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Bioefficacy of *Moringa oleifera* leaf extract: seed germination and growth of seedling of falcata (*Paraserianthes falcataria*)

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Abstract. This study explores the potential of *Moringa oleifera* leaf extract (MLE) in seed germination and growth of seedlings of *Paraserianthes falcataria*. Seed priming was done by soaking the seeds in hot water for 1 minute and in different dilutions of the MLE extracts in distilled water (MLE 1:10, MLE 1:20, MLE 1:30, MLE 1:40, MLE 1:50) for 24 hours. In the application of foliar fertilizer, MLE was prepared by dilutions with distilled water (MLE 1:1, MLE 1:3, MLE 1:5) applied to a 3-day old *P. falcataria* germinants and evaluated after 45 days. The experimental set up was laid-out in a complete randomized design with 30 experimental samples replicated 3 times. In the seed germination test, hot water treatment and seed priming treatment with dilutions of MLE 1:40 and ANAA (commercial growth hormone) were more effective methods to improve final germination percentage (FGP) to synchronize early germination. All seed priming treatments (MLE 1:10, MLE 1:20, MLE 1:30, MLE 1:40, MLE 1:50 and ANAA) and hot water treatment of *P. falcataria* seeds were more effective compared with control in all parameters except for time until 50% germination (T50) and germination energy period (GEP). Treatments MLE 1:40, hot water and ANAA were among the most efficient treatments that would improve the germination attributes of *P. falcataria* seeds. The application of MLE, commercial foliar fertilizer (Grow More) and control have the same effect on the growth vigor of *P. falcataria* seedlings as exhibited by the root collar diameter, height, root-shoot ratio, fresh shoot weight, oven-dry shoot weight, total fresh weight, total oven-dry weight and moisture content. However, it reveals that root nodule count and fresh root weight were significantly different from the control ($p < 0.05$) but the oven-dry root weight was found to be not significantly different from the control ($p > 0.05$). The MLE possess potential as seed priming treatment but as an organic fertilizer more in-depth study is needed.
Key Words: *Moringa oleifera,* leaf extracts*, Paraserianthes falcataria*, seed priming, zeatin, imbibition and root nodulation.

Introduction. Forest is one of the vital resources of our country. It plays an important part in the different biogeochemical cycle such as nutrient and water. Forests also provide a habitat to different macro and micro flora and fauna. Its high biodiversity provides a wide resource base for the basic necessities of life such as food, clothing, shelter and medicine.

As population increases rapidly, more forests have been converted to provide for more lands for agriculture and grazing and other developmental activities. More have also been used up to support the need for more forest products beyond its sustainable capacity.

The rapid decline of the natural forests of the Philippines has been experienced by the country. It is a cause for concern and massive efforts on enrichment, rehabilitation and development of the denuded areas have been undertaken. Different reforestation and afforestation activities have been conducted by different institutions and organizations, both public and private. This strategy will, at least, revegetate if not regain the natural state of our forest.

Plantation ecosystems may not be as diverse compared with the natural forests. However, this is the only option to alleviate the pressures on the remaining natural forests. It is primarily intended to produce pulpwood and timber to contribute to the national economy improving the condition of the lives of the Filipino people.

Plantation also plays a very important function in sequestering carbon from the atmosphere which improves the microclimate of a particular area. The maximum sequestration rates forfalcata (*Paraserianthes falcataria*) range between 63 and 73 Mg CO_2 ha^{-1} per year in plantation forestry of Indonesia (Subarudi et al 2003).

Caraga Region, the timber corridor of the country, for many decades made tree farming a way of life. The region contributes 60% of the production for wood of the country (FMB-DENR 2011).

Sixty seven percent (67%) of national log production is from falcata trees (FMB-DENR 2011). It is the major crop raised in the region. Its suitability to the site as exhibited by its fast growth rate; and the high market demand for plywood, lumber, boxes and crates production, encourage more and more farmers into falcata tree farming.

Demand for falcata seedlings is so high that seedling growing nurseries are flourishing like mushrooms along national highways. In the region based on the interview with growers, they are faced with the consistent problem of plant nutrient deficiencies resulting to more or less 50% mortality in their production thereby affecting costs and returns. They are totally dependent on the use of available commercial inorganic fertilizers which take additional costs in the seedling production. This condition is not beneficial to the growers alone but to the environment as well. According to Barak et al (1998), chemical fertilizers can gradually increase the acidity of the soil until it begins to impede plant growth. Chemically fertilized plots also show less biological activity in the soil food web (the microscopic organisms that make up the soil ecosystem) than do plots fertilized organically with manure or other biological sources of fertility (Raupp 1997).

Growers are now conscious of the need to shift from inorganic to organic but less information are available. Many potential plants are growing naturally, however, only a few have been studied and their significant uses known. Use of these sources is more cost-efficient, cheap, easy to use and safe.

A natural occurring biochemical in plants found to be rich in nutrients and growth regulating hormones. Moringa leaf extract (MLE), being rich in amino acids, K, Ca, Fe, ascorbate, and growth regulating hormones like zeatin, is an ideal plant growth enhancer (Makkar & Becker 1996; Basra et al 2009 a, b). Zeatin is the most naturally occurring cytokinin that not only promotes the growth of plants but also has anti aging potential and protective effects in plants (Marcu 2005). Zeatin plays an important role in cell division and cell elongation (Taiz & Zeiger 2006).

The use of plant leaves for the extract is non-destructive to the whole plant and harvested leaves can be quickly replenished. The use of plant leaf extract organic fertilizers would be a good alternative to and possibly as replacement for chemical fertilizers.

Material and Method. The study was conducted last November 15, 2013 to January 9, 2014 in a vacant lot of 120 sqm at Purok 18 San Vicente, Butuan City for nursery set-up and experimentation (125°32'33.98"E, 125°32'33.98"E). Laboratory works were done at the Macrosomatic Clonal Nursery Laboratory of Department of Environment and Natural Resources – Region 13, Ecosystems Research and Development Services (DENR 13 – ERDS), Bud Shrine Eco-Park, Brgy. Pinamangculan, Butuan City (8°57'9.79"N, 125°29'31.67"E) as shown in Figure 1.

Malunggay (*Moringa oleifera)* leaf extract (MLE) was used as organic fertilizer. Three kilograms of fresh leaves free from any form of pest infestation and disease were collected and washed thoroughly by running with tap water and then rinsed with sterile distilled water prior to leaf the extraction process.

Three kilograms of fresh leaves were extracted using a commercially fabricated juicer machine. The extract was strained four times through a cheese cloth and then

diluted with distilled water to prepare the required dilutions in different treatment proportion for seed priming for the germination test and seedling vigor test for seedlings.

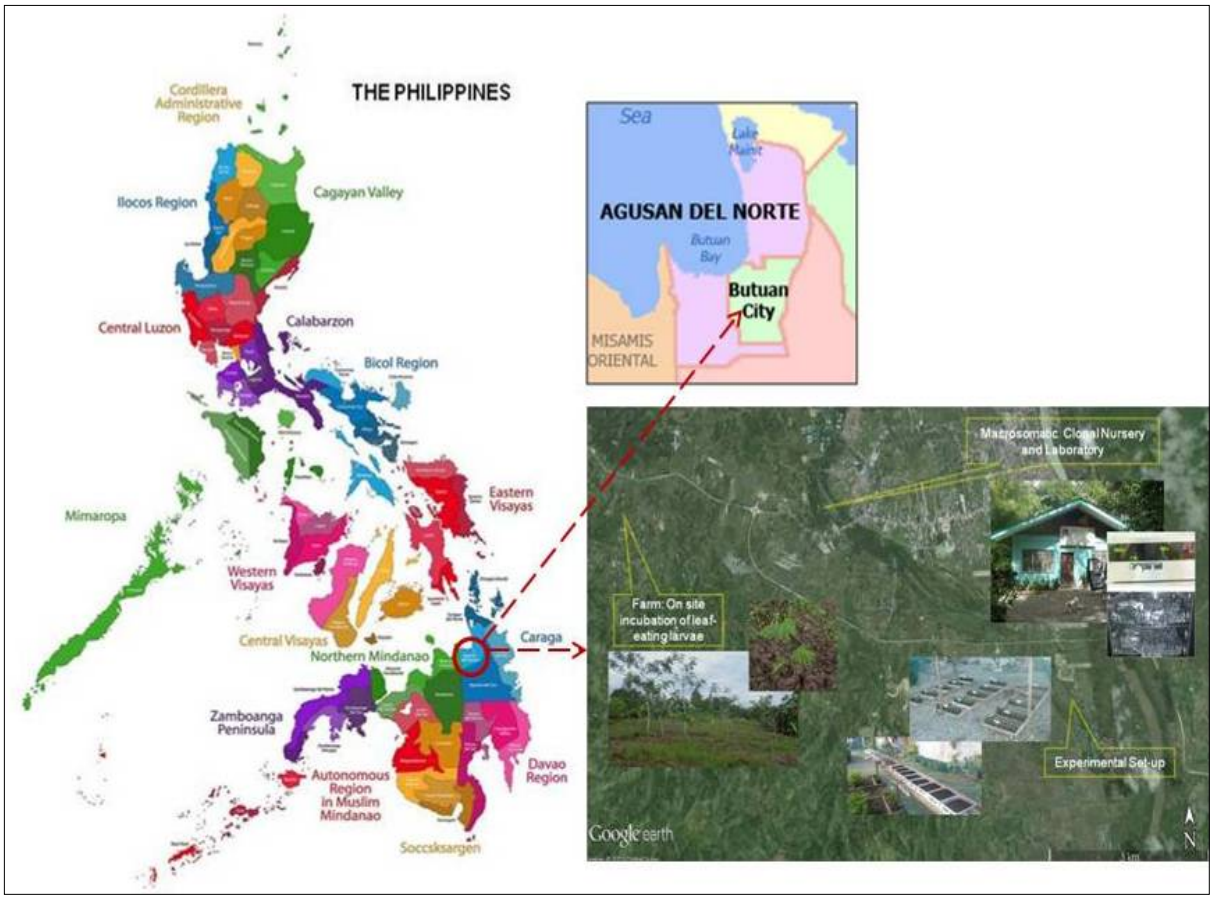

Figure 1. Map of Butuan City, Philippines showing the study area (Source: Google Earth Pro. Imagery Date: April 28, 2013).

Germination test. The *P. falcataria* seeds used in this study were secured and provided by the Mindanao Tree Seeds Center (MTSC) of DENR 13-ERDS with guaranteed highest purity and viability. In seed priming, all seeds except for the control were soaked in boiling water for 1 minute and then soaked in different treatment dilutions (Table 1) for 24 hours. After priming, seeds were washed three times with rainwater (distilled water) and re-dried near its original weight under shade at 23 ± 3°C (Basra et al 2002). In hot water treatment, seeds were soaked in boiling water for one minute, and subsequently soaked in tap water at room temperature for 24 hours. Germination testing was conducted after re-drying. Untreated seeds were used as control.

Table 1

List of treatments in germination test

Treatments	*Dilutions*
MLE*	1:10, 1:20, 1:30, 1:40 and 1:50
ANAA (Commercial Growth hormone)[1]	Prescription (50 ml L^{-1})
Hot water[2]	Without treatment dilution
Control	Untreated seeds

*Leaf extract concentrate and rainwater (distilled water) ratios;
[1]ANAA (Napthalene acetic acid – 0.10%, Vitamin B-1 – 0.05% and Inert 99.85%);
[2]Hot water – seeds undergo hydropriming but without treatment dilution during soaking.

For each treatment, thirty seeds were sown in germination tray (5 in x 8 in) with sandy clay loam soil as germination media laid-out in a complete randomized design in 3 replications. The final germination percentage (FGP), counts germinated seed daily according to the Association of Official Seed Analysts method (AOSA 1990) until the final seed emerged (Nouman et al 2012). The time until 50% germination (T50) was calculated using the following formula (Farooq et al 2005):

$$T50 = ti + \frac{\left(\frac{N}{2} - ni\right)(tj - ti)}{nj - ni}$$

where: N is the final number of seeds germinated; ni and nj are the cumulative numbers of seeds germinated at adjacent counts at times ti and tj when ni < N/2 < nj.

The mean germination time (MGT) was calculated for each treatment using the formula (Ellis & Roberts 1981):

$$MGT = \sum \frac{n\,T}{\sum n}$$

where: n = number of seeds newly germinated; T = hours from the beginning of the germination test, Σn = final germination.

Germination index (GI) was calculated using the formula of the Association of Official Seed Analysts (AOSA 1983):

$$GI = \frac{no.of\ germinated\ seeds}{Days\ of\ first\ count} + - - - - - - + \frac{no.of\ germinated\ seeds}{Days\ of\ final\ count}$$

Germination energy period (GEP), defined as the percent, by number, of seeds in a given sample which germinate within a given period e.g. in 7 or 14 days, under optimum conditions (Willan 1985). Seed coat is removed from the cotyledon.

Seedling vigor test. In every treatment, 3-day old *P. falcataria* germinant was planted individually in a 3 x 6 inches polyethylene black potting bag with sandy clay loam soil medium, and laid-out in a complete randomized design in 3 replications. The seedlings were exposed in partially shaded area and sprayed with different treatment foliar dilutions every three days (Table 2).

Table 2

List of treatments in seedling vigor test

Treatments	*Dilutions*
MLE*	1:1, 1:3 and 1:5
GrowMore (Commercial foliar fertilizer 20-20-20)[1]	Prescription (2.5 grams L^{-1}; applied at 10 days interval)
Control	Untreated seedlings

*Leaf extract concentrate and rainwater (distilled water) ratios;
[1]20% nitrogen, 20% phosphorus and 20% potassium.

Foliar spraying of treatment dilutions was conducted early morning from 6 am to 8 am. After 45 days, the growth performances of seedlings were evaluated measuring the root collar diameter, height, root-shoot ratio, occurrence of root nodule and biomass. Fresh plant biomass was weighed immediately after harvesting and then oven-dried at 70°C until it reaches a constant weight for the determination of dry weight (Nouman et al 2012).

Statistical analysis. Analyses of variance of the data for each experimental set-up were computed and analyzed using MS office Excel and Statistical Analysis Software (SAS). A Duncan's multiple range test (DMRT) at a 5% level of probability was used to test the differences among treatment means.

Results and Discussion

Germination test. This study aimed to investigate whether seed priming of *P. falcataria* seeds in combination with different dilutions of treatments (e.g. MLE) and commercial growth hormone could improve the germination attributes.

Table 3 shows that germination test results which revealed highly significant differences ($p < 0.01$) in all the treatments for the different measured parameters except for time 50% germination (T50) and germination energy period (GEP). The final germination percentage (FGP) showed highly significant ($p < 0.01$) variations among treatments. Seed priming with the treatment dilutions of MLE 1:40 (59.83%), ANAA (59.19%) and hot water treatment (59.13%), respectively, had higher FGP compared with the other treatments.

Table 3

Effect of seed priming on Final Germination Percentage (FGP), Germination Energy Period (GEP), Time 50% Germination (T50), Mean Germination Time (MGP) and Germination Index (GI) of *P. falcataria* seeds (DMRT)

Treatment	*FGP (%)*	*GI*	*MGT (days)*	*T50*	*GEP (days)*
MLE 1:10	46.97^{b}	3.34^{b}	4.98^{b}	4.86^{b}	8.33^{ab}
MLE 1:20	51.54^{ab}	4.74^{b}	4.15^{c}	5.48^{ab}	7.33^{b}
MLE 1:30	54.07^{ab}	4.79^{b}	4.12^{c}	4.65^{b}	7.67^{b}
MLE 1:40	59.83^{a}	6.58^{a}	3.59^{d}	6.61^{ab}	5.67^{b}
MLE 1:50	45.27^{b}	4.3^{b}	3.90^{cd}	5.75^{ab}	6.33^{b}
ANAA	59.19^{a}	6.51^{a}	3.98^{cd}	5.43^{ab}	7.00^{b}
Hot water	59.13^{a}	6.67^{a}	3.49^{d}	7.38^{a}	7.00^{b}
Control	32.48^{c}	1.01^{c}	8.89^{a}	4.74^{b}	11.67^{a}
P value (ANOVA)	0.0003	0.0001	0.0001	0.0889	0.1047
CV(%)	11.21	17.8	5.78	19.86	28.58

***** Means with the same letter are not significantly different at 5% probability level by Duncan's multiple range test (DMRT).

Moreover, seed priming and hot water treatment have a highly significant ($p < 0.01$) effect on germination speed by increasing GI in all the treatments applied. The hot water treatment, MLE 1:40, ANAA have a higher GI of 6.67, 6.58 and 6.51, respectively, compared with the control. In terms of MGT, there were highly significant differences ($p < 0.01$) among the treatment means. Treatment MLE 1:40, 1:50, ANAA and hot water recorded as the efficient treatment in reducing MGT by 3.59, 3.90, 3.98 and 3.49 days, respectively compared with the control (8.89) days. In the time until 50% germination (T50) and GEP, no significant ($p > 0.05$) differences among treatments means were observed. In the germination of *P. falcataria* seeds, hot water treatment and seed priming treatment with dilution of MLE 1:40 and ANAA were more effective methods to improve FGP to synchronize early germination. All seed priming treatments (MLE 1:10, MLE 1:20, MLE 1:30, MLE 1:40, MLE 1:50 and ANAA) and hot water treatment of *P. falcataria* seeds were more effective compared with the control in all parameters except for time until 50% germination (T50). Seed priming has been seldom used in forest tree seeds. It is already proven that seed priming can improve germination and plant vigor in vegetables (Brocklehurst & Dearman 1983; Brocklehurst et al 1984; Karssen et al 1989; Gray et al 1990; Bradford & Haigh 1994; Welbaum et al 1998), crops (Harris et al 1999, 2000; Musa et al 1999), and range grasses (Hardegree & Emmerich 1992a, 1992b; Nouman et al 2012). In the germination of *P. falcataria* seeds, hot water treatment and

seed priming treatment with dilution of MLE 1:40 and ANAA were more effective methods to improve final germination percentage (FGP) to synchronize early germination. All seed priming treatments (MLE 1:10, MLE 1:20, MLE 1:30, MLE 1:40, MLE 1:50 and ANAA) and hot water treatment of *P. falcataria* seeds were more effective compared with control in all parameters except for T50 and GEP. Seed priming enhances mobilization of reserves from the storage of seed (e. g. cotyledons or endosperms for partitioning to embryo (Wahid & Farooq 2012). Generally, treatments MLE 1:40, hot water and ANAA were among the most efficient treatments that would improved the germination attributes of *P. falcataria* seeds. Seeds that have not germinated may have undergone imbibitional damage due to rapid uptake of hot water and the number of hours of soaking in the treatment dilutions to hasten germination. According to the study of Mabhaudhi & Modi (2011) rapid uptake of water during priming may have caused imbibition injury, resulting in failure of maize (*Zea mays* L.) seeds to germinate. The results of this study are similar with previous studies on seed priming with diluted MLE from germination until seedling stage which effectively improved germination and seedling growth in lentil (Ghassemi-Golezani et al 2008) and sunflower (Basra et al 2009a).

Seedling vigor test (organic fertilizer). In the application of foliar spray treatments the results revealed that there were no significant differences in root collar diameter, height, root-shoot ratio, fresh shoot weight, oven dry shoot weight, total fresh weight, total oven dry weight, oven dry root weight and moisture content in *P. falcataria* seedlings ($p > 0.05$) (Table 4). However, there were significant ($p < 0.05$) variations in the application of foliar sprays treatments with respect to root nodule count and fresh root weight. The weight of the fresh root of the control (0.48 g) was heavier compared with the other treatments (Figure 2). The average root nodule count of the control and MLE 1:3 which are 20.44 and 10.56, respectively, were observed to be higher compared with the other treatments (Figure 3). This can be attributed by the occurrence of more root nodules in the control compared with the other treatments.

Table 4

Effect of foliar spray treatments on average root collar diameter (RCD), height, root-shoot ratio, root nodule count, fresh root weight, fresh shoot weight, oven dry root weight, oven dry shoot weight, total fresh weight, total oven dry weight of *P. falcataria* seedlings

	Treatment						
	MLE 1:1	*MLE 1:3*	*MLE 1:5*	*Grow More*	*Control*	*P value (ANOVA)*	*CV (%)*
RCD (mm)	1.71[a]	1.82[a]	1.73[a]	1.81[a]	1.93[a]	0.9511	20.63
Height (cm)	12.90[a]	13.90[a]	13.20[a]	15.36[a]	15.39[a]	0.9347	20.63
Root - shoot ratio	0.19[a]	0.24[a]	0.24[a]	0.23[a]	0.28[a]	0.8002	38.07
Nodule count	2.44[b]	10.56[ab]	3.44[b]	4.44[b]	20.44[a]	0.0164	69.42
Fresh root wt (g)	0.21[b]	0.24[b]	0.13[b]	0.20[b]	0.48[a]	0.0223	52.52
Fresh shoot wt (g)	1.05[a]	0.88[a]	0.85[a]	0.82[a]	1.56[a]	0.6472	64.82
Oven dry root wt (g)	0.04[b]	0.07[b]	0.05[b]	0.07[b]	0.10[a]	0.1867	40.99
Oven dry shoot wt (g)	0.27[a]	0.32[a]	0.31[a]	0.34[a]	0.38[a]	0.7545	41.28
Total fresh wt (g)	1.26[a]	1.12[a]	1.12[a]	1.12[a]	2.04[a]	0.4921	61.26
Total oven dry wt (g)	0.31[a]	0.38[a]	0.36[a]	0.41[a]	0.48[a]	0.8809	52.24
MC (%)	67.87[a]	64.53[a]	49.19[a]	61.92[a]	74.71[a]	0.3692	23.34

***** *Means with the same letter are not significantly different at 5% probability level by Duncan's multiple range test (DMRT).*

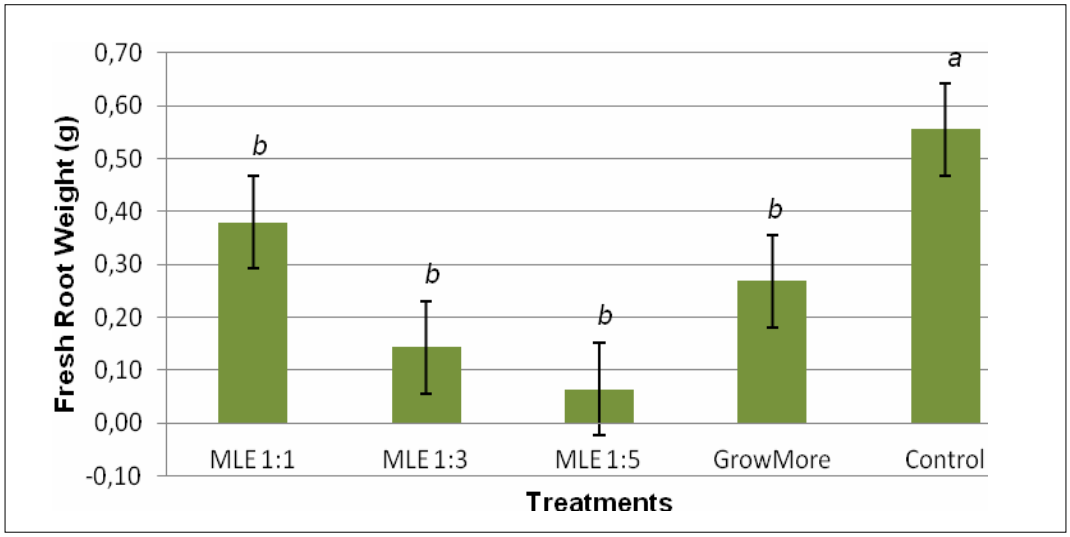

Figure 2. Effect of foliar spray treatments on fresh root weight of *P. falcataria* seedlings (mean ± SE; n = 30) (Means with the same letter are not significantly different at 5% probability level by DMRT).

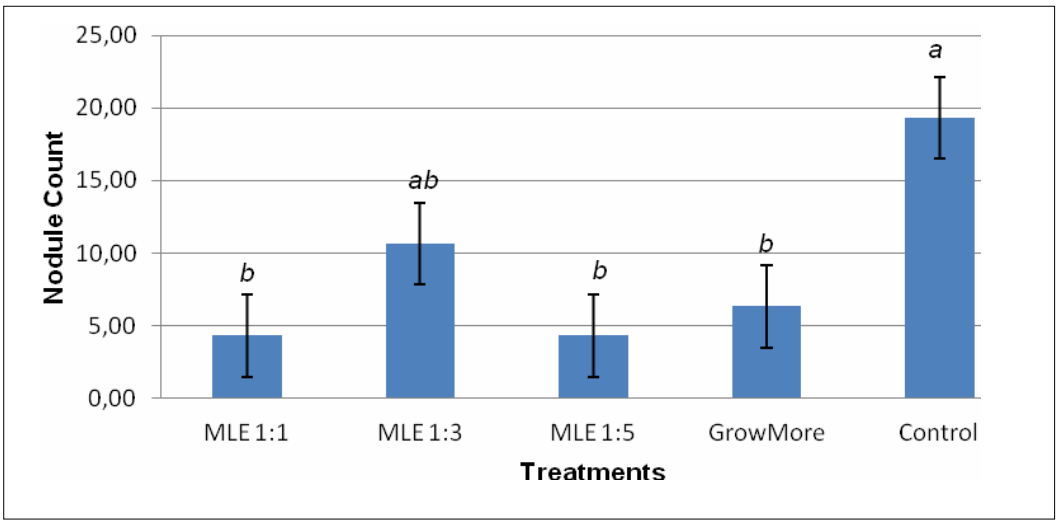

Figure 3. Effect of foliar spray treatments on nodule count of *P. falcataria* seedlings (mean ± SE; n = 30) (Means with the same letter are not significantly different at 5% probability level by DMRT).

The application of MLE and GrowMore have the same effect as with control in terms of growth of *P. falcataria* seedlings when the root collar diameter, height, root-shoot ratio, fresh shoot weight, oven dry shoot weight, total fresh weight, total oven dry weight and moisture content were determined. Conversely, it reveals that the seedlings applied with foliar spray resulted in lesser occurrence of root nodules compared with the control. The phytohormone abscisic acid (ABA) is known to be important for regulating the number of nodules established on the roots of legumes (Hirsch & Fang 1994). Tominaga et al (2010) found out that endogenous abscisic acid (ABA) concentration not only regulates root nodulation, but also nitrogen fixation activity by decreasing the production of root nodules of *Lotus japonicus* seedlings. It was reported that the stimulatory effects of abscisic acid mediated the presence of other plant growth regulating hormones like zeatin, which is commonly found in the moringa leaf extract. Ella & Zapata (1991) determined the effects of preculture in abscisic acid and exogenously applied zeatin in the regeneration of the plant from the calli of rice. It was found that abscisic acid increased regeneration of the plant in the medium that was zeatin-free. There is also evidence for the enhancement of nodulation by zeatin at a certain level of concentration. Taller & Sturtevant (1991) carried out growth pouch experiments of soybean plants

inoculated with the nitrogen fixing bacterium *Bradyrizobium japonicum* were given a one-time addition of trans-zeatin. Plants treated with 0.5, 1 or 5 µg L^{-1} zeatin showed twice as many root nodules as the control, while 10 µg L^{-1} zeatin was inhibitory, causing a 50% reduction in root nodule number.

According to Mangal et al (2013), the moringa leaves aqueous extract contain water soluble allelo-chemicals which could inhibit the growth of seedling and biochemical contents of chickpea crop. Similarly, Phiri (2010) reported that the application of *M. oleifera* leaf extract reduced radicle length of rice, hypocotyls of sorghum, seedling survival of sorghum, germination percentage of rice and delayed the germination of rice. Phiri & Mbewe (2009) conducted another series of experiments in which *Moringa* leaf extracts were applied on seeds of three legumes including beans, groundnut and cowpea and found that extracts obtained from *Moringa* delayed crop emergence and reduced root length and field survival of leguminous crops.

However, the study of Bandana et al (1987) proved that moringa leaf extracts have been found to increase *Rhizobium* root nodulation, nodule weight, and nitrogenise activity in mung bean (*Vigna mungo* (L.) Hepper), when applied to seeds or as a root dressing, but not in this study when applied to *P. falcataria* seedling as foliar spray. Iqbal et al (2013) suggested that *Moringa* leaf extracts (zeatin) foliar spray should be applied along with other fertilizers, making it clear that this hormone cannot serve in place of fertilizers but if applied along with other fertilizers produce better results.

Conclusions. Seed priming of *P. falcataria* seeds, dilutions of treatment *Moringa oleifera* leaf extracts (MLE 1:40) of *P. falcataria* seeds were effective in breaking the dormancy and induced germination as compared with hot water and ANAA.

There were no differences in the applications of MLE as foliar spray and the commercial foliar fertilizer (GrowMore) in *P. falcataria* seedlings except for root nodule count and fresh root weight. The control treatment is prominent in terms of root nodule count and fresh root weight. Hence, the application of MLE and the commercial foliar fertilizer (GrowMore) inhibited the growth performance of *P. falcataria* seedlings particularly the reduction in fresh root weight and root nodule count.

A comparative study on the growth performance of *P. falcataria* seedlings from germinated seeds treated with hot water and seed priming may further clarify and elucidate the use of either method. Economically, hot water treatment without dilution can be easily adopted by the tree farmers. However, the use of MLE dilution in seed priming holds great potential as an alternative to hot water and commercial synthetic growth hormone (ANAA). Hence, there is a need to observe further the efficiency of seed priming using MLE dilution from germination until the seedling stage of *P. falcataria*. In addition, there is a need to explore the variations in the length of time in soaking of *P. falcataria* seeds to break the dormancy. Furthermore, the imbibition damage due to rapid uptake of water which resulted to the failure in germination of some seeds is also worth investigating.

There is also need to further study other methods (e.g. frequency of foliar spray application, variations in the concentration of dilutions and seedlings are grown in partial shade and slowly exposed to sunlight until full hardening) to demonstrate the efficacy of *Moringa* as an organic fertilizer.

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