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Original Article

Synthesis and Optimisation of *Psidium guajava* Leaf-Based Titanium nanoparticles and their larvicidal effect against *Anopheles gambiae* s.l.

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ABSTRACT

With increasing resistance of malaria vectors to synthetic insecticides, sustainable and eco-friendly alternatives are needed. This study reports the green synthesis of titanium dioxide nanoparticles (TiO_2NPs) using aqueous leaf extract of *Psidium guajava* (guava) and evaluation of their larvicidal activity against *Anopheles gambiae* s.l. Titanium (IV) isopropoxide (TTIP)was used as precursor. The nanoparticle synthesis was optimised for three variables- pH, salt concentration and reaction time. The suitable combination for the variables in the rapid synthesis of TiO_2NPs was determined using the Design Expert software. Larvicidal bioassay was carried out following established protocols. Formation of TiO_2NPs was confirmed by UV-Vis spectrophotometry, revealing characteristic absorbance peaks ranging from 214 nm to 275 nm under varying conditions. Dynamic Light Scattering (DLS) revealed particle sizes ranging from 75–100 nm. Larvicidal bioassays revealed concentration- and time-dependent toxicity, with LC_{50} and LC_{90} of 19.57 and 34.02 mg/L, and 13.25 and 22.67 mg/L, respectively, for synthesized titanium nanoparticles, and crude aqueous extract of leaf of *P. guajava*, respectively. These results highlight the potential of *P. guajava*-mediated TiO_2NPs as a natural, environmentally benign alternative for mosquito vector control.

Keywords: Malaria, larvicide, nanoparticles, titanium, *Psidium guajava*

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INTRODUCTION

Mosquito-borne diseases, particularly malaria, continue to pose serious public health challenges in sub-Saharan Africa [1]. The primary vector, *Anopheles gambiae* s.l., has developed resistance to many conventional insecticides, diminishing the efficacy of control programs [2]. These synthetic chemicals persist in the environment and thus, affect non-target organisms and degrade the ecosystems [3]. Nanotechnology offers a promising alternative to these uses of synthetic chemicals, particularly when the nanoparticles are synthesised through green routes using plant extracts. Titanium dioxide nanoparticles (TiO_2NPs) are of particular interest due to their photocatalytic properties, biocompatibility, and environmental safety [4]. *Psidium guajava*, a medicinal plant rich in flavonoids, phenolics, and tannins, provides a natural reducing and stabilising agent for nanoparticle synthesis [5]. This study explores the synthesis of TiO_2NPs using *P. guajava* leaf extract and assesses their larvicidal efficacy against *Anopheles gambiae* s. l. in comparison with their crude aqueous leaf extracts.

MATERIALS AND METHODS

Collection and Preparation of Plant Extract

Fresh *Psidium guajava* leaves were collected from Shiroro-Zumba, Niger State, Nigeria. After washing and air-drying, the leaves were pulverised. Forty grams (40 g) of the powdered leaves were boiled in 400 mL of distilled water, filtered, and concentrated to obtain the aqueous extract [6].

Synthesis and Optimization of Titanium dioxide Nanoparticles

A 10 mM solution of Titanium (IV) isopropoxide (TTIP) was prepared as stock from which varying concentrations were prepared by serial dissolution. The guava leaf extract and TTIP solution were mixed in a 1:4 ratio and stirred at 350 rpm for 30 minutes at 50°C [7]. The synthesis was optimized for reaction time, pH and concentration of salt. Design Expert was used in determining the values for each variable. The initial yellow colour of the extract changed to dark brown, indicating nanoparticle formation. This preparation was repeated until the required quantity of nanoparticles was obtained.

Characterisation of Titanium Dioxide Nanoparticles

The absorption spectrum and formation of the nanoparticles were determined using UV-Vis Spectrophotometry. The formation of nanoparticles was confirmed using a UV-VIS spectrophotometer (Shimadzu UV-VISIBLE, 1800 series) at room temperature, operated at a resolution of 1 nm with fast scanning speed under x5 [6]. Typically, a distinct peak in the wavelength range of 250-350 nm with absorbance ranging from 0.5- 1.5 indicates the formation of TiO_2 NPs. Dispersion Light Scattering (DLS) analysis was used to assess the surface charge of nanoparticles, which is crucial for understanding their stability and behaviours in suspension.

Larvicidal Bioassay

Larvicidal tests were conducted using late third and fourth instar *Anopheles gambiae* s. l. larvae. Test solutions (0.1, 0.2, 0.3, and 0.4 mg/L) of the TiO_2 NPs were prepared in four replicates, while 2.0, 3.0, 4.0 and 5.0 mg/L of the crude aqueous extract solution were also prepared in eight replicates for the bioassay. Mortality was recorded at 6-, 12-, 18-, 24-, and 48-hours post-exposure [8]. Controls included distilled water and silver nitrate (0.01%). Lethal concentrations (LC_{50} and LC_{90}) were calculated using probit analysis.

RESULTS

Synthesis and Optimisation of Titanium Nanoparticles

The experimental design of the optimisation for the synthesis of TiO_2 NPs is presented in Table 1. The experiment uses three factors – pH, reaction time and the salt concentration. pH ranges from acidic to alkaline (4, 7, 10), the concentration of TTIP ranges used were 1, 5.5 and 10 mM, while the reaction times considered were 5, 17.5 and 30 minutes as determined by Design Expert. Each ‘Run’ represents a specific combination of these conditions, and the wavelength corresponds to the UV-Vis absorbance peak, which can indicate the size, band gap or morphology of the nanoparticles. Smaller nanoparticles usually absorb at lower wavelengths, implying higher surface area and greater biological activity, including larvicidal effect. Run 3 with pH of 10, 1 mM of TTIP and the reaction time of 17.5 minutes produced nanoparticles with the smallest wavelength of 214 nm.

For this study, 'Run' 6 (pH 7, 10 mM and 5 minutes) was considered for the mass production of TiO_2 NPs used in the larvicidal bioassay. The wavelength and the absorbance are in the expected range, 270-275 nm and 0.5- 1.0, respectively, typical of TiO_2 NPs. The Dispersion light scattering (DLS) and Zeta potential analysis of TiO_2 NPs nanoparticles produced under the conditions - pH 7, 10 mM and 5 minutes reaction time revealed that the main population of particles is centred around 90 – 100 nm which is typical of TiO_2 NPs and it falls within the effective nano-range (Figure 3). The presence of minor peaks at 10 nm and > 500 nm suggests a small portion of very fine nanoparticles and some degree of agglomeration or larger aggregates, respectively. The dominant size is well suited for biological applications with regard to penetration through larval cuticle and a high surface area to volume ratio, which enhances reactivity and toxicity. TiO_2 NPs in this range can generate reactive oxygen species (ROS) under UV light, contributing to mosquito larval mortality.

Table 1: Optimisation of the synthesis of TiO_2 NPs

	Factor 1	Factor 2	Factor 3	Response 1
Run	A: pH	B: Salt concentration	C: Time	Wavelength
		mM	Min	nm
1	4	5.5	30	271.5
2	7	5.5	17.5	274.5
3	10	1	17.5	214
4	4	10	17.5	270
5	7	10	30	278
6	7	10	5	275
7	7	1	30	225
8	4	5.5	5	270
9	10	5.5	30	274
10	10	5.5	5	276
11	10	10	17.5	276
12	4	1	17.5	214
13	7	1	5	225
14	7	1	30	223
15	10	10	17.5	272
16	7	5.5	17.5	274
17	7	10	5	272

Characterisation of Synthesised Titanium Nanoparticles

UV-Vis of the TiO_2NPs synthesised under the various conditions revealed further the runs in which the nanoparticles were formed. Despite that, pH 10 and 1 mM of TTIP at 17.5 reaction time produced the smallest wavelength of 214 nm, UV-Vis showed the absorbance to be very high (4.00), which is not typical of titanium nanoparticles (Figure 1). Despite the low wavelength, it is evident that TiO_2 NPs have not been produced. It is possible that with longer reaction time, the nanoparticles may be properly formed.

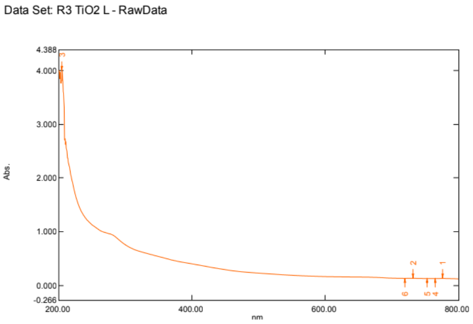

Figure 1: UV-Vis for Run 3 (pH 10 and 1 mM of TTIP at 17.5 minutes reaction time)

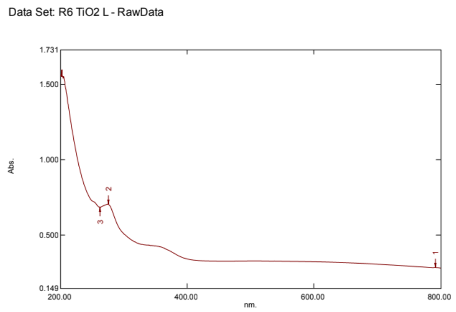

Figure 2: UV-Vis for Run 6 (pH 7, 10 mM and 5 minutes)

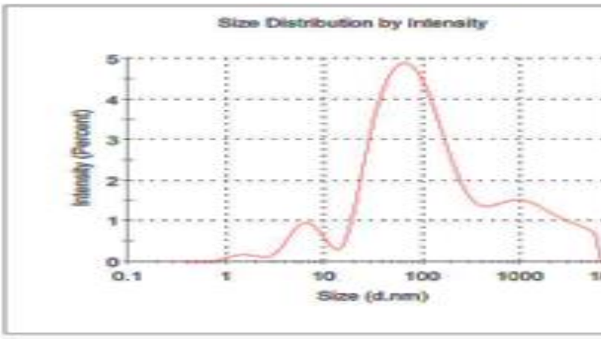

Figure 3: Size distribution by intensity of TiO2 NPs synthesised under pH 7, 10 mM and 5 minutes reaction time

Larvicidal Activity of crude aqueous extract of *Psidium guajava* leaves and **Synthesized titanium dioxide (TIT) nanoparticles**

The percentage mortality of *Anopheles gambiae* s.l. Larvae exposed to the crude aqueous extract of *Psidium guajava* leaves over 48 hours is presented in Figure 4. The x-axis represents the exposure time (6, 12, 18, 24, and 48 hours), while the y-axis denotes larval mortality (%). The tested concentrations range from 2 mg/L to 5 mg/L. The results show a gradual increase in mortality over time and with higher concentrations, indicating a dose-dependent and time-dependent effect of the extract. At 6 hours, no significant mortality was observed. By 12 hours, mortality reached approximately 10-15% for 3 mg/L and 4 mg/L, while 5 mg/L showed a slightly higher effect (~16%). At 18 hours, mortality remained relatively low, with only the highest concentration (5 mg/L) exceeding 20% mortality, whereas lower concentrations induced below 15% mortality. A significant increase occurred at 24 hours, where 5 mg/L caused over 30% mortality, followed by 4 mg/L (~20%), and 3 mg/L (~15%), while lower doses exhibited minimal effect. At 48 hours, 5 mg/L reached its highest mortality (~35%), followed by 4 mg/L (~20%) and 3 mg/L (~18%), while 2 mg/L remained below 10% mortality, indicating a weaker effect at lower doses. These findings suggest that crude *Psidium guajava* extract has moderate larvicidal activity but is less effective compared to synthesised nanoparticles. The gradual increase in mortality suggests a delayed toxic effect, possibly due to the slower action of plant-derived compounds compared to fast-acting metal nanoparticles. Although higher concentrations (≥5 mg/L) show some promise, complete larval mortality was not achieved even after 48 hours, indicating that the crude extract alone may not be potent enough for practical larvicidal applications unless used at significantly higher doses or in combination with other agents.

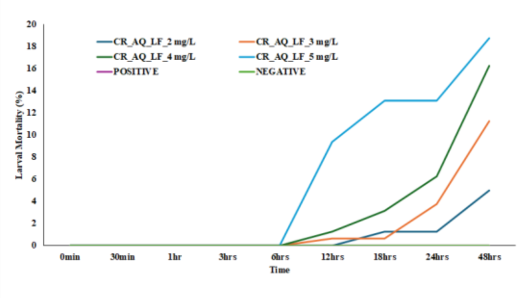

Figure 4: Percentage Mortality of the Larvicidal efficacy of crude aqueous (CR_AQ_AL) extract of *P. guajava* leaf against *An. gambiae* s.l. after 48 hours exposure period.

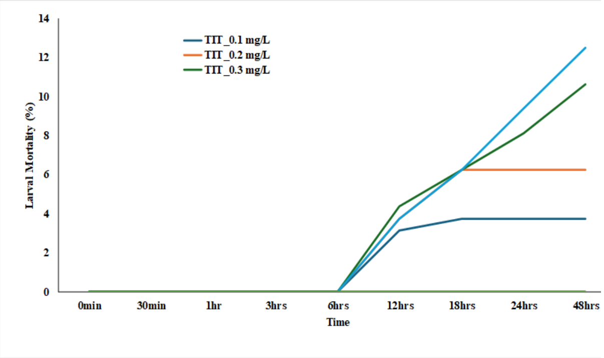

Figure 5: Percentage Mortality of the Larvicidal efficacy of Synthesized titanium dioxide (TIT) nanoparticles of *P. guajava* leaf against *An. gambiae* s.l. after 48 hours exposure period

Figure 5 illustrates the percentage mortality of *Anopheles gambiae s.l.* larvae when exposed to different concentrations (0.1 mg/L, 0.2 mg/L, 0.3 mg/L, and 0.4 mg/L) of synthesized titanium nanoparticles from *Psidium guajava* over a 48-hour exposure period, with positive and negative controls included for reference. The x-axis represents the exposure period (6, 12, 18, 24, and 48 hours), while the y-axis denotes percentage mortality. The results indicate a gradual, dose-dependent increase in mortality, with higher concentrations and longer exposure periods yielding greater mortality rates. At 6 and 12 hours, mortality was minimal, with all test concentrations and the positive control resulting in less than 10% mortality, while the negative control showed no mortality at any point. By 18 hours, mortality increased slightly, with 0.3 mg/L and 0.4 mg/L concentrations showing the highest effects (~10-15%), while lower concentrations and the positive control exhibited less than 10% mortality. At 24 hours, **a** more pronounced increase in mortality was observed, particularly at higher concentrations, where 0.4 mg/L showed nearly 20% mortality, followed by 0.3 mg/L (~15%), and lower doses with proportionally smaller effects. The positive control also increased to ~20% mortality, while the negative control remained at 0%. The most significant changes occurred at 48 hours, where 0.4 mg/L resulted in the highest mortality (~55%), followed by 0.3 mg/L (~40%), 0.2 mg/L (~25%), and 0.1 mg/L (~15%), while the positive control exhibited ~30% mortality**.** The negative control remained ineffective, confirming the larvicidal effect was due to titanium nanoparticles. The results suggest that synthesized titanium nanoparticles from *Psidium guajava* exhibit moderate larvicidal activity, with effectiveness increasing over time but not achieving complete mortality, even at the highest concentration. Compared to silver nanoparticles, titanium nanoparticles appear less potent, requiring longer exposure times and higher doses to reach significant mortality rates.

Table 2: Lethal Concentrations (LC) of the extracts of Plants post 24-hour exposure

Extract	24 Hours				48 Hours			
	LC_{50} (mg/L)	LC_{90} (mg/L)	R^2	Regression Equation	LC_{50} (mg/L)	LC_{90} (mg/L)	R^2	Regression Equation
Crude Aqueous Leaf	22.00	38.12	0.84	y = 2.4821x - 4.625	13.25	22.67	0.96	y = 4.25x - 6.3333
Synthesized Titanium Nanoparticles	25.05	44.03	0.95	y = 2.1071x - 2.7917	19.57	34.02	0.96	y = 2.7679x - 4.1667

NA = not applicable

DISCUSSION

The current study presents the green synthesis, optimization, and characterization of titanium dioxide nanoparticles (TiO_2NPs) using *Psidium guajava* leaf extract, with subsequent evaluation of their larvicidal potential against *Anopheles gambiae* s.l. larvae. The use of plant-mediated green synthesis provides an eco-friendly and sustainable approach to nanoparticle production, addressing the growing demand for safer alternatives to synthetic insecticides in mosquito control programs [9].

Green Synthesis and Optimisation of TiO_2 Nanoparticles

The synthesis of TiO_2NPs was successfully achieved by mixing guava leaf extract with titanium isopropoxide (TTIP) under varying conditions of pH, salt concentration, and reaction time. The colour changes from yellow to brown indicated nanoparticle formation, consistent with earlier reports where phytochemicals such as flavonoids, tannins, and phenolics act as reducing and stabilising agents [10]. The Design Expert-based optimisation highlighted the critical influence of synthesis parameters on nanoparticle formation, particularly pH and precursor concentration. This aligns with studies by Thakur *et al.*[11], who observed that slightly alkaline pH values enhance the nucleation and growth of nanoparticles due to increased ionisation of phytoconstituents.

Interestingly, although Run 3 (pH 10, 1 mM TTIP, 17.5 min) yielded the lowest UV-Vis absorbance wavelength (214 nm), the corresponding absorbance of 4.00 was beyond the optimal range for typical TiO_2NPs (250–350 nm), suggesting either the presence of unreacted precursors or measurement artifacts due to oversaturation [12]. This supports the assertion that low wavelength does not necessarily equate to successful nanoparticle formation if the absorbance is atypically high [13].

In contrast, Run 6 (pH 7, 10 mM TTIP, 5 min) produced nanoparticles with a characteristic UV-Vis peak at 270–275 nm and an absorbance value within the expected range (0.5–1.0), confirming successful synthesis. This finding correlates with the study by Rajakumar and Rahuman [7], who reported similar absorption behaviour for TiO_2NPs synthesized from *Cymbopogon citratus*. The observed peak corresponds to the band gap excitation of electrons from the valence band to the conduction band of TiO_2, confirming the nanoparticle's anatase phase [14].

Particle Size and Morphological Analysis

Dynamic Light Scattering (DLS) analysis of the TiO_2NPs from Run 6 revealed a dominant particle size distribution centred around 90–100 nm, which is optimal for biological interactions due to enhanced cellular uptake and surface reactivity (Bhattacharya & Mukherjee, 2008). Minor peaks at $\sim$10 nm and $>$500 nm indicated the presence of smaller particles and some degree of agglomeration, respectively, which is common in green-synthesised NPs due to incomplete capping or insufficient stabilisation [15].

The particle size range observed is consistent with findings from previous green synthesis studies using *Azadirachta indica* [11] and Aloe vera [16], both of which reported effective larvicidal activity for nanoparticles within the 70–120 nm size range.

Larvicidal Activity of Synthesised TiO_2NPs

The TiO_2NPs exhibited a clear dose- and time-dependent larvicidal activity against *Anopheles gambiae* s.l. larvae. At 48 hours, the LC_{50} and LC_{90} values were

19.57 mg/L and 34.02 mg/L, respectively, indicating moderate toxicity. Comparatively, the crude *P. guajava* leaf extract showed higher efficacy (LC_{50} = 13.25 mg/L), possibly due to the combined effect of phytochemicals acting as toxins or growth inhibitors [6].

The reduced toxicity of TiO_2NPs relative to crude extract may reflect their delayed release of reactive oxygen species (ROS), which requires UV activation to fully exert their larvicidal effects [17]. TiO_2NPs are known to generate ROS such as hydroxyl radicals (OH), superoxide anions (O_2^-), and hydrogen peroxide (H_2O_2) under UV or visible light, leading to oxidative stress and eventual larval death [18].

Despite this, the relatively high mortality rates observed at 0.3 mg/L and 0.4 mg/L nanoparticle concentrations suggest that TiO_2NPs have practical potential for mosquito control, particularly when formulated to enhance UV activation or in combination with UV-emitting light traps. Similar trends have been observed in TiO_2NPs synthesized using *Ocimum sanctum* and *Lantana camara*, which showed significant larvicidal effects against *Culex quinquefasciatus* and *Aedes aegypti* [11, 19].

The difference in mortality trends between crude extract and synthesized nanoparticles also supports the hypothesis that multiple mechanisms may be at play. For example, crude extract toxicity may arise from phenolic and flavonoid compounds that disrupt enzymatic activity or interfere with the larval nervous system [20]. In contrast, nanoparticle toxicity primarily relies on physical interactions, ROS-induced membrane damage, and oxidative stress [21].

Comparison with Other Nanoparticle-Based Larvicides

While TiO_2NPs show promise, their larvicidal potency appears to be lower than that of silver nanoparticles (AgNPs), which often achieve LC_{50} values below 10 mg/L [6, 9]. However, the lower ecological toxicity, higher stability, and reusability of TiO_2NPs make them a viable alternative for long-term, integrated vector management strategies [7]. Furthermore, TiO_2 is non-toxic to aquatic vertebrates at concentrations effective for mosquito control, making it safer for use in breeding sites [22]

A comparative analysis by Mane [14] on Ag-TiO_2 nanocomposites demonstrated enhanced larvicidal activity due to synergistic effects, indicating a future direction for increasing TiO_2 efficacy. Additionally, doping TiO_2NPs with metals like copper or zinc has been shown to improve ROS production and antimicrobial activity [21].

The environmental friendliness of the green synthesis method using *P. guajava* aligns with current trends in sustainable nanotechnology. The availability and low cost of guava leaves in tropical regions further enhance the scalability of this approach. Moreover, this synthesis avoids hazardous reagents, reduces toxic waste, and aligns with the principles of green chemistry [16]. However, challenges remain in terms of optimizing formulation stability, delivery mechanisms in field conditions, and ensuring consistent particle quality. Incorporating TiO_2NPs into slow-release formulations or combining them with larval habitat management could enhance field effectiveness [22]

REFERENCES

1. Kolawole, E. O., Ayeni, E. T., Abolade, S. A., Ugwu, S. E., Awoyinka, T. B., Ofeh, A. S.,

& Okolo, B. O. (2023). Malaria endemicity in Sub-Saharan Africa: Past and present issues in public health. *Microbes and Infectious Diseases*, 4(1), 242-251.

2. Chukwuekezie, O., Nwosu, E., Nwangwu, U., Dogunro, F., Onwude, C., Agashi, N., Ezihe, E., Anioke, C., Anokwu, S., Eloy, E., Attah, P., Orizu, F., Ewo, S., Okoronkwo, A., Joseph, A., Ikeakor, I., Haruna, S., & Gnanguenon, V. (2020). Resistance status of Anopheles gambiae (s.l.) to four commonly used insecticides for malaria vector control in South-East Nigeria. *Parasites & vectors*, *13*(1), 152. https://doi.org/10.1186/s13071-020-04027-z

3. Ranatunga, M., Kellar, C., & Pettigrove, V. (2023). Toxicological impacts of synthetic pyrethroids on non-target aquatic organisms: A review. *Environmental Advances*, 12, 100388.

4.Rashid, M. M., Forte Tavčer, P., & Tomšič, B. (2021). Influence of titanium dioxide nanoparticles on human health and the environment. *Nanomaterials*, 11(9), 2354.

5. Wang, L., Wu, Y., Xie, J., Wu, S., & Wu, Z. (2018). Characterisation, antioxidant and antimicrobial activities of green synthesized silver nanoparticles from Psidium guajava L. leaf aqueous extracts. *Materials Science and Engineering: C*, 86, 1-8.

6. Adeleye, E. A., Ukubuiwe, A. C., Adeniyi, K. A., Francis, M. O., & Aina, A. O. (2023). Effect of Different Light Regimens on Larvicidal Activities of *Vitellaria paradoxa* Leaf Mediated Silver Nanoparticles against Malaria Vector, *Anopheles gambiae* s. l. *FUAM Journal of Pure and Applied Science (FUAMJPAS),* 3(1), 20-32

7. Rajakumar & Rahuman (2015) – Efficacy of TiO_2 nanoparticles (using Mangifera indica) against blood feeding parasites in Parasitology Research, 114, 571–581

8. Adeniyi, K. A., Olayemi, I. K., Shittu, K. O., Ukubuiwe A. C., Oyibo-Usman, K. A. & Garba, Y. (2020). Entomotoxicological potencies of extracts of the weaver ant (*Oecophylla smaragdina*) against *Anopheles* spp. *Asian Journal of Biological Sciences*, 13, 223-227.

9. Ahmed, S., Ahmad, M., Swami, B. L., & Ikram, S. (2016). A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. *Journal of Advanced Research*, 7(1), 17-28.

10. Ibrahim, H. M. (2015). Green synthesis and characterization of silver nanoparticles using banana peel extract and their antimicrobial activity against representative microorganisms. *Journal of Radiation Research and Applied Sciences*, 8(3), 265-275.

11. Thakur, B. K., Kumar, A., & Kumar, D. (2019). Green synthesis of titanium dioxide nanoparticles using *Azadirachta indica* leaf extract and evaluation of their antibacterial activity. *South African Journal of Botany*, 124, 223-227.

12. Singh, S., Maurya, I. C., Tiwari, A., Srivastava, P., & Bahadur, L. (2022). Green synthesis of TiO2 nanoparticles using *Citrus limon* juice extract as a bio-capping agent for enhanced performance of dye-sensitized solar cells. *Surfaces and Interfaces*, 28, 101652.

13. Salam, H. A., Sivaraj, R., & Venckatesh, R. J. M. L. (2014). Green synthesis and characterization of zinc oxide nanoparticles from Ocimum basilicum L. var. purpurascens Benth.-Lamiaceae leaf extract. *Materials Letters*, 131, 16-18.

14. Li, M., Noriega-Trevino, M. E., Nino-Martinez, N., Marambio-Jones, C., Wang, J., Damoiseaux, R., Ruiz, F., & Hoek, E. M.

(2011). Synergistic bactericidal activity of Ag-TiO_2 nanoparticles in both light and dark conditions. *Environmental Science & Technology*, *45*(20), 8989–8995. https://doi.org/10.1021/es201675m

15. Kordy, M. G., Abdel-Gabbar, M., Soliman, H. A., Aljohani, G., BinSabt, M., Ahmed, I. A., & Shaban, M. (2022). Phyto-capped Ag nanoparticles: green synthesis, characterization, and catalytic and antioxidant activities. *Nanomaterials*, 12(3), 373.

16. Iravani, S. (2014). Green synthesis of metal nanoparticles using plants. *Green Chemistry*, 16(6), 4034–4044.

17. Chen, D., Cheng, Y., Zhou, N., Chen, P., Wang, Y., Li, K., Huo., S., Cheng, P., Peng, P., Zhang, R., Wang, L., and Ruan, R. (2020). Photocatalytic degradation of organic pollutants using TiO2-based photocatalysts: A review. *Journal of Cleaner Production*, 268, 121725.https://www.sciencedirect.com/science/article/pii/S0959652620317728

18. Sujitha, V., Murugan, K., Paulpandi, M., Panneerselvam, C., Suresh, U., Roni, M., Nicoletti, M., Higuchi, A., Madhiyazhagan, P., Subramaniam, J., Dinesh, D., Vadivalagan, C., Chandramohan, B., Alarfaj, A. A., Munusamy, M. A., Barnard, D. R., & Benelli, G. (2015). Green-synthesized silver nanoparticles as a novel control tool against dengue virus (DEN-2) and its primary vector Aedes aegypti. *Parasitology research*, *114*(9), 3315–3325. https://doi.org/10.1007/s00436-015-4556-2

19. Öztürk, U. Ş., & Citak, A. (2022). Synthesis of titanium dioxide nanoparticles with renewable resources and their applications. *Turkish journal of chemistry*, 46(5), 1345-1357.

20. Shi, Z., Liang, X., Zhao, Y., Liu, W., & Martyniuk, C. J. (2022). Neurotoxic effects of synthetic phenolic antioxidants on dopaminergic, serotoninergic, and GABAergic signaling in larval zebrafish (Danio rerio). *The Science of the total environment*, *830*, 154688. https://doi.org/10.1016/j.scitotenv.2022.154688

21. Mingmongkol, Y., Trinh, D. T., Phuinthiang, P., Channei, D., Ratananikom, K., Nakaruk, A., & Khanitchaidecha, W. (2021). Enhanced Photocatalytic and Photokilling Activities of Cu-Doped TiO2 Nanoparticles. *Nanomaterials*, *12*(7), 1198. https://doi.org/10.3390/nano12071198

22. Kojom Foko, L. P., Eya'ane Meva, F., Eboumbou Moukoko, C. E., Ntoumba, A. A., Ekoko, W. E., Ebanda Kedi Belle, P., Ndjouondo, G. P., Bunda, G. W., & Lehman, L. G. (2021). Green-synthesized metal nanoparticles for mosquito control: A systematic review about their toxicity on non-target organisms. *Acta tropica*, *214*, 105792. https://doi.org/10.1016/j.actatropica.2020.105792