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Enhancing plantlet quality by adding neem oil as an organic additive to the *in vitro* culture of Cavendish banana

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ABSTRACT

In this study, we investigated for the first time the effect of neem oil as an organic additive on the quality of *in vitro* banana plantlets during the rooting and acclimatization stages. Treatments at different concentrations of neem oil on rooting media were added and evaluated. The results showed that the addition of neem oil to the rooting medium had an effect on the establishment of *in vitro* shoots and the development of *in vitro* plantlets during the hardening stage. Among the different concentrations, when 0.5 mL/L neem oil was added to the rooting medium, the plantlet height, root number, root length, and plantlet weight were better than those of the control plantlets. Higher concentrations of neem oil (1–2 mL/L) in the medium inhibited the growth of *in vitro* plantlets. Consequently, these treatments affected their development in the acclimatization stage. The results showed that an appropriate concentration of neem oil had a significant effect on the quality of *in vitro* banana plantlets. Thus, this study highlights the potential application of a new organic additive in banana culture as well as in other species to improve the quality of *in vitro* plantlets.

1. INTRODUCTION

Banana (*Musa spp.*) is one of the most important food crops in the world due to its nutritional and economic values [1]. Bananas are mostly grown in subtropical and tropical countries [2]. According to FAOSTAT, in 2024, total world banana export quantities are estimated at around 19.1 million tonnes from main exporters such as South America, Central America, the Caribbean, Asia, and Africa. The demand for global banana production is increasing constantly [2]. Among popular banana varieties grown for production, the Cavendish banana is the most common and traded variety [3]. In the banana industry, it is required that the plants supplied to the growing areas be disease-free, have a large number of plantlets at the same developmental stage during the growing season, and be of high quality. The traditional method for propagation, such as suckers, is not suitable for the requirements of banana cultivation. Nowadays, using plant tissue culture technology for banana micropropagation is one of the effective alternatives to obtain high-quality and high propagation rates of banana plantlets. Starting from a single explant, propagators can produce up to 10,000 plantlets in 8 months [4]. These high-tech technologies have been applied in many countries and are constantly being improved to optimize *in vitro* plantlet quality and reduce production costs [5]. The effects of different components of propagation medium, such as vitamins, plant growth regulators, sugar, micronutrients, or amino acids, on banana production have been studied and demonstrated [6]. These components determine the quality of the plantlets, including the morphology, physiology, growth, and development. From the perspective of the various stages of banana micropropagation technology, the rooting stage is the period for regenerated plants to induce roots, improve shoot elongation, and increase the biomass of *in vitro* plants [7]. Therefore, the rooting stage has a great influence on the plantlet quality and consequently contributes to the plantlet growth in the field. Several studies have identified the optimal culture medium composition for the *in vitro* rooting stage of banana, such as selecting the culture systems [8], adding organic additives (coconut water) [9], supplementing with copper sulfate [10], or calcium nitrate [11]. Among these factors, the incorporation of organic additives into the culture medium may be an effective solution due to their cost-effectiveness, environmental sustainability, efficiency in micropropagation rates, and capacity for maintaining genetic stability in regenerated plants [12]. Therefore, it is necessary to find a suitable substance to improve the quality of tissue-cultured banana plants in a stable approach that can be applied on a large scale. Recently, neem oil was used as a "complex mixture" to increase the shoot proliferation rate and shoot quality of *in vitro* olive micropropagation [13,14]. Interestingly, the addition of neem oil (0.1 mL/L) into the proliferation medium could obtain well-developed olive shoots under lower zeatin concentration. Besides that, adding neem oil to different rooting substrates exhibited no positive effect on the rooting parameters of olive plantlets [14]. Neem oil is a natural

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substance that contains different components, such as triglycerides, triterpenoids, Vitamin E, phenols, and has been used as a fertilizer, pesticide, and organic additive for *in vitro* propagation [14,15]. Therefore, the application and effect of neem oil in plant tissue culture of different plant species remains to be explored. The aim of this research was to test the efficiency of neem oil on the establishment of *in vitro* banana shoots and the acclimatization of induced plantlets.

2. MATERIALS AND METHODS

The Cavendish banana shoots used in this study were subcultured on the multiplication medium, including MS salt [16] supplemented with 2 g/L 6-benzylaminopurine, 30 g/L sucrose, and 6 g/L agar [9]. Single uniform-sized shoots were selected to transfer to the rooting medium.

To evaluate the effect of neem oil on the development of banana shoots in the rooting stage, five different concentrations of neem oil (0-control, 0.25, 0.5, 1, and 2 mL/L) were added to MS medium, including 30 g/L sucrose and 6 g/L agar. Neem oil used in this study is a commercial product (Docneem®, Vietnam). The media with all compositions (MS medium, sucrose, and neem oil) except agar were adjusted to a pH of 5.8 before autoclaving. The media were autoclaved at 121°C for 20 min. Subsequently, the shoots that were 2 cm long were cultured in glass vessels with tested media (5 shoots/vessel). In this experiment, 90 single shoots (replicates) were used for each treatment with three replications. The cultures were maintained for 4 weeks in a growth room at 25°C±2 under a photoperiod of 16 h. After 4 weeks, the rooted plants were washed out of the agar, and the attached water was removed with tissue paper. Then, the growth parameters of explants were assessed, including plant height (cm), root number, root length (cm), and plant fresh weight (g).

To evaluate the effect of neem oil on plantlet development, *in vitro* rooted banana plantlets treated with different concentrations of neem oil were taken out from culture bottles and washed with distilled water to remove agar. Subsequently, all plantlets were transplanted into plastic trays containing a sterilized mixture of coco coir, vermicule, and horticulture soil (1:1:1). The trays were covered with clear plastic lids to maintain the relative humidity (80–90%) and kept in a greenhouse. After 2 weeks, the lids were removed, and the plants' survival percentages were evaluated. Next, the trays were continuously kept under greenhouse conditions for 2 weeks. Then, the growth pattern of plantlets was evaluated, including plant height (cm), root number, root length (cm), and plant fresh weight (g).

The following parameters were collected after 4 weeks of *in vitro* culture or greenhouse culture.

- Plant height (cm): Average length of generated shoot
- Root number (n): Average number of induced roots from generated shoots or plantlets
- Root length (n): Average length of five longest induced roots per generated shoots or plantlets
- Fresh weight (g): Average fresh weight per plantlet.

Data were presented as the mean with standard error. Statistical analysis was performed using One-way analysis of variance by GraphPad Prism (version 9.3.1). The comparisons of mean values were carried out through Tukey's multiple comparisons test.

3. RESULTS

In this experiment, the shoots were excised and transferred to rooting media with different concentrations of neem oil. After 4 weeks of culture, all shoots grown on MS medium supplemented with neem oil showed root induction and were fully transformed to plantlets [Table 1]. However, the morphology of plantlets, such as plant height, root number, root length, and plant weight, showed significant differences when different concentrations of neem oil were added to the rooting medium [Table 1, Figures 1 and 2].

Supplementation of 0.25 mL/L or 0.5 mL/L neem oil to the rooting medium can increase the height of the plantlets. There is no difference in the mean values of plant height in the medium supplemented with 1 mL/L of neem oil compared to the control (no neem oil added). In contrast, supplementation with a higher concentration of neem oil at 2 mL/L led to a decrease in the height of plants compared to the

Table 1: Effects of various neem oil concentrations on *in vitro* shoot performance in the rooting stage (4 weeks of culture).

Treatments	Neem oil (mL/L)	Root formation (%)	*In vitro* rooting stage		
			Height of plantlet±SE (cm)	Number of roots per explant±SE	Length of root±SE (cm)
T1	0	100	6.19±0.06^{b}	7.36±0.18^{bc}	10.56±0.07^{b}
T2	0.25	100	6.69±0.06^{c}	7.09±0.15^{b}	11.42±0.05^{c}
T3	0.5	100	7.20±0.03^{d}	7.87±0.10^{c}	12.73±0.05^{d}
T4	1	100	6.13±0.05^{b}	7.56±0.12^{bc}	11.20±0.06^{c}
T5	2	100	5.55±0.06^{a}	5.11±0.11^{a}	6.75±0.06^{a}

In each column, means with different letters are significantly different for $P \leq 0.05$

Table 2: Effects of various neem oil concentrations on *in vitro* plantlet performance in the hardening stage (4 weeks in greenhouse).

Treatments	Neem oil (mL/L)	Survival rate (%)	Hardening stage		
			Height of plantlet±SE (cm)	Number of roots per explant±SE	Length of root±SE (cm)
T1	0	100	8.73±0.17^{b}	11.30±0.15^{b}	14.52±0.16^{b}
T2	0.25	100	8.88±0.15^{b}	10.98±0.22^{b}	16.85±0.09^{c}
T3	0.5	100	9.47±0.15^{c}	12.12±0.24^{c}	17.67±0.12^{d}
T4	1	100	8.33±0.11^{b}	11.52±0.22^{bc}	16.33±0.08^{c}
T5	2	100	7.15±0.09^{a}	9.23±0.17^{a}	10.47±0.19^{a}

In each column, means with different letters are significantly different for $P \leq 0.05$

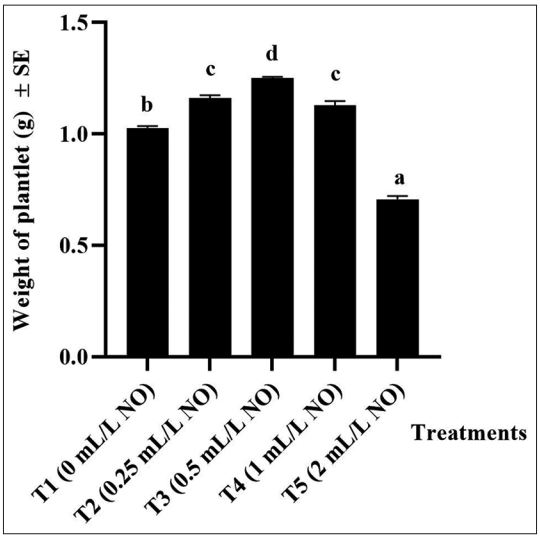

Figure 1: Weight of *in vitro* plantlets in different neem oil (NO) treatments.

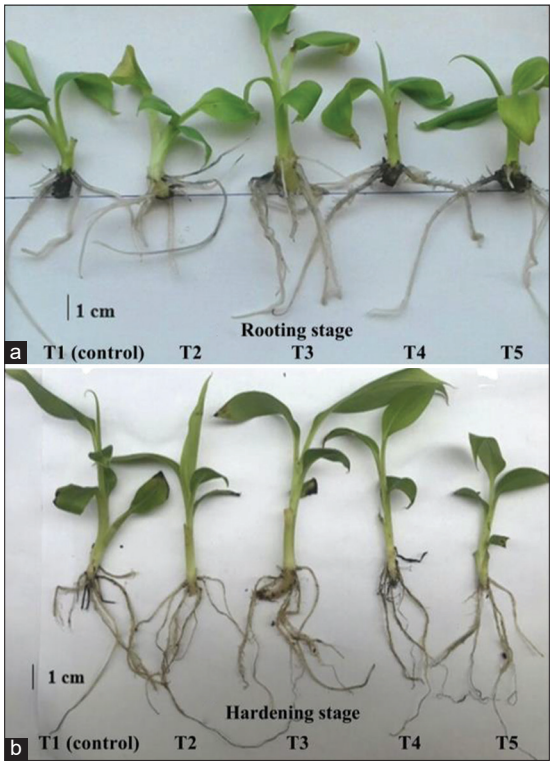

Figure 2: The morphology of *in vitro* banana plantlets at the rooting stage (a) and hardening stage (b) after treatments with different neem oil (NO) concentrations (T1: 0 mL/L NO, T2: 0.25 mL/L NO, T3: 0.5 mL/L NO, T4: 1 mL/L NO, T5: 2 mL/L NO).

plants in the control treatment. The addition of neem oil to the culture medium affected the number and length of roots in the plantlets. The root number and root length were highest in the medium supplemented with 0.5 mL/L neem oil. In the treatments with 0.25 mL/L or 1 mL/L neem oil, no effect of this substance on the root number was observed. Contrarily, the root length of plantlets was changed in all treatments. The number and length of roots decreased in the medium supplemented with 2 mL/L of neem oil. Similarly, neem oil in all tested concentrations in the medium also affected the biomass of *in vitro* plants after 4 weeks

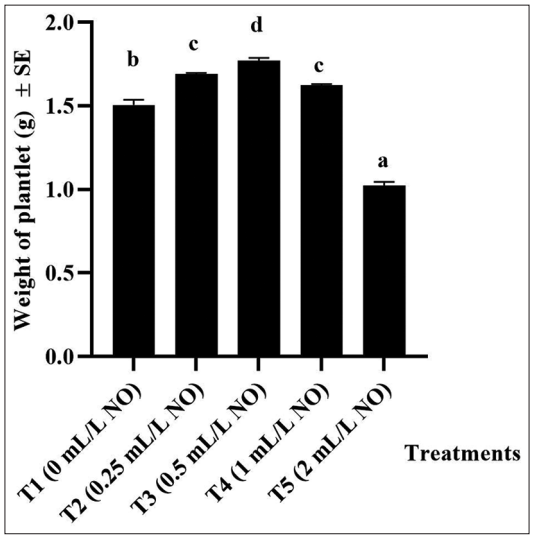

Figure 3: Weight of plantlets from different neem oil treatments after 4 weeks of acclimatization.

of culture. Rooting medium containing 0.25–1 mL/L of neem oil promoted the growth of *in vitro* plants. The highest weight of *in vitro* plantlets (1.25 ± 0.02 g/plant) was observed on the plantlets cultured on treatment with 0.5 mL/L neem oil ($P \leq 0.05$). Furthermore, it is notable that the growth of *in vitro* plantlets was inhibited in the media with 2 mL/L of neem oil. Overall, when 0.5 mL/L neem oil was added to rooting media, it resulted in significantly enhanced shoot development, root proliferation, and consequently, *in vitro* plant biomass. In this experiment, all plants with roots induced on media with different neem oil concentrations (Experiment 1) were planted in mixed horticulture soil and transferred to greenhouse conditions to observe the survival rate and growth pattern. The data showed that at the hardening stage, the survival rate was 100% for all treatments. In addition, plantlets in the treatments exhibited the same growth pattern as in Experiment 1 (plantlet height, root number, root length, and plantlet weight). Root length and plantlet weight of explants were increased under 0.25–1 mL/L neem oil treatment compared to the control group [Table 2, Figures 2 and 3]. As a result, the plantlets in these treatments were healthier than those in the control treatment. Plantlets rooted on medium with 0.5 mL/L neem oil (T3) showed rapid growth, reaching the highest average weight of 1.77 g per plantlet [Table 2]. Similar to the previous experiment, plantlets in the treatment with 2 mL/L of neem oil (T5) were observed to decline in quality. After 4 weeks of growth in the greenhouse, the plantlets (T5) were still smaller than those in the other treatments.

4. DISCUSSION

This study highlights the impact of neem oil on banana culture. The results clearly demonstrate that rooting medium supplemented with an appropriate amount of neem oil induced root formation and root development in banana. In addition, neem oil promoted the growth of *in vitro* banana plantlets during the hardening stage. Therefore, these data suggest that neem oil has a significant effect on the quality of *in vitro* banana. This is the first time the effect of a new natural substance (neem oil) on *in vitro* banana culture has been reported. Our results are in agreement with previous studies on olive (*Olea europaea* L.), which found that the addition of neem oil to the proliferation medium increased the fresh/dry weight as well as root development of regenerated shoots

[13,14]. These data suggested that neem oil could be used in multiplication and rooting stages to optimize the efficiency of micropropagation. To date, neem oil's chemical composition has been reported in different observations by many authors. Neem seeds from the Cameroon sample are dominated by fatty acids (39.02%), triterpenoids (36.58%, mostly azadirachtin), and sterols (24.40%) [17]. Diedhiou *et al*. [18] reported that the major compounds from Senegal neem were 5,6-dihydro-2,4,6-triethyl-(4H) 1-3,5-dithiazine (39.1%), cis 1,2,4-Trithiolane, 3, 5-diethyl (7.9%), 1-H-indole 2,3-dione (7.9%), trans 1,2,4-Trithiolane, 3,5-diethyl (6.2%), and ethyl thioisobutyrate (4%). In addition, this study also identified that most of the compounds were non-terpene sulfur and nitrogen compounds [18]. Another study found that neem oil is composed of more than 100 biologically active compounds [15]. Atta *et al*. [19] emphasized that crude neem oil is abundant with oil, natural antioxidants (phenol, flavonoid compounds, and unsaponifiable matter), and fat-soluble vitamins (Vitamins A, D, and K). These studies have indicated that neem oil is a complex of components and is different based on specific neem oil sources and geographical locations. With typical chemical characteristics, the natural extract "neem oil" has revealed a wide range of biological and pharmacological activities [20]. Thus, the variety of chemical compositions in neem oil could explain the stimulation of the shoots and roots of *in vitro* explants. In this study, neem oil was added to the rooting medium in small amounts and was found to influence the morphogenesis of explants. However, increasing the concentration of neem oil negatively affected the growth of *in vitro* shoots and plantlets. These results suggest that neem oil may affect the explants in a manner related to plant phytohormones. We hypothesize that certain compounds in neem oil may affect the activity and synthesis of endogenous plant phytohormones, leading to alterations in the morphogenesis of the explants. Moreover, natural antioxidants in neem oil could reduce oxidative damage and then improve the health of plantlets. In addition, the presence of different compounds such as vitamins, amino acids, and others could enhance nutrient availability in the medium for *in vitro* shoots. As a result, applying neem oil at the rooting stage with appropriate concentrations improved the quality of the *in vitro* bananas. The results obtained here are in line with those found by other studies that neem oil exhibited as a valuable natural complex in plant tissue culture to enhance the quality of *in vitro* plantlets [13,14]. As mentioned above, the quality of *in vitr*o plants is one of the most important parameters in commercial production. These data could be a novelty for future studies on neem oil to be used for the improvement of *in vitro* culture of banana and other species. However, further research is required to investigate the mechanisms by which neem oil promotes shoot and root development, facilitating its application in other crops.

5. CONCLUSION

The present study showed that the addition of 0.5 mL/L of neem oil to the rooting medium promoted the growth and vigor of banana plantlets during the rooting and acclimatization stages. Compared with the control, the treatment showed the enhancement of the root length, root number, height, and weight of the banana plantlets. The effect of neem oil in banana tissue culture emphasized the potential of this natural additive to micropropagation systems of different species to improve the quality of *in vitro* plantlets.

6. AUTHOR'S CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be authors as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All data generated and analyzed are included within this research article.

11. PUBLISHER'S NOTE

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12. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

REFERENCES

1. Pereira A, Maraschin M. Banana (*Musa* spp) from peel to pulp: Ethnopharmacology, source of bioactive compounds and its relevance for human health. J Ethnopharmacol. 2015;160:149-63. https://doi.org/10.1016/j.jep.2014.11.008
2. FAO. Banana Market Review Preliminary Results; 2024. Rome: FAO; 2025.
3. Kema GH, Drenth A, Dita M, Jansen K, Vellema S, Stoorvogel JJ. Editorial: *Fusarium* wilt of banana, a recurring threat to global banana production. Front Plant Sci. 2021;11:628888. https://doi.org/10.3389/fpls.2020.628888
4. Singh DH, Uma S, Selvarajan R, Karihaloo J. Micropropagation for Production of Quality Banana Planting Material in Asia-Pacific. New Delhi, India: Asia-Pacific Consortium on Agricultural Biotechnology; 2011. p. 92.
5. Kumar D, Chakradhar P, Ranganna G, Vimal VK, Raj R, Anusha C, *et al*. Tissue culture in banana cultivation: A review of its impact on disease management, yield improvement, and sustainable production. J Adv Biol Biotechnol. 2024;27:628-44. https://doi.org/10.9734/jabb/2024/v27i91336
6. Madhulatha P, Kirubakaran SI, Sakthivel N. Effects of carbon sources and auxins on *in vitro* propagation of banana. Biologia Plantarum. 2006;50(4):782-4. https://doi.org/10.1007/s10535-006-0131-0
7. Waman AA, Bohra P, Sarthyanarayana BN, Umesha K, Mukunda GK, Ashok TH, *et al*. Optimization of factors affecting

in vitro establishment, *ex vitro* rooting and hardening for commercial scale multiplication of silk banana (*Musa* AAB). Erwerbs-Obstbau. 2015;57:153-64.
8. Erol MH, Dönmez D, Biçen B, Şimşek Ö, Kaçar YA. Modern approaches to *in vitro* clonal banana production: Next-generation tissue culture systems. Horticulturae. 2023;9(10):1154. https://doi.org/10.3390/horticulturae9101154
9. Son DT, Hoa DT, Son VC, Hoa NT, Hien VT, Tam DT. Evaluating the effect of organic additives on the *in vitro* rapid multiplication of *Musa acuminata*. Vietnam J Agric Sci. 2023;21(5):597-604.
10. Elyazid DM, Salama AM, Zanaty AF, Abdalla N. *In vitro* propagation and acclimatization of banana plants: Antioxidant enzymes, chemical assessments and genetic stability of regenerates as a response to copper sulphate. Plants. 2021;10(9):1853. https://doi.org/10.3390/plants10091853
11. El-Mahrouk ME, El-Shereif AR, Dewir YH, Hafez YM, Abdelaal KA, El-Hendawy S, *et al*. Micropropagation of banana: Reversion, rooting, and acclimatization of hyperhydric shoots. HortScience. 2019;54(8):1384-90. https://doi.org/10.21273/HORTSCI14036-19
12. Hamdeni I, Louhaichi M, Slim S, Boulila A, Bettaieb T. Incorporation of organic growth additives to enhance *in vitro* tissue culture for producing genetically stable plants. Plants (Basel). 2022;11:3087. https://doi.org/10.3390/plants11223087
13. Micheli M, Fernandes Da Silva D, Farinelli D, Agate G, Pio R, Famiani F. Neem oil used as a "complex mixture" to improve *in vitro* shoot proliferation in olive. HortScience. 2018;53(4):531-4. https://doi.org/10.21273/hortsci12731-17
14. Regni L, Facchin SL, Da Silva DF, De Cesaris M, Famiani F, Proietti P, *et al*. Neem oil to reduce zeatin use and optimize the rooting phase in *Olea europaea* L. Micropropagation. Plants. 2023;12:576.
15. Campos EV, De Oliveira JL, Pascoli M, De Lima R, Fraceto LF. Neem oil and crop protection: From now to the future. Front Plant Sci. 2016;7:1494. https://doi.org/10.3389/fpls.2016.01494
16. Murashige T, Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum. 2006;15:473-97. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x
17. Sonhafouo VM, Kana JR, Dongmo KN. Effects of graded levels of *Azadirachta indica* seed oil on growth performance and biochemical profiles of broiler chickens. Vet Med Sci. 2019;5:442-50. https://doi.org/10.1002/vms3.162
18. Diedhiou D, Faye M, Candy L, Vandenbossche V, Raynaud C, Sock O, *et al*. Chemical composition of the essential oil of Neem (*Azadirachta indica* A. Juss) seeds harvested in Senegal. Indian J Sci Technol. 2023;16(2):118-22. https://doi.org/10.17485/IJST/v16i2.1650
19. Atta NM, Ismaiel GH, Hashish AE, Mohamed ES. Physical and chemical characteristics of neem oils extracted from seed, whole fruit and flesh. Egyptian J Agric Res. 2015;93:887-99. https://doi.org/10.21608/ejar.2015.155469
20. Biswas K, Chattopadhyay I, Banerjee R, Bandyopadhyay U. Biological activities and medicinal properties of Neem (*Azadirachta indica*). Curr Sci. 2001;82:1336-45.

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