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INHIBITION OF SEED GERMINATION BY THE ENDOCARP IN *NEEM* (*Azadirachta indica* A. JUSS.)

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The endocarp inhibited the seed germination in neem (Azadirachta indica A. Juss.) *by delaying the initiation of germination and lowering the germination percentage to about three to four times. The endocarp not only acted as a physical barrier but also seemed to contain some inhibitory substance (s) which possibly acted as a metabolic barrier during seed germination.*

Neem (*Azadirachta indica* A. Juss.) is native to India and has found several applications in indigenous medicines, soaps and insecticides. Although neem tree provides abundant fruits seasonally, yet limited work has been done on the germination aspects of neem seed (orthodox in nature) especially the role of endocarp during seed germination. Only adverse effect of temperature on germination of neem seeds above 30° C had been reported (Ezumah, 1986). It was not clearly understood whether endocarp prevented germination entirely or delayed it considerably (Roberts *et al.*, 1984). This paper reports the results of the investigations to determine the effect of various factors like light, water and endocarp on seed germination in neem.

MATERIALS AND METHODS

Ripe yellow fruits naturally fallen on the ground and shown to be physiologically mature with maximum germination capacity (Maithani *et al.*, 1989) were collected from neem trees in the campus area of the Indian Agricultural Research Institute, New Delhi. The seeds were pressed out from the fruits and the pulp was removed by water washings. The seeds were given hot water treatment at 47° C for 10 minutes, and treated with fungicide (captan 0.5%). Seeds were air dried and packed in polythene bags before storing at room temperature till use.

For germination studies, both intact seeds and excised seeds (without endocarp) were plated in between the folds of moistened filter paper in plastic petridishes of 11 cms diameter and maintained at 27° C with 16 hour photoperiod (Ezumah, 1986). Germination was recorded based on emergence of well formed 1 cm long radicle. Three replicates of 50 seeds each were used per treatment. To determine the water uptake, three replicates of ten weighed seeds each with/without endocarp were imbibed in distilled water at 30° C and the increase in their weights was recorded at regular intervals until 24 hours when the first sign of germination was visible in seeds without endocarp.

The effect of light was studied by plating 50 seeds for germination under continuous light after removal of endocarp and controls were maintained in dark at

27° C. Scarification of the seeds was done by removal of the endocarp at different positions of the seed i.e. from micropylar end, distal end, pricking in middle of the seed and complete removal of endocarp to allow the free entry of water into the seed.

Extract of the endocarp (Thapliyal and Nautiyal, 1989) was obtained by grinding 3 g of the material in 50 ml of distilled water and filtering it through double layers of muslin cloth. In another set, 20 intact seeds were suspended in 50 ml of distilled water for 18 hours to obtain the endocarp leachates. Both the extract and leachate were used for irrigating the seeds put for germination in petridishes.

RESULTS AND DISCUSSION

Germinability of intact and excised seeds of neem is depicted in Fig. 1. There was a marked difference in the rate of germination and final germination percentage of seeds kept with and without endocarp. Removal of endocarp not only brought an early onset of germination but the percentage germination also improved about three times. The excised seeds germinated rapidly, radicle appeared within 24 hours accomplishing maximum germination of 95 per cent within 8 days of imbibition, whereas, in intact seeds, the rate of germination was slow and the maximum germination of 28 per cent was attained only after 30 days of imbibition.

Although the excised seeds imbibed considerable amount of water about as much as its own weight, the intact seeds imbibed only about 9 per cent of its original weight (Table 1). The water imbibed by the intact seeds was mainly confined to the endocarp since the seeds excised from intact imbibed seeds did not show any increase in their original weights. The initiation of germination was slower in seeds kept in dark when observed after 2nd and 6th day of plating (Table 2). However, by

Table 1. Water uptake in *neem* seeds with endocarp intact and without endocarp

Treatment	Weight of 10 seeds (g)	
	Before imbibition	24 hrs after imbibition
Seeds with intact endocarp	3.10 ± 0.84	3.39 (1.94)* ± 0.63 (0.02)
Seeds without endocarp	1.90 ± 0.020	3.22 ± 0.028

* Wt. of the seed after endocarp removal.

Table 2. Effect of light on the germination of *neem* seeds without endocarp

Treatment	Germination percentage* Days after seed plating			
	2	6	10	12
Light	48	92	94	94
Dark	20	74	94	93

* Average value of three replicates.

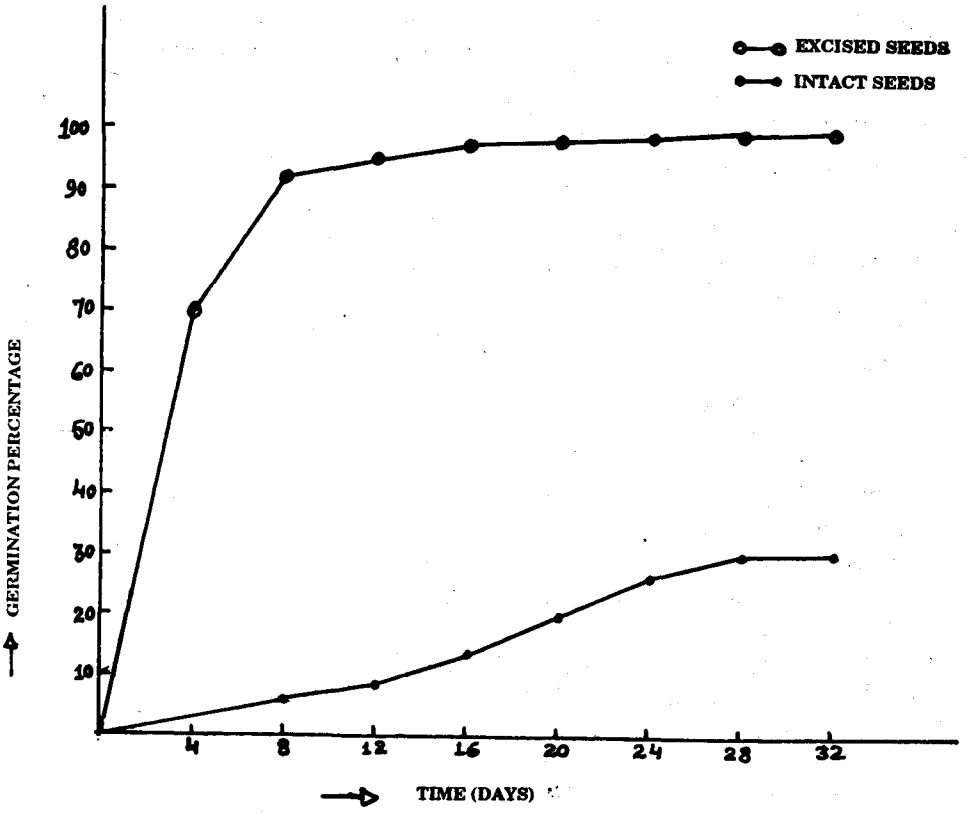


Fig. 1. Germination behaviour in *neem* seeds.

10th day, this was found to be at par with the seeds kept under light conditions. Studies on effect of scarification indicated that germination percentage (Table 3) was improved by 11 per cent in seeds pricked from lower surface, 61 per cent in seeds

Table 3. Effect of scarification of endocarp from various positions of seed on the germination percentage

Treatment	Germination percentage*	Days for germination
Intact seeds	9.0 ± 3.65	30
Endocarp pricked from the middle of seed	20.0 ± 3.53	20
Endocarp removed from micropylar end	82.0 ± 2.82	10
Endocarp removed from distal end	70.0 ± 10.6	12
Endocarp completely removed	98.0 ± 1.4	8

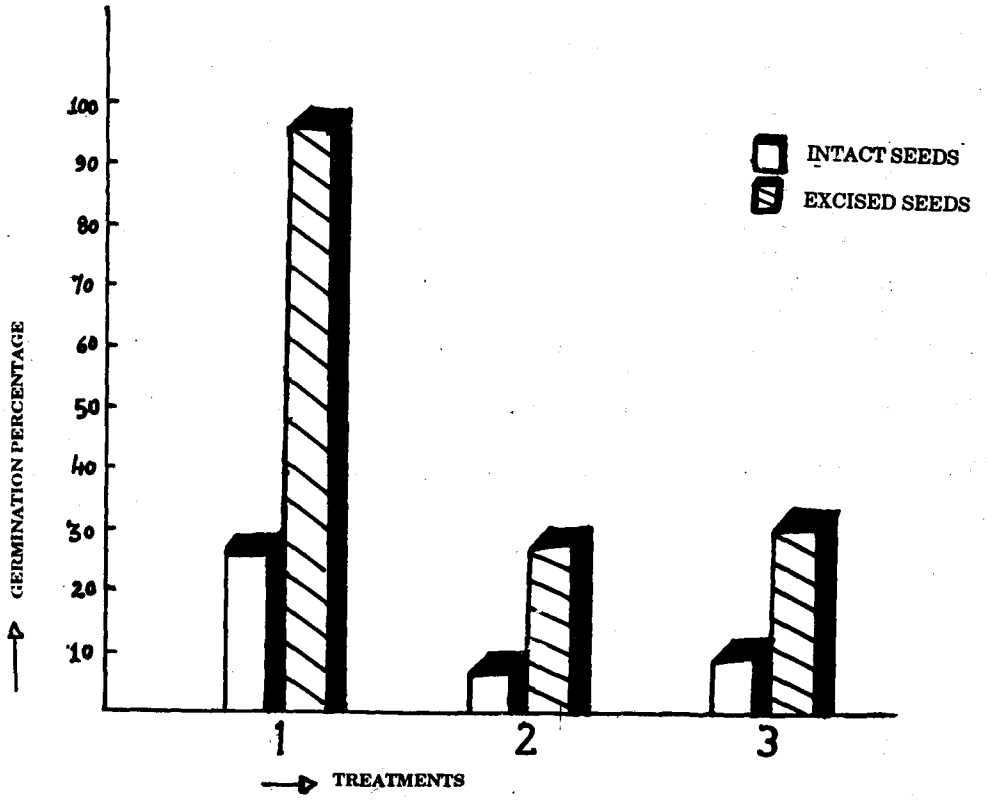
* Average value of three replicates

where the endocarp was removed from distal end of seed, 73 per cent in seeds where endocarp was removed from micropylar end and 90 per cent in seeds where the whole endocarp was removed compared to the control seeds with intact endocarp.

The results revealed that germination was not adversely affected in dark implying that endocarp did not inhibit germination in dark thereby not acting as a barrier for light penetration. However, the endocarp delayed the onset and per cent germination considerably by delaying the uptake of water (Table 3). Thus, water does not become readily available for the seeds (cotyledons) to initiate germination process. The fact that even after 32 days of imbibition, the germination percentage of intact seeds was significantly lower than that obtained with excised seeds. This suggested that the endocarp imposes mechanical resistance, to the extent that about 60 per cent of the seeds remained ungerminated. This response was mainly because of the resistance imposed on the growth of the embryo as evident from the results obtained by scarification of seeds. Although in all the scarification treatments, water was readily available to the cotyledons, the highest germination percentage was obtained where the endocarp was removed from micropylar end i.e. nearest to the embryo. Besides improving water uptake, scarification of the seeds would also improve gas exchange to take place which in turn might have improved the germination percentage in comparison to seeds sown with endocarp intact. Interestingly, seeds sown with endocarp removed from micropylar end, showed 16% lower germination percentage compared to seeds where the whole endocarp was removed. This ruled out the possibility of presence of any inhibitor (s) in the embryo alone which otherwise would have leached out slowly during imbibition in both the treatments bringing the germination percentage to the same level. Inhibition of germination by endocarp is known to be accomplished by disallowing water uptake, gas exchange, penetration of light, escape of any inhibitor(s) if present from embryo (Villiers and Wareing, 1965 and Valio, 1976), as abscissic acid is one of the prominent identified inhibitor which is not only found in covering tissue but also in embryo (Sondheimer *et al.*, 1968) or by exerting a mechanical resistance to embryo growth or by presence of any inhibitor (Bewley and Black, 1982; Valio, 1976). Since most of the factors studied could not prove fully the cause for the low germination in intact seeds, a test for the presence of an inhibitor in the endocarp was carried out.

The germination of seeds in test solutions was strongly inhibited and the values for the germination percentages (Fig. 2) were found to be as low as 27 per cent in excised seeds and 7 per cent in intact seeds with extract and leachate, respectively. This suggested presence of water soluble inhibitor in the endocarp. This inhibitor alone would have been responsible for the lower germination in seeds sown with endocarp removed from micropylar end, since under such condition neither water, gases or light were limiting nor there was any mechanical barrier imposed on the embryo when compared to excised seeds. An inhibitory extract has been reported to be isolated from embryos of *Fraxinus excelsior* (Villiers and Wareing, 1965). The reduction in the germinability of the intact seeds in the presence of endocarp extract and leachate over the control is indicative of the fact that the concentration of the

probable inhibitor present may be determining the extent of inhibition. The results revealed that inhibition in germination of neem seeds was due to the nature of thick endocarp which acted both as a mechanical barrier and also a barrier for water.



Treatment 1: Seeds germinated on filter paper disc moistened with distilled water
Treatment 2: Seeds germinated on filter paper disc moistened with endocarp extract
Treatment 3: Seeds germinated on filter paper disc moistened with endocarp leachate
Fig. 2. **Germinability of intact and excised *neem* seeds in the presence and absence of endocarp extract and leachate.**

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