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Original Research Article

Sustainable Synthesis and Characterization of Plant-mediated Nanobiopesticides and Assessment of their Pesticidal Potential

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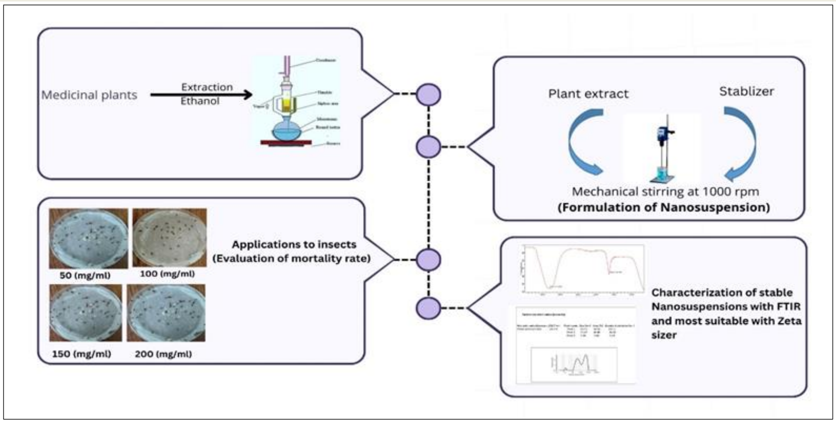
Abstract



Graphical Abstract

The excessive consumption of chemical pesticides has negative consequences for men, non-target creatures, and the surrounding environment. Pest control tactics are evolving towards biopesticides, which offer a viable and ecologically friendly sustainable approach to the insect challenges. The primary goals of this study are to investigate the potential of nanotechnology in revolutionizing pest management through the development of nanopesticides and to address the environmental and health concerns associated with traditional agrochemicals. The main objective of the current research work was to prepare medicinal plants based nanobiopesticides having improved stability, and pesticidal activity by following the method of antisolvent precipitation. Nanobiopesticides showed increased pesticidal activity and might be employed as an effective substitute to conventional chemical pesticides. The antisolvent precipitation process was used to create nanobiopesticides from medicinal plant extracts. Different medicinal plants (Mentha piperita, Lawsonia Inermis, T. Arjuna bark, Withania Somnifera roots and Ocimum basilicum) was used for the formulation of nanobiopesticides. Soxhlet apparatus was used to formulate medicinal plants extract. Different stablizers was utilized until the stable nanosuspension was formulated. The most suitable nanoformulated pesticides was characterized using FTIR, and Zeta sizer. Almost all nanobiopesticides demonstrated the existence of the O-H stretch at 3300 cm which is characteristic of alcohol and carbon-oxygen double bond at 1636.3 cm. Lawsonia Inermis based nanosuspension revealed intense peak at 3317 cm. The average particle size and polydisperity index of Lawsonia Inermis based nanobiopesticide is 228.7 nm and 24.5%. Medicinal plants extracts, nanosuspensions and pyriproxyfen was applied on Tribolium castaneum insects and mortality rate of insects was determined after different time inervals. Lawsonia Inermis plant extract showed highest ($p < 0.05$) mortality rate after 72 hours of treatment 51.83 ± 0.76 by utilizing 200mg/ml concentration. Results demonstrated that the Lawsonia Inermis nanosuspension had an 61.83% death rate after 72 hours of exposure due to their nanosized structure, which is higher than the plant extract but lower than the synthetic pesticide.

Keywords: Medicinal Plants, Nanoprecipitation, Nanobiopesticides, Nanosuspensions, Tribolium Castaneum.

INTRODUCTION

Pesticides are applied to plants to keep them healthy and manage pests. These chemicals most commonly contain organophosphates, carbamates, derivatives of carbamides, and chlorinated hydrocarbons as active ingredients (Cuenca *et al.*, 2020). These substances are frequently employed in agriculture, health, and veterinary care. They cause environment pollution and harm to humans and animals by ingestion, inhalation, and skin absorption (Abbasi-Jorjandi *et al.*, 2020). The limitations of traditional pesticide formulations are numerous and include dust drift, limited dispersibility, long soil retention times, high organic solvent content, and other related issues. Because of these restrictions, just 1% of pesticides remain on surfaces and get released into the atmosphere. The environment is severely contaminated as a result of this inefficiency. Consequently, minimizing pesticide waste, production expenses, and emissions into the environment is important, especially prolonging the time that pesticides are effective on crops (Sun *et al.*, 2016).

Pest control agents known as biopesticides are derived from plants and microbes. Although synthetic pesticides still provide significant protection for agricultural crops, prolonged use of them can have detrimental impacts on the environment, including long-term stability and carcinogenicity. It became important to produce new pesticides in order to address these challenges (Jampílek and Kráľová, 2019). Biopesticides originate from natural sources, including plants, animals, microbes, and minerals. Eco-friendly alternatives to traditional pesticides include those that are less toxic, biodegradable, specialised to pests, and need less application (Barua *et al.*, 2020). Medicinal plants are high in phytochemicals, which are capable of being structurally improved and converted into new medications. Nigeria has a varied assortment of medicinal herbs, and cooperative study has demonstrated their usefulness. Medicinal plants are also a major component of many contemporary medications (Rasool *et al.*, 2020).

Within the Lamiaceae family, Mentha piperita is a significant fragrant and medicinal plant. Secondary metabolites were discovered to be abundant in Mentha piperita extract. Menthone, methyl acetate, terpinene, menthofuran, tannin, the compound pinene, menthol, isomenthol, and so on are the principal constituents of essential oils (EOs) (Roshanpour *et al.*, 2021). Ancient Egyptians cultivated mentha piperita leaves, which were acknowledged in the 13th-century Icelandic pharmacopoeia. Growing widely in temperate regions of the world, especially in North Africa, and Europe, but currently grown everywhere in the globe. (Jahan *et al.*, 2024). The Arjun tree, Terminalia arjuna, yields arjunic acid, arjungenin, and arjunglucoside, among other triterpenoid secondary metabolites that are derived from the fundamental oleanane triterpene skeleton. Native to India and the surrounding nations, T. arjuna bark has long been used in traditional medicine (Shalini *et al.*, 2015).

Insect control using natural compounds, such as plant extracts, has been investigated recently in an effort to reduce the consumption of synthetic pesticides and prevent environmental damage. In many parts of the world, Lawsonia inermis, also known as henna, has been widely utilized for ages as a medicine. Lawsone (C10H6O3), the active component, and a naturally generated naphthoquinone are both present in henna extract. The creation of novel antibacterial substances is a crucial field of study. Globally, the prevalence of antimicrobial resistance in important microbial diseases is rising at an alarming rate. Numerous laboratories have taken on the issue of creating derivatives of naturally occurring antibacterial naphthoquinones in order to enhance their medicinal qualities (El-Hag *et al.*, 2007).

Ashwagandha, or Withania somnifera (Family: Solanaceae) is a plant which is widely employed in the Indian Ayurvedic and Unani medical systems, due to its therapeutic qualities, Numerous different phytochemicals, including alkaloids, steroidal and flavanoids, are present in this therapeutic plant (Dar *et al.*, 2015).Considering the economic significance of T. castaneum and its extremely detrimental effects on grains that result in massive losses of stored goods, it has been deemed worthwhile to examine the effects of W. somnifera root extract on this infamous pest's metamorphosis in order to determine whether or not it can be used in an insect management programme (Gaur and Kumar, 2020).

Biopesticides are categorised into three types based on their active ingredients and method of action: microbial pesticides, biological pesticides, and plant-incorporated safeguards (Daraban *et al.*, 2023). For a variety of reasons nano-biopesticides are superior to biopesticides and traditional methods (Sayed *et al.*, 2017). One of the main sources of biopesticides based on nanoparticles is plants, which use various forms of nanoparticles being a component of pesticides in agricultural applications (Krishnamurthy *et al.*, 2020). Nanobiopesticides outperform traditional biopesticides in terms of accessibility, biocompatibility, and sustainability, with no precocious degradation. These variables improve pesticide stability and promote sustainable use, certain environmental triggers cause the release of active substances. Nanopesticides need minimal doses and are environmentally beneficial (Mali *et al.*, 2020; Hazafa *et al.*, 2022). The safe and environmentally benign nature of biopesticides led to their consideration (Abdollahdokht *et al.*, 2022).

Biopesticides work by a variety of ways, such as blocking and destroying the pathogens' or pests' plasma membranes and translation of proteins. In contrast to synthetic pesticides, biopesticides are highly targeted, have limited shelf life, remain less stable within the soil environment, and are made from biodegradable raw materials, despite a few disadvantages that have decreased their acceptance and commercial application (Kumar *et al.*, 2022). A few of the previously listed benefits of biopesticides could also be considered their drawbacks. For instance, if controlling multiple insects at once is the goal, then the specificity of their response to pests may be hazardous. Additionally, because of their short shelf life, they degrade more quickly and are less persistent in the atmosphere, but this becomes a disadvantage if the aim is to eradicate all current pests and stop future pest growth following the application of biopesticides. It is now crucial to evaluate these benefits and downsides critically and consider potential solutions to these apparent shortcomings (Ayilara *et al.*, 2023).

Nanosuspensions are micron-sized colloidal mixtures of nanoparticles stabilised by surfactants. Numerous studies have demonstrated that nanoemulsions of oranges with a sweet flavor, garlic, trees made from e citronella, and neem extracts may act as botanical pesticides (Hassanshahian *et al.*, 2020).The fluctuating state of nanosuspension production is a complicated interplay between a number of variables, namely functional groups and surface free energy. The durability of the nanosuspensions and the effectiveness of the chosen methodology may be determined by the stabilizer's faster surface adsorption rate as compared to the freshly formed surface during different preparation techniques (Jacob *et al.*, 2020).

2. MATERIALS AND METHODS

2.1 Formulation of Mentha Piperita based Nanobiopesticide

2.1.1 Preparation of Plant Extract

The Mentha piperita leaves were purchased from local market Faisalabad. The leaves were glabrous and had shades of green on top and pale green below. For this process, a round-bottom flask was filled with 200 mL of ethanol (solvent), 20 g of plant leaves powder was put in the Soxhlet apparatus, and the extraction process was carried out for 10 hours. Ethanol was evaporated in open air prepared plant extract was paced in refrigerator at4 °C (Jahan *et al.*, 2024).

2.1.2 Formulation of Nanosuspensions

The solvent/antisolvent precipitation approach was used to create Mentha piperita extract nanosuspensions (Aslam *et al.*, 2020). In short, 0.2g of Mentha piperita extract was diluted in 10 ml of ethanol. An aqueous solution (100 mL) containing a stabilizer (0.4g), such as sodium lauryl sulfate, Tween-80, polyvinyl alcohol or polyethylene glycol was filled with ethanolic extract (organic phase) with double than the concentration of plant extract, dropwise (1 drop/s) using a dropper. The mixture was continuously stirred for 10 hours at 1000 rpm. Previous method was used with some modifications (Zafar *et al.*, 2022).

The improved nanosuspensions were tested for physical stability during a three-month time period at two different temperatures: ambient temperature (25–30 °C) and refrigerator temperature (4 °C) (Baliyarsingh *et al.*, 2021).

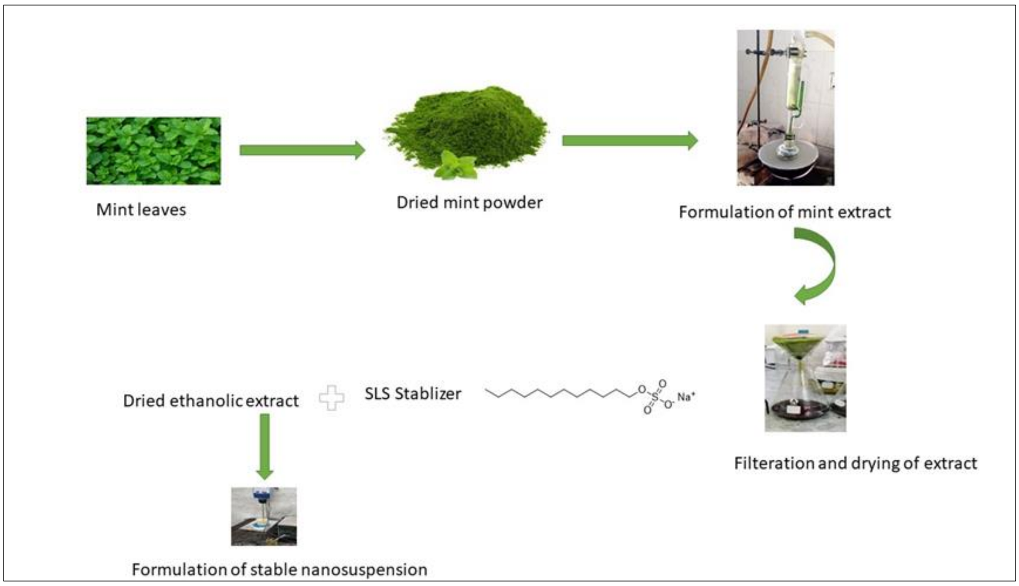


Figure 2.1.2: Formulation process of Mentha piperita based nanobiopesticides

2.2 Formulation of Lawsonia inermis based Nanobiopesticide

2.2.1 Preparation of Plant Extract

Lawsonia inermis leaves were purchased from local market Faisalabad. After being cleaned Lawsonia inermis leaves were allowed to dry in the shade and crushed into a powder form. Plant extraction was carried out with ethanol as a solvent using Soxhlet apparatus and the extraction process was carried out for 10 hours.

Ethanol was evaporated in open air and prepared plant extract was paced in refrigerator at 4 °C.

2.2.2 Formulation of Nanosuspensions

The solvent/antisolvent precipitation approach was used to create Lawsonia inermis leaves nanosuspensions. 0.2g of Lawsonia inermis extract was diluted in 10 mL of ethanol. An aqueous solution (100 mL) with stabilizer, such as sodium lauryl sulfate, Tween-80 or polyethylene glycol was filled with ethanolic extract (organic phase) dropwise (1 drop/s) using a dropper. The solution mixture was continuously stirred for 10 hours at 1000 rpm. The formulated nanosuspensions' particle size (nm) and the polydispersity index (PDI) values were measured with a Malvern zeta sizer. A stable nanosuspension of Lawsonia inermis leaves was observed with SLS.

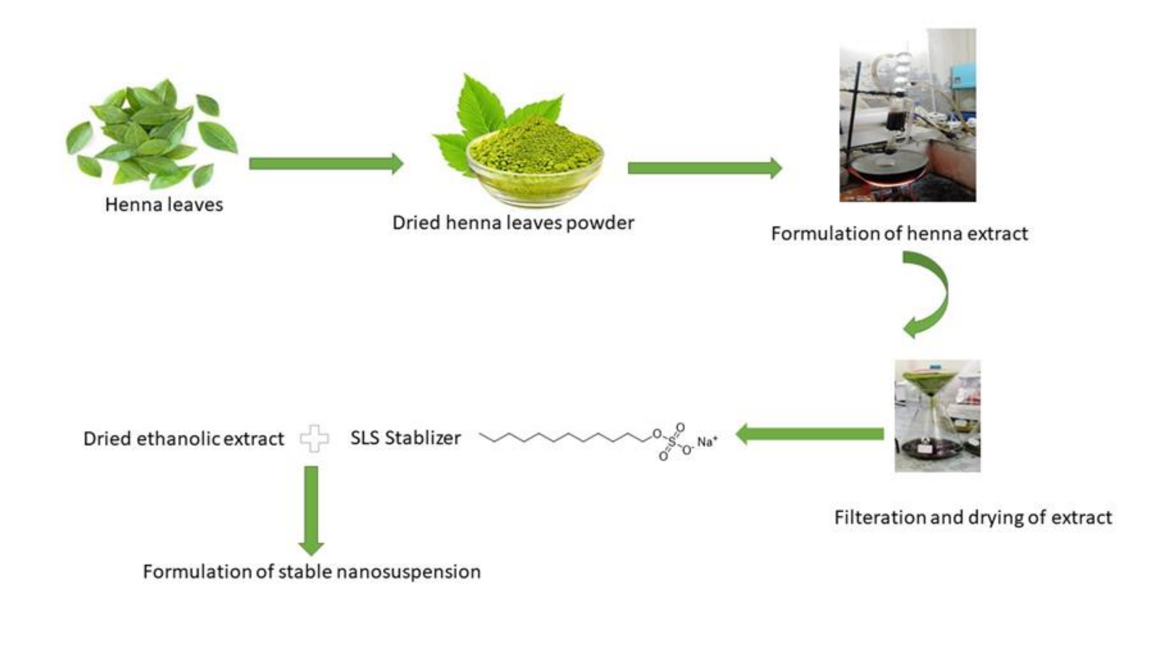


Figure 2.2.2: Synthesis of Lawsonia inermis based nanobiopesticides

3.1 Preparation of Plant Extract

The annual plant known as sweet basil (Ocimum basilicum L.) is native to many parts of the world. One of the most significant ingredients in food is the plant. Ocimum basilicum leaves were collected form horticulture farm of University of Agriculture, Faisalabad. After being cleaned, Ocimum basilicum were allowed to dry in the shade and crushed into a powder form. Plant extraction was carried out with ethanol as a solvent using Soxhlet apparatus and the extraction process was carried out for 10 hours. Ethanol was evaporated in open air and prepared plant extract was paced in refrigerator at 4 °C (Zafar *et al.*, 2019).

2.3.2 Formulation of Nanosuspensions

The solvent/antisolvent precipitation approach was used to create Ocimum basilicum nanosuspensions. In short, different concentrations of basil extract was diluted in 10 mL of ethanol. An aqueous medium (100 mL) containing a stabilizer, such as sodium lauryl sulfate, Tween-80, polyvinyl alcohol or polyethylene glycol was filled with ethanolic extract (organic phase) dropwise (1 drop/s) using a dropper. The mixture was constantly agitated for 10 hours at 1000 rpm. The improved nanosuspensions were tested for physical stability during a three-month period at two different temperatures: ambient temperature (25–30 °C) and refrigerator temperature (4 °C) (Baliyarsingh *et al.*, 2021).

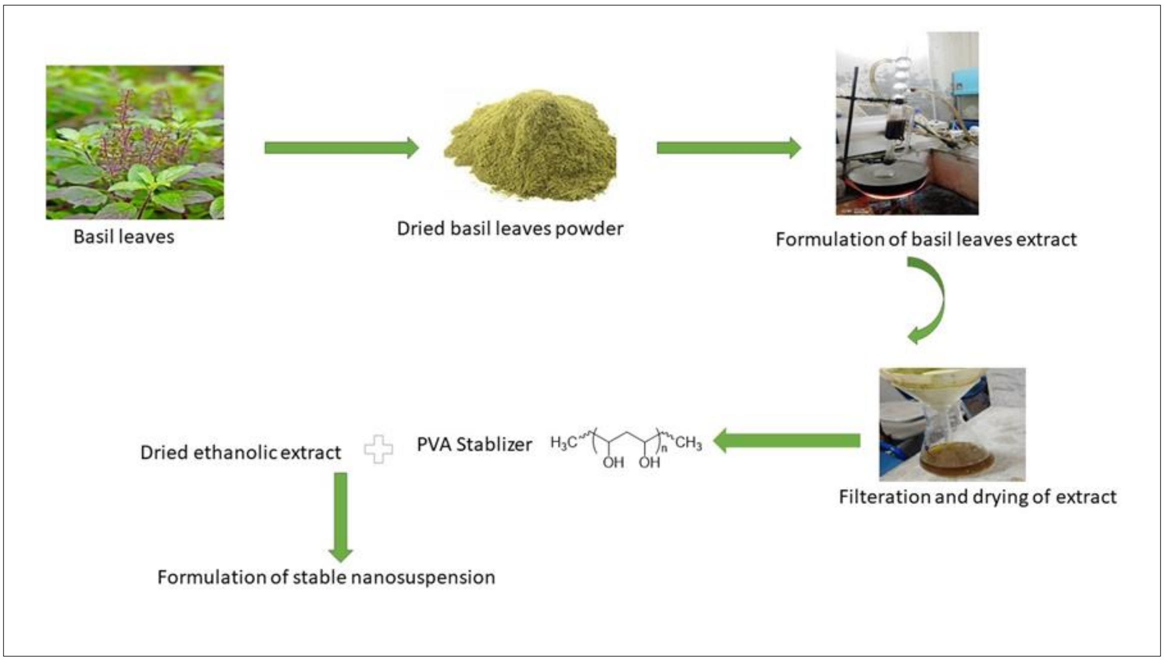


Figure 2.3.2: Formulation of Ocimum basilicum based nanobiopesticides

2.4 Formulation of Terminalia Arjuna Based Nanobiopesticide

2.4.1 Preparation of Plant Extract

T. arjuna (bark) was gathered form Botanical garden University of Agriculture, Faisalabad. After being cleaned, T.arjuna bark were allowed to dry in the shade and crushed into a powder form. Plant extraction was carried out with ethanol as a solvent using Soxhlet apparatus and the extraction process was carried out for 10 hours. Ethanol was evaporated in open air and prepared plant extract was paced in refrigerator at 4 ◦C (Zafar *et al.*, 2019).

2.4.2 Formulation of Nanosuspensions

The solvent/antisolvent precipitation approach was used to create T. arjuna nanosuspensions. In short, different concentrations of T. arjuna extract was diluted in 10 mL of ethanol. An aqueous medium (100 ml) containing a stabilizer, such as sodium lauryl sulfate, Tween-80, polyvinyl alcohol or polyethylene glycol was filled with ethanolic extract (organic phase) dropwise (1 drop/s) using a dropper. The mixture was mechanically stirred for 10 hours at 1000 rpm.

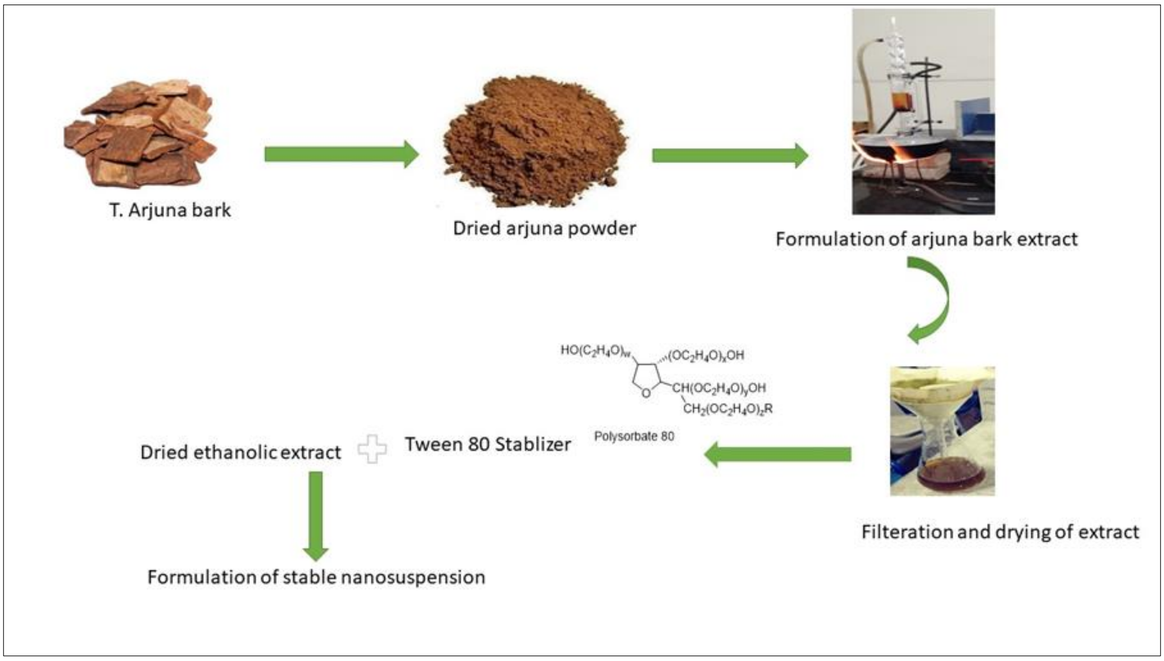


Figure 2.4.2: Formulation of Terminalia arjuna based Nanobiopesticide

2.5 Formulation of Withania Somnifera Roots Based Nanobiopesticide

2.5.1 Preparation of Plant Extract

Withania somnifera roots were purchased from local market Faisalabad. After being cleaned, withania somnifera roots were allowed to dry in the shade and crushed into a powder form. Plant extraction was carried out with ethanol as a solvent using Soxhlet apparatus and the extraction process was carried out for 10 hours. Ethanol was evaporated in open air and prepared plant extract was paced in refrigerator at4 °C.

2.5.2 Formulation of Nanosuspensions

The solvent/antisolvent precipitation approach was used to create Withania somnifera roots nanosuspensions. In short, different concentrations of basil extract was diluted in 10 ml of ethanol. An aqueous medium (100 ml) containing a stabilizer, such as sodium lauryl sulfate, Tween-80, polyvinyl alcohol or polyethylene glycol was filled with ethanolic extract (organic phase) dropwise (1 drop/s) using a dropper. The mixture was mechanically stirred for 10 hours at 1000 rpm.

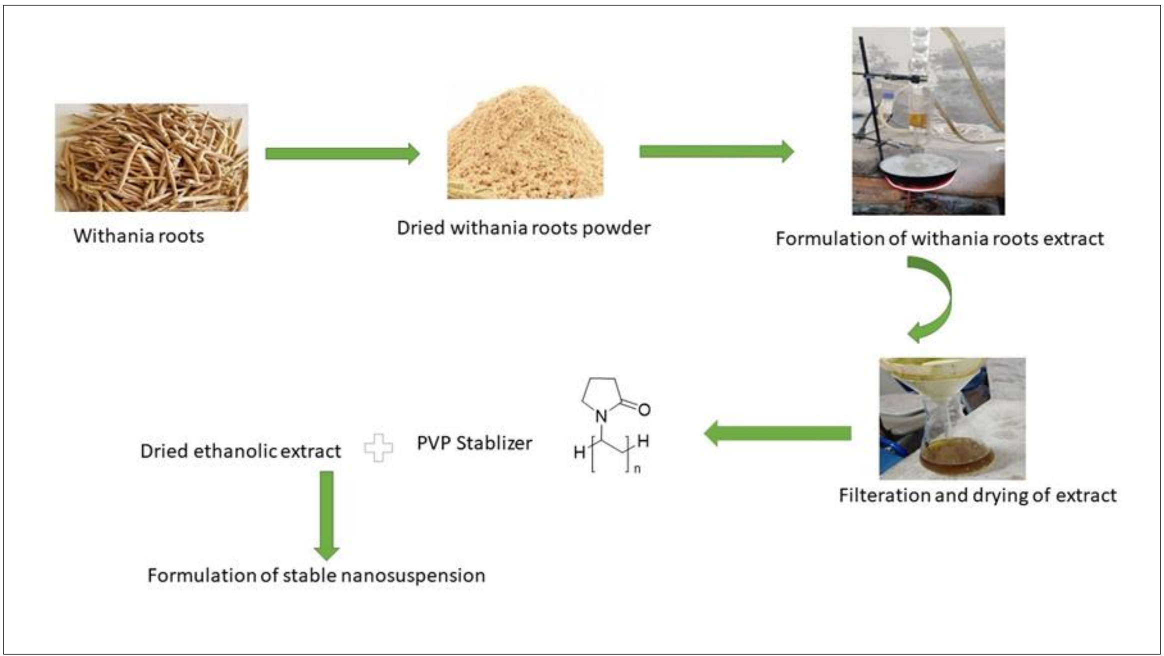


Figure 2.5.2: Synthesis of Withania somnifera roots based Nanobiopesticide

2.6 Applications of Formulated Nanobiopesticides and Plant Extracts

The formulated plant extracts and nanosuspension were applied on tribolium casteneum insects and the efficiency of formulated plant extracts and nanosuspensions were compared with synthetic pesticide.

2.6.1 Collection of Insects

The applications of formulated nanobiopesticides and plant extracts was performed on tribolium castaneum insects. Red flour beetles (tribolium castaneum) was acquired from the Department of Enatamolgy of Agricultural university Faisalabad. The most damaging insect to items that are kept in storage is Tribolium castaneum (Herbst 1797), also referred to as the red flour beetle. It is distributed globally and has a higher frequency of subjects (Faroni and SOUSA, 2006)

2.6.2 Preparation of Plant Extracts and Nanopesticides Dilutions

In addition to a control group, several concentrations of medicinnal plants leaves extract and nanosuspensions (50ung, 100mg/dl, 130mg/ml, and 200mg/ml) were used.

2.6.3 Bioassays for Contact Toxicity of Insects

Fitter sheets, cut to the size of petri plates, were sprayed with various quantities of plant extract (50mg/ml, 100mg/ml, 150mg/ml, and 200mg/ml), as well as nanosuspension (50mg/ml, 100mg/ml, 150mg/ml, and 200mg/ml). After that, the treated filter sheets were put into the petri dishes. Distilled water was utilized in the process for best outcomes. Adult Tribolium castaneum underwent a preliminary screening at various doses in order to get 0% to 100% mortalities. After that, insects were carefully placed on separate labeled petri dishes. The Insects in the petri plates were then moved to a growth chamber, and their contact toxicity was measured at 12, and 24-hour intervals, respectively.

2.7 Statistical Analysis

Analysis of variance (ANOVA) was used to assess the effectiveness of nanosuspension and the plant extract dilution. These trials were repeated three times to measure accuracy and reliability. Insect mortality was determined by the mathmetical equatin given below.

Percentage mortality = no of insects killed/Total no of insects taken × 100

4. RESULTS AND DISCUSSIONS

4.1 Utilizing Stabilizers to Formulate Stable Nanosuspension of Lawsonia Inermis Leaves Extract

Nanobiopesticides based on Lawsonia inermis were developed as nanosuspensions. Various nanosuspensions of the Lawsonia inermis leaf extract were prepared using medicinally suitable stabilizers, such as Tween-80, PVP, and SLS. At room temperature, the nanosuspensions made with PVP, and PVA were unstable, whereas the ones with SLS stayed stable.

4.1.2 FTIR Spectrum of Lawsonia Inermis Based Nanobiopesticide

FTIR spectroscopy is utilized to identify the various functional groups.

It's efficacy and capacities enable the detection of adulterants and suspicious components in drugs beverage and food materials e.t.c. Previous contributions revealed that mid-infrared spectroscopy might be used to control the quality of high-valued organic extracts including essential oils (Bounaas *et al.*, 2018).

The FTIR study of Ag-NPs exhibited broad peaks at 1647 cm−1, indicating carboxyl groups of C-C stretching vibrations and amide I bands in proteins (Said *et al.*, 2024). Broad bands from 3167 to 3640 cm−1 indicate the N-H group of protein and phenolic O-H stretching vibrations of alcohols (Baptista *et al.*, 2018).

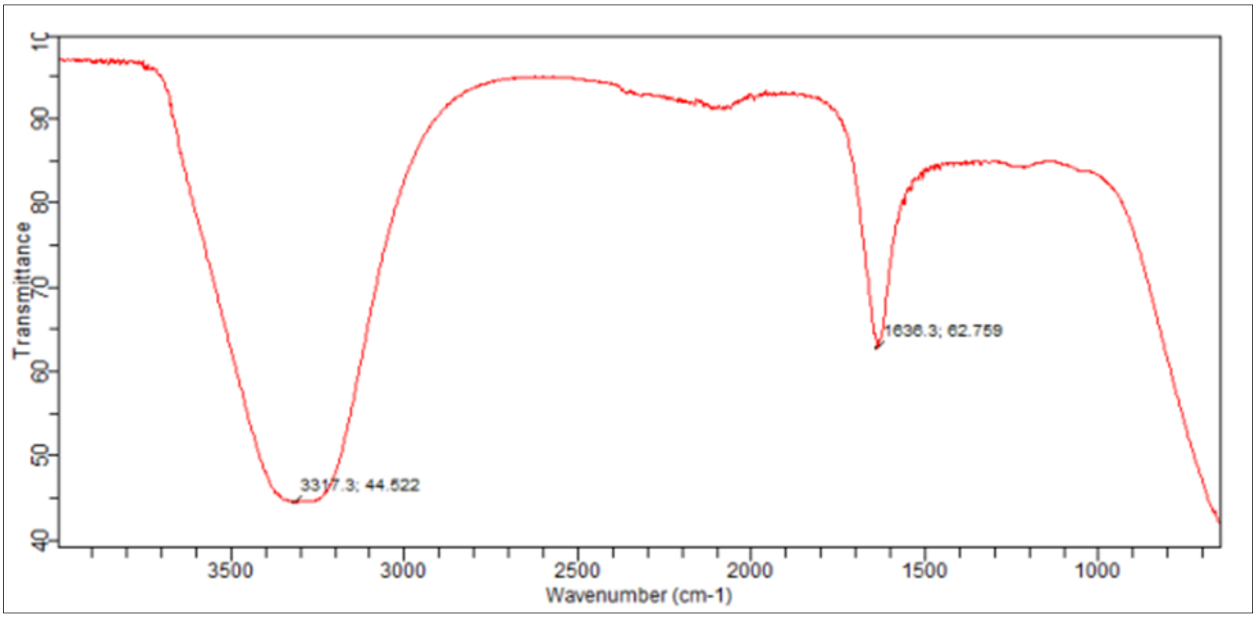


Figure 4.1.2: FTIR spectrum of Lawsonia inermis based nanobiopesticide

4.1.3 Zeta Size Analysis of L. Inermis Based Nanobiopesticide

The FTIR spectrum of Lawsonia Inermis nanosuspension revealed intense peak at 3317 cm, demonstrating the existence of the O-H stretch characteristic of alcohol. Another prominent peak at 1636.3 cm revealed the existence of the carbon-oxygen double bond, with the C-O (carbonyl group) stretch observed in this region. Additionally, the broad peak at 1077.69 cm demonstrate the C-OH bond, that is equivalent to the main alcohol found in the ethanolic extract of Lawsonia Inermis. This finding demonstrates that L. inermis based nanopesticides have greatest pesticidal activity among all the synthesized nanobiopesticides.

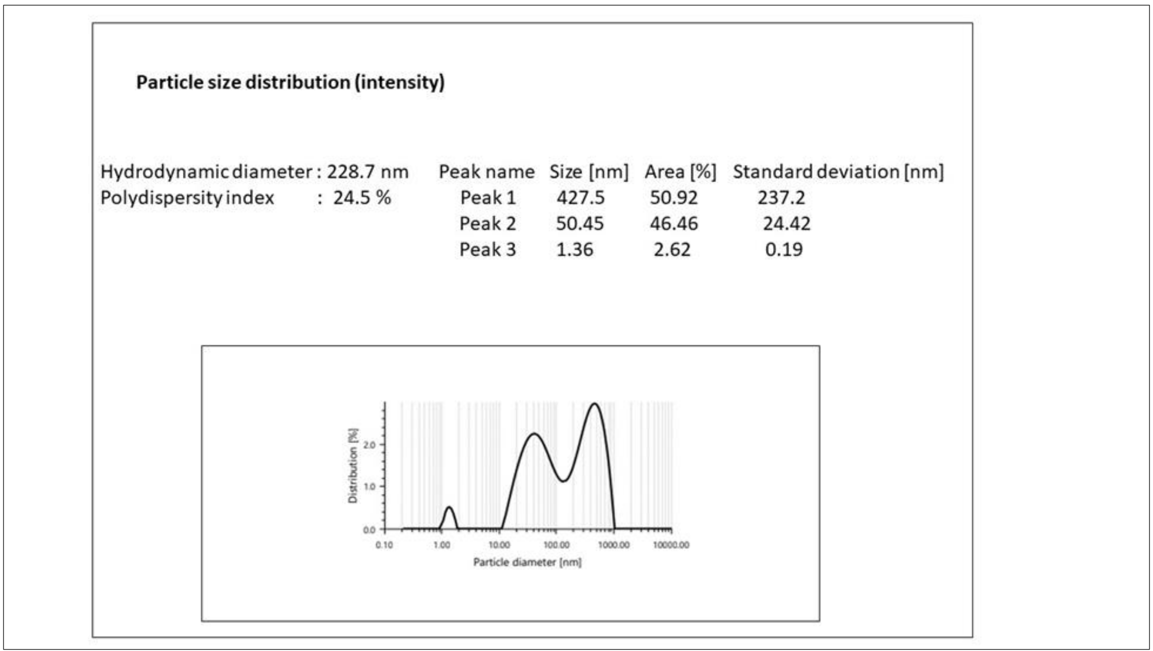


Figure 4.1.3: PDI and particle size values of most suitable L. inermis based nanosuspension

This research comprises the creation, optimization, and assessment of Cilnidipine (CLN) nanoformulation to improve rate of dissolution and accessibility. Furthermore, the effectiveness of the enhanced formulation was studied and compared to the marketed product. The improved nanoformulation showed excellent outcomes for average particle size 280.1 nm and 0.176 % PDI value (Shaikh *et al.*, 2022).

The aim of this study was to develop a posaco nazole nanosuspension through the wet milling technique. Posaconazole belongs to the triazole class of antifungal drugs. The refined nanoparticles had an average particle diameter of 395.7 nm and a PDI of 0.30% (Kolipaka *et al.*, 2023). This work evaluated the state of equilibrium solubility, and dissolution factor of Isoxanthohumol (IXN), as well as the formulation of IXN nanoparticles (IXN-Nps) utilizing a small-scale media milling process. The study analyzed the nanoparticles' particle size, shape, polydispersity index, and structure to determine the best cryoprotectant. IXN-Nps' showed an average particle size of 249.500 nm, with 0.149% polydispersity index (Zhang *et al.*, 2024).

4.2 Pesticidal Activity

Effect of Different Concentration of Lawsonia Inermis Extract and Nanosuspensions on Red Flour Beetle (T. castaneum)

Effect of different concentration of nanosuspensions and plant extract on red four beetle, for the assessment of the mortality rate of them, adult Insects were released into every petri dish brearing filter sheets that had been coated with nanosuspension and prepared cont of plant extract, mortality data was noted accordingly. Different concentrations were employed. (50mg/ml, 100mg/ml, 150mg/ml, and 200mg/ml) and mortality rate was taken after exposure of 24,48 and 72 hours (da Silva *et al.*, 2021).

The mortality rate (%) was calculated using the following formula:

$Ma(\%) = (Mp - Mc) \times 100$

Where Mp is the observed mortality rate of insect treatments (%), Mc is the death rate of the control (%), and Ma is the actual death rate (%).

Table displays the results of the experiment where the pesticidal activity of Lawsonia inermis plant extract, nanosuspension, and Pyriproxyfin against T. castaneum was tested There was a substantial pesticidal effect seen in the evaluation of the various goups being treated, including the plant extract, nanosuspension, and control. As the conc of plant extract, nanosuspension, and synthetic control increased, the average death rate rose. The mortality rate was also higher when the exposure time was longer. The pest known as Tribolium castaneum, or red flour beetle, is found in flour mills, cereal goods, and dried food storage areas. It is a worldwide pest. Synthetic insecticides are the major tool used to control pest infestations, but if they are applied carelessly, insects become resistant to them and may have an impact on both the environment and health. The significant concentrations of pesticidal bioactive phytochemicals in the nanobiopesticides provide them good promise as green biopesticides. A reduced particle size is essential for insecticidal action because it facilitates faster pesticide uptake through the insect membrane (Heydari *et al.*, 2020; Mohafrash *et al.*, 2020).This indicates that as the exposure time to Lawsonia inermis extracts increased, the mortality rate of T. Castaneum gradually, reaching its peak at the highest concentration.

Table 4.2.1: Average % mortality of Tribolium Casteneum (Lawsonia inermis)

Treatments	**Concentration (mg/ml)**	**% Average morality**		
		24 hour	**48 hour**	**72 hour**
Plant extract	50	9.16 ± 1.04^{At}	18.16 ± 1.25^{Bs}	25.33 ± 1.04^{Cpq}
	100	13.16 ± 0.76^{As}	21.5 ± 0.5^{Br}	32.5 ± 0.5^{Cn}
	150	23.5 ± 0.5^{Aqr}	32.5 ± 0.5^{Bn}	41.5 ± 0.5^{Ckl}
	200	33.5 ± 0.5^{An}	42.66 ± 0.58^{Bk}	51.83 ± 0.76^{Chi}
Nanosuspension	50	18.5 ± 0.5^{As}	27.5 ± 0.5^{Bo}	38.17 ± 0.29^{Cm}
	100	26.5 ± 0.5^{Aop}	32.5 ± 0.5^{Bn}	41.83± 0.76^{Clm}
	150	31.5 ± 0.5^{An}	41.67 ± 0.58^{Bkl}	52.83 ± 0.76^{Ch}
	200	42.5 ± 0.5^{Ak}	56.5 ± 0.5^{Bg}	61.83 ± 0.76^{Ce}
Pyriproxyfen	50	27.57 ± 0.5^{Ao}	38.5 ± 0.5^{Bm}	46.83 ± 0.76^{Cj}
	100	39.83 ± 0.76^{Alm}	48.17 ± 0.29^{Bij}	57.83 ± 0.76^{Cfg}
	150	49.5 ± 0.5^{Ai}	59.33 ± 0.58^{Be}	65.5 ± 0.5^{Cd}
	200	72.5 ± 0.5^{Ac}	80.83 ± 0.76^{Bb}	89.17 ± 0.76^{Ca}

The data is presented as mean SD, which represents the average inhibition zone Means in each column with identical capital letters are not substantially different from or mother, and within every row, means with the same lowercase letter are also significantly different. The Tukey test, with a degree of significance of P<0.05, is the statistical analysis performed to determine these differences.

The analysis revealed that at a higher concentration, the mean mortality rate of T. castaneum caused by Lawsonia inermis plant extract was 51.83 ± 0.76 and for Lawsonia inermis nanosuspension, it was 61.83 ± 0.76. This indicates that as the exposure time to Lawsonia inermis nosuspensions and plant extracts increased, the mortality rate of T. castaneum gradually increased, reaching its peak at the highest concentration. This finding supports the idea that exposure time determines. the susceptibility of T. castaneum to treatments.

Results demonstrated that the Lawsonia inermis nanosuspension had an 61.83% death rate after 72 hours of exposure, which is higher than the plant extract but lower than the synthetic control. Pyriproxyfin had a maximüm death rate of 89.17%. Pesticidal action is attributed to active elements of plant such as lawsone, flavonoids and saponins As a result, it concluded that the percentage of mortality had a direct relationship with the concentration of plant extract.

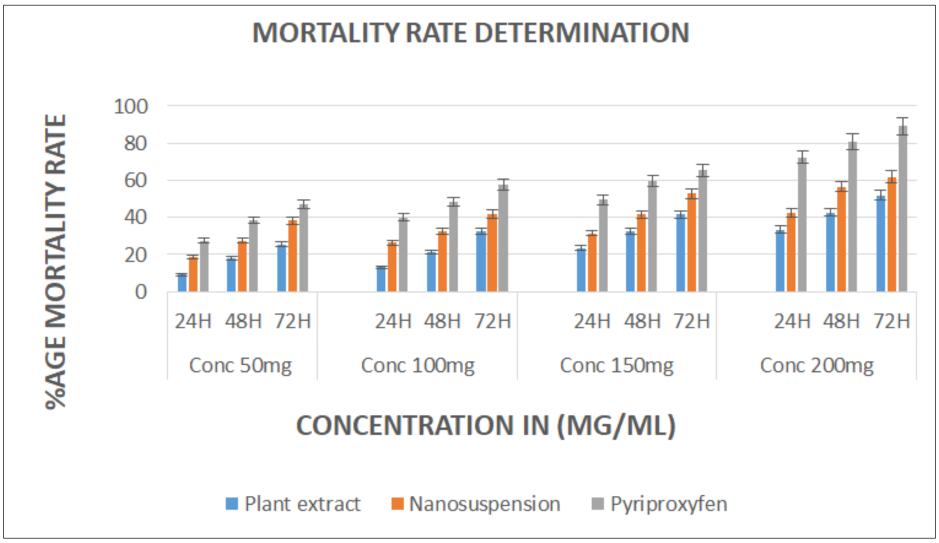


Figure 4.2.2: Average % mortality rate of Lawsonia inermis extract, nanobiopesticide and pyriproxyfen against T. castaneum at different concentration after 24 hours,48 hours and 72 hours

4.3 Utilizing Stabilizers to Formulate Stable Nanosuspension of Mentha Piperita Leaves Extract

Nanobiopesticides based on Mentha piperita were developed as nanosuspensions. Various nanosuspensions of the Mentha piperita leaf extract were prepared using medicinally suitable stabilizers, such as Tween-80, PVP, SLS, and PEG. At room temperature, the nanosuspensions made with PVP, PVA, and polyethylene glycol were unstable, whereas the ones with Tween-80 and SLS stayed stable. The stabiliser is an important component in keeping nanosuspensions physically stable. A larger surface area particle size may raise the system's free energy (ΔG) and interfacial tension, which are in charge of the aggregate of particles or the formation of crystals. By lowering surface tension and free energy, surfactants stop particle aggregation and avert Ostwald ripening (Aslam *et al.*, 2020). The formulation of nanobiopesticides requires the use of a stabilizer because they create steric inhibition or electrostatic repellent to stabilize the smaller particles.

4.3.1 FTIR spectrum of Mentha Piperita Based Nanobiopesticide

Previous research indicated that the spectrum bands of 3100 to 3000 cm−1 and 3150 to 3050 cm−1 comprised vibrations coming from the stretching of C-H groups (Boughendjioua and Boughendjioua, 2017).

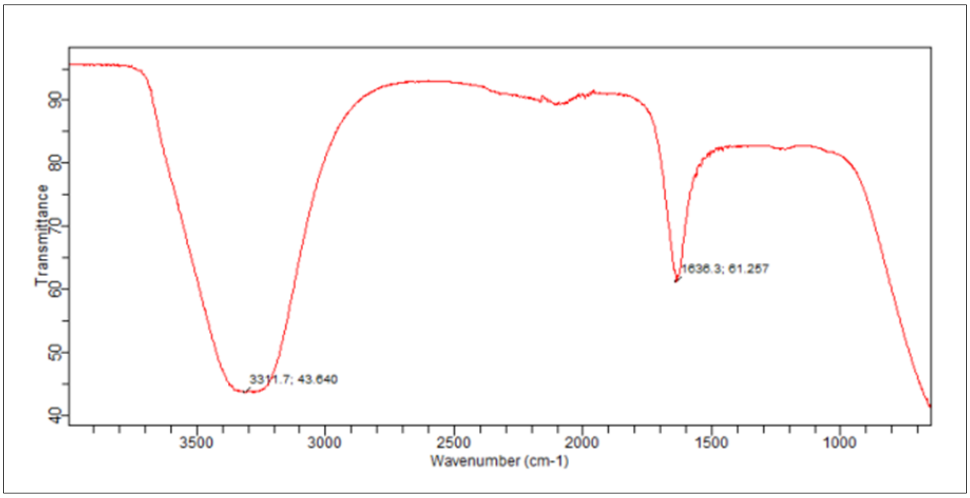


Figure 4.3.1: FTIR spectrum of Mentha piperita based nanobiopesticide

The FTIR spectrum of Mentha piperita nanosuspension revealed significant peak at 3311 cm, demonstrating the existence of the O-H stretch characteristic of alcohol. Another prominent peak at 1636.3 cm revealed the existence of the carbon-oxygen double bond, with the C-O (carbonyl group) stretch observed in this region. Additionally, the broad peak at 1077.69 cm demonstrate the C-OH bond, that is equivalent to the main alcohol found in the ethanolic extract of Mentha piperita.

4.3.2 Mortality Rate Determination

Table 4.3.3: Average % mortality of *Tribolium Casteneum (*Mentha pipertia*)*

Treatments	Concentration (mg/ml)	% Average morality		
		24 hours	48 hours	72 hours
Plant extract	50	7.5 ± 0.5 A	16.33 ± 1.15 Bt	24.67 ± 0.58 Cr
	100	12.5 ± 0.5 A	19.5 ± 0.5 Bs	27.17 ± 0.29 Cpq
	150	17.83 ± 0.76 Ast	25.5 ± 0.5 Bqr	32.17 ± 1.26 Co
	200	26.33 ± 0.58 Apqr	34.83 ± 0.76 Bmn	41.83 ± 0.76 Cjk
Nanosuspension	50	17.5 ± 0.5 Ast	24.33 ± 0.58 Br	37.5 ± 0.5 Cm
	100	27.83 ± 1.04 Ap	39.83 ± 0.76 Bkl	47.33 ± 0.58 Chi
	150	42.33 ± 0.58 Aj	48.5 ± 0.5 Bhi	59.83 ± 0.76 Cf
	200	46.83 ± 0.76 Ai	56 ± 1 Bg	61.67 ± 0.58 Ce
Pyriproxyfen	50	27.57 ± 0.5 Ao	38.5 ± 0.5 Bm	46.83 ± 0.76 Cj
	100	39.83 ± 0.76 Alm	48.17 ± 0.29 Bij	57.83 ± 0.76 Cfg
	150	49.5 ± 0.5 Ai	59.33 ± 0.58 Be	65.5 ± 0.5 Cd
	200	72.5 ± 0.5 Ac	80.83 ± 0.76 Bb	89.17 ± 0.76 Ca

The data is presented as mean SD, which represents the average inhibition zone Means in each column with identical capital letters are not substantially different from or mother, and within every row, means with the same lowercase letter are also significantly different. The Tukey test, with a degree of significance of P<0.05, is sutistical analysis performed to determine these differences. Table displays the results of the experiment where the pesticidal activity of Mentha pipertia plant extract, nanosuspension, and Pyriproxyfin against T. castaneum was tested. There was a substantial (p<0.001) pesticidal groups being treated, including the plant extract, nanosuspension, and control. As the conc. of plant extract, nanosuspension, and synthetic control increased, the average death rate increased. The mortality rate was also higher when the exposure time was longer.

The analysis revealed that at a higher concentration, the mean mortality rate of 7. castaneum caused by *Lawsonia Inermis* plant extract was 41.83 ± 0.76 and for Mentha pipertia nanosuspension, it was 62.67 ± 0.58. This indicates that as the exposure time to Mentha pipertia nanosuspensions and plant extracts increased, the mortality rate of 7. castaneum gradually rose, reaching its peak at the highest concentration. This finding supports the idea that exposure time determines. the susceptibility of 7. castaneum to treatments. Results demonstrated that the Mentha pipertia nanosuspension had an 61.67% death rate after 72 hours of exposure, which is higher than the plant extract but lower than the synthetic control. Pyriproxyfin had a maximüm death rate of 89.17%.

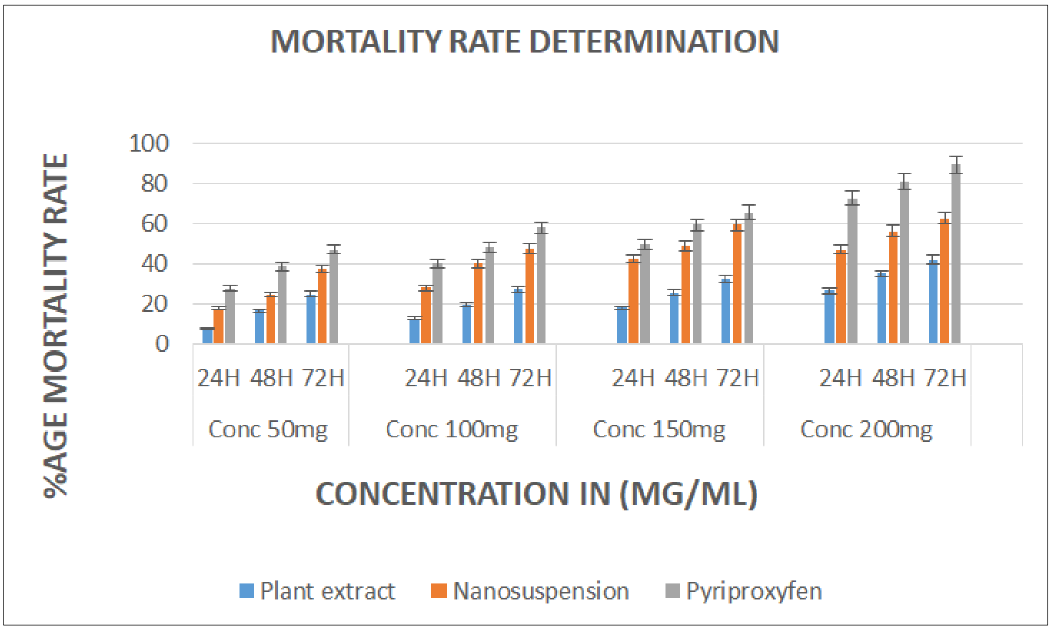


Figure 4.3.2: Average % mortality rate of Mentha pipertia extract, nanobiopesticide and pyriproxyfen against T. castaneum at different concentration after 24 hours,48 hours and 72 hours

4.4 Utilizing Stabilizers to Formulate Stable Nanosuspension of Ocimum basilicum

Nanobiopesticides based on Ocimum basilicum were developed as nanosuspensions. Various nanosuspensions of the Ocimum basilicum leaves extract were prepared using pharmaceutically suitable stabilizers, such as Tween-80, PVA, PVP and SLS. At room temperature, the nanosuspensions made with Tween-80, PVP and SLS were unstable, whereas the ones with PVA stayed stable.

4.4.1 FTIR Spectrum of Ocimum Basilicum Based Nanobiopesticide

The occurrence of the band at 2930 cm-1 and 1387 cm-1 may be attributed to CH2 stretching in hemicellulose and CH vibrations, which normally occur in carbohydrates and lignans present in great quantities in leafy materials, especially basil (Ramos *et al.*, 2020).

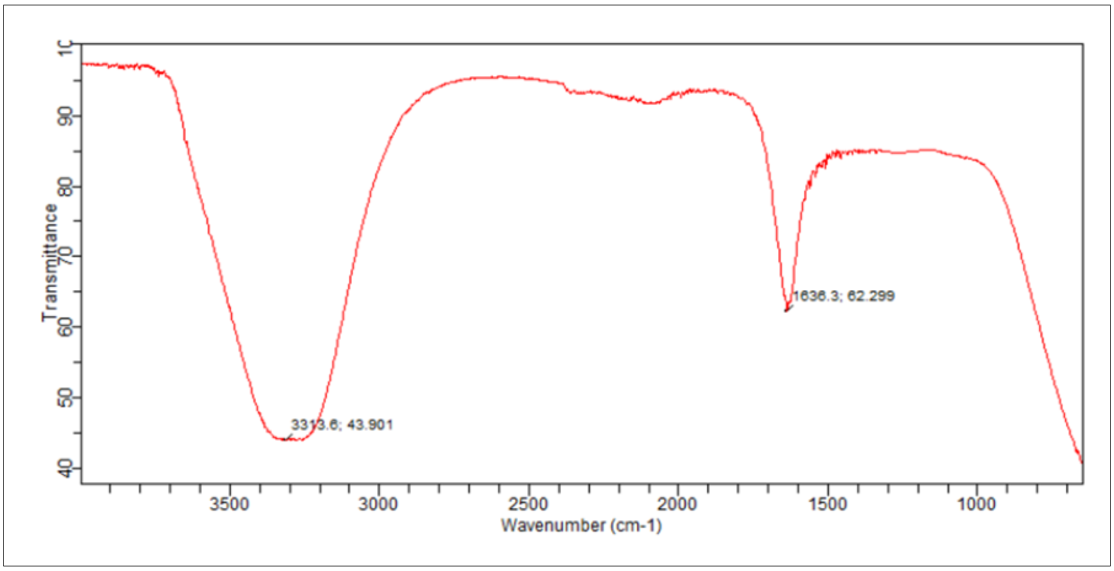


Figure 4.4.1 FTIR spectrum of Ocimum basilicum based nanobiopesticide

The FTIR spectrum of Osimum basilicum nanosuspension revealed significant peak at 3313.6 cm, demonstrating the existence of the O-H stretch which is characteristic of alcohol. Another prominent peak at 1636.3 cm revealed the existence of the carbon-oxygen double bond, with the C-O (carbonyl group) stretch observed in this region.

4.4.2 Mortality Rate Determination

Table 4.4.2: Average % mortality of Tribolium Casteneum (Osimum bacilium)

Treatments	Concentration(mg/ml)	% Average morality		
		24 hours	48 hours	72 hours
Plant extract	50	6.83 ± 0.76^{As}	14.5 ± 0.5^{Br}	21.5 ± 0.5^{Cq}
	100	12.83 ± 0.29^{Ar}	17.33 ± 0.58^{Bq}	27.5 ± 0.5^{Co}
	150	17.83 ± 0.76^{Aq}	25.5 ± 0.5^{Bo}	32.33 ± 1.52^{Clm}
	200	26.33 ± 0.58^{Ano}	32.83 ± 0.76^{Blm}	41.83 ± 0.76^{Cij}
Nanosuspension	50	14.5 ± 0.5^{Ar}	23 ± 1^{Bp}	30.83 ± 0.76^{Clm}
	100	17.83 ± 0.76^{Aq}	25.5 ± 0.5^{Bo}	33 ± 1^{Cl}
	150	26.5 ± 1.32^{Ao}	32.83 ± 0.76^{Bl}	41.83 ± 0.76^{Cij}
	200	30.17 ± 1.26^{Amn}	42.83 ± 0.76^{Bi}	51.5 ± 0.5^{Cf}
Pyriproxyfen	50	27.57 ± 0.5^{Ao}	38.5 ± 0.5^{Bm}	46.83 ± 0.76^{Cj}
	100	39.83 ± 0.76^{Alm}	48.17 ± 0.29^{Bij}	57.83 ± 0.76^{Cfg}
	150	49.5 ± 0.5^{Ai}	59.33 ± 0.58^{Be}	65.5 ± 0.5^{Cd}
	200	72.5 ± 0.5^{Ac}	80.83 ± 0.76^{Bb}	89.17 ± 0.76^{Ca}

Means that do not share a letter are significantly different. The analysis revealed that at a higher concentration, the mean mortality rate of 7. castaneum caused by osimum basilicum plant extract was 41.83 ± 0.76 and for osimum basilicum nanosuspension, it was 51.5 ± 0.5. This indicates that as the exposure time to osimum basilicum nosuspensions and plant extracts increased, the mortality rate of 7. castaneum gradually increased, reaching its peak at the highest concentration. This finding supports the idea that exposure time determines. The susceptibility of 7. castaneum to treatments.

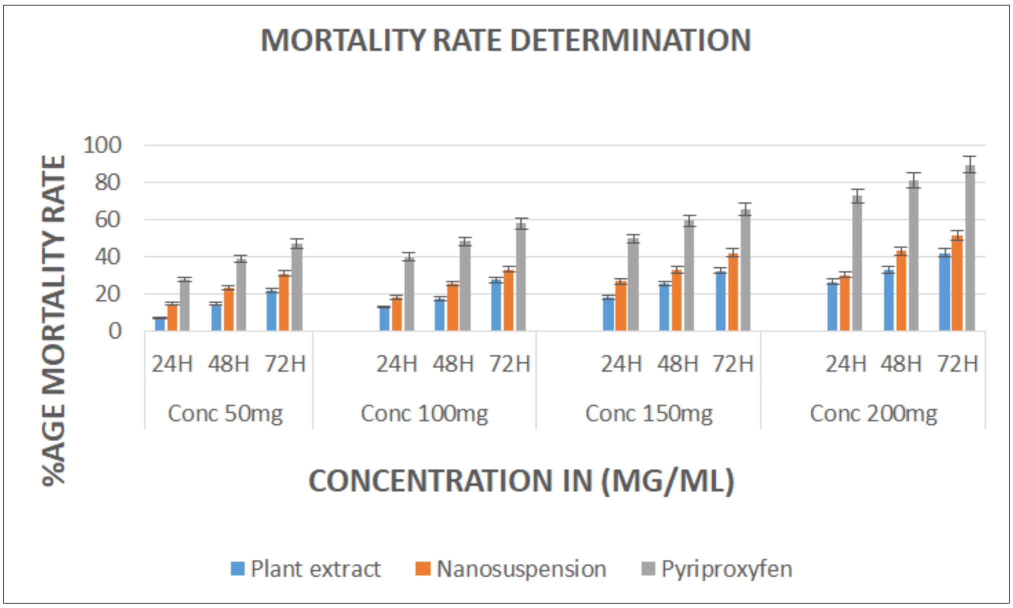


Figure 4.4.3: Mortality rate determination at different concentrations of Ocimum basilicum plant extract nanosuspensions and pyriproxyfen after different time intervals

4.5 Utilizing Stabilizers to Formulate Stable Nanosuspension of Terminalia Arjuna Bark Extract

Nanobiopesticides based on Terminalia arjuna were developed as nanosuspensions. Various nanosuspensions of the Terminalia arjuna bark extract were prepared using medicinally suitable stabilizers, such as Tween-80, PVP, SLS, and PEG. At room temperature, the nanosuspensions made with PVP, SLS, PVA, and PEG were unstable, whereas the ones with Tween-80 stayed stable.

4.5.1 FTIR Spectrum of T. Arjuna Based Nanobiopesticide

The hydroxyl group's broad peak at 3363.24 cm-1 was reduced to 3445.42 cm-1. The peaks at 2918.32 cm-1 and 2850.62 cm-1 in the plant extract spectrum were similar to those of nanosuspension. There was no substantial shift in peak positions or intensity when compared to the remaining peaks (Zafar *et al.*, 2019).

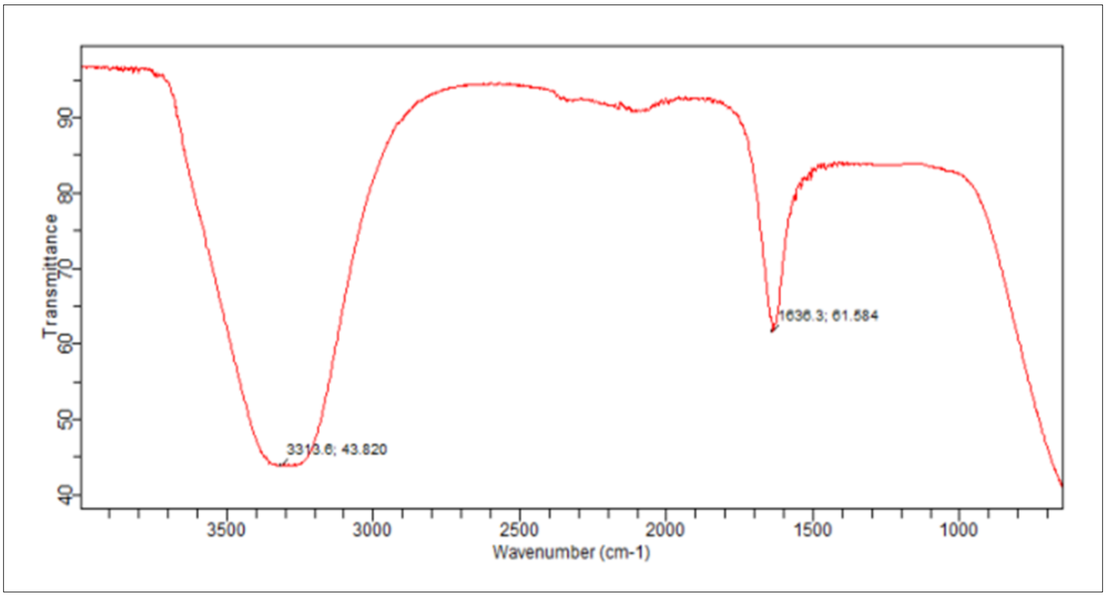


Figure 4.5.1: FTIR spectrum of T. arjuna based nanobiopesticide

The FTIR spectrum of T. arjuna nanosuspension revealed significant peak at 3313.6 cm, demonstrating the existence of the O-H stretch which is characteristic of alcohol. Another prominent peak at 1636.6 cm revealed the existence of the carbon-oxygen double bond, with the C-O (carbonyl group) stretch observed in this region.

4.5.2 Mortality Rate Determination

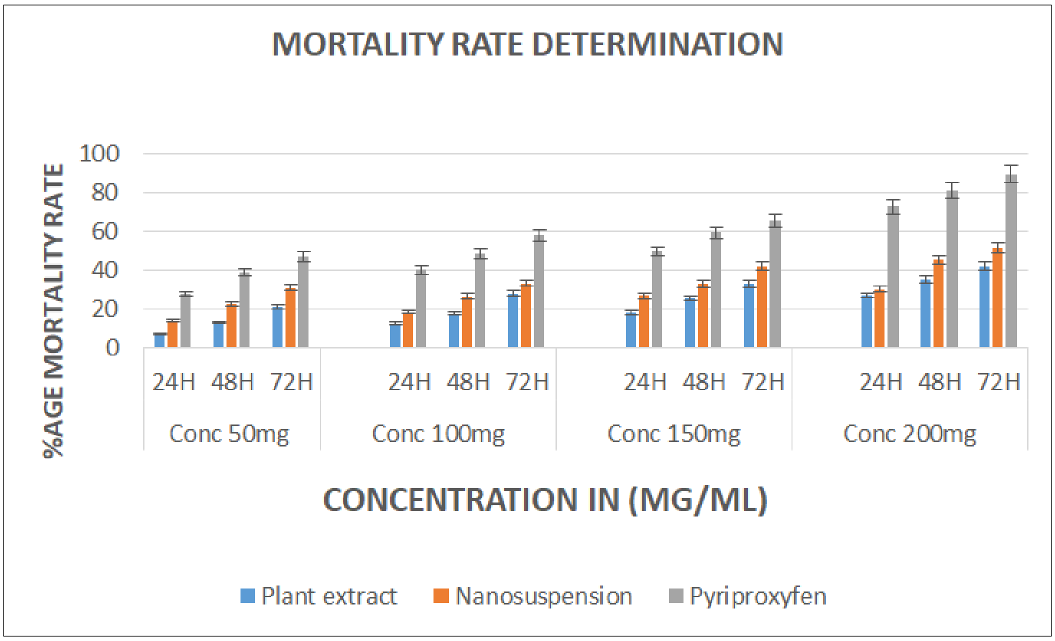


Figure 4.5.2: Average % mortality rate of T. arjuna extract, nanobiopesticide and pyriproxyfen against T. castaneum at different concentration after 24 hours,48 hours and 72 hours

4.6 Utilizing Stabilizers to Formulate Stable Nanosuspension of Withania Somnifera Roots Extract

Nanobiopesticides based on Withania somnifera were developed as nanosuspensions. Various nanosuspensions of the Withania somnifera roots extract were prepared using pharmaceutically suitable stabilizers, such as Tween-80, PVP, SLS, and PEG. At room temperature, the nanosuspensions made with PVA, SLS, HPMC, and PEG were unstable, whereas the ones with PVP stayed stable.

4.6.1 FTIR Spectrum of Withania Somnifera Based Nanobiopesticide

FTIR evaluation of different volumes of Withania somnifera extracts in ethanol, indicated the presence of distinct functional groups. Stretch vibrations of O-H, C-H, and N-H were observed between 4000 and 2500 cm−1. The 1500-400 cm−1 area contained functional groups including C-C, C-O, C-I, S-S, and N-O vibrations. The discovered FTIR spectra of several extracts of Withania somnifera indicate the existence of many bioactive chemicals with varied therapeutic activities. FTIR spectrum Withania somnifera of based nanobiopesticide (Kashyap *et al.*, 2023).

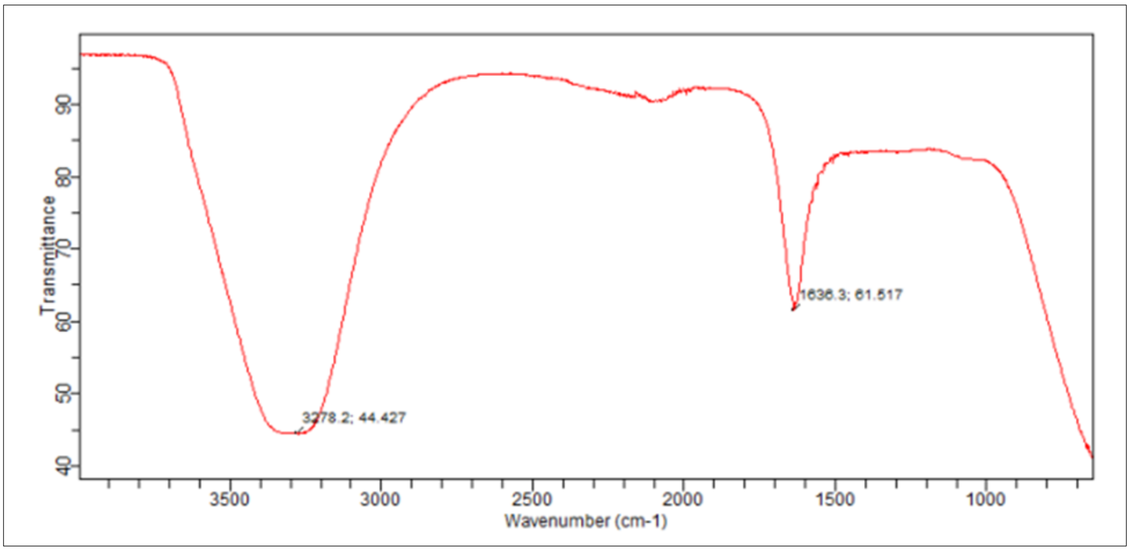


Figure 4.6.1: FTIR spectrum of Withania somnifera based nanobiopesticide

The FTIR spectrum of Withania somnifera nanosuspension revealed significant peak at 3278.2 cm, demonstrating the existence of the O-H stretch which is characteristic of alcohol. Another prominent peak at 1636.3 cm revealed the existence of the carbon-oxygen double bond, with the C-O (carbonyl group) stretch observed in this region.

4.6.2 Mortality Rate Determination

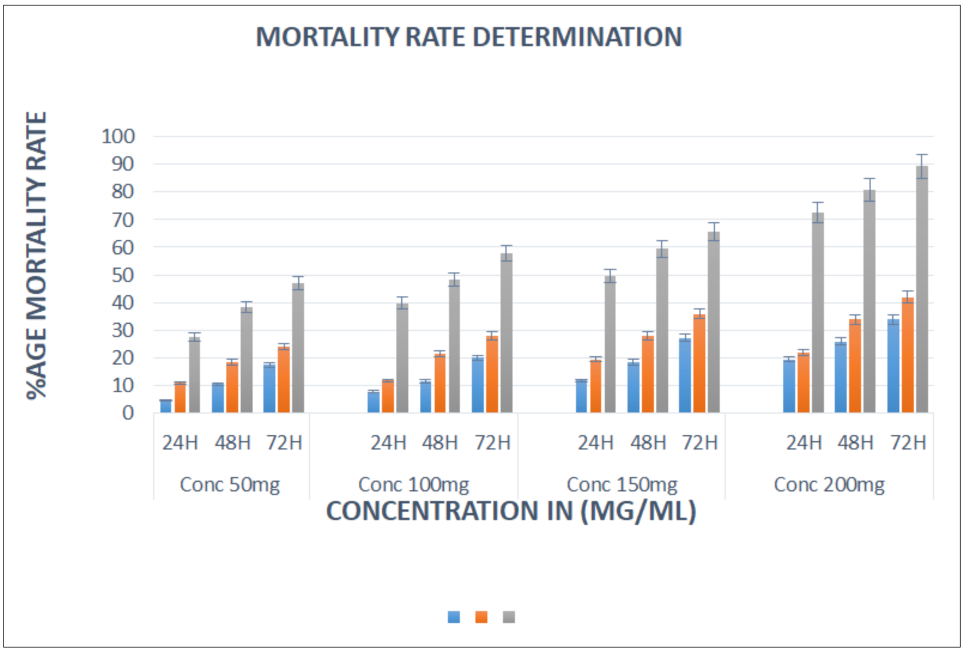


Figure 4.6.2: Average % mortality rate of Withania somnifera extract, nanobiopesticide and synthetic pesticide against T. castaneum at different concentration after 24 hours,48 hours and 72 hours

The analysis revealed that at a higher concentration, the mean mortality rate of 7. castaneum caused by Withania somnifera plant extract was 33.83 and for Withania somnifera nanosuspension, it was 42 at 200(mg/ml).

CONCLUSION

The main purposes of this study are to investigate the potential of nanotechnology in revolutionizing pest management through the development of nanopesticides and to address the environmental and health concerns associated with traditional agrochemicals. The main objective of the current research work was to prepare medicinal plants based nanobiopesticides having improved stability, and pesticidal activity by following the method of antisolvent precipitation. Nanobiopesticides showed increased pesticidal activity and might be employed as an effective substitute to conventional chemical pesticides. The pesticidal activity of the plant extracts and their nanosuspensions against stored-grain insects was evaluated. Lawsonia Inermis based nanobiopesticides demonstrated significantly high average mortality against Tribolium castaneum, respectively. Lawsonioa inermis plant extract showed highest mortality rate i.e, 51.83% while its nanosuspension showed pesticidal potential of 61.83% after 72 hours of exposure, Mentha pipertia nanosuspension had an 61.67% death rate, Ocimum basilicum nanosuspension showed mortality rate of 51.5%, T.arjuna nanosuspension showed mortality rate of 51%, and Withania somnifera based nanosuspension showed lowest mortality rate of 42.5%.

Nanotechnology presents promising opportunities for sustainable pest management by addressing the limitations of traditional agrochemicals. The formulation of nanoscale pesticides offers enhanced efficacy and reduced environmental impact. However, careful consideration of the potential adverse effects and responsible waste management strategies are necessary to ensure the safe and sustainable implementation of nanopesticides in agriculture.

Highlights

A. Synthesis of medicinal plants based nanobiopesticides by following the method of antisolvent precipitation
B. Charactrization of stable nanosuspensions with FTIR and most suitable and effective with Malvern zeta sizer
C. Applications of prepared plants extract and nanosuspension to Tribolium castaneum insects
D. Comparison with the efficiency of synthetic pesticides

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