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The effect of different soil and shade regime on germination and growth pattern of *Dodonaea viscosa* (Linn.) Jacq., in Malakand division

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Abstract

Both soil and shade are the most essential factors for the establishment and growth of plants. The study investigated two stages of *Dodonaea viscosa* reproduction, seed germination and seedling growth on various screening effects of four combinations i.e. B1S1-seeds sowed in pure Garden soil; B2S2-seeds sowed in field soil substrate containing organic manure (Humus); B3S3-seeds sowed in original soil collected from the field (OS-Original soil); B4S4-seeds sowed in undergrowth soil substrate and placed under shade of trees. The seeds were sowed according to their polarity in the bags, which were arranged according to a randomized complete block design (RCBD), with four treatments per block and four repeats. Each experimental unit included 30 plant pots, each of which contained three seeds. The results showed significantly different impacts on *D. viscosa* seed germination in the soil types with various textural, physiochemical compositions and the influence of shade. Various soils type and shade regimes from different sites of the study area have significant differences ($p < 0.05$) for germination percentage, leaf morphology, stem diameter, height, cover, root length and above ground biomass respectively Germination percentage was adversely influenced by shade. In the comparisons among original soil with same environmental factor, it was always showed that germination rate of seeds was higher in the order of Garden soil, Humus mixed soil, and soil placed in shade. It was concluded from the results that *Dodonaea viscosa* have variable response to different soil substrate and shade and well adopted to original soil with open sunshine.

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Introduction

Dodonaea viscosa Jacq., belong to Sapindaceae is a dominant shrub species in the sub-tropical dry temperate forests located in the northern Hindukush and Himalayan ranges mountains in Pakistan (Champion *et al.* 1965; Sheikh *et al.* 1994). It is a long lived woody perennial spreading or erect shrub or tree up to about 5 to 8 m (Walsh and Entwisle 1996). *Dodonaea viscosa is* very effective in sand dune fixation and controlling coastal erosion since its roots are excellent soil binders (Khan *et al.* 2010; Khan *et al.* 2011). The species is also used to reclaim soil erosion, grown as an ornamental plant for its shiny foliage and pink-red winged fruit and Poles are useful in fencing (Personal observations). The timber is hard and durable and generally used for roof thatching materials of houses in northern areas of Pakistan (Ali *et al.* 2007). Studies have shown that roots are used in the preparation of medicinal oil, which is used to treat rheumatism whereas, leaves are also used in the treatment of rheumatism and bone fracture.

A seed will go through many tests (e.g. predation, freezing, drought, physical damage and water stress) and stages (e.g. pre-dispersal, dispersal, germination, seedling and sapling) until being a survived sapling (Khan 2011; Wahab 2011). Although seed dispersal is a key process in plant population dynamics (Harper 1997), the post-dispersed environment has a powerful screening effect on the survival probability of seed and its subsequent periods. As an outstanding germplasm resource in Inner Hindukush region, it is being seriously threatened by the deterioration of the sub-tropical environment (Ahmed *et al.* 2011). *Dodonaea viscosa* cannot be successfully regenerated by seeds in natural conditions, but it also could regenerate by a large production of roots. It's extremely urgent for us to solve the problem of seed regeneration failure because of the lower diversity leading by long-term seed dormancy in the soil due to various environmental factors.

Knowledge of seed movement and fates of *Dodonaea viscosa* are essential for ecosystem restoration and conservation efforts and for the control of alien species in all biomes (Khan *et al.* 2010), especially in the extreme arid regions. The fates of seed are affected both by abiotic and biotic factors as well in the sub-tropical dry and moist areas of located in Hindukush range of Pakistan. Some previous researches have figured out that mother shrub/trees of *Dodonaea viscosa* can produce hundreds to thousands of seeds every year, however, many seedlings hardly being found in the forest. We assumed that the constantly changing and threatening environment was the key reason for sexual regeneration failure based on the fact of large production of vigorous seeds per year and temporal heterogeneity of seed rain. Besides, considering that plants exhibit a variety of behaviors in response to environmental stimuli (Karban 2008), this paper focused on the influence of two important natural factors (i.e., common soil substrate and shade regimes on the seed germination and seedling survival in *Dodonaea viscosa* by manipulating their soil gradients or soil types and shade governance. Our goal was to figure out why *Dodonaea viscosa* failed to regenerate naturally by seeds to some extent, and then to provide some useful measures of management for *Dodonaea viscosa* shrub-land forest restoration and conservation in subtropical dry temperate areas of Hindukush range of Pakistan.

Materials and methods

Study site

This experiment was conducted at the Botanical Garden Post Graduate Jahanzeb college Saidu Sharif Swat spanning between longitude 060 22' 40" north; latitude 020 37' 08" east and altitude 13m), which lies 20 km east of River Swat. The Botanic Garden is located at altitude of 700 m above sea level, which is suitable range for sowing seeds of *Dodonaea viscosa* because it's populations at this altitude are stable in Hindukush and Himalayan regions of northern Pakistan (Fig. 1). Soils of the region are of both transported and residual type (Ali 2012) and mostly the foot hills and low lands have deep soil profile with large pore space due to large particle size (Khan and Bibi 2013).

In forest floors the soils are quite rich in organic matter which is due to rapid humification and ranges from light brown to deep brown (Beg 1984).

The ground water is very close to the surface along the small tributaries and river Swat. The study area lying in the temperate zone in northern mountainous ranges and the weather is affected by the climatic factors, latitude, longitude and monsoon. In summer the area comes under the influence of monsoon and cyclonic current in the winter from Mediterranean Sea (Ahmed 2012).

The climate of area is generally continental with an average rainfall of 50 inches per year where, December to February is the winter rains that remain continuous to one or two weeks usually with snowfall. March to May is regarded as spring rains and after one dry month (June) the summer rains begin and end in September (Sher *et al.* 2009). The mean annual temperature is 8.2°C, with the mean monthly maximum and minimum air temperatures are 40°C and –26°C, respectively. January is the coldest month (2 °C to -2 °C) while July is the hottest month in which temperature rises to more than 32 °C in lowlands. The sunshine is 2075 hours per year according to 1980-2014 climate data. The mean annual temperature is 8.2°C, with the mean monthly maximum and minimum air temperatures are 40°C and –26°C, respectively. The vegetation is characterized by broad leaved and coniferous forest (Ahmed *et al.* 2006). *Q. baloot*, *Q. incana* and *O. ferruginea* are common broad leaved whereas, *P. roxburghii*, *P. wallichiana*, *C. deodara*; *A. pindrow* and *P. smithiana*are common conifer species in the study area (Champion *et al.* 1965).

Experimental manipulation

Seed collection and selection

Seed were placed under neutral shade for 10 days. Initial germination trails showed that *Dodonaea* seeds germinate rapidly, within 3-5 days, seeds were monitored for 3 weeks and no additional germination was noted beyond 10 days (Personal observation).

Seeds for germination trails were collected from four different districts of Malakand division at different altitudinal ranges in July and August in three consecutive years i.e. 2011, 2012 and 2013. Seeds were collected from at least 50 healthy *D. viscosa* plants with various diameters, height and crown cover from natural forest stands. The seeds were brought to the laboratory in paper bags and stored at approximately 25-30 °C temperature and 50-60% humidity until used. Prior to experimentation, seeds were sieved and exposed to sunlight for ventilation and later on sorted and damaged seeds were removed in order to remove undue effect in determining viability. On average 20 % of seeds were found with damaged embryo and were removed from the experiment. The selected seeds were treated with fungicide (Thiram, 80%) before the germination test following Avci and Kaya (2013).

For all field experiments, caryopses remained within the dispersal unit so as to best understand the germination ecology of these seeds in their natural state (Baskin and Baskin 2001). A subset of the sorted caryopses was placed on moist sand petri dishes with sealed parafilm (Baskin and Baskin 2001) and allowed to germinate in order to verify the percent of viable seeds. For all laboratory germination trails, 5 replicates of 10 caryopses were used. For these germination trials, petri dishes

Soil samples collection and preparation

At the same time, soils were dug-out at a depth of 30 cm at three randomly selected areas within *D. viscosa* shrub-land following Khan *et al.* 2013 (Fig. 1; Table 1). At least 100 kg soil were collected from individual site and pooled to form a homogenized composite sample following Khan *et al.* (2014). Before sowing seeds 90 perforated black polythene bags of 30 cm x 199 cm^2 size were filled with two types of soil.

The seed thus obtained were sowed out in 30 cm x 199 cm^2 perforated black polythene bags. Two types of substrate were used to fill the bags. The topsoil at the planting sites was mixed with organic manure (2/3 soil to 1/3 organic manure).

The second substrate was made from soil taken from the forest undergrowth at the research station in Botanical Garden Postgraduate Jahanzeb College Swat, which is the substrate generally used for young nursery plants. After the bags were filled, they were placed in open flat surface and in shade of trees.

Watering was conducted 2-3 times per week in order to keep the substrate moist. The seeds were sowed according to their polarity in the bags, which were arranged according to a randomized complete block design (RCBD), with four treatments per block and four repeats. Each experimental unit included 30 plant pots, each of which contained a seeds. The test was conducted from 9 July 2010 - 4 July 2011. The various treatments were as follows: B1S1: seeds sowed in field soil substrate containing organic manure; B1S2: seeds sowed in undergrowth soil substrate; B2S1: seeds sowed in soil substrate containing organic manure; B2S2: seeds sowed in undergrowth soil substrate and placed under shade of trees.

Observations

Every week for 2 years, the number of seeds geminated in each type of substrate was recorded, together with the number of leaves and morphology of the first leaf as soon as it developed. A final count was taken after three months for each type of substrate. Three months after sowing, the plants height was measured, based on a sample of 10 plants per treatment between the point of sprouting and the terminal bud, one year after planting out.

Statistics indexes and analysis

The average values for the different parameters studied were calculated. After the whole test, we calculated the germination percentage (G) and germination index (GI, an index of germination speed) according to Liu *et al.* (2011). We also calculated the root to shoot ratio, the absolute growth rate, the absolute elongation rate of shoot and the absolute elongation rate of root by the following equations: R/S = DWR / DWS, where R/S is the root to shoot ratio, DWR the dry weight of root biomass and DWS the dry weight of shoot biomass.

AGRS = W/Dt, where AGRS is the absolute growth rate of seedlings and W the dry weight of seedlings in day t.

AERS = H / Dt, where AERS is the absolute elongation rate of shoot and H the height of seedlings in day t. AERR = ln (L / Dt) where AERR is the absolute elongation rate of root and L the length of main root in day t. SPSS 17.0 for The significance or insignificance of the differences observed was evaluated by means of a variance analysis (ANOVA) and the Newman-Keuls test for the classification of averages.

Windows was used in one-way and two-way ANOVA with multiple comparisons, using least significant difference tests (LSD). The variance analysis model used was the 3-factor crossed model: a random factor (block) and two fixed factors: substrate and cutting type. The variance analysis was conducted with SAS software (version 9.1).

All the soils were chosen as three kinds of substrates for seed germination and seedling growth, and each was homogenized, sterilized (134°C) and sieved (5 mm). Soil chemical parameters are shown in Table 1. Moreover, 3 gradients of soil water content (i.e., 10%, 15% and 20%) were also set. The water contents were controlled twice a day (i.e., 9:00 AM and 9:00 PM) by weight ratio. To know the complete germination rate, we chose the pure water (i.e. distilled water, hereafter, CG) as an additional germination substrate. In the germination experiment, there were 5 replicates for each group and 30 seeds in each replicate.

The seeds were observed in a 4-hour-interval and the germinated ones were immediately taken into the other containers (3 seeds evenly in one container) with the same conditions for the subsequent growth experiment. The term of “germination” means that we observed a seed radicel breaks its coat. Then, we counted the survived seedlings every day. After the 45th day from the treatment of the whole experiment, we randomly chose 5 seedlings from each group and recorded their heights, leaf area and taproot lengths.

We also recorded the fresh and dry weights of above- and below-ground biomass by analytical balance (MS104S, Mettler-Toledo International Inc., USA).

In total, 1500 seeds (30seeds × 5 replicates × 3 soilsubstrates × 3 water gradients + 30seeds × 5 replicates× 1 pure water substrate) were used for the whole experiment. The containers for seed germination test and examining seedling growth were all the plasticbowls (150mm in diameter and 100mm in height) with full of different substrates placed in a room with aconstant temperature of 25°C and a 16:8 L: D lightcycle with incandescent lumps.

Results

Soil and stand characteristics

The distribution, geo-physical and ecological characteristics of the four sampling site for the seed collection of *Dodonaea viscosa*in Malakand division is shown in Table 1. The species is distributed between an attitudinal ranged from 500 m 1800 m and generally show their dominance below Pinus zone forming an eco-tone. The range in the annual mean temperature was -3– 38 °C, and the range in the mean precipitation surplus 3 to -145 mm (Table 1). Subsoil texture and subsoil pH ranged from coarse sand to loam and from acid (4.6±0.7) to neutral (7.0±0.2) pH.

Water holding capacity (WHC) was ranged from 5.2±0.6 to 11±4.4 per 10 gram of soil while organic matter varied between 1.4±0.4 to 2.3±0.8% in the soil. The average lime content was in the ranged of 10.9±1.2 to 14±1.4% in the four sampling sites.

Total nitrogen pools to soil depth 50 cm ranged from 2.0±0.5 to 2.9±0.5 g/kg^{-1}, whereas Potassium ($K^{2+)}$) and Phosphorus (P^{2+}) pools varied by factor 10-20 and about factor 50 for Phosphorus. Among the four sites C: N ratios in 0-15 cm mineral soils were generally low (7 to 20) probably owing to the former agricultural use.

Table 1. Percent seed germination and reproductive capacity (seed output) of *Dodonaea viscose* in four different soil types.

Soil type	SG	%	RC (SO)	%	R.C	M. Height
G. Soil	48	53	39.5	53.33	21.06	29.4±1.74
H. Soil	38	42	39.5	42.22	16.67	28.2±2.43
O. Soil	62	69	39.5	68.88	27.20	31.5±2.97
S. Soil	10	11	39.5	11.11	4.38	

Note: G. Soil = Garden Soil; H. Soil = Soil with Humus; O. Soil = original Soil; S. Soil = Shade Soil.
SG = Seed Germination; % = Percentage; RC (SO) = Reproductive Capacity (Seed Output); M. H = Mean Height.

Seed germination

Significantly different impacts on *D. viscosa* seed germination was observed in the soil types with various textural, physiochemical compositions and the influence of shade (Table 2). Various type soils and soil under shade regimes from different sites of the study area have significant differences ($p < 0.05$) for germination percentage, leaf morphology, stem diameter, height, cover, root length and above ground biomass (Table 2) respectively in the four experimental trials using CRBD (Complete Randomized Block Design).

Table 2. Average (±SE) values of different parameters of *Dodonaea viscosa* in four different soil types.

Soil type	RL Mean ±SE	RC	RFW	RDW	CC	SFW	SDW
G. Soil	17±2.11	17.2±2.32	5.96±0.80	2.56±0.39	29.9±4.32	22.6±2.90	12.5±1.64
H. Soil	15.35±3.12	14.17±2.9	16.03±3.13	9.96±2.00	23.8±5.24	38.6±7.46	9.96±2.00
O. Soil	14.52±2.21	16.2±2.6	4.94±0.31	3.82±0.24	19.76±3.01	19.88±0.43	8.47±0.38
S. Soil							

Note: RL = Root Length; RC = Root cover; RFW = Root Fresh Weight.
RDW = Root Dry Weight; CC = Canopy cover; SFW = Shoot Fresh Weight.
SDW = Shoot dry weight.

Among the *Dodonaea* seeds collected from open areas and uphill slope there was wide variation in seed characteristics and germination patterns. The germination percentage of the taxa ranged from 11.11% to 53.33%, and the lowest germination was observed in shade pots. Germination percentage was adversely influenced by shade, where germination percentages were below 11% of the total 90 seeds sown.

Taking the germination rate (G) and the germination index (GI)into account, there were differences in the terms of nutrient contents of the same substrate and substrates with the same water content (Fig. 2, 3).Table 2 shows that, to all the four soil types, the germination rates of *Dodonaea viscosa* went larger with the increasing soil contents in the original soil, and the curves became sharp from 11%for both the Humus mixed soil and the Garden soil that is, 43% to 52% respectively. In the comparisons among original soil (OS) with the same environmental factor, it was always showed that the germination rate of seeds was higher in the order of on Garden soil, Humus mixed soil, and soil placed in shade.

Seedling survival

As shown in Table 2 and 3, different types of soil and treatment had diverse screening effects on seedling survival. Few seedling of *Dodonaea viscosa* were survived in original soil under shade stress but couldn't pass through the '20-day-barrier' at all. The reproductive capacity (RC) of seeds were highest for original soil (27.20%) followed by Garden (21.06%) and humus mixed soil. A somewhat, similar pattern was observed for seedling survival in the three soil types except original soil under shade. However, the seedling survival percentages were always higher in the condition of open pots exposed to light.

Seedling growth

Because the seedlings could not survive over 20 days in some conditions (Table 2), it was only necessary to test the growth indexes of seedlings on Garden, Humus mixed and original soil and with different composition of soils. A two way ANOVA was conducted to determine whether soil and water influenced the indexes of seedling growth (Table 3 and 4). The values of each index served as the response variable, with soil type, water content, and their interaction serving as fixed effects.

Table 3. Average (±SE) fresh and dry biomass of *Dodonaea viscose* in four different soil types.

Soil type	NOL	LFW	LDW	LL	LW	ASH
G. Soil	55.16±6.82		3.53±0.56	5.78±0.15	1.4±0.07	1.69±0.30
H. Soil	139.2±26.81		9.53±1.90	4.9±0.27	1.2±0.09	2.78±0.57
O. Soil	49.47±1.14		1.51±0.08	5.9±0.12	1.5±0.03	0.56±0.07
S. Soil						

Note: RL = NOL = Number of Leaves.
LFW = Leave Fresh weight; LDW = leaves Dry weight.
LL = Leaves Length; LW = Leaves Width; Ash = Ash.

Almost all the indexes showed that seedlings on RB significantly grew better than those on SM (Table 3; Fig. 5). As to soil types, the P values of all the indexes except R/S were less than 0.01, which means that soil types have a significant effect on the seedling growth; however, as to the water content and the interaction between soil type and water content, only the P values of TL were less than 0.01 (Table 4). Besides, it was worth being mentioned that the taproot of seedlings at the 15% level could elongate longer and faster than those at the 20% level on the same substrates (Table 3; Fig. 5).

Discussions

This study showed that the original soil was the most suitable soil substrate in the natural condition with sufficient amount of organic matter for *Dodonaea viscosa* seed germination and seedling growth. Original field soil always consists with lower salty content and higher nutrients than garden soil and Soil mixed with humus. Wu *et al.* (2007) have proved that lower concentration of NaCl solution has little effect on the total seed germination rate; and when the concentration reaches 90mmol/L, the rate will obviously decrease.

Besides, there were another two reasons for higher germination rate in Original Field Soil (OFS): (1) because of its more organic constituents and higher compaction, Original field soil retention was greater than those of Soil of garden and Soil mixed with humus oils, for example, the seedlings growing on the original soil with 20% moisture cannot survive over 20 days, l*et al*one with lower moisture in natural conditions; (2) many arid plants and weeds, such as *Amaranthusviridus*, *Euphorbia helioscopia* etc., usually exude allele-chemicals into soil, inhibiting other plants' seed germination or growth. Apart from these weeds some species are exotic theat adversely affect the germination and growth and development of native species. One of these in the northern Pakistan is *Partheniumhysterophorus* that effect the germination of various native species. Zhang *et al.* (2005) figured out that low moisture, high salt content and allelopathy chemicals, which were the characteristics of S soil and SM soil, had negative effect on germination. Therefore, we argued that soil type in nature conditions could significantly influence the seed germination and seedling growth of *Dodonaea viscosa*, which is consistent with the results of two-way ANOVA.

Although higher soil moisture in original soil with high content of organic matter could effectively promote the seedling growth on the whole, less moisture availed the root elongation and AERR instead of the shoot height, leaf largeness or biomass accumulation of *Dodonaea viscosa* seedling. However, Peters (1957) found that at a soil suction (i.e. the relative vapor pressure of the soil moisture) of 1/3 bar and a bulk density of 1.25g·cm-2, corn roots elongated faster in a soil mixture with a gravimetric water content of 27% than in one with a water content of 8%, which was different with our results in *Dodonaea viscosa*. The actual reason for this may be the fact of different plant with different characteristics but still need further study.

All the values in the experimental group of '20%, RB' showed that this condition could guarantee the higher seed germination rate and seedling survival percentage.

This condition made most seeds germinate faster and longer and seedlings grow stronger, which may be the results of lower salty content, higher nutrients and less allelo-chemicals as above.

Many studies have focused on *Dodonaea viscosa* growth and its reliance on groundwater (Gries *et al.* 2003; Rüger *et al.* 2005; Liu *et al.* 2007; Fu *et al.* 2010), but still few on its seed and seedling stage. In natural conditions, though there are favorable conditions for *Dodonaea viscosa* seed germination during their long lives (Zhang *et al.* 2005), and the channeling has changed the natural hydrologic regimes and processes leading to the failure of regeneration of *Dodonaea viscosa* (Cao *et al.* 2009b).

However, we argued that the suitable habitat for its seed germination and seedling growth really existed in the nature and found that many branches of Swat and Dir River were not cemented except the main channel. The areas found being covered by many seed-originated seedlings are usually low and flat flood plains, which can store river water to keep high moisture and low salinity. But there were few seedlings in the other areas, such as in the sandy areas or in the main *Dodonaea viscosa* forests. That fact was just coincident with our experimental results.

Seed dispersal process was generally divided into two periods: Phase I, the movement of germinable seeds from the plant to a surface and Phase II, the secondary horizontal and vertical movements of seeds (Chambers and MacMahon 1994). As to the anemophilous *Dodonaea viscosa* seeds, wind is the key factor in Phase I. Considering the adhesive effect of abundant vegetation in the forests on seeds, we thought river bank and the other shallow sites with sufficient water were the most suitable ones for seed germination among all of the surfaces (e.g. river, soil and plants).

That may be the reason why *Dodonaea viscosa* forests are always called 'riparian'. After quick germination in the water, seeds adhered to the flooded river bank as 'belt' where were suitable for seedling growth.

Although Cao *et al.* (2009b) revealed that the supplemental flows were sufficient during the seed rain period of July 14th to August 28th; we still found that the water could not reach the Nature Reserve in that period every year. It was proved that the most steady water supplement was during the two periods of March to April and September to October every year, but unstable in the seed rain period (Chen, 2010), which leaded to the discontinuous suitable habitats for seedlings in the preliminary key years and might be the main reason for the failure of *Dodonaea viscosa* reproduction.

Due to the huge economic effect of the hot and developing tourism of *Dodonaea viscosa* forest from September to October, the precious and rare river flow was usually supplied to create beautiful landscape in autumn and more impossible to be got from July to August in this extreme arid region, which accelerated the vegetation deterioration. It was reported that there was a 'biennial fruiting variation' phenomenon among many plants (Li *et al.* 1998; Borgardt and Nixon 2003; Hirayama *et al.* 2008).

Based on the assumption that the whole community gets the equal energy and nutrients every year, some thought that there might also be a biennial seed production in *Dodonaea viscosa* which severely affected the seed quality and then the subsequent growth stage. However, the high seed germination rate of our experiments in recent years proved this idea was not true (Zhang *et al.* 2005; Wu *et al.* 2007; Liu *et al.* 2011). Furthermore, light condition may be another limiting factor for *Dodonaea viscosa* regeneration (Zhang *et al.* 2005; Liu 2011). Therefore, we did a simple additional experiment about the effect of lightness on seed germination. It is obvious that light significantly lowered the seed vitality. In nature, the extremely high illumination intensity there made the ground temperature rise to over 70°C with rare vegetation, definitely leading to the death of weak seeds and seedlings.

Along the river bank, the conditions of vegetation were various and only the suitable shading condition provided by the abundant plants could pretend them from being burnt by sun.

In a word, the water supply amount and time were the keys for the successful sexual regeneration of *Dodonaea viscosa* It is badly in need of the reasonable water adjustment and the foundation of river bank vegetation to protect the *Dodonaea viscosa* forest in Hindukush range Oasis.

Suggestions for artificial seedling cultivation

Through our experiment connecting two essential environmental factors with seed and seedling periods, we provided some useful suggestions for artificial seedling cultivation of *D. viscosa*:

1) The harvested seeds should be germinated in the original field soil which can guarantee the highest germination rate;

2) The seeds should be kept in the water for 1-2 days before germination;

3) Seed should not be sow in original, garden and original soil mixed with humus under shade because, the seeds remain dormant in shade regimes though there will be the availability of nutrients in enough quantity in the soils.

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