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RESEARCH ARTICLE

STUDY ON SEED GERMINATION AND VEGETATIVE PROPAGATION OF *SALVADORA OLEOIDES* DECNE

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ABSTRACT

Because of its high vulnerability, seed germination is considered to be the most critical phase in the plant life cycle. In an attempt to find the possible reason for extremely fast decline in population of *Salvadora oleoides* in north-west India; the experiments were conducted on seed germination, seedling growth and vegetative propagation. Seeds were sown in different soils types. Desert sand was best in terms of higher percent seed germination and also a shorter time taken for germination. Whereas vermicompost was better in terms of seedling growth parameters such as height, number of node per seedling, fresh and dry weights of seedlings and number of leaves per seedling. The study showed that the seed viability is very short with only 20 per cent germination after three months of storage. Seeds with fruit pulp resulted in to very poor germination as compared with de pulped seeds indicating an ecological adaptation for dispersal by fruit eating animals. Effect of pH on seed germination and seedling growth was maximum (100%) near neutral pH and declined both at lower and higher pH levels (20% at pH 4 and 50% at pH 10), the effect being more drastic at lower pH. *Salvadora oleoides* did not respond to stem cuttings and air layering vegetative propagation techniques.

INTRODUCTION

Salvadora oleoides is a multipurpose tree distributed in north western Indian states. It is locally known as Jhal and Piloo. It is a small tree attaining 6-9 meter height under favorable conditions. This tree has great ethno-medicinal values. Leaves of this species are used to relieve cough and are given to horses as purgative. The fruits have considerable amount of glucose, fructose, sucrose and are good source of calcium (Duhan, 1992). Fruits are also useful in the treatment of enlarged spleen, rheumatism and low fever. The seeds are rich in non edible oil and their fat is used in the treatment of rheumatic pains and as a base of many ointments (Anonymous, 1972). Purified seed oil is used in soap and candle making and in detergent industries as a substitute for coconut oil. Trees of *Salvadora oleoides* provide an habitat for a variety of birds (nesting), rodents (burrows), snake and lizards (hollow stem) and mammals (thickets). The fruits of tree are also consumed by a variety of insects, rodents and birds (Khan 1996). *Salvadora oleoides* Decne is decreasing very rapidly due to over exploitation, indiscriminate collection (Khan, 1997; Khan *et al*., 2001; Singh, 2004). Due to over exploitation, indiscriminate collection and low regeneration of this plant in the wild, the population of this species has decreased very rapidly over the last decades in various parts of North-West India. The success of seed germination and the establishment of a normal seedling are determining features for the propagation of plant species, which are of both economic and ecologic importance. Because of its high vulnerability to injury, disease, and environmental stress, germination is considered to be the most critical phase in the plant life cycle. Various causes may result in low seed germination which is one of the basic problems, resulting in low propagation of a species. Therefore, experiments were conducted to determine factors affecting seed viability and germination, in an attempt to understand the declining population and low regeneration of *Salvadora oleoides* in the study area and also to find the optimum conditions for seed germination and methods of vegetative propagation.

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MATERIALS AND METHODS

Fruits were collected in the month of May - June from village 'Sawand' distt. Bhiwani, Haryana. Half of the collected seeds were de pulped by hand rubbing in bucket filled with tap water to remove all traces of fruit pulp followed by shade drying for a day. Seeds were stored under natural conditions in laboratory. Different types of soil media (desert sand, river sand, alluvial soil, clay and vermicompost) were collected from nearby areas and dried. Soil was crushed with the help of wooden log and sieved to remove large soil debris and gravels. Soil samples

were sterilized for 20 min at 121 ^{0}C in autoclave and thereafter passed through a sieve. Soil samples were classified at Regional Krishi Vighyan Kendra, Rohtak, a centre of C.C.S.Haryana Agricultural University, Hissar. Experiments were conducted at Herbal Garden of Maharshi Dayanand University, Rohtak (latitude 28^0 52'48.90" N, longitude76^0 37'09.93" E) in Haryana.

One seeds was sown at a depth of 5-7 mm in small pots of three inch diameter. Ten pots were used for each type of soil media. All experiments were conducted in triplicate. Pots were kept inside a screen house under natural conditions. Sowing was done in second week of June. The emergence of shoot was considered as seed germination. After 35 days of growth, the seedlings were uprooted and washed carefully to remove all soil particles. The fresh and dry weights of each seedling were taken. The dry weight was measured after keeping seedlings in an oven at 60^{0}C for five days. Observations were taken on day to day basis for all parameters. Observations were made on per cent germination of seed, seedling length, number of leaves per seedling, number of nodes per seedlings. As seed germination percentage was reported highest in sandy soil therefore further experiments were conducted using sandy soil to study the effect of pH, storage time and de pulping on seed germination.
For evaluating the effect of different levels of pH on emergence of seedlings, experiments were conducted by sowing of de pulped seed in sandy soil and irrigating with solution of different pH levels, prepared using 0.lN NaOH and 0.1 N HCl in distilled water.

To investigate the effect of storage time on seed viability, sowing of de pulped seeds was carried out at different intervals up to three months from the date of harvesting of seeds. As viability was found to decrease very rapidly on storage, all experiments were carried out on freshly harvested seeds.

Vegetative Propagation

Stem cutting

The semi-hard wood cuttings collected from the secondary branches of current season growth from mature tree were used to develop roots. Stem cuttings were implanted in pots for root formation with and without growth regulators. Two size of cuttings were used ,15-20 cm in length with a basal diameter 4-5 mm and 15-20 cm length and basal diameter of 6-8 mm. Basal ends of cuttings were dipped for 35 minutes in the growth regulator solution to the depth of five centimeters. Cutting without any hormonal treatment served as control. IBA and NAA were used in different concentrations starting from 500 mg l^{-1}, 1000 mg l^{-1}, 1500 mg l^{-1} and 2000 mg l^{-1}, 2500 mg l^{-1} and 5000 mg l^{-1}. The terminal end of each cutting was sealed with cow dung to prevent desiccation. Mixture of desert sand, clay and dung manure in the ratio of 1:1:1 was used as rooting medium. Antifungal Bavistin solution 0.1 % (w/v) was applied to the rooting media.

Air layering

Air layering experiments were conducted on mature tree. Branches of current season growth having diameter of 7-8 mm were layered. A ring of bark about 2.5 to 3.5 cm length was removed from the shoot and solutions of IBA and NAA with different concentrations (500 mg l^{-1}, 1000 mg l^{-1}, 1500 mg l^{-1}, 2000 mg l^{-1}, 2500 mg l^{-1} and 5000 mg l^{-1}) were applied with the help of a piece of absorbent cotton. Ringed part of stem was covered with medium consisted of Sphagnum moss and sterilized clay soil followed by strapping with transparent polythene sheet and further covered by aluminum foil. The rooting media were watered by syringing once in weekly.

RESULTS AND DISCUSSION

During present investigation various soils media were tried for assessing the parameters of seed germination. Seed germination percentage was reported highest in desert sand. Hundred percent germination in sandy soil was followed by vermicompost (90%), alluvial soil (90%), river sand (90%) indicating no significant role of soil in seed germination except clay soil which showed only 50% germination. In addition to germination percentage, least time for germination of seeds was also noticed in sandy soil (Fig.1).

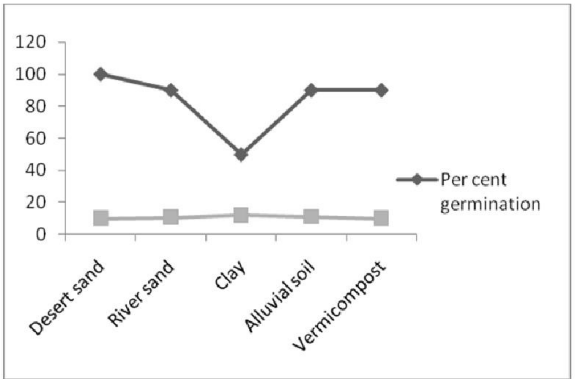

Fig. 1. Percent germination and number of days taken for seed germination

Clay soil gave very poor seed germination percentage as compared to all other soils media, as 50 per cent germination was reported. Eldarier and Youssef, 2010 noticed higher germination percentage in sandy soil than clay soil. Domenech and Villa, 2008 predicted that seed germination was higher in sandy soil and decreased in soil which contained increased level of clay in *Cortaderia selloana*. Urgessa, 2011 also favored the role of sandy soil for enhancement of germination percentage in *Ficus vallischaude.* Okunomo, 2010 reported that sandy soil was most suitable for early germination of seed in *Annona muricata.* Sandy soil is commonly found in dry areas as its water holding capacity is very little so favours the concept of drought hardy species during planting and as mature tree (Hocking, 1993). Water holding capacity of sandy soil being low results in percolation of excess water in deeper soil zone making the root zone free from water logging, resulting in better oxygen supply, enhancing seed respiration leading to faster germination. Higher porosity and less mechanical strength of sandy soil also enhance root growth. Vermicompost was best in terms of growth parameters such as, root length, shoot length, seedlings length, number of nodes per seedlings, fresh and dry weight of seedlings and number of leaves per

seedlings (Table 1). These findings agree with the study by Giwa and Ojeniyi, 2004 that organic manure significantly increased the plant height, number of leaves and root growth during early seedling growth.

Germination of *Salvadora oleoides* seeds was also tested in relation to five storage times viz. 0,7,15,30,90 days after harvest (DAH). The seeds were stored in paper bags under laboratory conditions at 28^0-40^0 C temperature until

Table 1. Effects of different types of soils on seed germination and early seedling growth of *Salvadora oleoides*

Type of soils	Root length (cm)	Shoot length (cm)	No. of node per seedlings	No. of leaves per seedling	Seedling length (cm)	Total fresh weight of seedling (gm)	Total dry weight of seedling (gm)
Desert sand	7.0±0.28	12.5±0.20	7.0±0.24	14.0±0.56	19.5±0.18	1.474±0.36	0.482±0.65
River sand	6.8±0.39	10.0±0.38	6.0±0.16	12.0±0.27	16.8±0.30	1.349±0.33	0.352±0.14
Clay	5.5±0.44	14.7±0.54	7.2±0.12	14.0±0.35	20.2±0.44	1.490±0.65	0.510±0.18
Alluvial soil	6.0±0.50	14.8±0.68	7.0±0.28	15.0±0.17	20.8±0.38	1.970±0.48	0.780±0.32
Vermicompost	7.0±0.54	16.2±0.64	8.5±0.42	17.0±0.16	23.3±0.26	2.140±0.34	0.829±0.48

Values ±S.E.

In addition to effect of different soil media, effects of fruit pulp on seed germination were also studied. Seeds with fruit pulp gave very poor results of germination. Twenty per cent germination was noticed on seed with pulp, where as depulped seed gave hundred per cent germination (Fig. 2). Beneficial effect of depulping of seeds to promote early seed germination has been demonstrated by many workers (Mertia and Kunhamu, 2000; Cipollini and Levey, 1997; Barnea *et al*., 1991; Tsutomu *et al*., 1998; Rodrigo *et al*., 2011). Ingestion of fruits containing seeds by mammals favours the germination of *Prosopis flexousa* because pulp may have some germination inhibitor (Campos and Ojeda, 1997). Better seed germination of depulped fruit is an ecological adaptive mechanism which is affected by dispersal organisms like birds. Eating of the fruits by birds or some other dispersal organism leads to depulping of fruits. Dispersal leads to random dispersal of seeds reduces overcrowding of growing seedlings in the vicinity of parent tree that ensure better survival.

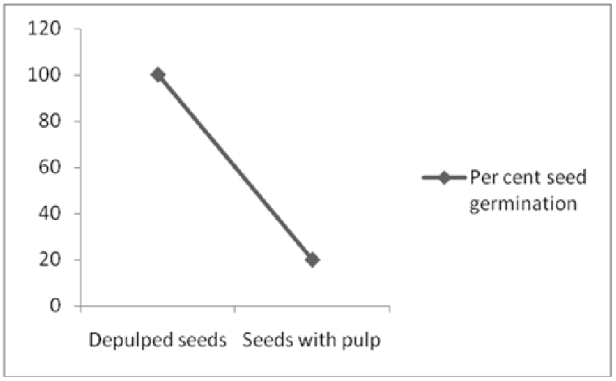

Fig. 2. Percent germination of depulp and pulped seeds

During present studies the rate of seed germination was reported to decrease at both end of pH scale (Fig. 3). The maximum percent germination occurred in soil irrigated with water having pH 7. Germination in *Salvadora persica* increased with increase in pH up to 8.0 but started to decline thereafter as reported by Dagar *et al*. (2004). pH may affect better uptake of minerals from soils and high rate of enzyme and hormone action. Severe acidic as well as strong alkaline conditions of the medium inhibit seed germination (Ahlawat and Dagar, 1980).

germination tests were performed. Seed germination percentage in *Salvadora oleoides* decrease as the storage time increased (Fig. 4).

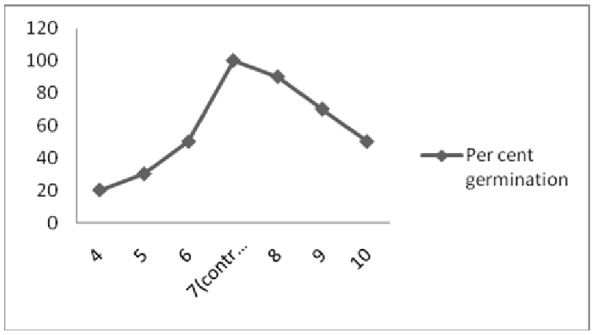

Fig. 3. Effect of pH on percent germination of seeds

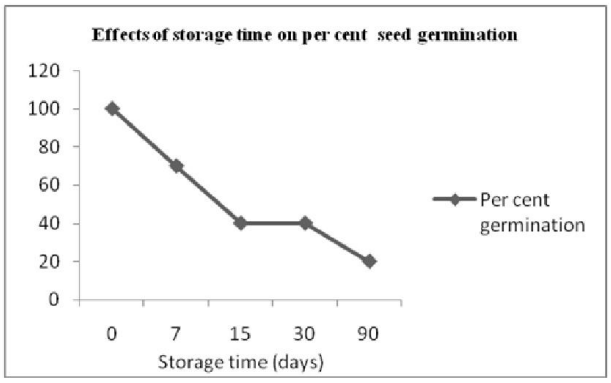

Fig. 4. Effect of storage time on percent seed germination

Similar reports have been noticed by Gresta *et al*., 2007 in *Scorpiurus subvillosus*, Poluyanova and Lyubarskii, 2008 in *Adonis vernalis*. The results indicate the highly recalcitrant nature of the *Salvadora oleoides* seeds. Hence this plant cannot be conserved through seed storage and regular propagation is required. During vegetative propagation through stem cuttings experiments, IBA and NAA have been used for rooting because these are more active than IAA (Zimmermann and Wilcoxon, 1935). Root formation was not reported in any stem cuttings of *Salvadora oleoides* during present studies, possibly due to high level of secondary metabolites in *S. oleoides*

(Yadav *et al*., 2006). Similarly, Cuir *et al*., 1993 reported that difficult- to root hardwood cuttings of *Chamaelaucium uncinatum* had high level of cinnamic acid derivative that inhibits rooting, while no detectable levels of this phenolic compound were found in easy to root soft wood cuttings. Rooting inhibitors have also been reported in other plants also e.g. *Eucalyptus* (Paton *et al*., 1970), chesnut (Vieitez *et al*., 1987).

Air layering techniques were also employed for root formation with and without exogenous application of auxins (NAA and IBA). During air layering, stem is still attached to mother plant for food and water so there are many reasons for increase in regeneration capacity. In the present investigation air layering practices did not show any root formation. There are many species which are difficult to propagate by vegetative methods (Martin *et al*., 2011). Mingguang *et al*., 2009 investigated that *Merremia biosiana* was incapable to propagate by stem cutting and air layering techniques. Non rooting may be due to sensitivity of plant to auxins or disruption of auxins transport as reported by (Caboche *et al*., 1987). The results of the study show the highly recalcitrant nature of the *Salvadora oleoides* seeds. Hence this plant cannot be conserved through seed storage and regular propagation is required. The seed germination is highly affected by pH and prone to acid rain and other pollutants affecting the soil pH. Possibly low seed germination is a major factor for the rapid decline in the population of this plant.

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