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EFFECTS OF GIBBERELLIC ACID (GA_3), INDOLE-3-ACETIC ACID (IAA) AND WATER TREATMENTS ON SEED GERMINATION OF *MELİA AZEDARACH* L.

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Abstract

Melia azedarach L. in addition to ornamental purposes and traditional usage of the plant, some potentially occured bioactive compounds from all parts of the plant that recently studied on can cause an increasing demand of the plant seedling. The present study aims to determine the effects of gibberellic acid (GA_3), indole-3-acetic acid (IAA) and water treatments on breaking seed dormancy, obtaining germination and to reach healthy acclimatized seedlings. The two plant growth regulators, water and different treatment durations have been applied for some plant genus or species as obtaining different germination activities. In the study for germination tests, seeds treated with 500, 1000, 2000, 3000 ppm GA_3 or IAA separately for 6 hours or 24 hours besides water treatments and control in two different seed ages. Among the all treatments in both of the two seed ages (seeds from fresh fruits from tree and seeds from fruits after one year hanging on tree); GA_3 doses gave the best germination and seedling results while controls and IAA treatments had no possitive effects on germination rates. The data was analysed using analysis of variance (ANOVA) of completely randomized block design and the groups that showed variance were then subjected to Duncan's Multiple Range Test with a significance value at P=.05. The percentage data was transformed by Arc Sin √% before carrying out ANOVA.

Key words: *Melia azedarach, GA_3, IAA, seed germination, seed age.*

INTRODUCTION

Melia azedarach L. is a perennial, deciduous woody plant species, belongs to Meliaceae family. The plant has an important role in the nature with valuable fruite, stem, bark, root, leaves, leaf juice, flowers, seeds that used in different purposes in traditional medicine, plant protection and ornamental usage (Mishra et al., 2013). There are some scientific studies (Alché et al., 2002; Saleem et al., 2002; Carpinella et al., 2003; Prophiro et al., 2008; Defagó et al., 2009; Cavoski et al., 2012; Sen and Batra, 2012; Leelavathi and Doss, 2014) which reached positive findings, studied on the plant in last decades related on the mentioned topics.

For the reasons demands for *M. azedarach* would be increased for pharmaceutical and pesticide industries that need raw material without any interruption. For this purposes researhers have worked on clonal propagation or sprouting capacity of the plant with vegetative tissue (Tourn et al., 1999; Scocchi and Mroginski, 2004; Khosh-Khui and Kaviani, 2010; Sen et al., 2010). Furthermore this was emphasized that the plant has very poor seed germination rate due to seed dormancy (Azad et al., 2010) and considerable differences can occure among seed source for growth and yield characters in *M. azedarach* and genetically controlled and selected seed can be an effective tool in the improvement of this economically and ecologically important tree species (Meena et al., 2014).

There are a few reached studies focused on *M. azedarach* generative propagation via seed germination (Banerjee, 1998; Thakur et al., 1998; Sharry et al., 2006; Azad et al., 2010; Khosh-Khui and Kaviani, 2010). During seed germination studies, some external plant growth regulators and another pretreatments like water treatment in different treatment durations are well-known, constantly used and applicable techniques. Especially treatments of GA_3 and IAA are mostly preferred chemicals among all plant growth regulators in seed germination of woody plants because of revealed physiological effects on seed. Some of seeds can be hard to germinate themselves because of external or internal factors (Lang, 1965). The factors well defined and classified in the previous study (Nikolaeva, 1969) and modified by Baskin and Baskin (2004). Physiological dormancy is the most frequent dormancy class (Baskin and Baskin, 2004). The adjustment of physiological dormancy seeds to their external environment is highly specific, and increased germination occurs in response to specific temperature, chemical, or light signals and conditions required for breaking dormancy include application of GA_3 or other hormones such as ethylene, dry storage (after-ripening) warm stratification, and cold stratification (Baskin and Baskin, 2014).

Therewithal, there are some studies conducted on woody species and found positive effects of IAA on seed germination (Mostafa and Abou-Alhamd, 2011; Sinhababu and Banerjee, 2013).

At the same time seed age has an importance on germination rate (Oziegbe et al., 2010).

The study aimed to determine the effects of the GA_3 and IAA concentrations besides water treatment and control on germination rate with two different treatment duration in two different seed ages on germination and seedling capability of *Melia azedarach* seeds under laboratory condition.

MATERIALS AND METHODS

All of the seed materials were collected from a detected single tree which has adapted naturally to Kocaeli City (Turkey) ecological condition. This can be said that the plant is a naturalized species for this area. In the first step of the study *Melia azedarach* seeds from fruits that were gathered at semi-mature yellowish-green step (6 months after the beginning of the fruit development) and called "young seed" in November. In the second step of the study seeds were taken from the fruits that were gathered at over-mature pale yellow step (12 months after the beginning of the fruit development) hanging on the tree called "old seed" in May (Figure 1A,B).

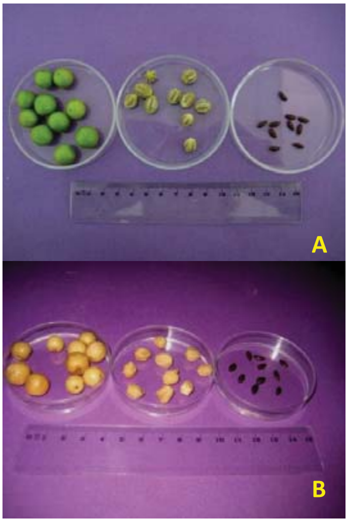

Figure 1. (A) Newly formed fruits, endocarps and seeds; (B) Over-mature fruits after one year hanging on tree, endocarps and seeds

Seeds with the same size selected after fruit flesh and endocarp removing manually without any intermission after fruit gathering in both of the two fruit maturity steps. The experiments (Figure 2A) were conducted during (November, 2013 - August, 2014) at Plant Research Laboratory, Arslanbey Campus (Kocaeli University, Turkey).

After removing, the seed (with seed coat) they were treated with gibberellic acid (GA_3) or indole-3-acetic acid (IAA) one of the concentration of 500, 1000, 2000, 3000 ppm or distilled water under room temperature for 6 or 24 hours. Control seeds were not treated with any chemicals or water. The treated and control seeds were germinated on 6 mm *Petri* plate on moist filter paper beds, irrigated with a fungicide solution once and watered equally in every four days. The step was maintened in an incubator at 20^{0}C in darkness.

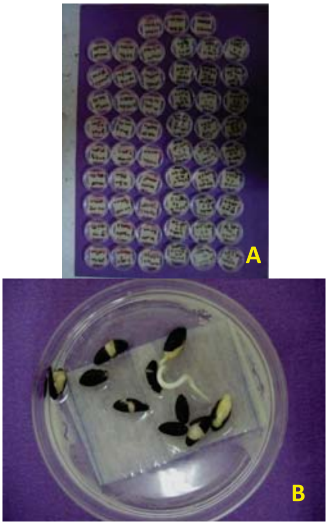

Figure 2. (A) First step of the study in germination experiments; (B) Seed germination in 500 ppm GA_3-6hr treatments in old seed at 21. Day

When root reached 1 cm (more than seed lenght) (Figure 2B), weekly germination percentage (7., 14. and 21. day) and final germination percentage (28. day) were calculated. Final germination anomaly percentage was recorded at 28. day of germination. Following germination, seedling growth was provided in pots consisted peat under day light at laboratory conditions. Seedling survival capability were also calculated as percentage after 2 months acclimatization period of seedlings. In the study each treatment replicated trice, 10 seed were consisted in each replication. The results were calculated as percentage and transformed by Arc Sin √% prior to statistical analysis. Factorial analysis was used based on Randomized Complete Block Design and all data were subjected to analysis of variance (ANOVA). Mean of treatments tested by Duncan's Multiple Range Test. Significance level of source *P*=.05 have been determined.

RESULTS AND DISCUSSIONS

While germination started in the first week in old seeds, young seed gave response to germination in the second week. In the first week, firstly old seeds to which were applied 2000 and 3000 ppm GA_3 for 6 hr and 3000 ppm GA_3 for 24 hr treatments have started to germination. In the second week, young seeds treated with 500 ppm GA_3 6 hr and 2000 ppm GA_3 24 hr showed first germination. There are not significant interaction between the seed age and the treatments in this experiments. In the first 7 and 14 days of germination there were not a clear differences between the mean germination rate of treatments while the differences were statistically important in 21. day of germination. There was any germinated seed in water or IAA applied seeds. Among the GA_3 doses, 3000 ppm GA_3 when treated for 6 hr gave the highest germination rate in mean of seed age but this effect was not statistically significant then other doses of GA_3 (Table 1, Figure 3A).

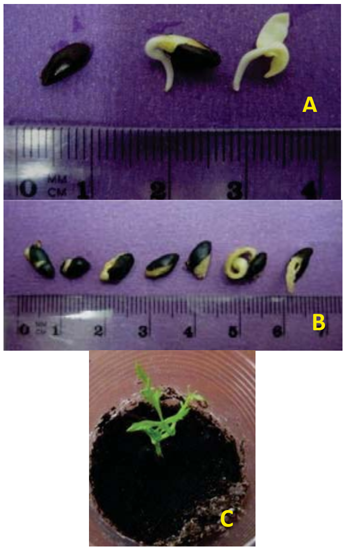

Figure 3. (A) Healty germination steps in 3000 ppm GA_3-6 hr; (B) Different germination anomalies; (C) Seedling acclimatization in peat under lab.condition at day light

Additionally among seed ages, all GA_3 doses showed more germination rates in old seeds than young seeds numerically. Oziegbe et al. (2010) found high percentage germination in six month old seeds as compared to freshly shed seed of some *Ludwigia* species and this was revealed as some degree of dormancy in fresh seeds.

Some germination anomalies were observed during the germination period (Figure 3B). For example, seed coat was cracked and endosperm was observed in some seeds but roots or cotylodone formation did not occure in the later stage. In the other cases cotyledones developed but there were no root development and malformed root developed but there were no arial parts. In these mentioned posture we call them as "germination anomalies" (Pêgo et al., 2012). At the end of the germination period, the germination anomalies were calculated take into account the treatments effects. According to the statistical analysis, being young or old seed were not important but in avarage most of GA_3 concentrations (6 hr or 24 hr) have also responce on seed germination anomalies. In this topic seeds treated with IAA doses, water and control also showed some response. The germination anomalies increased in the old seeds however the difference is not statistically important. The highest anomalies were observed in 500 ppm GA_3-6 hr treated seeds (38.4%) while it was lowest in seeds that was treated water for 6 hr (6.7%).

Germinated seeds and anomaly developed seeds was called as "total vitality". GA_3 concentrations showed higher percentage of total vitality of seeds than control, water or IAA treatments (Table 1).

At the end of the germination period all germinated seeds were transferred in peat media in pots and acclimatized under laboratory condition at day light period. Two months after acclimatization 100% seedling growth observed (Figure 3C).

Banerjee (1998) studied on germination of *M. azedarach* seed with IAA, IBA and GA_3 at duration 24 hr or 48 hr durations. Duration was not found significantly important similarly our result but 200 ppm IAA gave higher germination rate (85.25%) than used GA_3 concentrations showed germination rate between 66.17-74.58%. In contrast to the mentioned results, IAA concentration showed no germination rate in our study. Miransari and Smith (2014) clearly reviewed studies on IAA effects on seed germination and seedling growth. Bialek et al. (1992) and Hentrich et al. (2013) emphasized that although IAA may not be necessary for seed germination, it is necessary for the growth of young seedlings.

Azad et al., (2010) studied on some pre-sowing treatments on seed germination in a mixture media of *M. azedarach*. The treatments were cold water, hot water, scarification and H_2SO_4 concentration besides control. They found that the germination started between 8. and 11. days and 3 weeks after the beginning of the study germination ended in all treatment. Similarly most of germination ended 21. days and after than a few treatments continue to germinate 1 week more in both of seed ages. While the mentioned results supported our germination time, in the study germination rates were found between 39-80% in treated media which is higher than our findings between 0-20% in all treated concentrations or water and control at two seed ages. The seed age, media type and plant origin could be cause the different results. Khosh-Khui and Kaviani (2010) determined that concentration of 800 ppm GA_3 resulted in highest germination percentage (34%) in *Melia azedarach* among all traetments which were water, running water, scarification, dark or light, and 200, 500, 800 ppm GA_3. This findings supported our results that GA_3 effectiveness.

Table 1. Weekly Germination Percentage, Germination Anomalies and Vitality Rate of *Melia azedarach* seed (%)

TREATMENTS	7. day			14. day			21. day			28. day End of Germination			Germination Anomalies			Total Vitality		
	Young seed	Old seed	**Mean of Treat-ments**	Young seed	Old seed	**Mean of Treat-ments**	Young seed	Old seed	**Mean of Treat-ments**	Young seed	Old seed	**Mean of Treat-ments**	Young seed	Old seed	**Mean of Treat-ments**	Young seed	Old seed	**Mean of Treat-ments**
Control	0	0	**0**	0	0	**0**	0	0	**0 b***	0	0	**0**	10	30	**20 abc***	10	30	**20 abcd***
Water																		
Water 6 h.	0	0	**0**	0	0	**0**	0	0	**0b**	0	0	**0**	6.7	6.7	**6.7 c**	6.7	6.7	**6.7 d**
Water 24 h.	0	0	**0**	0	0	**0**	0	0	**0b**	0	0	**0**	3.3	20	**11.7 abc**	3.3	20	**11.7 bcd**
GA_3																		
500ppmGA_3 6 h.	0	0	**0**	3.3	6.7	**5**	6.7	13.3	**10 a**	6.7	13.3	**10**	26.7	50	**38.4 a**	33.4	63.3	**48.4 a**
1000ppmGA_3 6 h.	0	0	**0**	0	0	**0**	3.3	6.7	**5 ab**	3.3	6.7	**5**	30	33.3	**31.7 ab**	33.3	40	**36.7 abc**
2000ppmGA_3 6 h.	0	6.7	**3.4**	0	6.7	**3.4**	0	10	**5 ab**	3.3	10	**6.7**	20	40	**30 ab**	23.3	50	**36.7 abc**
3000ppmGA_3 6 h.	0	6.7	**3.4**	0	6.7	**3.4**	3.3	20	**11.7 a**	3.3	20	**11.7**	20	40	**30 ab**	23.3	60	**41.7 ab**
500ppmGA_3 24 h.	0	0	**0**	0	0	**0**	0	10	**5 ab**	3.3	10	**6.7**	13.3	46.7	**30 ab**	16.6	56.7	**36.7 abc**
1000ppmGA_3 24 h.	0	0	**0**	0	6.7	**3.4**	6.7	13.3	**10 a**	6.7	13.3	**10**	23.3	43.3	**33.3 ab**	30	56.6	**43.3 ab**
2000ppmGA3 24 h.	0	0	**0**	3.3	6.7	**5**	3.3	10	**6.7 ab**	3.3	13.3	**8.3**	16.7	20	**18.4 abc**	20	33.3	**26.7 abcd**
3000ppmGA_3 24 h.	0	6.7	**3.4**	0	6.7	**3.4**	0	13.3	**6.7 ab**	3.3	13.3	**8.3**	13.3	40	**26.7 abc**	16.6	53.3	**35 abcd**
IAA																		
500ppmIAA 6 h.	0	0	**0**	0	0	**0**	0	0	**0 b**	0	0	**0**	6.7	20	**13.4 abc**	6.7	20	**13.4 abcd**
1000ppmIAA 6 h.	0	0	**0**	0	0	**0**	0	0	**0 b**	0	0	**0**	20	26.7	**23.4 abc**	20	26.7	**23.4 abcd**
2000ppmIAA 6 h.	0	0	**0**	0	0	**0**	0	0	**0 b**	0	0	**0**	16.7	16.7	**16.7 abc**	16.7	16.7	**16.7 abcd**
3000ppmIAA 6 h.	0	0	**0**	0	0	**0**	0	0	**0 b**	0	0	**0**	10	16.7	**13.4 abc**	10	16.7	**13.4 abcd**
500ppmIAA 24 h.	0	0	**0**	0	0	**0**	0	0	**0 b**	0	0	**0**	6.7	13.3	**10 bc**	6.7	13.3	**10 bcd**
1000ppmIAA 24 h.	0	0	**0**	0	0	**0**	0	0	**0 b**	0	0	**0**	10	13.3	**11.7 abc**	10	13.3	**11.7 bcd**
2000ppmIAA 24 h.	0	0	**0**	0	0	**0**	0	0	**0 b**	0	0	**0**	10	10	**10 bc**	10	10	**10 bcd**
3000ppmIAA 24 h.	0	0	**0**	0	0	**0**	0	0	**0 b**	0	0	**0**	6.7	10	**8.4 bc**	6.7	10	**8.4 cd**

*Value within the same column (mean of the treatments) with different lowercase letters are significantly different at P=.05

In our study different types of germination anomalies were observed. The anomalies types were activation and growth in endosperm and cotyledone development without any roots or without the formation of aerial part with malformed roots. In this respect, GA_3 concentrations gave higher responses than IAA doses in mean and in old seed germination anomalies were observed more than young seeds. Narantsetseg (2014), Queiroz et al. (2000) and Pêgo et al. (2012) studied on seedling abnormalities using seed germination tests in different plants. They emphasized that abnormalities are related with plant region, used chemicals-genotypes or treated temperature respectively.

According to the scientific findings studied by Meena et al. (2014) genotypic correlation coefficient values are higher than corresponding phenotypic values and they emphasized that considerable differences exist among seed sources that they worked for growth and yield characters in *M. azedarach* and appreciable improvement in growth parameters can be achieved by collecting seeds from selected plus trees on a short-term basis.

CONCLUSIONS

The study reached the following findings as follows:

- ✓ Germination has occured in 4 weeks after beginning of the experiments.
- ✓ While GA_3 concentrations were effective on germination; control, water or IAA treatments had no response on germination.
- ✓ Germination rate in old seeds was higher than young seeds so the seed may need a physiologic maturation period.
- ✓ The maximum germination capability was found in 3000 ppm GA_3-6 hr treatment (20%) in old seeds.
- ✓ GA_3 concentrations had more effect on seed vitality.
- ✓ *M.azedarach* seed germination rate was very low and needed forcing with GA_3 under the experimental conditions.
- ✓ The study requires ongoing experiments on this plant at different seed collecting time, different media, pretreatments and environmental conditions.

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