Korean J. Plant Res. 22(6) : 528～534 (2009)

Physiological Characteristics and Seedling Growth Patterns of Neem (*Azadirachta indica* A. Juss) under Different Soil Conditions

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Abstract - The study of germination and seedling growth characteristics of Neem under different soil and environment conditions was undertaken. The seed germination started 8 days after sowing in commercial bed soil, whereas, delayed germination was observed in sandy-loam (15 days) and sandy (19 days) soil. The highest germination (73.33%) was observed in commercial bed soil in green house, whereas, the lowest germination was observed in sandy soil (16.67%) and sandy-loam soil (8.33%). The seeds in the open field (sandy soil) also showed poor (10%) germination. The mean number of germination seed/day (GD) and seed germination vigor rate (GV) both were highest in the commercial bed soil with 0.733% and 16.67% respectively in the green house, whereas sandy and sandy-loam soil in green house and open field (sandy soil) all showed much lower GD and GV values. The seedling characteristics of nursery revealed that the seedling grown in the growth chamber in commercial bed soil was significantly higher in all the parameters comparing to others grown in green house and open field. The growth was nearly 7 fold in the chamber compared to that of the green house nursery observed in three months old seedlings. Likewise, HPLC analysis revealed that the green house grown seedling contain higher quantity of pigments compare to the chamber grown seedlings. Among the soils used the commercial soil alone or in combination with sandy and sandy-loam soil in the ratio of 2:1:1 respectively with the temperature of 27±2℃ showed better for Neem nursery preparation.

Key words - Neem, Dehulled seed, Germination value, Growth pattern, Soil, Temperature

Introduction

Azadirachta indica A. Juss, commonly known as Neem, belong to the family meliaceae. Due to its high medicinal importance Neem is described as a Nature's drugstore, Village pharmacy, Divine tree, Heal All, and Panacea of all disease (Rawat, 1995). Almost all parts of the Neem extracts have been therapeutically used as folk medicine to control diseases like leprosy, intestinal helminthiasis, respiratory disorders, constipation, and skin infections (Biswas *et al.*, 2002). There are several reports on the biological activities and pharmacological actions of Neem based on modern scientific investigations, such as antiviral (Gogati and Marathe 1989), antibacterial (Singh and Sastry 1997), anti-inflammatory and antipyretic (Okpanyi and Ezeukwu 1981), antioxidant (Sultana *et al*., 2007) etc. In addition, Neem is used as a wood source, reforestation, provider of shade, an animal and poultry feed, fertilizer and natural pesticides (Parmar and Ketkar 1993). Therefore, because of its immense potential, it is cultivated in most of the tropical and subtropical countries of the world (Puri, 1999; Sombatsiri *et al*., 1995).

The Neem is a fast growing, tropical evergreen tree (Werner and Müller 1990). The tree is tall and spreading with short trunk and commonly reaches 15 m and occasionally 25 m height. This plant grows well in many of the dried areas where the rainfall is between 400-1299 mm per annum. It grows best between 21-32℃ on dry, stony, clay, and shallow soils having a pH range of 5.0 to 8.5 (Puri, 1999). It is well known for its resistance to drought and poor soil. However, it is reported that the Neem plant cannot tolerate frost or excessive cold conditions (Chaturevedi, 1993). The tree can be propagated naturally via the seed, and seedlings may be readily grown in the nursery (Utz, 1993).

South Korea has diverse climate with four seasons. It has

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different types of soil with large variations. The winter temperature is cold and snowy throughout the country (except some part of southern island) and the summer is hot and humid. Due to its diverse distribution of climatic conditions and its topographical complexity, Korea exhibits alpine (mountain region), temperate and warm-temperate (southern island) types of vegetation (Oh *et al*. 2000). Taking into account of this climatic variation of Korea, we are trying to cultivate the Neem tree especially in the warm temperate climate of South Korea. Therefore, in this paper, we have described the Neem seed germination pattern and seedling characteristics of Neem nursery in locally and commercially available soil and environment before its plantation in the desired field.

Materials and Methods

Seed collection and growing media

The seeds were collected in 3rd week of August in 2008 from Mechi Anchal of Nepal following the protocol of Nagaveni *et al*. (1987) and transported within a week to Kangwon National University, S. Korea, where the study was undertaken. The Sandy (89.08% sand, 6.43% silt, 4.49% clay, pH 5.5), sandy loam (69% sand, 17.53% silt and 13.47% clay pH 6.5) soil available around the campus (identified by professor Joo Jin-Ho, Soil science, Kangwon National University) and commercial bed soil (containing, peattmoos 20-30%, zeolite 8-12%, cocopeat 50-60%, vermiculite 8-12%, NH_4^+-N 120-150 mg L^{-1}, NO_3^--N 210-270 mg L^{-1}, P_2O_5 270-330 mg L^{-1}, K_2O 60-90 mg L^{-1}, CEC 9-11 $Cmol^+/l$, moisture content 50-60%, pH 5.5-6.5, Korean company) was used for the experiment.

Seed germination test

A total of 60 dehulled seeds (3 replicates of 20 seeds) were sown at 1.5-2 cm depth (following the protocol of Bahuguna, 1997) in each pot containing sandy, sandy-loam and commercial bed soil and placed in a greenhouse. Pots were watered once every 2 days (100 mL). The temperature regime in the green house was maximum 30℃ at day and minimum 20℃ at night in the month of late August 2008. The field experiment was carried out on the available sandy soil (69% sand, 17.53% silt and 13.47% clay), in the campus province around the university. The raised beds of 5×1 m in area and 30 cm in height were prepared to avoid water lodging, because Neem is sensitive to excess water (Gunasena and Marambe 1998). In each plot, 20 seeds (dehulled) were sown in 3 rows about 2 to 2.5 cm depth and at a distance of 25 cm each. Seeds were sown on last week of August and the seed germination was assessed periodically by counting germinated seeds. The visible protrusion on the surface of soil was considered as seed germination. The germination was counted daily and mean number of germination seed/day (GD), mean of seed germination vigor rate (GV), mean of seed germination rate (GR) and total seed germination (TSG) in percent were calculated (Table 1).

Growth performance of seedling after germination up to one month

After germination, some germinated seedlings grown in the commercial bed soil were taken to a growth chamber (GC)

Table 1. Total seed germination, mean seed germination percent, seed germination vigor rate and seed germination per day under different soil types and environment

	Soil type	TSG	GD (%)	GV (%)	GR (%)
Green House	C-soil	14.67a	0.733a	16.67a	73.33a
	S-soil	3.33b	0.0167b	0.000b	16.67b
	S-L-soil	1.67c	0.0833c	0.000b	8.33c
Open Filed	S-soil	2.00c	0.100c	0.000b	10.00c
	Cv (%)	10.2	10.21	138.56	10.2
	LSD	1.105	0.0631	11.53	5.522

Where, C-soil=commercial bed soil, S-soil=sandy soil, S-Loam=sandy loam soil, GD=mean number of germination seed/day (%), GV=mean of seed germination vigor rate for first 8 days (%), GR=mean of seed germination rate (%), TSG=total seed germination up to 20 days. Number of seeds used was 20 in three replications.

having constant temperature of 27±2℃ with 16/8 h light. And other seedlings were allowed to grow altogether under same environment conditions for one month and their growth characteristics (stem height, number of leaves, leaf length, number of nodes and number of internodes) were evaluated (Table 2).

Seedling growth characteristics in nursery

One month old seedlings were transferred carefully to the different soil composition and environment and the growth performance (stem height, stem diameter, number of leaves, leaf diameter, number of branches, auxiliary leaf, number of nodes, number of internodes and first internode height) of two months (data not shown) and three months old seedlings were evaluated (Table 3).

Pigment analysis

Pigments were analyzed following the protocol of Zulfugarov *et al.* (2007). Briefly, Three month old plants leaf segments for both green house (GH) and growth chamber (GC) grown were harvested and quickly frozen in liquid nitrogen and grounded. Then pigments were extracted by gently agitating the leaf powder in ice-cold 100% acetone for 1 hour. To

Table 2. Growth characteristics of one month old seedling in different soil in green house (GH), open field and growth chamber (GC)

	Soil used	PH (cm)	NL	LL (cm)	NN	NI
Green house	C-soil	6.960b	3.20b	5.54b	3.00b	2.00b
	S-soil	4.840c	3.20b	3.00c	2.400c	1.40c
	S-L-soil	3.920c	2.00c	2.02cd	2.00c	1.00c
Open filed	S-soil	1.680d	2.00c	1.42d	2.00c	1.00c
G-Chamber	C-soil	9.060a	7.60a	6.82a	4.80a	3.80a
	Cv (%)	17.14	18.63	23.65	14.3	22.08
	LSD	1.216	0.899	1.192	0.545	0.545

Where, PH=plant height (shoot height), NL=number of leaves, LL=leaf length, NN=number of nodes, NI=number of internodes, C-soil=commercial bed soil, S-soil= sandy soil, S-L-soil=sandy loam soil.

Table 3. shows the growth performance of three months after sowing *Azadirachta indica* seedling grown under different soil and environment condition

Soil used	PH (cm)	SD (mm)	NL	LL (cm)	LW (cm)	NN	NI	NB	AL	HI (cm)
S-soil	6.20b	2.20c	4.67b	4.43b	4.23b	4.0bc	3.0bc	0	0.00c	2.50c
S-L-soil	5.40b	2.40bc	4.33b	4.07b	4.53b	3.00c	2.00bc	0	0.00c	2.10c
C-soil	8.23b	2.93bc	6.0b	5.8b	6.10b	5.67b	4.67b	0	4.33ab	3.77b
CSL (1:1:1)	6.10b	2.27c	6.0b	3.97b	4.20b	4.67bc	3.67bc	0	0.00c	2.00c
CSL (2:1:1)	7.27b	2.43bc	4.67b	5.5b	4.33b	6.33b	5.33b	0	3.67ab	2.43c
CLS (1:2:1)	5.70b	2.10c	5.33b	3.93b	4.17b	4.44bc	3.44bc	0	2.33bc	2.43c
CLS (1:1:2)	6.27b	2.50bc	5.33b	5.0b	4.30b	5.00bc	4.00bc	0	6.00c	3.00bc
C-soil (ch.)	54.67a	3.27a	28.8a	16.80a	13.17a	28.0a	27.67a	0	7.00a	5.03a
Cv (%)	13.6	15.02	16.86	20.33	23.65	17.84	20.02	0	96.27	20.19
LSD	2.971	0.659	2.375	2.203	2.331	2.383	2.352	0	3.653	1.029

Where, PH=plant height (shoot height), SD=stem diameter, NL=number of leaves, LL= leaf length, LW=leaf width, NN=number of nodes, NI=number of internodes, NB=number of branches, AL=Number of auxiliary leaf, HI=height of first internode, C-soil=commercial bed soil, S-soil=sandy soil, S-L-soil=sandy loam soil, C-soil (ch)=Commercial bed soil in growth chamber. In the soil composition, C=commercial soil, L=sandy loam soil and S=sandy soil.

minimize pigment degradation, the extraction was performed in darkness at 4℃. Cell debris was removed by centrifuging at 4℃ for 10 minute at 14,000 rpm. The extracts were filtered and the pigment separation was performed in an HPLC system (HP 1100 Series; Hewlett Packard, Waldbronn, Germany) on a Spherisorb ODS-1 column (Alltech, USA) with a solvent mixture of acetonitrile: methanol: 0.1 M Tris−HCl (pH=8, 72: 12:7, v/v), and a 10-min linear gradient (methanol:hexane, 4:1, v/v). The Chlorophyll content of the leaf discs was determined in 80% acetone extracts using the extinction coefficients and wavelengths as described by Porra *et al*. (1989).

Statistical analysis

The data were statistically analyzed using one-way ANOVA; Duncan's multiple range tests at 0.05 probability using MSTA-TC (1986) package.

Results

Seed Germination period

Seed germination period was varied according to the soil types and environment condition (green house and open field). The data are shown in Table 1. The germination started 8 days after sowing and continued up to 19 days. The types of soil and environment significantly affected the germination period. The fastest germination was found in commercial soil (8 days) and the delayed germination was observed in sandy loam soil (15 days) and sandy soil (19 Days).

Seed germination percentage and pattern

The seed germination percentage and patterns are shown in Table 1. The highest germination percentage (73.33%) was observed in commercial bed soil in green house, whereas, the lowest germination was observed in sandy-loam soil (8.33%). The seeds in the open field (sandy soil) also showed poor germination (10%). Likewise, the mean daily germination (GD) and mean seed germination vigor rate (GV) was varied in different days in different soils. The GD and GV both were highest in the commercial bed soil with 0.733% and 16.67% respectively in the green house, whereas sandy and sandy-loam soil showed much lower GD value with 0.0167% and 0.0833% respectively. Similarly, the open field (sandy soil) also showed lower GD value with 0.10%. However, the GV values were negligible in sandy and sandy loam soil in green house and in open field.

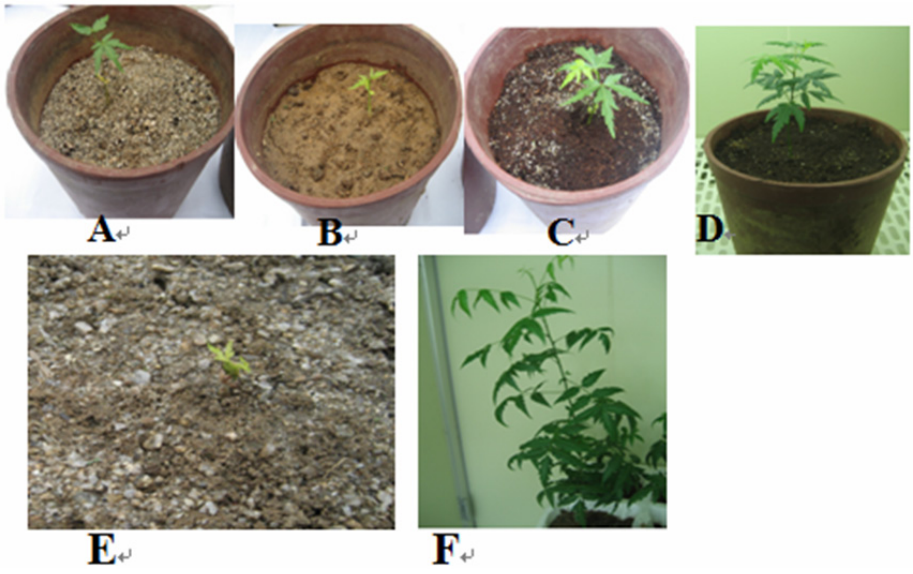

Fig. 1. Growth performance of *Azadirachta indica* A Juss grown under different soil and environment condition. One months old seedling in sandy soil (A), sandy loam soil (B), commercial bed soil (C) in green house. One months old seedling in growth chamber (27±2℃, 16/8 h light) under commercial bed soil (D) and open field (sandy soil) (E). Three months old seedling grown in commercial bed soil inside growth chamber (F).

Growth performance of seedlings after one month of germination

The plant growth in commercial bed soil inside the growth chamber was found to be vigor compared to the other soils used in the experiment (Table 2). The number of leaves, leaf length, number of nodes and internodes all were significantly higher in the commercial bed soil grown in the chamber than in the other soils. The shoot length of the seedlings developed in the commercial bed soil was highest (9.06 cm). Similarly, the seedlings grown in commercial bed soil in green house also showed better growth (6.96 cm) compared to sandy (4.84 cm) and sandy-loam soil (3.92 cm) in the green house. The seedling growth in the open field in sandy soil was retarded in all the parameters observed (Table 2). The cotyledon characters were also evaluated in all the germinated seedlings and found to be intact and green in color in commercial bed soil in both green house and in chamber. However, it turned yellow in sandy and sandy-loam soil.

Seedling growth characteristics in Nursery

The seedling characteristics of three month old nursery grown in different soil and environmental conditions are given in the Table 3. The results revealed that the seedling grown in the chamber (temperature 27±2℃) in commercial bed soil was significantly vigorous in all the parameters (shoot growth, stem diameter, number of leaves, leaf length, leaf width, number of nodes, internodes, auxiliary leaves and first internode height). The growth was nearly 7 fold in the chamber compared to that of the green house nursery. The average maximum growth of the seedlings in the chamber was 26 cm in a month. However, the green house grown seedling did not show vigorous growth like that of the chamber. Among the soils used the commercial soil alone or in combination with sandy and sandy-loam soil in the ratio of 2:1:1 respectively in green house showed better growth compared to the others. However, in any of the cases there were no branches developed in the seedlings but auxiliary leaves were seen especially in the commercial bed soil both in the chamber and green house nursery.

Pigment analysis

Pigment plays an important role in the environmental adaptation of the plants, therefore, the pigment composition were evaluated between the green house (GH) grown seedlings and growth chamber (GC) grown seedling Nursery. Table 4 shows the HPLC analysis of pigments in the Neem seedling leaves grown in the green house and growth chamber. The data revealed that the green house grown seedling leaf contains slightly higher pigments (Lutein, Zeaxanthin, Chlorophyll b and β-Cartenoids) compare to the chamber grown seedlings. However, Violaxanthin, Neoxanthin and Antheroxanthin contents did not show much different in both GH and GC grown seedlings.

Table 4. HPLC pigment analysis of Neem seedling leaves grown under Green House (GH) and Growth Chamber (GC)

Pigments	GH grown seedling	GC grown seedling
Neoxanthin	70.13 ± 2.6	63.63 ± 14
Violaxanthin	22.00 ± 1.3	29.60 ± 3.6
Antheraxanthin	14.10 ± 1.2	17.66 ± 1.3
Lutein	270.40 ± 13	256.6 ± 11
Zeaxanthin	115.00 ± 14	85.04 ± 6.0
Chlorophyll b	299.93 ± 15	276.43 ± 12
Chlorophyll a	1000.00	1000.00
β-Carotene	175.36 ± 8	150.76 ± 6

The values are given in mmol of pigments per mol Chl a.
Mean value ± SD are given for at least 3 independent experiments.

Discussion

Conventionally, Neem is propagated by seed. In our experiment, we have tried to sow seeds within a week after collection from the source; otherwise, Neem seed loses its germination capacity considerably after 2-3 weeks (Webb *et al*. 1984). Further, to achieve higher percentage of germination we depulped Neem seeds (Chaney and Knudson 1988) because it has been postulated that the endocarp develops a physical barrier for water, gases, enzymes and inhibitors and for the metabolism of fats (Radhamani *et al*. 1990). However, the different types of soil used in the experiment showed variation in the seed germination. This variation is directly proportional to the nutrients content in the soils and may be due to the recalcitrant na-

ture of the Neem seed (Ezumah, 1986). In a previous study, Singh *et al*. (1995) observed that the fresh Neem seed germinates 7 days after sowing and is completed after 25 days. However, in our experiment, the seed germination started 8 days after sowing in commercial bed soil, whereas, delayed germination was observed in sandy-loam (15 days) and sandy (19 days) soil. Therefore, seed germination rate per day (GD) and mean seed germination vigor rate (GV) were calculated in the period of 8 days and 20 days (maximum), respectively. The commercial bed soil showed the highest percentage of germination compared to the locally available sandy and sandy-loam soil. This may be due to the fact that the commercial bed soil contains all the necessary minerals and nutrients (N.P.K.) which is required for the plant growth and development, and gives vigor seed germination and growth, whereas, sandy and sandy-loam soil does not contain sufficient amount of nutrient required for the germination or plant growth. These results are almost in agreement with the previous reports by Gupta (1992) in which he stated that the growth of nursery plants of Neem was influenced by the soil mixture and fertilizers. Likewise, Mohan *et al*. (1990) found that the Neem plant growth was vigor in the mixture of farmyard manure with 30 ppm Nitrogen and 20 ppm phosphorus, sandy and clay soils.

Neem is a tropical plant; therefore, temperature plays a crucial role for the development of seedling growth in the nursery. The nursery in the chamber with the temperature of 27±2℃ and 16/8 h light showed highly significant growth. The average growth of plant height in a month was 26 cm in the chamber. However, in the green house nursery the plant growth height was nearly 1.3 cm (Table 3). This significant variation in growth showed that Neem growth depends on temperature. Likewise, the pigment composition between the GH and GC grown plant were also different (Table 4), indicating that plants grown under green house (high light) have the capacity to synthesize greater amounts of β-carotene and xanthophylls as an adaptive strategy to protect plants from high light (Thayer and Bjorkman 1990).

Overall, the data revealed that the sandy soil and sandy-loam soils both are not suitable for Neem seed germination as well as nursery preparation either in the green house or in the open field. However, commercial bed soil (containing N,P,K) alone or mixture of commercial bed soil with sandy or sandy-loam soil is preferred for the seed germination and seedling growth. Likewise, the data also showed that the temperature plays a crucial role for seedling growth, therefore, the temperature of 27±2℃ with mixture of soil with fertilizer can be a better protocol to grow Neem seedling in the nursery either in green house or in growth chamber.

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(Received 2 July 2009 ; Accepted 6 December 2009)