https://doi.org/10.48047/AFJBS.6.15.2024.11658-11678

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

PREPARATION OF LEMONGRASS OIL-LOADED BANANA STARCH NANOPARTICLES TO CONTROL ECTOPARASITES IN CATTLE

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Volume 6, Issue 15, Sep 2024

Received: 15 July 2024

Accepted: 25 Aug 2024

Published: 25 Sep 2024

doi: 10.48047/AFJBS.6.15.2024.11658-11678

ABSTRACT

India is renowned as one of the world's top producers of livestock and milk. The cattle industry exerts significant influence on the economy, providing essential sustenance and diverse commodities to the populace. Livestock serve as vital sources of both nutrition and materials, extending beyond mere food production. However, a pressing concern within this sector is the prevalence of ticks, blood-sucking parasites that pose a significant threat, drastically reducing the productivity of dairy animals and presenting formidable economic and welfare challenges in dairy farming. Historically, the prevailing approach involved the use of acaricides to eradicate attached larvae, nymphs, and adults. Nevertheless, the escalating proliferation of parasites, coupled with the emergence of resistant strains, has become increasingly prevalent. Hence, there is a crucial need to explore alternative systems that are cost-effective, environmentally friendly, and sustainable. Biological methods are preferred for their ability to ensure toxin-free milk and meat, thereby minimizing potential impacts on human health, the environment, and animal welfare. Essential oils offer a promising alternative due to their natural origin, therapeutic properties, and lower toxicity. Lemon grass oil (*Cymbopogon citratus*) has emerged as a particularly effective essential oil against ticks. This study primarily focuses on formulating lemon grass oil (*Cymbopogon citratus*) loaded banana starch nanoparticles to combat *Rhipicephalus microplus* in cattle. The particle size of starch and lemon grass oil-loaded starch nanoparticles was determined to be 431.6 and 527.1 nm, respectively, further confirmed using Scanning Electron Microscopy. The bio-efficacy of nano encapsulated lemon grass oil with starch nanoparticles was assessed using the Larval Packet Test (LPT) and Adult Immersion Test.

Keywords: Adult Immersion Test; Banana starch nanoparticle; Larval Packet Test; Lemon grass oil; *Rhipicephalus microplus*.

1. Introduction

India's cattle industry, comprising a significant portion of the national income, aids in poverty alleviation and job creation, particularly for women, youth, and entrepreneurs. As the top milk producer globally, it's pivotal for economic growth. Challenges like disease outbreaks hinder productivity, alongside infrastructural and resource deficiencies. Tick infestation exacerbates these issues, threatening livestock health and productivity. Rectifying low productivity, inadequate resources, and marketing hurdles is imperative. Addressing tick-related problems is paramount due to their blood-feeding nature. Enhancing livestock sector infrastructure, resources, and disease management strategies are vital for sustained growth and socio-economic development. The Asian blue tick, *Rhipicephalus microplus*, is a significant ectoparasite infesting cattle worldwide. Originating with Asian zebu cattle, it spread globally with European breeds. *R. microplus*, a species complex of 5 clades, including *R.australis* and *R.annulatus*, inflicts economic damage by transmitting diseases like anaplasmosis and babesiosis. European cattle, lacking immunity like tropical breeds, suffer greater infestations.

Ticks, specifically Acari: Ixodidae, are parasitic arachnids classified into families like Ixodidae (hard ticks) and soft ticks such as Nuttalliellidae and Argasidae. They locate hosts through odor, moisture, heat, and vibrations. Hard ticks have a thick dorsal scutum, unlike soft ticks. All ticks are obligate temporary parasites requiring blood for survival, undergoing larval, nymph, and adult stages. They integrate into pathogen transmission chains via trans-stadial or transovarial passages. Males have scutum-covered bodies for feeding. Eggs hatch into larvae, then develop into nymphs, which feed on hosts before molting into adults. Female blood meals are crucial for egg production, with soft ticks generally having longer lifespans than hard ticks. Tick-borne diseases (TBDs) significantly hinder livestock production, imposing economic burdens on rural communities by compromising food supply and daily income. Although ticks primarily feed on blood and do not directly harm animals, they indirectly affect their well-being. Livestock, particularly cattle, suffer reduced weight gain and milk production due to blood loss from tick infestation. Moreover, parasites transmitted by ticks impede young animals' growth, resulting in thinness, weakness, and stunted development. Tick paralysis, typically caused by Ixodes and Ornithodoruslaboremis genera, further exacerbates these challenges by injecting toxins into hosts. Hyalommatruncatum infestations induce sweating sickness in cattle and other domestic species in regions like South, Central, and East Africa. Financial losses occur due to skin and hide depreciation and persistent irritation. Severely affected cattle may develop oral and stomach cavities. Tick control is crucial due to losses from infestation, aiming to prevent disease transmission and physical damage. Chemical acaricides like arsenicals, pyrethroids, and

organophosphates are traditionally used but lead to resistance and health concerns. Alternatives include biological control, herbal solutions, green nanotechnology, and vaccination, with herbal acaricides showing promise as substitutes.

The optimal approach for tick control lies in utilizing plant-derived products, particularly essential oils, known for their acaricidal activity due to varied chemical compositions (Selles et al., 2021). This method offers advantages such as toxin-free milk and meat, promoting human health, environmental safety, and animal welfare. Essential oils exhibit low toxicity, water solubility, and efficacy ranging from 5-100% against ticks. Obtained through distillation or solvent extraction, these oils contain compounds with pesticidal, growth-regulating, and repellent properties. With low costs, minimal residual effects, and a reduced risk of resistance, essential oils inhibit tick feeding, chitin synthesis, growth, and reproduction through neurotoxic, cytotoxic, and mechanical effects (Selles et al., 2021)

Cymbopogon citratus, or lemongrass oil, demonstrates exceptional efficacy (98%) against ticks when applied for 12 days, surpassing synthetic acaricides without adverse effects on humans or the environment, particularly in the nymphal stage of infestation. Adult Immersion Test and Statistical Analysis affirm its efficiency (Pazinato et al., 2016). Lemongrass oil, renowned for antibacterial, anti-inflammatory, and anti-fungal properties, emerges as a potential substitute for synthetic acaricides, addressing environmental concerns (Boukhatem et al., 2014). Its longterm use shows no resistance development, a common issue with synthetics (Dutta et al., 2018).

Starch, a ubiquitous natural polymer, stands out for its abundance, low cost, non-toxicity, renewability, and biodegradability. Recognized for its versatility, starch serves as an ideal

encapsulation material for bioactive compounds, owing to its biocompatibility and straightforward synthesis (Ismail & Gopinath, 2017; Qiu et al., 2020). Widely employed in pharmaceuticals, starch plays roles as a binder, diluent, and filler in drug delivery, with starch nanoparticles proving effective for this purpose. Green banana starch, rich in starch content, is particularly chosen due to its transformation into sugars as bananas ripen. Essential oils, unsuitable for direct use, find a carrier in banana starch, facilitating their application (Katherine et al., 2021). Carbohydrates, including starch, sugars, and dietary fiber from fruits like plantains, contribute to diverse nutritional sources (Englyst et al., 2007). This study focuses on creating starch nanoparticles from lemon grass oil-loaded banana starch, exhibiting high acaricidal efficiency against ticks in cattle without harming animals or the environment.

2. MATERIALS AND METHOD

2.1 MATERIALS

The bio-derived oil utilized for nano-emulsion was sourced from Himedia Labs in India. MilliQ water (with a resistivity of 18.2 MΩ.cm) was acquired from the Cascada BIO-water Purification System. All additional chemicals procured for the experiment were of analytical quality.

2.2 METHOD

2.2.1 Preparation of starch from green banana

Unripe bananas are peeled and sliced into 2cm sections. These pieces are immersed in a citric acid solution with a concentration of 2% w/v for approximately 5 minutes before being blended. The resulting mixture is then filtered through various sieves until the rinse water is clear of any solid particles. The residual white sediments consist of starch, which is subsequently dried in a hot air oven at 40°C for a period of 48 hours. The dried solids are then pulverized with a pestle, sifted, and finally stored in a container. (Fontes et al 2017).

2.2.2 Preparation of banana starch nanoparticle

By using the nanoprecipitation method unmodified starch nanoparticles are prepared. From the pre-prepared starch, 1 to 4 grams of the starch sample is combined with 200 milliliters of acetone utilizing a magnetic stirrer set at 600 revolutions per minute (rpm). After stirring for 15 minutes, 400 milliliters of water is gradually added drop by drop with continuous agitation. Mixing is carried out at room temperature until the acetone completely evaporates. The mixture is then spun in a centrifuge at 4000 rotations per minute (rpm) for 40 minutes to isolate the nanoparticles. The supernatant is removed and the pellet is dried in a hot air oven for 48 h at 30°C.

2.2.3 Nano encapsulation of banana starch with lemon grass oil

As lemongrass oil has to be encapsulated, 4g of prepared starch is mixed with 200mL of acetone using a magnetic stirrer at 600 rpm for 15 min. 400mL water is added drop by drop for encapsulating 1:1 ratio of water, and lemongrass oil is replaced with the water at the last step. To isolate nanoparticles, the suspension undergoes centrifugation at 4000 revolutions per minute (rpm) for a duration of 40 minutes. Subsequently, the resulting pellet is subjected to desiccation in a hot-air oven maintained at 30°C for a period of 48 hours.

2.3 Characterization of nano-encapsulated lemon grass oil-loaded BSN

2.3.1 Dynamic light scattering

The size distribution of starch nanoparticles and starch nanoparticles loaded with lemon grass oil was examined using a Particle Size Analyzer. (90 Plus Particle Size Analyzer, Brookhaven Instruments Corporation, USA)

2.3.2 Zetapotential Analysis

The zeta potential of the starch nanoparticle and lemon grass oil-loaded starch nanoparticle were measured using an SZ100 nanoparticle analyzer (Horiba Scientific Ltd,Japan).

2.3.4 Scanning Electron Microscopy

Using a Hitachi S-4700 High-Definition Scanning Electron Microscope (Hitachi High Technologies America, Inc., Dallas, TX, USA), the morphological examination was done.

2.4 In -vitro studies

2.4.1 Bacterial strains

The efficacy of starch nanoparticles against Gram-positive (*E.coli*) and Gram-negative (*Pseudomonas aeruginosa*) bacteria was assessed using strains obtained from the Microbial Type Culture Collection and Gene Bank (MTCC). The confirmation of bacterial cultures followed the biochemical protocol outlined in Bergey's Manual of Systematic Bacteriology (Vol 2, Second Edition) (Brenner et al 2005)

2.4.2 Inoculums preparation

Bacterial cultures were generated by transferring microorganisms onto nutrient agar slants and incubating them at 37°C for 18 hours. Following incubation, the bacterial growth was collected using 5 ml of sterile saline solution, then diluted accordingly. Subsequently, the absorbance of the solution was set to 25% at 580 nanometers using a spectrophotometer. At this optical density, the approximate viable cell count for each bacterial strain was 10^7 colonyforming units per milliliter.

2.4.3 Well Diffusion-Antibacterial assay

To evaluate the antibacterial or antifungal properties of extracts derived from plants or microorganisms, researchers commonly employ the agar well diffusion technique. This method

involves inoculating the agar plate surface, similar to the disk-diffusion method, where a volume of microbial inoculum is evenly spread across the agar surface. Subsequently, a measured volume (typically ranging from 20 to 100 microliters) of the antimicrobial substance or extract solution is carefully dispensed into a well created aseptically by using a sterile cork borer or pipette tip, with a diameter typically ranging from 6 to 8 millimeters. The targeted microorganism is then streaked onto a suitable agar plate, and the plates are incubated under appropriate conditions. The antimicrobial compound diffuses through the agar medium, inhibiting the growth of the tested microorganism.100 µL of samples were added into each well and Ampicillin is used as the positive control. The different sample used here includes starch nanoparticle, lemongrass oil-loaded starch nanoparticle, and lemongrass oil. The Petri dishes were placed in an incubator set at 37°C for a duration of 24 hours. Following incubation, the presence of inhibition zones was observed and recorded. These zones were then measured using a Vernier caliper to determine the extent of antibacterial activity.

2.5 In-vitro Bio-efficacy of lemon grass oil-loaded BSN against cattle ticks

2.5.1 Collection of *Rhipicephalusmicroplus*

The *Rhipicephalus microplus* were collected from farms that are both organized and unorganized. They were washed in tap water and dried. It is then kept in a glass bottle with the mouth covered with muslin cloth. The environment is maintained at a temperature range of 28±1 °C and a relative humidity range of 85±5%. Under these conditions, the eggs are permitted to develop into larvae. It is kept under incubation for 15 – 25 days.

2.5.2 Larval Packet Test (LPT)

Five different concentrations of prepared concentration of nano-emulsions were taken. Five replicates that are 10 ticks for each replicate were selected for each concentration. The ticks are immersed in the 10mL sample tested solution for 2min in a petri dish. It is desiccated using filter paper and placed in an incubator set at 26-28°C with a relative humidity of 80%. Distilled water is used as a negative control. And Deltamethrin as a positive control in the concentration of 2mL/L. mortality was observed for 14 days and live and dead ticks were counted.

2.5.3 Adult Immersion Test Larval Tarsal Test (LTT)

Five different concentrations of prepared concentration of nano-emulsions were taken. Fivereplicatesthatare10ticksforeachreplicatewereselectedforeachconcentration. The ticks are immersed in the 10mL sample tested solution for 2min in a petridish. It is dried with filter paper and incubated at 26-28°C and 80% relative humidity. Distilled water is used as a negative

control. And Deltamethrin as a positive control in the concentration of 2mL/L. mortality was observed for 14 days and live and dead ticks were counted.

3. RESULTS AND DISCUSSION

3.1 Synthesis of starch from green banana

Green banana was collected from the local market, cleaned, and sliced into small pieces of 2cm. Further, the samples are dipped immediately into a citric acid solution for about 5 min. Then it was blended and sieved and the solid mixture was further kept in a hot air oven at 40^{o}C for 48h which resulted in the formation of fine dried powder

Starch was prepared from green bananas using the acid hydrolysis method. Acid hydrolysis is known to be a highly efficient method for the hydrolysis of starch, which can lead to a high yield of starch nanoparticles from green bananas. It is a relatively simple method that does not require specialized equipment, making it more accessible and cost-effective than other methods. Acid hydrolysis is used to control the size of the resulting starch nanoparticles by adjusting the reaction conditions such as temperature, time, and acid concentration. This allowed for the production of uniform nanoparticles with desirable properties. The acid hydrolysis method improves the physicochemical properties of the starch nanoparticles. such as increased solubility, stability, and bioavailability, which enhances their potential applications in various industries (Jana et al 2016). Acid hydrolysis does not generate any toxic by products, making it an environmentally friendly method for the preparation of starch nanoparticles. Citric acid was commonly used in starch preparation as an acidulant and chelating agent. Citric acid reduces the pH of the starch solution, promoting the cleavage of glycosidic bonds, which in turn results in the generation of shorter chains. It was used to modify the properties of starch by crosslinking the starch molecules to form ester bonds, resulting in a more stable and resistant starch (Singh et al 2014). Overall, incorporating citric acid into starch preparation enhances the functional attributes of the starch and broadens its possible uses across diverse sectors, including food, pharmaceuticals, and cosmetics. The citric acid solution was used to break down the cell walls of the green banana and solubilize the starch, which was then extracted and purified using sieves and washing with deionized water. Finally, the starch was dried and ground to obtain a purified starch powder (Figure 3.1). This technique was frequently employed for extracting starch from different plant sources and adapted by varying the type and concentration of acid utilized, along with adjusting the processing parameters, to enhance both the yield and characteristics of the obtained starch.

Starch presence was confirmed using the iodine test, when the unripe banana powder was mixed with iodine, it changed its color to blue. In this research, two sets of bananas are used for starch preparation. One set contained a ripened banana whereas the other set belonged to an unripened banana. The Iodine study revealed that unripened banana powder contained more amount of starch compared to ripened one.

3.2 Banana starch nanoparticle

The banana starch nanoparticle was synthesized from the banana starch along with the mixing of acetone and drying of the pellet after centrifugation in a hot air oven. The resulting sample was ground with the pestle to make it a fine powder. Acetone frequently served as a solvent in the production of starch nanoparticles, as it dissolved both amylose and amylopectin, the two

main components of starch. Acetone was used to dissolve banana starch and form a starchacetone mixture. Following this, the mixture underwent centrifugation to eliminate any insoluble components, and the ensuing pellet was subjected to drying in a hot air oven (Singh et al 2018; Li et al 2016; Jana et al 2016; Singh et al 2014). The dried pellet was then ground with a pestle to obtain a fine powder of banana starch nanoparticles (Figure 3.2). The use of acetone in this method was important because it aided in the dissolution of the starch and promoted the generation of nanoparticles throughout the drying procedure.

3.3 Nanoencapsulation of banana starch nanoparticle with lemon grass oil

To avoid aggression as well as controlled release of essential oil, lemongrass oil was encapsulated using a starch nanoparticle. The starch nanoparticle prepared from the banana was further mixed with lemongrass oil at a particular ratio (1:1). Then the suspension was subjected to centrifuge and kept in a hot air oven which resulted in a fine powder of lemon grass oilloaded starch nanoparticle (Figure 3.3).

Nanoencapsulation of banana starch nanoparticles with lemon grass oil refers to the process of enclosing or entrapping lemon grass oil within the matrix of banana starch nanoparticles to protect it from environmental factors and to control its release. This process involves the formation of a complex between the oil and the starch nanoparticles, which was stabilized by intermolecular forces such as hydrogen bonding, electrostatic interactions, and hydrophobic interactions. The nanoencapsulation of lemon grass oil in banana starch nanoparticleshas potential applications in the food and pharmaceutical industries. It can be used as a natural flavoring agent or as a therapeutic agent for the treatment of various diseases. The controlled

release of lemon grass oil from the banana starch nanoparticle was achieved by adjusting the properties of the nanoparticles, such as their size, shape,and surface charge. Several studies have been conducted on the nanoencapsulation of lemon grass oil in banana starch nanoparticles, and the results have shown that this method can improve the stability and bioavailability of the oil. Moreover, the use of natural materials such as banana starch and lemon grass oil made this process environmentally friendly and sustainable (Kumar et al 2019; Sarker et al 2021; Juhaimi 2020; Singh et al 2018).

3.4 CHARACTERIZATION OF STARCH NANOPARTICLE

3.4.1 Dynamic Light Scattering

Starch nanoparticles and lemongrass oil-loaded starch nanoparticles are analyzed using Dynamic Light Scattering. The dimensions of starch and lemongrass oil-incorporated starch nanoparticles were measured at 431.6 nm and 527.1 nm, respectively, exhibiting polydispersity index (PDI) values of 0.391 and 0.380, correspondingly (Figure 3.4 (a) & (b)). The PDI value indicates the uniformity in the size distribution of synthesized nanoparticles.

DLS can be used to measure the hydrodynamic diameter of the starch nanoparticles, which takes into account the size of the particle and the thickness of the hydration layer around it (Kumar et al 2019; Sarker et al 2021; Juhaimi 2020; Singh et al 2018).

3.4.2 Zeta potential

The zeta potential of the lemongrass oil-loaded banana starch nanoemulsion was found to be 41.29 ± 3.23 mV, indicating a highly stable system (Figure 3.5). A zeta potential beyond ±30 mV (absolute value) suggests strong electrostatic repulsion between droplets, preventing aggregation and phase separation. The negative charge likely arises from anionic groups on the banana starch or interface molecules, and may be enhanced by the pH of the medium. The small measurement error (±3.23 mV) further supports the consistency of the results. This high zeta potential confirms that banana starch effectively stabilizes the dispersion by both steric and electrostatic mechanisms, maintaining the nanoemulsion's uniformity over time.

3.4.2 Scanning Electron Microscopy

The morphological characterization of the starch nanoparticle, encompassing its dimensions, geometry, and permeability, was assessed utilizing Scanning Electron Microscopy (SEM). The SEM enables the examination of nanoparticle surface morphology by detecting electrons

scattered from the particle's surface. This instrument generates highly magnified images by utilizing electrons rather than light to create visual representations. Figure 3.6 shows starch nanoparticles and modifications brought about by the inclusion of lemongrass oil. The starch particles 1+5 granular structure was smooth and round or oval. The Scanning Electron Microscopic image shows the initial granule population of about 10 µm in diameter consisting of the structural elements of the average size in the range of 400600 nm (Figure 3.6 (a)). The microscopic image (Figure 3.6 (b)) unveiled that starch nanoparticles infused with essential oil exhibited granules that were consistently shaped and sized, displaying a uniform arrangement. These findings are consistent with the droplet size measurement and the Polydispersity Index (PDI) value observed in both samples. The mean size of the essential oilloaded starch was determined to be around 500 nm and it is spherical in structure (Ahmad et al 2019; Ashwar et al 2016).

3.5 IN-VITRO ANALYSIS

The starch nanoparticle and lemon grass oil-loaded starch nanoparticles were assessed for their applicative purpose as an antibacterial agent against two predominant bacteria *E.coli* and *Pseudomonas aeruginosa*, shown in Table 3.1. For Gram-positive bacteria, the outcomes of well diffusion assays with starch nanoparticles and lemon grass oil-loaded starch nanoparticles showed that treated wells demonstrated a zone of inhibition measuring 8±1.2 and 18±1.1 mm, respectively. The well treated with lemon grass oil alone showed a good zone of inhibition measuring 15±1.2 mm. When compared to lemon grass oil-loaded starch nanoparticles, ampicillin employed as a positive control resulted in a zone of inhibition measuring 19±0.9 mm. When compared to the positive control, lemon grass oil-loaded starch nanoparticles also showed a prominent antibacterial activity against the host pathogen.

The well treated with lemon grass oil alone showed a good zone of inhibition measuring 17±1.2 mm. oil-loaded starch nanoparticles, ampicillin used as a positive control yielded a zone of inhibition measuring 20±1.3 mm. Lemongrass oil and its bioactive compounds may disrupt the cell membrane of bacteria, leading to leakage of intracellular components and eventual cell death (Tiwari & Tiwari 2014). Some bioactive compounds in lemongrass oil may inhibit the activity of enzymes essential for bacterial growth and metabolism, leading to growth inhibition and cell death. This mechanism may be more effective against Gram-positive bacteria, which have thicker cell walls and are less susceptible to cell membrane disruption (Nostro et al 2004; Khan et al 2016). Lemongrass oil and its bioactive compounds may act synergistically with

conventional antibiotics, enhancing their antibacterial activity and reducing the risk of antibiotic resistance (de Sousa et al 2012).

3.6 ACARICIDAL ACTIVITY

The Adult Immersion Test (AIT) can be used to evaluate the efficacy of insecticides against tick *Rhipicephalus microplus*. This test is also used to studying the acaricidal activity of lemongrass oil loaded banana starch nanoparticle. In the case of lemon grass oil loaded starch nanoparticles, the AIT would involve immersing adult ticks in a solution containing the nanoparticles for a specified amount of time, followed by transfer to holding containers to assess mortality. The Larval Packet Test (LPT) can also be used to evaluate the efficacy of essential oils on *Rhipicephalus microplus* larvae, should be considered when performing an LPT with lemongrass oil. These include the concentration of the oil, the solvent used, and the exposure time. It is important to ensure that the concentration of the lemongrass oil is appropriate to achieve the desired efficacy, but not as high as to cause harm to the test organisms or to the environment

The larvicidal activity of the essential oil studied is presented in Table 3.2. The lemongrass oil loaded banana starch nanoparticle produces 100% mortality of larvae at concentrations tested. The starch nanoparticle produced respectively 30.27%, 40.29% and 50.94% mortality respectively at 1%, 5% and 10% dilutions against the 14-to-21-day-old *R. microplus* tick larvae.

The LC50 value of this starch and lemongrass oil loaded banana starch nanoparticle on larvae was 3.279 and 0.48 µg/mL (Table 3.2).

Studies have demonstrated that lemongrass oil can significantly reduce the viability and development of *Rhipicephalus microplus* larvae, with higher concentrations generally resulting in greater efficacy. The active compounds in lemongrass oil, such as citral and geraniol, are believed to be responsible for its acaricidal effects. These compounds may act on the nervous system of the larvae, causing paralysis and death, or disrupt hormonal balance and interfere with the growth and development of the larvae. Several methods of application have been tested for lemongrass oil, including immersion, spraying, and impregnation. Immersion in a lemongrass oil solution has been shown to be particularly effective at reducing *Rhipicephalus microplus* larvae viability, with higher concentrations resulting in greater mortality rates.

4. CONCLUSION

In conclusion, our study demonstrated that lemongrass oil-loaded banana starch nanoparticles possessed potent acaricidal activity against *Rhipicephalus microplus* larvae. Starch prepared from bananas was used to synthesize starch nanoparticles and further, it was converted into starch nanoparticles. The examination through scanning electron microscopy indicated that the granules exhibited consistent shape and size, displaying a homogeneous pattern. This observation was subsequently corroborated by dynamic light scattering analysis, alongside the determination of the Polydispersity Index (PDI). Lemon grass oil-incorporated banana starch nanoparticles demonstrated potent antibacterial efficacy against various bacterial strains, encompassing both Gram-positive and Gram-negative types. The antimicrobial effectiveness of the nanoparticles was ascribed to the existence of bioactive constituents within lemongrass oil, notably citral, which exhibited significant antibacterial characteristics. Employing banana starch as a vehicle for lemongrass oil augmented the oil's antibacterial effectiveness by enhancing stability and facilitating the sustained release of its bioactive components. The LC50 value of the nanoparticles was found to be significantly lower than that of pure lemongrass oil, indicating that the nanoparticles enhance the efficacy of the oil. The Adult Immersion Test (AIT) and Larval Packet Test (LPT) results further confirmed the acaricidal activity of the nanoparticles. These findings suggest that lemongrass oil-loaded banana starch nanoparticles have potential as a natural and effective acaricide for controlling *R. microplus* infestations.

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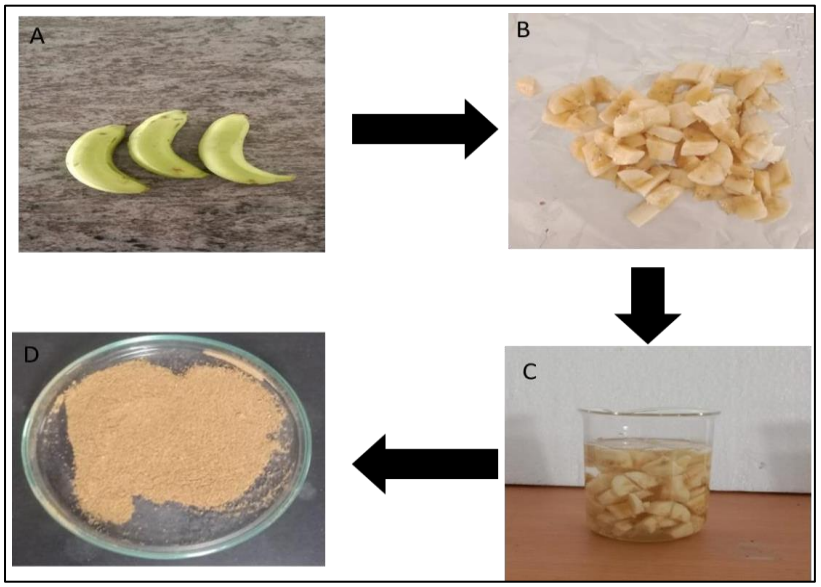
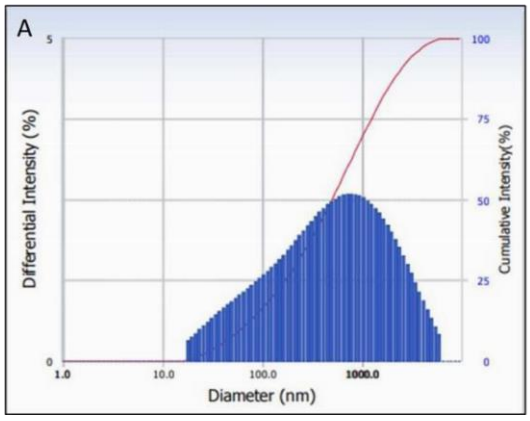
3.1. Schematic representation of starch preparation from A) green banana B) Sliced banana C) Banana dipped in citric acid solution D) starch powder

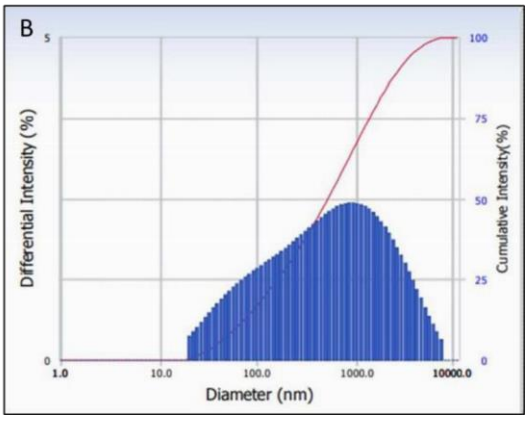


3.2. Banana starch nanoparticle powder

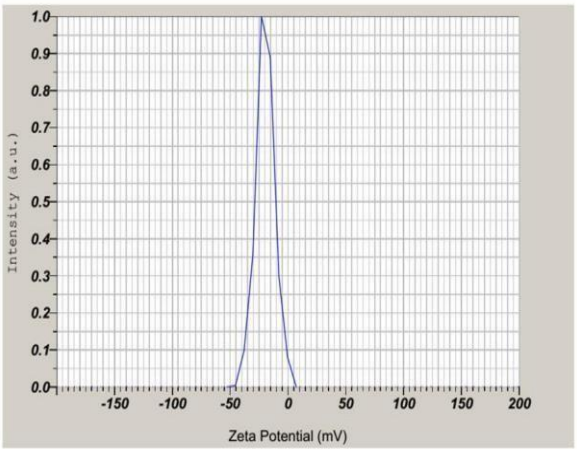
3.3 Encapsulation of banana starch nanoparticle with lemon grass oil

3.4: Particle size distribution of (a) starch nanoparticle (b) lemongrass oil-loaded BSN.





3.5 The zeta potential for the formulated lemongrass oil-loaded BSN



3.6 Scanning electron microscopy image of (A) Starch nanoparticle (B) lemongrass oilloaded Banana Starch Nanoparticle

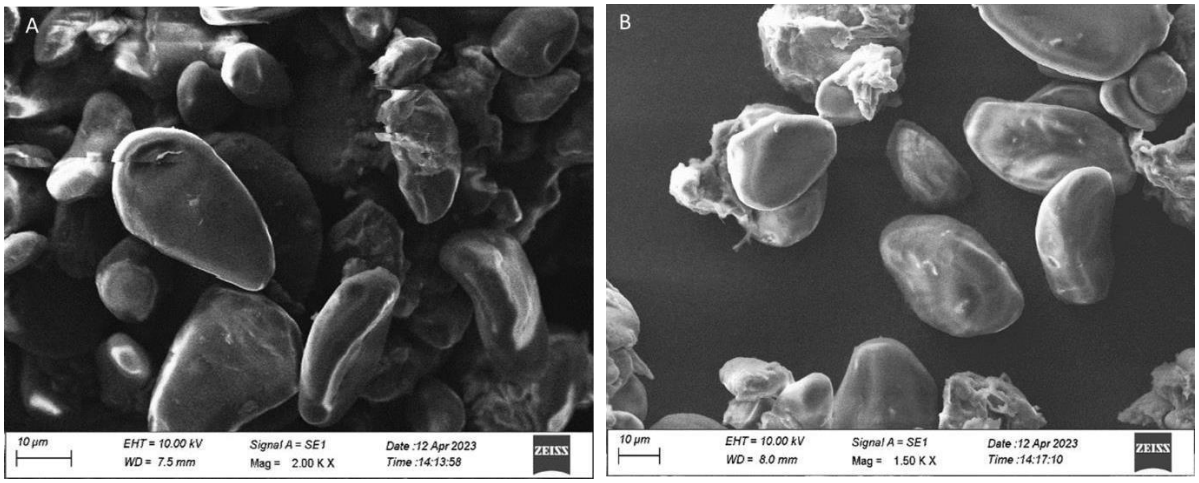


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Table 3.1 Antibacterial activity of samples against various bacteria using well diffusion

SI NO	SAMPLE	ZONE OF INHIBITION (diameter in mm)	
		E.Coli	*Pseudomonas aeruginosa*
1	Starch nanoparticle	8±1.2	8±1.1
2	Lemon grass oil	15±1.2	17±1.2
3	Lemon grass oil loaded banana starch nanoparticle	18±1.1	19±1.2
4	Ampicillin	19±1.3	20±1.3

.

Table 3.2 Efficacy of lemongrass oil-loaded banana starch nanoparticle against *R. microplus.*

Sl.No	Samples	Concentration (%)	Mortality (%)		LC 50 value (µg/mL)
			24 hrs	48 hrs.	
1.	Starch nanoparticle	1	20.1	30.27	3.279
		5	35.9	40.29	
		10	41.3	50.94	
2.	Lemongrass oil loaded banana starch nanoparticle	1	80.9	95.6	0.48
		5	90.1	98.7	
		10	95.6	99.1	
3.	Lemon grass oil	1	60.9	75.1	0.34
		5	70.9	85.6	
		10	90.9	90.6	