

## SEED GERMINATION STUDIES IN *AEGLE MARMELLOS*

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Viability, imbibitions and germination behaviour was studied in *Aegle marmelos*. Tetrazolium test showed 98% seed viability. Imbibition percentage increased from 24 to 72 hours with sufficient uptake upto 96 hours. Hot water treatment showed maximum imbibition but rate of germination was completely inhibited. Cold water did not show positive response towards imbibition as well as seed germination. 150 ppm GA<sub>3</sub> and BAP gave best response on percentage of seed germination. Higher percentage of GA<sub>3</sub> and BAP (200ppm) inhibited germination percentage. H<sub>2</sub>SO<sub>4</sub> and HNO<sub>3</sub> (40% and 80%) treatment did not prove to be successful in seed germination. Non - polar solvents showed positive increase in imbibition percentage but rate of germination was not satisfactory.

**Keywords :** *Aegle marmelos*; Seed germination.

### Introduction

*Aegle marmelos* is a popular medicinal plant in the Ayurvedic system of medicine and used to treat a wide variety of ailments. All parts of tree are highly medicinal. The leaves are astringent, laxative, febrifuge and expectorant useful in ophthalmia, deafness, inflammation, diabetes and asthmatic complaints. The tender fruit is bitter astringent, anti laxative, digestive and promotes digestion and strength, overcomes colics and diarrhea. The roots as well as the bark are used in the form of a decoction as a remedy in melancholia, intermittent fevers and palpitation of heart.

It is widely accepted that physiological quality of seed, defined in terms of percentage, rate and uniformity of germination, has a major impact on the efficiency and production. The use of plant growth substances and chemicals to break seed dormancy and to synchronize seed germination is well known<sup>1,2</sup>. Physical parameters such as light and temperature also influence the germinability of the seeds. Seed size widely influences seedling fitness and small seeds with very low reserves of nutrients could be potentially disadvantageous to plant as in *Aegle marmelos*. Pulp of fruit of *A. marmelos* used to prepare juice by juice makers and seeds thrown in garbage is an important factor in reducing the percentage of seed germination. Taking into consideration all above factors, present investigation was undertaken to examine the effect of various physical and chemical agents and plant growth regulators for uniform and enhanced seed germination.

### Material and Methods

Naturally growing mature fruits located from nearby areas were collected in the month of May, June. Fruits were broken and seeds were separated from pulp and washed

thoroughly with tap water and dried. Healthy, fully matured, large sized seeds were separated from the bulk. **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 :-

**Hot water treatment** - 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<sub>2</sub>SO<sub>4</sub> and HNO<sub>3</sub> for 30 minutes with stirring at regular intervals.

**Growth hormone treatment** - Solutions of GA<sub>3</sub> (Gibberellic 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 1. Seeds were taken out after fixed time period wiped and again weighed to calculate imbibition percentage. Imbibed and weighed seeds were transferred into sterilized test tubes under

**Table 1.** Effect of different solvents on imbibition and germination of *Aegle marmelos* seeds (30 minutes treatments given to seeds).

	Time Period (Hours)	NW	HW	CW	DEE	EA	Pyr	40% H <sub>2</sub> SO <sub>4</sub>	GA <sub>3</sub> (100 ppm)	GA <sub>3</sub> (150 ppm)	GA <sub>3</sub> (200 ppm)	BAP (100 ppm)	BAP (150 ppm)	BAP (200 ppm)	80% H <sub>2</sub> SO <sub>4</sub>	40% HNO <sub>3</sub>	80% HNO <sub>3</sub>
		Imbibition % after	24	25.2 ±1.8	36.5 ±2.0	20.2 ±1.9	20.1 ±1.3	22.2 ±1.0	24.0 ±1.3	40.6 ±2.3	42.2 ±2.0	45.5 ±2.6	32.3 ±1.3	37.7 ±1.5	40.4 ±1.4	29.2 ±1.0	32.4 ±1.6
	48	31.4 ±2.0	62.7 ±2.9	28.6 ±1.6	25.5 ±1.7	24.3 ±1.6	26.5 ±1.5	45.8 ±2.8	50.7 ±2.3	60.7 ±2.8	40.4 ±1.4	43.4 ±1.7	45.3 ±1.6	32.3 ±1.3	35.5 ±1.5	24.6 ±1.3	23.2 ±0.5
	72	47.7 ±2.4	81.8 ±3.1	39.8 ±2.6	39.3 ±2.1	36.6 ±1.9	40.1 ±2.1	58.1 ±3.0	63.3 ±2.8	71.0 ±3.1	43.6 ±1.7	51.2 ±2.0	59.8 ±2.1	40.7 ±1.7	36.2 ±0.9	31.3 ±1.6	24.6 ±0.9
	96	49.8 ±2.3	99.1 ±3.3	40.3 ±2.1	40.0 ±2.0	38.2 ±1.8	42.3 ±2.4	77.6 ±3.4	86.6 ±3.3	98.9 ±3.3	46.4 ±1.8	68.6 ±2.9	77.7 ±2.7	43.6 ±1.6	36.6 ±1.0	34.7 ±1.9	25.9 ±1.1
Seed Germination % after	24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	48	31.1 ±1.1	-	-	-	-	-	11.3 ±0.7	41.0 ±0.9	46.4 ±1.3	31.7 ±1.2	30.3 ±1.6	37.3 ±1.7	34.3 ±1.4	06.6 ±0.4	9.5 ±0.6	4.4 ±0.3
	72	39.3 ±1.4	-	29.6 ±1.7	11.6 ±0.9	-	19.1 ±0.5	31.4 ±1.1	55.3 ±1.1	60.5 ±2.0	35.3 ±1.3	47.7 ±1.7	62.6 ±2.4	37.8 ±1.8	11.3 ±1.1	11.6 ±0.5	5.2 ±0.6
	96	40.4 ±1.7	-	30.5 ±1.5	32.3 ±1.4	20.6 ±1.2	20.3 ±1.0	52.2 ±2.0	60.7 ±2.3	73.7 ±2.6	+46.6 ±2.3	59.8 ±2.3	70.7 ±2.9	41.1 ±1.9	13.3 ±1.3	12.2 ±0.7	7.1 ±0.7

NW=Normal Water

CW = Cold Water

H<sub>2</sub>SO<sub>4</sub>=Sulphuric acid

DEE=Diethy Ether

GA<sub>3</sub>=Gibberellic Acid

HW=Hot Water

Pyr=Pyridine

HNO<sub>3</sub>=Nitric acid

EA = Ethyle Acetate

BAP=Benzyl Amino Purine

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 plugged with cotton and autoclaved at 1.05 kg/cm<sup>2</sup> 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 -

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

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

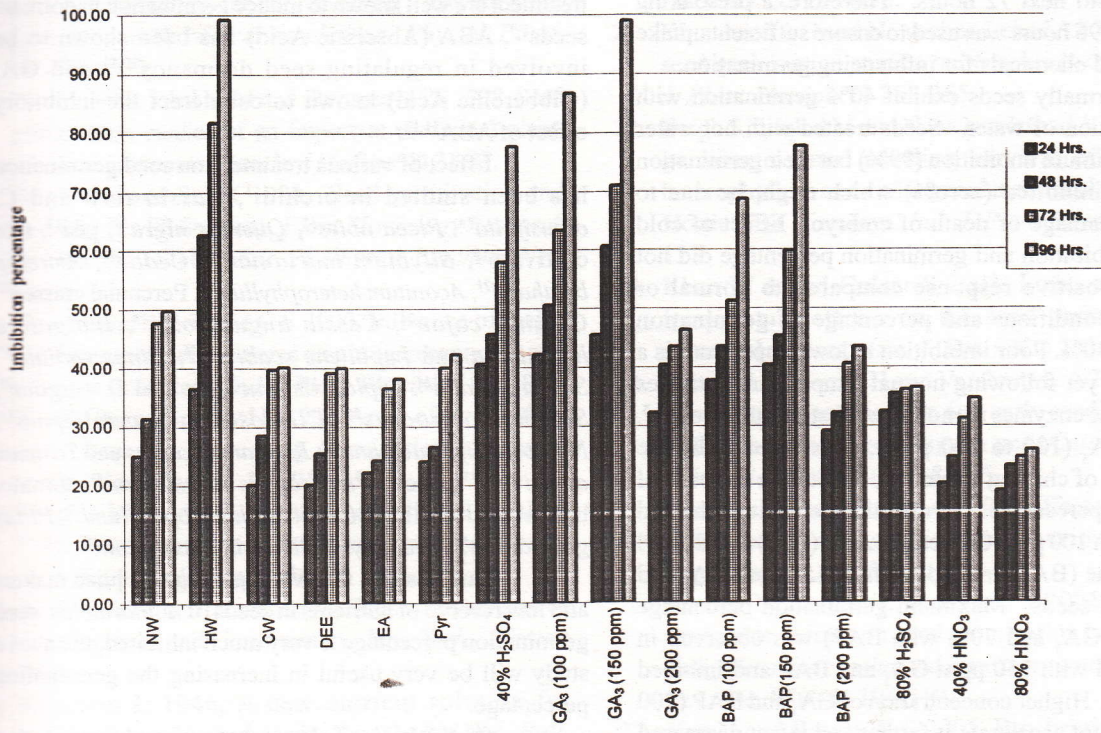
$$\text{Seed germination percentage} = \frac{\text{No. of seeds germinated}}{\text{Total no. of seeds taken}} \times 100$$

### Results and Discussion

Fruit of *A. marmelos* contain creamish to brown, rough coated seeds. Each seed weighs nearly 3 to 3.5 mg with high oil content. Fruits and seeds mature in the month of May, June in next year after formation.

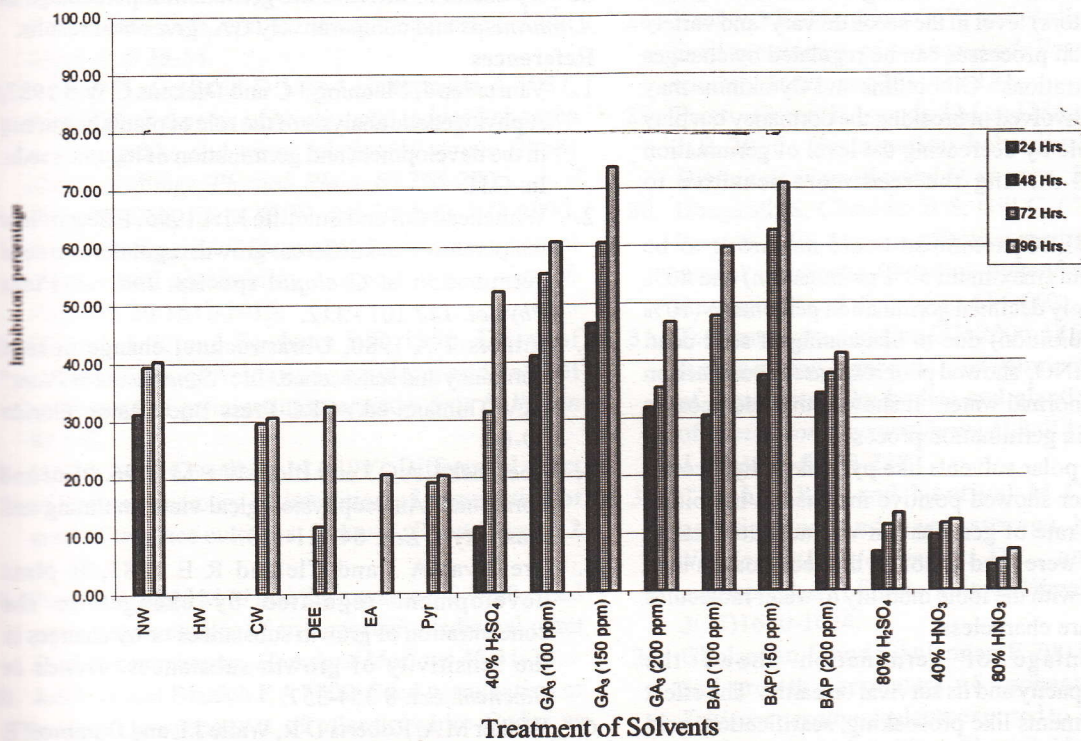
The tetrazolium test showed 98% viability in the seeds used. It was observed that freshly collected seeds germinate immediately signifying that seeds do not require dormancy period after ripening. However percentage of germination was nearly 40%.

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



Treatment of Solvents

Fig.1. Effect of various treatments on percentage imbibition of water in *A. marmelos* seeds.



Treatment of Solvents

Fig.2. Effect of various treatments on germination of *A. marmelos* seeds.

increased upto next 72 hours. Therefore, a presoaking treatment of 96 hours was used to ensure sufficient uptake of PGR's and chemicals for influencing germination.

Normally seeds exhibit 40% germination with 50% imbibition of water. Seeds treated with hot water showed maximum imbibition (99%) but their germination was nearly inhibited (zero%) which might be due to membrane damage or death of embryo. Effect of cold water on imbibition and germination percentage did not show any positive response compared to normal or controlled conditions and percentage of germination 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<sup>3</sup>. Effect of GA<sub>3</sub> (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 Gibberellic Acid (GA<sub>3</sub>) and Benzyl Amino Purine (BAP) treated seeds to 150 ppm GA<sub>3</sub> and BAP treated seeds. Maximum germination percentage (73% with GA<sub>3</sub> and 70% with BAP) was observed in seeds treated with 150 ppm GA<sub>3</sub> and BAP and imbibed for 96 hours. Higher concentration of GA<sub>3</sub> and BAP (200 ppm) could not accelerate it further and infact decreased the rate of germination (46% and 41%, respectively) with 96 hours imbibition. The endogenous PGR's (Plant Growth Regulators) level in the seeds do vary<sup>4</sup> and variety of developmental processes can be regulated by changes in their concentrations<sup>5</sup>. Gibberellins 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<sup>6</sup>.

40% H<sub>2</sub>SO<sub>4</sub> treatment could not prove to be highly successful (maximum 50% germination) and 80% H<sub>2</sub>SO<sub>4</sub> completely declined germination percentage (10% at 96 hours imbibition) due to blackening of seed coat. 40% and 80% HNO<sub>3</sub> showed poor response in comparison to H<sub>2</sub>SO<sub>4</sub> 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 with the ionic mobility of water molecules because these are chargeless.

Percentage of germination shows the reproductive capacity and its survival on earth<sup>7</sup>. The effect of various treatments like presoaking, scarification, acid treatment, chilling, hot water treatment, growth regulators

treatment are well known to induce germination in dormant seeds<sup>8,9</sup>. ABA (Abscisic Acid) has been shown to be involved in regulating seed dormancy<sup>10,11</sup> and GA<sub>3</sub> (Gibberellic Acid) known to counteract the inhibitory effect of ABA<sup>11,12</sup>.

Effect of various treatment on seed germination has been studied in orchid<sup>13</sup>, *Cassia tora* and *C. obtusifolia*<sup>14</sup>, *Picea obies*<sup>15</sup>, *Quercus nigra*<sup>16</sup>, pea<sup>17</sup>, rice cultivars<sup>18</sup>, *Silybium marianum*<sup>19</sup>, Cedar<sup>20</sup>, *Arnebia benthami*<sup>21</sup>, *Aconitum heterophyllum*<sup>22</sup>, Perennial grasses<sup>23</sup>, *Cajanus cajan*<sup>24</sup>, *Cassia angustifolia*<sup>25</sup>, *Indigofera heterantha* and *Impatiens scabrida*<sup>26</sup>, *Pinus radiata*<sup>27</sup>, *Sierra Nevada*<sup>28</sup>, *Diplotaxis eruroides* and *D. vergata*<sup>29</sup>, *Sonchus oleraceus*<sup>30</sup>, *Cleisostoma racemiferum*<sup>31</sup>, *Nardostachys jatamansi*<sup>32</sup>, *Raphanus sativus* and *Triticum aestivum*<sup>33</sup>, rice<sup>34</sup>, *Pterocarpus marsupium*<sup>35</sup>, tomato, brinjal and chilli<sup>36</sup>, *Aconitum heterophyllum*<sup>37</sup>, *Pinus gerardiana*<sup>38</sup>, *Zea mays*<sup>39</sup>, *Tamarix aucheriana*<sup>40</sup>.

Due to seeds thrown in garbage by juice makers and low reserve of nutrients in seeds of *A. marmelos*, seed germination percentage is very much inhibited, the above study will be very useful in increasing the germination percentage.

On the basis of above study it can be concluded that treatment of BAP and GA<sub>3</sub> at the rate of 150ppm will be very useful to increase the germination percentage in *A. marmelos* and comparatively GA<sub>3</sub> gives best results.

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