field soil which was collected at greater depths than in pots filled with the shallow soils. The reason for higher germination of the seeds in deep soils may be that such soils may be having more soil nutrients than the shallow soils.

Stofella and Kahn (2001) [35] Szmidt *et al*. (2003) [36] reported that the deep soil usually have more soil organic matter and other nutrients which promote seed germination in most *Acacia* species and other woody plants. The best the germination percentage the seeds that were showed near soil surface. This finding concurs with that of (Cox *et al.*1993) [6], who found the emergence of *Acacia constricta* Benth and *Prosopis velutina* seedlings was greatest from seeds sown at 1 to 2 cm depths in sandy loam soil. This may reflect a general survival strategy adopted by *Acacia spp.*; that they have the ability to accumulate large quantities of viable seeds in the soil (Cole, 1986; Holmes *et al.*, 1987 and Sabiiti *et al.* 1987) [5, 13].

Shoot of the seedlings of the species weighed more both in the fresh and dry form than roots after their germination in the field soils. The greater weights of shoots of seedlings may be because of production of leaves, branches, hard stem and thorns over the period of 14 months. The roots of the seedlings on the other hand were soft and had relatively lower amounts of fibrous material than the shoots of the seedlings. Seedlings under limited watering and in different sandy soils developed longer roots to uptake limited water available in the soil. The rapid development of a deep root system that can access water stored lower in the soil profile may be essential for successful seedling establishment (Joffre *et al.*, 2002; Otieno *et al.*, 2005) [15].

When grown in the field soils, seedlings of the species had greater elongation of their roots than the shoots over the period of 14 months. The higher root elongation rate may be because of the adaptation of the species to search moisture deep into the ground. Kandpal *et al.* (2005) [2] reported that the continuous moisture availability for longer duration in deep soil is probably allow *Acacia*s and woody plants protoplasm to multiply at maximum rate and utilize photo synthetically active radiation efficiently to produce higher growth of shoot and root. Fitzpatrick, (2001) [11] stated that results from previous study confirm the numerous assertions outlined in the literature about the positive effects of soils on the growing media characteristics, in terms of probably ameliorating their physical (bulk density, porosity, water holding capacity) and chemical (reactivity, conductivity, CEC, nutrient content, C/N) conditions of the *Acacia* plants (Mexal and Fisher, 1987) [29].

Abundant plant growth in deep soils in comparison to that in moderately deep and shallow soils is attributed to its preference for sandy plain or high dune habitat where the roots could easily penetrate and use subsurface soil moisture. It appears that successive improvements in soil from shallow to deep have improved the duration for which moisture is available. The continuous moisture availability for longer duration in deep soil is probably allow plants protoplasm to multiply at maximum rate and utilize photo synthetically active radiation efficiently to produce higher growth of shoot and root results given by Kandpal *et al.* (2005) [22].

These results are supported by Zimmer and Grose (1958) [39] who studied the physiology of woody plants and stated that, in dry areas, species tend to develop a long tap root with few lateral roots on good sites. Mathers *et al.* (2007) stated that Growing plants in containers, however, alters root growth and function and can change root morphology. Numerous factors influence root growth in containers. Roots of container-grown plants are subjected to temperature and moisture extremes not normally found in field production. The species respond differently to the experimental conditions or allocation of photosynthetic. In addition to differences in the distribution of resources, growth may also differ in the way available carbohydrates may be used within each organ system by Ledig (1983). Plants vary considerably in their capacity to sustain root growth as soil water diminishes by Kramer (1983) [18]. Moreover plants that are capable of sustaining root growth in drying soil have roots that are adapted to soil water deficits; accordingly root growth is ceased as soil dries by Davis *et al.* (1989) [7].

Seedlings' shoot weight and height in these substrates grew at similar patterns and with very close lengths all along the monitoring period. Towards the end of the monitoring period, seedlings' shoot heights growth in the silty soil medium slowed down and deflected from the parallels of compost media. Growth of root lengths developed in the same pattern as described for the shoot heights. Shoot heights for most of the species were more than double the root lengths except for *A. seyal*, which generally gave identical lengths. This indicates that seedlings did not expend much energy on root development because the substrates in which they grew were rich in nutrients (Marschner 1986; Kraske and Fernandez 1990) [25, 21].

Similar finding are found that seedlings biomass (shoot and root fresh weight) was invariably higher in the compost media and proportionate to the amount of compost doses added. The shoot/root biomass ratios revealed that *A. nilotica* and to some extend *A. tortilis* shoots responded to compost application better than the roots, with these ratios figuring to more than five and two folds, respectively (Kraske and Fernandez 1990) [21].

The ability of plants to cope with conditions of low soil moisture depends on their capacity to undertake some genetic traits involving a range of physiological mechanisms. Some of these mechanisms have been identified and include a shift in the partitioning of dry matter from shoot to root, osmotic adjustment and the closure of stomata (Khalil and Grace, 1992) [19].

Similar results are investigated by Joffre *et al*. (2002) [15] the reduction recorded in *A. tortilis* spp. *raddiana* seedlings dry weight under 50 and 25% FC was mainly attributed to leaf dry weight reduction which was caused by reduction in leaf area and number under both treatments and increasing in roots dry weight did not offset that reduction but the increase in roots dry weight explained the allocation of dry matter to roots under limited watering regime. Seedlings under limited watering regime developed longer roots to uptake limited water available in the soil. The rapid development of a deep root system that can access water stored lower in the soil profile may be essential for successful seedling establishment (Joffre *et al*., 2002; Otieno *et al*., 2006) [15].

Although root: shoot dry weight ratio showed no response to water stress, however, *A. sclerosperma* in water stressed treatment had root: shoot ratio was 2.85 as much as that in well-watered treatment whereas some other species had similar ratios in both treatments. The other variable calculated to describe the relative capture of above and below ground resources of trees was total leaf area/total root length. This decreased significantly due to water stress, but the reduction differed from species to another. For instance, it reduced in *A. sclerosperma* by 20% whereas reduced by 26% in *A. victoria b*y Ibrahim (1995) obtained similar result for poplar trees. As plants reduce their foliage more than root growth to endure water stress then decreasing leaf area/total root length ratio would be expected.

The decline in height, diameter growth, and dry matter production of species for some time may be related to the insufficiency of available nutrients. N, P and K uptake significantly diminished with stress. In addition, according to Kramer (1969) [17], absorption might be reduced because of the slow movement of minerals in drying soil, decreased root extension, and decreased root permeability due to suberization. But according to Ng (1992), usually the initial growth of *P. indicus* is relatively slow and it is deciduous for a short time during dry season (Cadiz and Mizal 1995) [4].

It appears that in medium and shallow soils water supply is not sufficient to meet plant water requirement. This results in water stress leading to poor plant growth. Growth behavior of plants in different soil conditions was studied by working out relationship between the age and mean height, stem diameter and number of stems/thicket that grew over a period of five year. (Mertia *et al.,* 2007). He also conducted the study on *Acacia jacquemontii* results showed that plants growing in deep soils yielded highest above ground biomass whereas, plants in shallow soils the least.

Accumulation of stem wood and branches/twigs was the maximum in deep soils followed by medium and shallow soils. However, foliage yield was at par in medium and shallow soils. Reduction in foliage percentage and corresponding increase in branch/twigs wood was evident in deep soils. Slight reduction in foliage allocation and corresponding increase in branch wood formation indicates that stem wood formation was at peak in deep soils (Kunhamu *et al*., 2005) [20].

5. References

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