World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

EFFECT OF VARIOUS TREATMENTS ON SEED GERMINATION IN *M.EMARGINATA*

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Article Received on 01/07/2020 **Article Revised on 22/07/2020** **Article Accepted on 12/08/2020**

ABSTRACT

Study aims various treatments on seeds of M.*emarginata.* Seeds treated with Hot water showed maximum imbibition (80%) but their germination was nearly inhibited (zero%) which might be due to membrane damage or death of embryo. Effect of GA_3 (100 to 200 ppm), considered to be the replacement of chilling treatment, resulted in increase of germination percentage. Germination percentage showed increase from 100 ppm Gibbrellic Acid (GA_3) and Benzyl Amino Purine (BAP) treated seeds to 150 ppm GA_3 and BAP treated seeds. Maximum germination percentage (75.7% with GA_3 and 70.7% with BAP) was observed in seeds treated with 150 ppm GA_3 and BAP and imbibed for 96 hours. Higher concentration of GA_3 and BAP (200 ppm) could not accelerate it further and infact decreased the rate of germination (46.6% and 41% respectively) with 96 hours imbibition. H_2SO_4 (40%) treatment could not prove to be highly successful (maximum 52.2% germination) and H_2SO_4 (80%) completely declined germination percentage (12.3% at 96 hours imbibition) due to blackening of seed coat. 40% and 80% HNO_3 showed poor response(12.2% and 7.1% respectively) in comparison to H_2SO_4 and normal water. Various effects like prosoaking, scarification, acid treatment, chilling, hot water treatment, growth regulators treatment are well known to induce germination in dormant seeds. ABA (Abscissic Acid) has been shown to be involved in regulating seed dormancy and GA_3 (Gibberellic Acid) known to counteract the inhibitory effect of ABA .Growth hormones are the best activators to increase the rate of germination in *M emarginata*.

KEYWORDS: M.*emarginata*, Imbibition, Germination Growth Hormones.

INTRODUCTION

Maytenus emerginata* (Willd.)** is an ever green tree that tolerates various types of stresses of the desert and is found in drier parts of central, south –western and north western India. ***Maytenus have been used for fever, asthma, rheumatism and gastrointestinal disorders, carcenoma and leukemia, gastrointestinal troubles etc. Medicinal plants are rich source of secondary metabolites, biosynthetically derived from primary metabolites but restricted to specific taxonomic genera of plant kingdom and specific part of plant body. Secondary plant products are of major interest because of their biological activities ranging from antibacterial, antibiotic, insecticidal, hormonal, pharmacological and pharmaceutical.

MATERIAL AND METHODS

Naturally growing mature fruits were collected in the month of March April.Large sized seeds were used for various treatments.

Seed Viability Test

The seed viability was assessed by performing tetrazolium (TZ) test. Hundred seeds were incubated in 50 ml of 1% (W/v) solution of 2, 4, 5 – triphenyl tetrazolium chloride (TTC) prepared in Sorensen's buffer (pH 7.0) for 24 hours at 28°C.After incubation seeds were longitudinally bisected and embryo was observed. The seeds in which embryo turned reddish pink were considered as viable and seeds that remained light yellow were treated as non-viable.

Seed Treatments

Five replicates were selected for each treatment and each replicate contained twenty large sized, healthy seeds. Room temperature water control (25 to 30°C), hot water (60°C), boiling water (100°C), chilling water (-15°C), HNO_3 and H_2SO_4 (40%, 80% each), GA_3 (Gibbrellic Acid 100, 150 and 200 ppm) BAP (Benzyl Amino Purine 100, 150 and 200 ppm) were used for seed treatments. Each treatment was given for 30 minutes.

Hot water treatments
Five replicates of seed treatment were submerged in hot distilled water (60°C) and boiling water (100°C) beakers separately, kept on temperature controlling water bath, for 30 minutes.

Chilling treatment
Five replicates of seeds were kept in freezer at – 15°C temperature for 30 minutes.

Acid Scarification of Seeds
Five replicates each of seeds were treated with 40% and 80% H_2SO_4 and HNO_3 for 30 minutes with stirring at regular intervals.

Growth Hormone Treatment
Solutions of GA_3 (Gibbrellic Acid) and BAP (Benzyl Amino Purine) were prepared for the concentrations of 100, 150 and 200 ppm each. Treatment of each concentration was given to each set of five replicates of seeds for 30 minutes.

Imbibition Percentage
Seeds of each replicate were weighed and marked. Weighed seeds were given various treatments and imbibed in distilled water for 24, 48, 72 and 96 hours as shown in table. Seeds were taken out after fixed time period wiped and again weighted to calculate imbibition percentage. Imbibed and weighed seeds were transferred into sterilized test tubes under aseptic conditions.

Preparation And Sterilization Of Test Tubes
Paper bridge of blotting paper strip were prepared and kept in test tubes (size 50 ml) containing distilled water (10 ml). Test tubes were pluged with cotton and autoclaved at 1.05 kg/cm^2 pressure for 15 to 20 minutes. One set of ten test tubes was prepared for each replicate of imbibed seeds.

Transfer of Imbibed Seeds
Transfer of imbibed seeds was carried out under aseptic conditions in sterilized laminar air flow cabinet. Treated imbibed seeds were surface sterilized with 0.1% mercuric chloride solution for five minutes and then washed thoroughly thrice with sterilized water. Two seeds were transferred on paper bridge, inside test tube, with the help of forceps, in the vicinity of spirit lamp flame. Test tubes were labelled and kept in culture chamber for observation after every 24 hours. Temperature of culture chamber was maintained at 26±2°C with relative humidity of 55% and 300 lux diffused light. Viability, imbibition and seed germination percentage was calculated as follows

$$\text{Viability percentage} = \frac{\text{No. of seeds turned pink}}{\text{Total no. of seeds taken}} \times 100$$

$$\text{Imbibition percentage} = \frac{\text{Wt. of seeds before treatment}}{\text{Wt. of seeds after treatment}} \times 100$$

$$\text{Seed germination percentage} = \frac{\text{No. of seeds germinated}}{\text{Total no. of seeds taken}} \times 100$$

RESULTS AND DISCUSSION

Seeds of ***M. emarginata*** are ellipsoidal, 3 X 2 mm,chestnut brown, glabrous, rugose and arilloid orange. Each seed weighs nearly 3 to 3.5 mg. Fruits and seeds mature in the month of March April. The tetrazolium test showed 98% viability in the seeds used. It was observed that freshly collected seeds germinate immediately signifying that seeds do not require after ripening. However percentage of germination was nearly 40%. Being main factor to proceed for germination, effect of treatments on imbibition was first studied.The imbibition data reveals that the seeds absorb water quite rapidly within 24 hours. Percentage of imbibition increased upto next 96 hours.Therefore, a presoaking treatment of 96 hours was used to ensure sufficient uptake of PGR's and chemical for influencing germination.

Table 1: Effect of different treatments on Imbibition and Germination of *Maytenus emarginata* seeds (30 Minutes treatments of each given to seeds).

	Time Period	NW	HW	CW	DEE	EA	Pyr	GA_3 (100 ppm)	GA_3 (150 ppm)	GA_3 (200 ppm)	BAP (100 ppm)	BAP (150 ppm)	BAP (200 ppm)	40% H_2SO_4	80% H_2SO_4	40% HNO_3	80% HNO_3
Imbibition % after	24 hours	15.2 ±1.8	26.5 ±2.0	10.2 ±1.9	7.1 ±1.3	8.2 ±1.0	9.0 ±1.3	32.2 ±2.0	35.5 ±2.6	25.3 ±1.3	27.7 ±1.5	30.4 ±1.4	19.2 ±1.0	30.6 ±2.3	22.4 ±1.6	10.1 ±0.8	09.0 ±0.3
	48 hours	21.4 ±2.0	42.7 ±2.9	18.6 ±1.6	12.5 ±1.7	11.3 ±1.6	13.5 ±1.5	40.7 ±2.3	50.7 ±2.8	30.4 ±1.4	33.4 ±1.7	35.3 ±1.6	22.3 ±1.3	35.8 ±2.8	25.5 ±1.5	14.6 ±1.3	13.2 ±0.5
	72 hours	30.7 ±2.4	61.8 ±3.1	25.8 ±2.6	18.3 ±2.1	16.6 ±1.9	20.1 ±2.1	53.3 ±2.8	61.0 ±3.1	33.6 ±1.7	41.2 ±2.0	49.8 ±2.1	30.7 ±1.7	48.1 ±3.0	26.2 ±0.9	21.3 ±1.6	14.6 ±0.9
	96 hours	39.8 ±2.3	80.1 ±3.3	30.3 ±2.1	25.0 ±2.0	22.2 ±1.8	28.3 ±2.4	76.6 ±3.3	88.9 ±3.3	36.4 ±1.8	58.6 ±2.9	67.7 ±2.7	33.6 ±1.6	67.6 ±3.4	26.6 ±1.0	24.7 ±1.9	15.9 ±1.1
Seed Germination % after	24 hours	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	48 hours	30.1 ±1.1	-	-	-	-	-	39.0 ±0.9	41.4 ±1.3	30.7 ±1.2	20.3 ±1.6	35.3 ±1.7	24.3 ±1.4	10.3 ±0.7	10.6 ±0.4	7.5 ±0.6	4.4 ±0.3
	72 hours	36.3 ±1.4	-	20.6 ±1.7	10.6 ±0.9	5.2 ±0.5	11.1 ±0.5	52.3 ±1.1	60.5 ±2.0	35.3 ±1.3	37.7 ±1.7	62.6 ±2.4	31.8 ±1.8	31.4 ±1.1	12.3 ±1.1	10.6 ±0.5	5.2 ±0.6
	96 hours	39.4 ±1.7	-	29.5 ±1.5	15.3 ±1.4	11.6 ±1.2	13.3 ±1.0	62.7 ±2.3	75.7 ±2.6	46.6 ±2.3	52.8 ±2.3	70.7 ±2.9	41.1 ±1.9	52.2 ±2.0	12.3 ±1.3	12.2 ±0.7	7.1 ±0.7

NW = Normal Water CW = Cold Water H_2SO_4 = Sulphuric acid DEE = Diethyl Ether GA_3 = Gibberellic Acid
HW = Hot Water Pyr = Pyridine HNO_3 = Nitric acid EA = Ethyl Acetate BAP = Benzyl Amino Purine

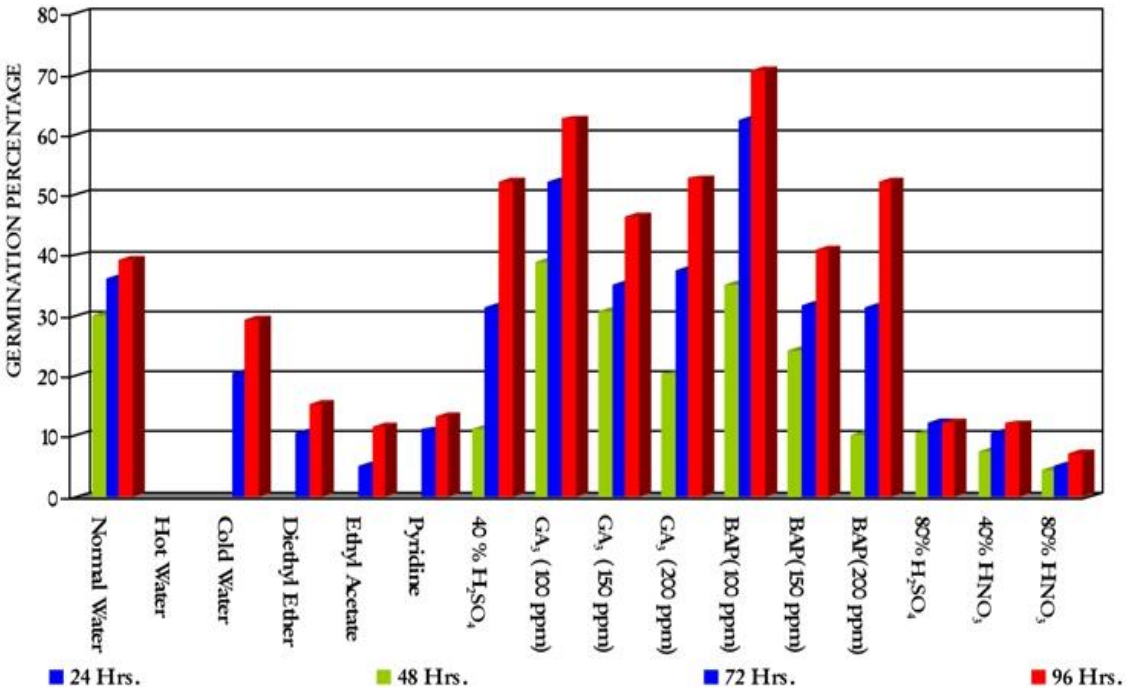

Fig. 1: Effect of different treatments on Germination of *Maytenus emarginata* seeds (30 Minutes treatments of each given to seeds).

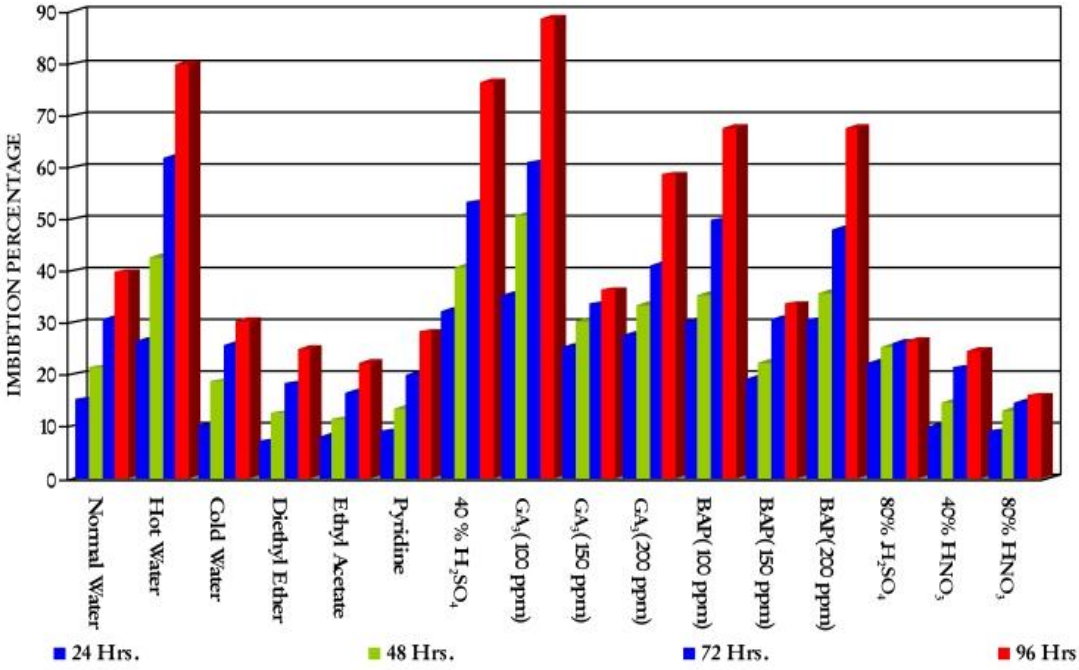

Fig. 2: Effect of different treatments on Imbibition of *Maytenus emarginata* seeds (30 Minutes treatments of each given to seeds).

Normally seeds exhibit 40% germination with 40% imbibition of water. Seeds treated with hot water showed maximum imbibition (80%) but their germination was nearly inhibited (zero%) which might be due to membrane damage or death of embryo. Effect of chilling on imbibition and germination percentage did not show any positive response compared to normal or controlled conditions and percentage of germination was declined to 30%. Poor imbibition at low temperature is a known fact, yet following normal temperature activates the hydrolytic enzymes to mobilize substrate and growth (Villiers, 1980).Effect of GA_3 (100 to 200 ppm), considered to be the replacement of chilling treatment, resulted in increase of germination percentage. Germination percentage showed increase from 100 ppm Gibbrellic Acid (GA_3) and Benzyl Amino Purine (BAP) treated seeds to 150 ppm GA_3 and BAP treated seeds. Maximum germination percentage (75.7% with GA_3 and 70.7% with BAP) was observed in seeds treated with 150 ppm GA_3 and BAP and imbibed for 96 hours. Higher concentration of GA_3 and BAP (200 ppm) could not accelerate it further and infact decreased the rate of germination (46.6% and 41% respectively) with 96 hours imbibition.The endogenous PGR's (Plant Growth Regulators) levels in the seeds do vary (Voesenek and Blom, 1996) and variety of developmental processes can be regulated by changes in their concentrations (Trewaves and Cleland, 1983). Gibbrellins and Cytokinins may not be directly involved in breaking the dormancy but play a permissive role by decreasing the level of germination inhibitors and making the seed more sensitive to gibberellins (Walker *et al*., 1989). 40% H_2SO_4 treatment could not prove to be highly successful (maximum 52.2% germination) and 80% H_2SO_4 completely declined germination percentage (12.3% at 96 hours imbibition) due to blackening of seed coat. 40% and 80% HNO_3 showed poor response (12.2% and 7.1% respectively) in comparison to H_2 SO_4 and normal water.It showed that seed coat is not the barrier in germination process. Non polar solvents like pyridine, ethyl acetate and diethyl ether showed positive increase in inhibition percentage but rate of germination was not satisfactory. These solvents were used to soften the seed coat as they do not interfere the ionic mobility of water molecules because these are chargeless. Percentage of germination shows the reproductive capacity and its survival on earth (Saba, 1998).The effect of various treatments like prosoaking, scarification, acid treatment, chilling, hot water treatment, growth regulators treatment are well known to induce germination in dormant seeds (Brad-beer, 1968; Basu and Sur, 1988; Bose and Sharma, 2000). ABA (Abscissic Acid) has been shown to be involved in regulating seed dormancy (Berry and Bewley, 1992; Mughal *et al*., 1998) and GA_3 (Gibberellic Acid) known to counteract the inhibitory effect of ABA (Bewley and Black, 1994; Mughal *et al*., 1998).

CONCLUSION

Study showed that growth hormones are the best activators to increase the rate of germination in ***M emarginata***.

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