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Assessment of effect of *Prosopis juliflora* litter extract on seed germination and growth of rice

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Abstract

It has been reported that mesquite (*Prosopis juliflora* (Sw.) DC.), which is widespread in Saudi Arabia, the United States of America and India, inhibits the germination or growth of many plant species growing in its vicinity, through by releasing allelopathic substances into the environment. Due to this Therefore, it has not been put to good use despite enormous biomass production. The present study was attempted to observed the effect of aqueous extract of mesquite on the growth of rice seedlings. For this To this end, two different concentrations of an aqueous extract (0.1 and 1%) of the aqueous extract was were added as treatments and various parameters of seedling growth like seed germination, root length, shoot length, length and total number of adventitious roots, fresh and dry weight of root and shoot were recorded. We did not eliminate the effect of microbial component in our treatments and during incubation (from lasting 1 day to one week): the phyllosphere microflora had sufficient opportunity to influence the allelopathic outcome after extract addition. The Our results indicated that except for some treatments, both at low concentrations; most of the treatments had led to comparable or better growth of seedlings than did the control treatment. Even when there was less reserve mobilization from seeds during germination; seedlings were able to make up the loss in due course of time and showed better growth than control.

Key words: Allelopathy, Rice Seedling growth, Prosopis juliflora.

1. Introduction

De Candolle (1832) speculated that some plants specifically noxious weeds exude chemicals from their roots that are detrimental to the growth of other plants. Later, Molisch (1937) coined the term allelopathy using it in reference to indicate biochemical interactions, both detrimental and beneficial, between all types of plants including microorganisms. The concept of allelopathy covered both detrimental and beneficial reciprocal biochemical interactions. Rice (1974) however differed from this concept initially and stressed that the term should be used for any direct or indirect harmful effect of one plant (including microorganisms) on another, through production of chemical compounds that escape into the environment. Supporting conceptually the same idea as put forth by Rice, much of the research in allelopathy was centered around the concept of detrimental effects of one plant on another, through release of chemicals in the environment. But, Khailov (1974) proved that the effect of any given compound may be inhibitory or stimulatory determined largely by the concentration of the compound in the surrounding medium. Returning back to Molisch's idea, later Rice (1979) also acknowledged the findings of Khailov and further added that many of the important ecological roles of allelopathy have been overlooked because the concern of many of the researchers was just for the detrimental effects of added chemicals.

Since allelopathy refers to the effect of a chemical compound added to the environment, it differs from competition which involves the removal or reduction of some factor (such as water, minerals, food and light) from the environment that is required by some other plant sharing the habitat, wherein the factor that is reduced could be water, minerals, food and light. Even then the confusion remained, this time between allelopathy and competition, because some biologists in some cases, considered allelopathy to be part of competition. To lessen the confusion, the new term 'interference' (given by Muller, 1969) can be used which encompasses both allelopathy and

competition. Recently Callaway (2002) has considered allelopathy as one form of non resource interaction among plants.

During the 1970s, allelopathy was used to explain community processes and patterns (Muller, 1969, Rice, 1974, Whittaker & Feeny, 1971), and many times it has been suggested to contribute to the ability of some exotic plant species to become dominant in invaded plant communities (Abdul-Wahab & Rice 1967, El-Ghareeb 1991, Fletcher & Renney 1963, Osvald 1948, Vaughan & Berlow 1999, Ridenour & Callaway 2001). It is reported that the invader plants exude some allelochemicals that are relatively ineffective against long time neighbours in their natural communities but to which the plants in the invaded communities lack co - evolved tolerance (Callaway & Aschehoug, 2000). One such plant which is reported to be both invasive and allelopathic (mostly in the detrimental sense) is mesquite (Prosopis juliflora (Sw.) DC), which is widespread in Saudi Arabia, United States of America and India. It is found to inhibit germination or growth of many plant species growing in its vicinity, through the release of allelopathic substances into the environment (Al – Humaid &Warrag, 1998, Pandit *et al.,* 1995). Recently Reigosa *et al.* (1999) assessed the effect of six known allelopathic phenolic compounds (p-coumaric acid, ferulic acid, vanillic acid, p-hydroxybenzoic acid, gallic acid and p-vanillin) on seeds of Chenopodium album, Plantago lanceolata, Amaranthus retroflexus, Solanum nigrum, Cirsium sp. and Rumex crispus. They found that the effects of the assayed allelochemicals on radicle growth and seed germination were very weak, and suggested that allelochemical effects can only be important in special situations, mainly in combination with other competitive effects.

There is suggestion that aqueous extracts derived from non-sterilized soil, leaf litter, and live leaves soaked in water are more realistic and ecologically relevant (Orr *et al.,* 2005) and that ground and macerated materials are ecologically less meaningful (Inderjit & Callaway, 2003), for the reason that they exaggerate the allelopathic effect.

Our earlier findings (Mehar *et al.*, 2002 a, b, c, Mehar *et al.*, 2003, Mehar *et al.*, 2008, Purohit *et al.,* 2002, Sundaramoorthy *et al.*, 2005) suggest that some of the allelopathic tree species growing in the Indian desert increase the soil nutrient status, improve soil microbial biomass and promote the growth of microbial groups. P. juliflora was found to promote the growth of microbial groups with specific physiological capabilities like degradation of cellulose, lipid, lignin and proteins even at very low soil moisture levels (Sundaramoorthy *et al.,* 2010) and also promoted the activity of soil enzymes in the similar low soil moisture conditions (unpublished data). Previously, Sen and Chawan (1970) observed that the functioning of desert ecosystems is such that whatever toxic substance is released imparts greater influence at the site of release as leaching does not take place rapidly because of restricted rainfall. As a result of this, the toxic substances accumulate at higher concentrations (much higher than in case of availability of water and proper leaching) and could well be the reason why many of the trees in deserts are recognized as allelopathic. But the situation in other non-desert ecosystems and areas is different and demands some study to evaluate it for use to improve the organic matter content of the soil.

P. juliflora grows luxuriantly in all parts of India, producing abundant biomass which is not put to any use, except for the pods which are fed to cattle; remaining tree biomass is in fact a problem wherever it is growing, because the biomass is not usable hence people leave it undisturbed, thereby there is no check to its spreading new areas also. Since the biomass is not used for any other purpose, and the evidence is there that it can improve the soil nutrient content and microbial activity, it should be tried in agricultural situations where proper irrigation would dilute the adverse chemical effect of the litter on the crops. Keeping this in mind I have tried to study the effect of litter of P. juliflora on rice, which is grown in submerged field conditions in the southern parts of the country. I have taken Kurnool Sona (BPT 5204) variety of rice as test crop, which is quite popular among the agrarians of the region and is cultivated in large parts of the Andhra Pradesh, India. To provide the conditions as natural as possible, in laboratory incubations there was no attempt to prevent the microbial effect, and the litter was incubated up to 7 days at room temperature to allow the microbes to degrade it and release whatever intermediate degradation products could be formed during the

process of decomposition. As reviewed earlier, for laboratory incubations generally macerated litter and higher concentrations are taken, I have also used the powdered litter for preparation of aqueous extracts to keep the detrimental allelopahthic effect towards the higher side. The hypothesis is that if, in these relatively harsh incubation conditions, the seeds germinate and their growth is comparable with the control treatment, than in field conditions the leaf litter from the mesquite could be used to ameliorate the soil fertility, thereby reducing if not eliminating the need for chemical fertilizers.

2. Material and methods

Leaf litter in the form of mature leaves from mature trees of P. juliflora growing in and around Sri Venkateswara University Tirupati, Andhra Pradesh was collected. After collection leaves were air dried in shade at room temperature. Afterwards 10g of dried leaves were macerated in 200ml of distilled water and kept for incubation at room temperature for different durations i.e. from 1 to 7 days, these are referred as 1DE to 7DE i.e. 1 Day incubation extract to 7 Day incubation extract, in the text hereafter. At the end of incubation the solution was filtered with double layer of whatman no. 1 filter paper and the extract was stored at 4o C till use.

Two different dilutions viz. 0.1% and 1%, of the filtered extract were made using distilled water for dilution and used for the experiment. Rice seeds were surface sterilized with 0.1% mercuric chloride for 1 min., after which the seeds were washed with distilled water for several times to remove residual mercuric chloride. Experiment was carried out by taking five seeds, and placing them on a sheet of whatman no. 1 filter paper in petri dishes (10cm diameter), taking four replicates for each treatment. The petri dishes were kept under light, illuminated with cool fluorescent tubes (14.4 W m-2) at 28 ± 2.4o C with a 12hr light and 12hr dark photoperiods. On the first day of incubation 5 ml of extract was added to completely wet the filter paper, thereafter 3ml extract was added every day for 15 days.

Observations were made for eight days during the incubation period, and seed germination rates were recorded taking due care to note the pattern of emergence of radicle and plumule, as to what emerged first (radicle, plumule or both together). This was felt necessary, because in an earlier screening experiment it was observed that at higher filtrate concentrations (25% and 50%) the seeds germinated but the plumule instead of the radicle emerged first. Furthermore, it was observed that it was the radicle that was worst affected by the treatments with higher concentration of extract. Most of the seeds germinated within the first 5 days of incubation but the observation continued up to 8 days as seeds in some of the treatments germinated late also. After 15 days the seedlings were collected and parameters like root length, shoot length, number of adventitious roots, length of adventitious roots, fresh and dry weight of seedlings were recorded. Strip plot analysis was carried out taking incubation days and concentration of extract as vertical and horizontal factors respectively. The analysis of variance was carried out as per Gomez and Gomez (1984).

3. Results and discussion

Seed germination is a widely used parameter in allelopathic bioassays (Rice, 1984), and there is suggestion to consider the growth of seedlings also which is more responsive to certain categories of allelochemicals (Einhellig & Rasmussen, 1978). However, some workers found oven dry weight of radicle (Leather & Einhellig 1985), root length and root weight to be more important (Cope, 1982, Pederson, 1986). In the present study we have tried to include all these parameters to have an understanding of the effect of *P. juliflora* litter on the growth and performance of rice seedlings.

Percent germination calculated on the basis of number of seeds germinating out of the total seeds ranged from a minimum of 75% (2 DE, 1% filtrate) to 100% in some of the extract treatments like (2 DE, 0.1% filtrate; 3 DE, 1% filtrate; 6 DE, 1% filtrate; and 7 DE, 0.1% filtrate; see Fig. 1). Most of the treatments had comparable or slightly better germination performance than the control treatment.

It was found that germination of seeds did not always result in emergence of the radicle first. In the control treatment, also 55.5 % of the seeds showed the emergence of radicle first and the remaining showed emergence of plumule first. But in remaining treatments the situation varied, 83.3

% of seeds in 1 DE 0.1% treatment showed the first emergence of radicle but in longest incubation at higher concentration (7 DE 1 %) none of the seed had radicle emerging first (Fig. 2).

Rice is a monocot plant and the main root is relatively short lived. In due course of time the function of absorption of water and nutrients is taken over by adventitious roots. The number of adventitious roots emerging out from the seedlings were recorded and the results show that they varied from 1 to 10; hence they were divided in different frequency classes to have a better understanding of the effect on growth. Although in control 50% of the seedlings had 2-4 adventitious roots, on average, the most common frequency class was the one with frequency of adventitious roots between 4-6 per seedling. This frequency class accommodated 38% of the seeds from all the treatments, and only 0.8% of the seeds showed the highest number class i.e. 8-10 (Fig. 3) Significant variation in the number of adventitious roots per seedling due to addition of the filtrate was caused due to concentration of the treatments (P>1%).

Main root length varied from 3.9 cm (7 DE 1%) to 13 cm (2 DE 1%), while the shoot length ranged from 7.1 cm (6 DE 0.1%) to 8.8 cm (5 DE 1%). Total number of adventitious roots were recorded from each seedling and there lengths were also measured individually. 7 Days incubated extract at the higher dose (7 DE, 1%) had maximum number of adventitious roots i.e. 55 and the total length of adventitious roots was also maximum in the same treatment (118 cm). Root lengths being an indicator of seedling growth, the root/shoot ratio values reflect the condition of seedling. We observed that the ratio was minimum in 7 DE 1% treatment and maximum in the intermediate incubation treatments viz. 2 DE 1% and 6 DE 0.1 %. The ratio of root length to total length of adventitious roots indicated that when both the numbers and the total length of adventitious roots were more, (7 DE 1% treatment), it resulted in minimum value for the ratio i.e. 0.03, while the maximum ratio was recorded for 5 DE 1% treatment (Table 1).

Minimum shoot fresh weight was recorded in 6 De 0.1% treatment, where it contributed 37% to the total seedling weight, and maximum values was recorded in 5 DE 1 % treatment (61% of the total weight of seedling). Incubation period was found to cause significant variation in shoot fresh weight (P>1%). The contribution of root fresh weight to total seedling weight was lower in both the control and the other treatments also. The contribution of root fresh weight (total of main and adventitious roots) ranged from 19% in 4 DE 1% treatment to 25% in 4 DE 0.1% and, 7 DE 1% treatments. Here it should be noted that the later treatment had the maximum number and length of adventitious roots. Seeds held from 31% (6 DE 0.1%) to 41% (3 DE 0.1%) of the total fresh weight of seedling (Table 2).

The results obtained for dry weights suggested that some of the variations observed in the seedling growth could be due to lack of reserve mobilization from the seeds during germination and growth process. This is indicated by higher dry weight of the seeds. Shoot dry weight ranged from 0.007 g (control and 1 DE 1%) to 0.009 g in 5 DE 1% treatment. Root dry weight was minimum in 2 DE 0.1% and 6 DE 1% treatment (0.003 g) and rest of the treatments had higher values than this (Table 2). It was also noted that although some of the treatments had higher values for seed dry weight, it did not mean that those seedlings were severely affected, instead the comparison suggested better growth than control in many of them, when other parameters like root length, shoot length, root and shoot dry weight were considered together. This means that those seedlings which failed to mobilize reserves from the seeds during germination in the initial period, made up the loss by using the improved fertility coming from the added litter extract and synthesizing the biomass through other physiological processes.

Orr *et al*. (2005) used both intact live leaves collected from living plants and also minced leaves collected from the same source. They reported that the effect of minced leaves was not always inhibitory, in fact there was reduction in the number of days to emergence for all species taken as targets in the study and also the survival of seedling in some cases was marginally increased. The stimulatory effect on germination and also on the growth of seedlings as observed in our study also could be due to a fertilizing effect resulting from nutrient release from damaged or decayed tissue (as *P. juliflora* is nitrogen fixing also). Similar observations were also reported by Simon and Seated

(1999). There are other explanations also for the stimulatory effect like release of hormones such as gibberellins that may stimulate germination (Brady & McCourt, 2003), or a release of toxic compound that breaks down the seed coat (Cohn 1996).

Some of the studies have attributed the negative effect of litter on target plants to be caused by soil microbes. Xingjun *et al.* (2005) studied the effect of Eupatorium adenophorum on the growth of Broussonetia papyrifera and soil (nutrient content and microbial population). They found that available N, P and K increased in the treatments. Moreover, when the allelopathic substances were neutralized by use of activated carbon, it was noticed it had no significant effect on growth. The only significant variation that they could find after application of allelopathic litter to the soil was that it changed the composition of soil microbial community. Based on this observation they suggested that the negative effect if any of the allelopathic plants on the target species is mediated due to change in the composition of microbial communities, indicating that the microbes inhibiting the growth of target species were promoted after the treatment. In our study we did not eliminate the effect of phyllosphere microbes during the incubation of litter for sufficiently long durations. But we did not notice significant negative effect of extract on seed germination and growth of seedlings.

One more argument put forth by Turner and Rice (1975) is that release of allelochemicals occurs continuously, affecting the adventitious flora during different physiological stages of their development and accumulating at certain times at concentrations high enough to reduce plant development. In our study as mentioned in the materials and methods section, the extract addition was continuous (3ml extract added each day), and in fact seedlings were exposed to increasingly higher concentrations of extract. Hence, whatever reduction in growth parameters of seedlings was there, could be attributed to the cumulative effect of extract addition which cannot be the case, when the litter is added in the fields for a limited number of times. Hence I would suggest that the concept of allelopathy should be looked at with broad perspective and attempts should be made to harness the available biomass of allelopathic tree and herb species in the form of organic amendments to soil so that we can reduce our dependence on the chemical fertilizers. For farmers with limited resources it is otherwise also not possible to spare money for chemical fertilizers, besides search for alternatives of chemical fertilizers becomes more pertinent when we take into account the adverse effects of chemical fertilizers on soil microbial functioning.

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Fig. 1. Percentage germination of seeds.

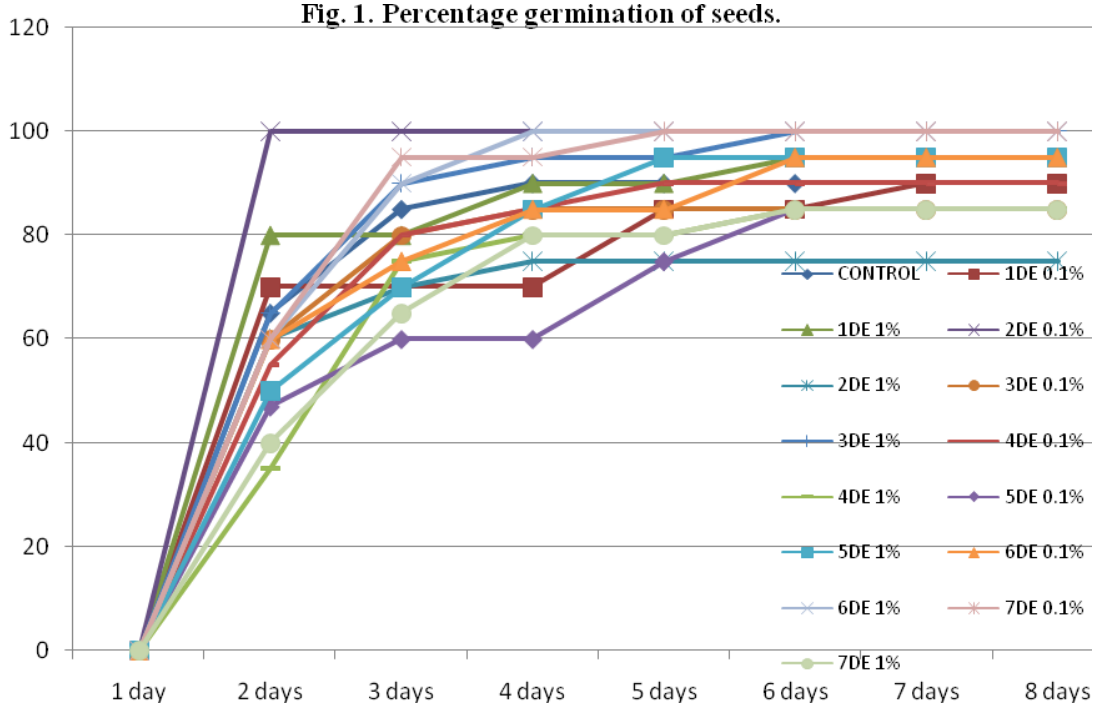
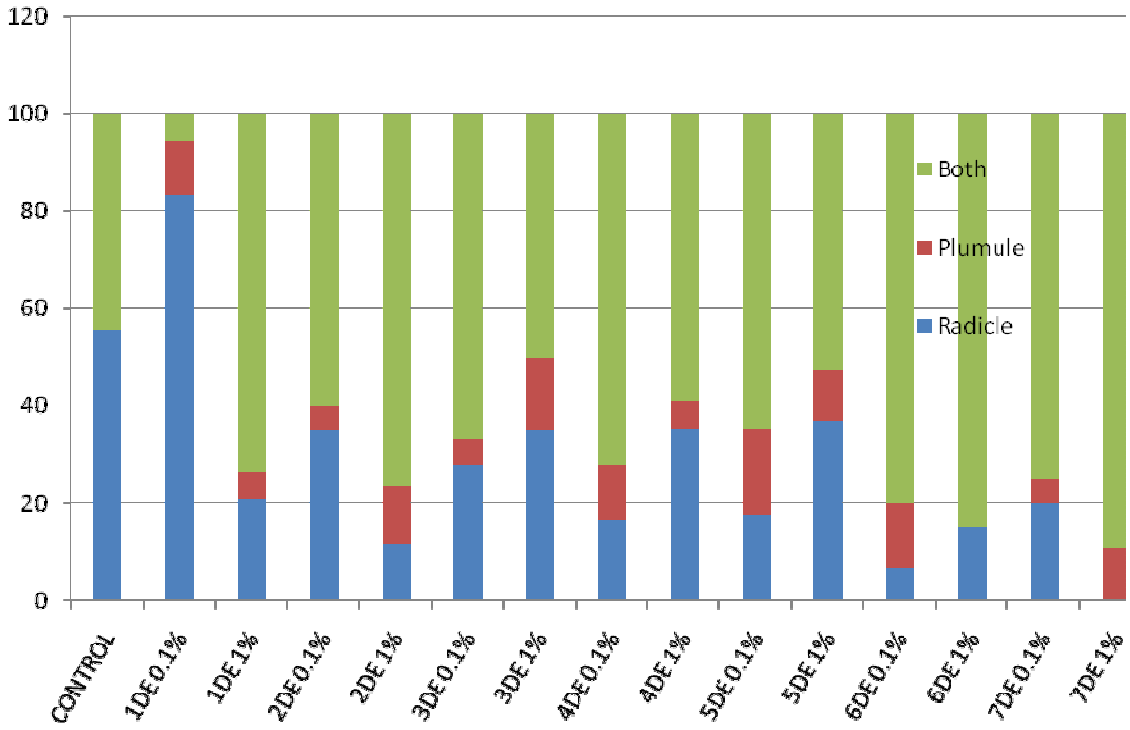


Fig. 2. First emergence percentage during germination.



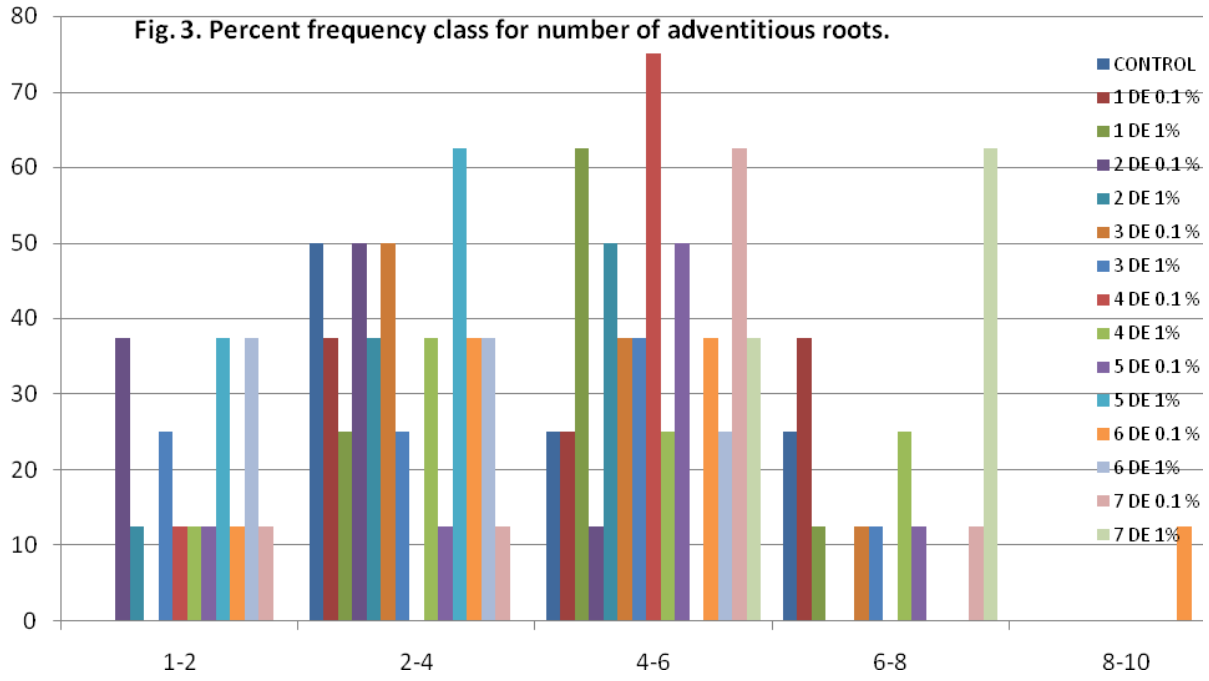


Fig. 3. Percent frequency class for number of adventitious roots.
80
70
60
50
40
30
20
10
0
1-2
2-4
4-6
6-8
8-10
CONTROL
1 DE 0.1 %
1 DE 1%
2 DE 0.1 %
2 DE 1%
3 DE 0.1 %
3 DE 1%
4 DE 0.1 %
4 DE 1%
5 DE 0.1 %
5 DE 1%
6 DE 0.1 %
6 DE 1%
7 DE 0.1 %
7 DE 1%

Table 1. Seedling growth parameters for different treatments.

	Radicle Length (cm)	Shoot Length (cm)	Total length of adventitious roots (cm)	Total number. of adventitious roots
CONTROL	8.86 ± 1.49	7.38 ± 1.23	57	43
1 DE 0.1 %	10.7 ± 2.94	8.5 ± 1.28	70.4	43
1 DE 1%	11.9 ± 2.10	8.21 ± 0.94	52.2	42
2 DE 0.1 %	10.6 ± 4.07	7.86 ± 1.69	31.3	24
2 DE 1%	13.1 ± 2.21	8.11 ± 0.88	66.3	31
3 DE 0.1 %	8.51 ± 3.18	8.45 ± 0.84	71.8	38
3 DE 1%	9.21 ± 3.94	8.48 ± 1.03	75	33
4 DE 0.1 %	11.5 ± 3.66	7.86 ± 1.69	50.4	32
4 DE 1%	10.2 ± 3.09	9.06 ± 1.04	84	38
5 DE 0.1 %	11.4 ± 3.56	7.88 ± 2.25	57.9	36
5 DE 1%	12.1 ± 3.57	8.83 ± 1.18	29.6	22
6 DE 0.1 %	11.2 ± 3.15	7.09 ± 1.83	58.1	39
6 DE 1%	11 ± 3.67	8.8 ± 1.59	47.8	26
7 DE 0.1 %	7.89 ± 3.31	7.94 ± 0.81	46	37
7 DE 1%	3.94 ± 1.57	8.08 ± 1.34	118	55

Table 2. Fresh weight and dry weight different parts of seedlings.

	Fresh weight (g)			Dry weight(g)		
	Shoot	Root	Seed	Shoot	Root	Seed
CONTROL	0.054±0.010	0.030±0.004	0.044±0.005	0.008	0.004	0.007
1 DE 0.1%	0.054±0.005	0.030±0.002	0.050±0.007	0.007	0.004	0.009
1 DE 1 %	0.049±0.011	0.030±0.004	0.040±0.003	0.008	0.004	0.007
2 DE 0.1%	0.058±0.009	0.029±0.005	0.050±0.006	0.008	0.003	0.008
2 DE 1 %	0.056±0.010	0.028±0.003	0.045±0.004	0.007	0.005	0.007
3 DE 0.1%	0.056±0.006	0.026±0.007	0.052±0.012	0.007	0.004	0.018
3 DE 1 %	0.051±0.009	0.029±0.004	0.043±0.006	0.007	0.004	0.008
4 DE 0.1%	0.060±0.005	0.033±0.017	0.044±0.006	0.007	0.006	0.009
4 DE 1 %	0.063±0.009	0.024±0.017	0.045±0.006	0.008	0.004	0.011
5 DE 0.1%	0.065±0.012	0.028±0.003	0.049±0.005	0.008	0.005	0.009
5 DE 1 %	0.078±0.014	0.027±0.004	0.044±0.015	0.009	0.004	0.008
6 DE 0.1%	0.047±0.009	0.025±0.006	0.039±0.007	0.007	0.006	0.008
6 DE 1 %	0.070±0.002	0.029±0.003	0.049±0.006	0.008	0.003	0.007
7 DE 0.1%	0.052±0.003	0.025±0.003	0.045±0.003	0.008	0.004	0.013
7 DE 1 %	0.064±0.006	0.033±0.004	0.047±0.003	0.008	0.006	0.012