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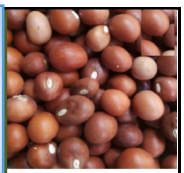

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Bambara groundnut

Determining the Median Lethal Concentration (LC_{50}) and Seedling Growth Effects of MARDI Nano-fertilizer on Rock melon (*Cucumis melo*)

N.I. Fadzil[1,2], M.N. Mohd Rosmi[1], N.S. Wahid[1], N.S. Mohd Nor[1], M.S. Abdul Karim[1], M.F. Mohd Anuar[2] and N.A. Masdor[1]*

[1]Biotechnology and Nanotechnology Research Centre, MARDI HQ, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.
[2]Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia,
43400 UPM Serdang, Selangor, Malaysia.

*Corresponding author:
Dr. Noor Azlina Masdor,
Biotechnology and Nanotechnology Research Centre,
MARDI HQ,
Persiaran MARDI-UPM,
43400 Serdang,
Selangor,
Malaysia.
Email: azlina@mardi.gov.my

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ABSTRACT

New fertilizer formulations and nitrogen delivery systems are needed to address deteriorating nutrient-use-efficiency and environmental hazards due to farmers' dependency on chemical fertilizers. Nano-fertilizers (NF) made from tailored smart nano materials are a viable alternative to inefficient and harmful traditional fertilizers. Nanotechnology and NFs may help solve chemical fertilizers formulation difficulties. To boost rock melon yields, MARDI has created a nano-fertilizer made from nano-emulsions. The relations between seed germination inhibition (%) of rock melon seed and different concentrations of MARDI nano-fertilizer showed that when nano-fertilizer concentrations increased from 10 to 45% with inhibition 3.3% to 100%, seed germination decreased. The LC_{50} value was calculated at 32.2% equal to 322,000 ppm, with upper and lower limits at 25.346% and 40.953%, 96 hrs after treatments, indicating the formulation is not toxic with a high LC_{50} value. To further study the effects of the created nano-fertilizer on the growth of rock melon plants across the adult, reproductive, and developmental stages, it is suggested that future field trials work use a concentration of 0.5% of the developed MARDI nano-fertilizer.

INTRODUCTION

For many years, fertilizers have been utilized in agriculture to increase the fruit yield and quality of various crops, increasing the nutritive content of products [1]. Increased food grain production has been called for as the developing world's population continues to soar. Sustainable and healthy crop production can be improved with the help of fertilizers, provided they are applied correctly. However, the excessive and haphazard use of chemical fertilizer (CF) results in not only increased costs for farmers but also serious environmental impureness. Nitrate-nitrogen and phosphorus leaching loss from agriculture systems into the environment creates various other risks, including eutrophication, in addition to reducing nutrient-use-efficiency. Groundwater quality declines as a result of leached nitrogenous compounds, leading to serious health problems for humans. Given farmers' reliance on CFs, finding solutions to the issues of declining nutrient-use-efficiency and environmental risks necessitates the development of new fertilizer formulations and nutrient delivery technologies. As a result, it is critical to rapidly implement alternative techniques to lessen reliance on ineffective CF formulations. An effective and promising alternative technique for lowering reliance on inefficient and dangerous conventional fertilizers is the use of engineered smart nano materials and the development of nano-fertilizers (NF) [2–6].

Using nanotechnology and NFs to combat issues brought on by CF formulations is a promising new avenue. Crop scientists are interested in NFs because they offer various benefits over CFs, including higher yields, more soil fertility, and lower pollution. NFs transport nutrients with nano-scale dimensions between 1 and 100 nm, with the average being between 30 and 40 nm. Because of their large surface area, NFs may absorb and store a lot of nutrient ions, releasing them slowly to match the plant's needs. High solubility, stability, efficiency, and time-

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oriented release are only few of the properties incorporated into NFs in addition to a nutrient ion concentration optimized for their intended use [7–13]. MARDI has developed a nano-fertilizer based on nano-emulsions to increase the productivity of rock melon [14].

Nano-emulsion fertilizer is a nano-fertilizer made by combining a surfactant with a liquid and mixing it with high energy to create a stable, homogeneous combination. The surfactant aids in the suspension of liquid droplets in the water-soluble matrix, allowing them to remain equally dispersed throughout the solution [15,16]. Much attention has been raised to nano-emulsion based agrochemicals, assessed by their environmental risk assessment (ERA) before being widely distributed on the market. It has been suggested that formulation synthesis by nanotechnology methods may impact the systems' toxicological or safety profile by modifying the systems' additives and properties.

Surfactants in general including nano-emulsion may pose a significant risk to the environment and human health [17]. Thus, under laboratory settings, the median lethal concentration at 50% (LC_{50}) value for the MARDI nano-fertilizer with the germination parameter of rock melon seeds was determined to build the experiment for assessing the potential phytotoxicity of the test chemical at rock melon seedling stage growth.

MATERIALS AND METHODS

The rock melon F1 hybrid, the Sakata Glamour variety, was utilized for this study. The experiment was conducted in greenhouses in the Department of Biotechnology and Nanotechnology, MARDI. Rock melon seed was sowed into a peat moss on the seedling tray and kept under a controlled environment with 75% relative humidity and temperature of 26±2°C for 2 weeks for seedling growth analysis.

Production of MARDI nano-fertilizer

The nano-fertilizer formulation which consists of neem oil (provided by Agrow Synergy Sdn. Bhd, Malaysia), surfactant polysorbate, and water was developed based on the selected points from the ternary phase diagram constructed and incorporated into the composition of NPK-TE using a combination of low and high-energy emulsification procedures. Neem oil was first mixed with a surfactant to form an oil phase. After that, the NPK-TE and water were added and homogenized at 1500 rpm for 25 minutes (min) (Ika T25 digital Ultra Turrax, USA). Then, the mixture was mixed and sonicated (Q500 sonicator, Qsonica, USA) for 30 min at room temperature [18,19].

Acute median lethal toxicity (LC_{50}) of MARDI nano-fertilizer to the rockmelon seeds

A preliminary range finding test was carried out for 96 h (hour) by diluted concentrations of MARDI nano-fertilizer from 0.02% to 45% (v/v), and the tests were conducted with triplicate samples. This method determined the concentration at which the toxicant was lethal to the test organisms. In 12-cm petri dish plates, ten seeds were placed on double-layer filter paper (3 mm, Whatman, Maidstone, UK) and wetted with 10 mL of distilled water (dH_2O) as control or 10 mL of test solutions. The seeds in a covered petri dishes were incubated at 25±5°C. The seeds were considered germinated when the seed coat breakage was visible and where 50% of seeds germinated.

Germination inhibition (%) was determined by reference to the control samples after 96 h and analyzed using Probit analysis. The LC_{50} values have been computed and calculated using the [3] Schneider-Orelli's formula [20], where the proportions are corrected for control mortality if it is more than 10% (**Equation 1**).

Mortality = % Responded −% Responded in control 100−% Responded in control × 100 **(Eqn. 1)**

Effect of MARDI nano-fertilizer on seedling growth in the pot experiment

The rock melon seeds were treated for 4 h with varying concentrations of MARDI nano-fertilizer at 0.2, 0.5, 1, 5, 10, 15, 25 and 35% (v/v) in an aqueous solution. The treated seeds were dried and potted in plastic containers (180×180 mm) filled with peat moss and maintained in a greenhouse under 16/8 h dark and light at 27±2 °C with periodic watering.

At the first trifoliate stage, foliar spray of the nano-fertilizer formulation (until run-off, i.e., 100 mL on each replication) was applied to seedlings. After 2 weeks of sowing, multiple growth variables were measured, including germination percentage, shoot-root length, root weight, hypocotyl length, fresh weight, dry weight, and plant height. The seedling vigour index (SVI) was calculated according to the formula of Abdul-Baki & Anderson [21] (**Equation 2**):

Seedling vigor index = (Germination %) × (Seedling length) **(Eqn. 2)**

Statistical analysis

The percentage of mortality for rock melon seeds treated with MARDI nano-fertilizer was subjected to Probit analysis [22] for calculating LC_{50} statistics at 95% confidence limits of lower and upper values. Using GraphPad Prism® version 6.04 for Windows software, the LC_{50} was calculated. The experiment was conducted following the Randomised Complete Block Design (RCBD) in quadruplicates, and a minimum of three (for laboratory tests) and nine (for field investigations) randomly selected plant samples comprised each replication. Data were subjected to Analysis of Variance (ANOVA) using IBM SPSS Statistics Version 25.0 (Armonk, NY: IBM Corp). Also, the means were compared using the Post Hoc-Tukey test at the 0.05 significance level.

RESULT AND DISCUSSION

LC_{50} determination using Probit analysis of MARDI nano-fertilizer

The relations between seed germination inhibition (%) of rock melon seed and different concentrations of MARDI nano-fertilizer are presented in **Fig. 1**. The level of seed germination was 100% from control to 5% of treatment, demonstrating the high quality of the tested seeds. It was discovered that when nano-fertilizer concentrations increased from 10 to 45% with inhibition 3.3% to 100%, seed germination decreased. The high concentration effect on germination is because they prevent water diffusion into seeds, resulting in a low growth rate and, consequently, a reduced germination rate and survival. The LC_{50} value was calculated at 32.2% equal to 322,000 ppm, with upper and lower limits at 25.346% and 40.953%, 96 h after treatments, indicating the formulation is not toxic with a high LC_{50} value.

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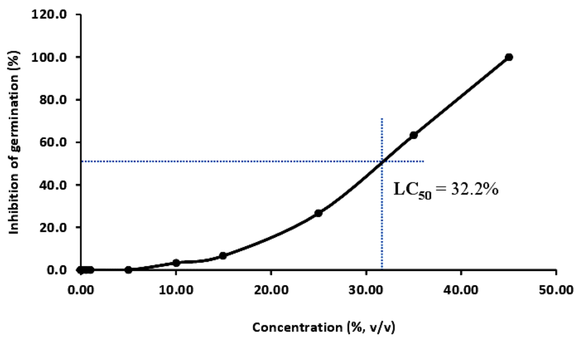


Fig. 1. Graphs showing the relationship between MARDI nano-fertilizer concentrations and the inhibition of germination percentage of rock melon seed and displaying LC_{50} concentrations for a nano-fertilizer.

Like other nanomaterials, it has been established that metal-containing nano formulations have dual impacts on plants, including seed germination. For seven days, solutions containing 62 µg/mL Au NPs stimulated cucumber and lettuce seeds germination [21]. Comparable results of Pennisetum glaucum (pearl millet) seeds soaked for 2 hrs in Au NP (20 and 50 µg/mL) were found [22]. Similarly, Almutairi & Alharbi [23] study results of watermelon and zucchini seeds were treated with low concentrations (0.5–2.0 g/L) of Ag NP for 2 h showed the seeds germinated at the same doses, significantly increased the germination percentage and stimulated root elongation. It is crucial to ascertain how developed MARDI nano-fertilizer emulsion can affect seed germination because it is the first and most vulnerable stage of plant ontogenesis, particularly in the case of crops.

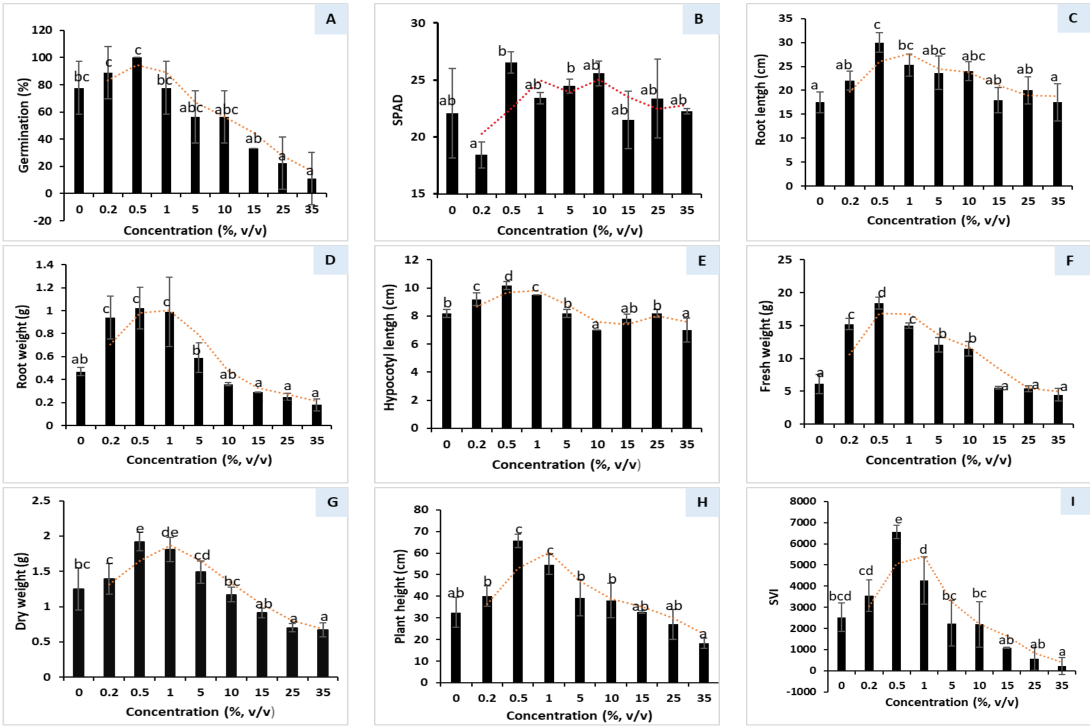
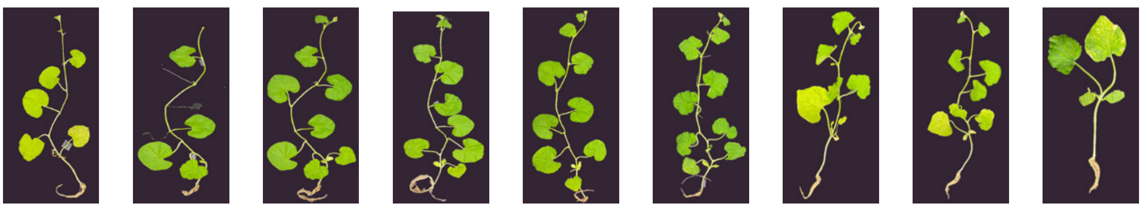


Fig. 2. Various seedling growth parameters were recorded after 2 weeks of sowing. Each value is the mean of triplicates, and each replicate consisted of 9 plants. Tukey's HSD Post Hoc Test indicates that mean±SEM followed by the same letter does not differ significantly at $p \leq 0.05$.



Control (Water) 0.2% 0.5% 1.0% 5% 10% 15% 25% 35%

Fig. 3. Impact of MARDI nano-fertilizer on the development of rock melon seedlings. The plant development with MARDI nano-fertilizer concentrations ranging from 0.2 to 35% (v/v) displayed visible differences.

From the result, the stimulative effects of the nano-fertilizer on seed germination at low concentrations are hypothesized to be accomplished through breaking seed dormancy, enhancing germination rate, and seedling vigor on an individual level. The deleterious effects of high concentrations of nanomaterials observed by Szőllősi et al. [24] are likely related to internal chromosomal aberrations and mitotic abnormalities, resulting in reduced cell division, hormonal imbalance, ROS/RNS overproduction, and an increase in lipid peroxidation level and lead to germination inhibition as observed in this study.

The effects of MARDI nano-fertilizer on seedling growth

Besides, it has been thoroughly documented by reviews and studies how various nanomaterials may influence the biomass, photosynthetic activity, and yields of adult plants, either positively or negatively [24–26]. Still, there is a notable paucity of knowledge regarding the earliest stages of development, such as early seedling growth. To determine the influence of MARDI nano-fertilizer on the growth characteristics of rock melon, germination percentage, leaf chlorophyll concentrations by SPAD unit, root length and weight, hypocotyl length, fresh-dry weight, plant height, and seedling vigor index (SVI) were evaluated in **Fig.** 2(A-I).

Statistical results revealed that the developed MARDI nano-fertilizer significantly increased the development of rock melon plants in pot experiments compared to the control, as observed exhibited a visual difference in plant growth, **Fig.** 3. In addition, higher values of various seedling growth parameters were observed in 0.2-0.5% treated plants compared to the control.

Nano-fertilizer emulsion was studied as a growth promoter since it can significantly affect plant seedlings as a promoting growth or seed treatment [27]. It was found that research conducted by [19] showed that the zinc-functionalized thymol essential oil, and saponin nano-emulsion could inhibit bacterial growth while enhancing the development of soybean plants in pot experiments at 0.02-0.05%. In other studies, eugenol oil nano-emulsion boosted germination on cottonseed plants and produced excellent resistance to fusarium wilt disease [28].

As a seed stimulant, methylcellulose nano-emulsion has enhanced the growth of maize seedlings by increasing root and shoot lengths by 18 and 33%, respectively [29]. Seed priming with turmeric nano-emulsion has also improved watermelon germination and growth [30]. Then, combining pectin with neem oil nano-emulsion enhances soybean seed development [31]. Reviewed by [32], nano-priming of seedlings with nanoparticles synthesized from metals has demonstrated considerable potential for agricultural uses.

For example, [33] priming chili seeds with metal oxide nanoparticles (Zn, titanium, and Ag) improved germination and seedling growth (shoot and root length), and iron oxide nanoparticle priming of sorghum seeds enhanced seed germination, water content, and photosynthesis [34]. Nanoparticles can operate directly against pathogens and affect the metabolism of seeds and plants, boosting the innate immune system, altering hormone synthesis, and increasing the plant's resistance to disease or abiotic stress [35].

CONCLUSION

The LC_{50} value of MARDI nano-fertilizer was 32.2% at 96 h. This indicated that the optimized nano-fertilizer emulsion had low phytotoxicity on germination inhibition of rock melon seeds. Further, notably higher values of seedling early growth parameters were observed at 0.5% of treatment in the pot experiment. Therefore, 0.5% of MARDI nano-fertilizer was recommended to field experiments to explore the effects of the developed nano-fertilizer on rock melon plant growth throughout the adult, reproductive, and developmental stages.

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