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Larvicidal Activity of Biofabricated Iron Nanoparticles

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Abstract: The Green synthesis of nanoparticles being cost effective and ecofriendly treatment technique, is gaining importance nowadays. An attempt was made for the green synthesis of iron nanoparticles (FeNPs) using the seed extract of *Avena sativa* and the nanoparticles were analyzed using UV-Vis spectroscopy, Scanning electron microscopy (SEM), and X - ray diffraction analysis was performed for its potential to act as larvicide against mosquito larvae. The larvae were exposed to different doses of FeNPs (0.025, 0.05, 0.1, 0.25, and 0.5 µg/ml) for 24 h. The concentration of 0.5 µg/ml caused 100% mortality. Probit analysis revealed the value of Dose50 of synthesized iron nanoparticles as 0.08562 µg/ml which indicates its toxicity. The histological analysis of the body of larvae exposed to FeNPs showed great variations with that of control. Overall, this study demonstrated that the larvicidal efficacy of green synthesized FeNPs can be an ideal eco-friendly and cost-effective in biocontrol of mosquitoe larvae and could serve as an alternative to chemical methods.

Keywords: *Avena sativa*, Iron nanoparticles, Larvicide, Mosquitoes, GCMS

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Introduction

The development of ecofriendly methods for the synthesis of nanoparticles is evolving as an important branch of nanotechnology (Hasan, 2015). Plants and microbes are the main biological materials used for the green synthesis. These biomaterials play the role of reducing, capping, stabilizing and fabricating agents in green synthesis of nanoparticles (Priya *et al*., 2021). The biosynthetic route is a safe, biocompatible, environment-friendly green approach to synthesize nanoparticles using plants and micro-organisms for biomedical applications (Razavi *et al*., 2015). This synthesis can be carried out with fungi, algae, bacteria, and plants. Some parts of plants such as leaves, fruits, roots, stem and seeds have been used for the synthesis of various nanoparticles due to the presence of phytochemicals in its extract, which act like stabilization and reducing agent (Narayanan and Sakthivel 2011). Magnetic nanoparticles like Fe_3O_4 (magnetite) and Fe_2O_3 (maghemite) are known to be biocompatible. They have been actively investigated for targeted cancer treatment (magnetic hyperthermia), stem cell sorting and manipulation, guided drug delivery, gene therapy, DNA analysis, and magnetic resonance imaging (Hasan, 2015).

There are several green approaches to synthesize iron-based nanomaterials using different bio-chemicals and bio-reducing agents. Iron nanomaterials are significantly important for abatement of environmental pollution such as degradation of organic dyes, chlorinated organic pollutants and removal of heavy metals, wastewater treatment and antibacterial activity (Saif *et al*., 2016). Iron nanoparticles (FeNPs) have unique characteristics like catalytic activity, optical, electronic and antibacterial properties. Thus, it has a wide range of applications, including cosmetics, biomedicine, bioremediation, clinical materials and engineering. Due to its enhanced properties and multifunctional application, it highly encourages us to choose FeNp synthesis. The conventional synthesis of FeNPs used a variety of organic solvents and reducing agents like sodium borohydride ($NaBH_4$), hydrazine, sodium dodecyl sulphate, etc.. These reducing agents pose great risks to the environment; it also creates harmful by-products to human health. In spite of this difficulty, high amounts of organic compounds in the plant extracts are used for the reduction process; it stabilizes nanoparticles by preventing agglomeration, and nanoparticles produced from biogenic synthesis compared to the traditional methods that demonstrate less toxicity. The problems associated with conventional methods initiate us to synthesise biocompatible FeNps using leaf extracts (Devatha *et al*., 2016).

In this study, biosynthesis of nanosized iron particles reduced from $FeCl_3$ were carried out using the aqueous extract of Oats (*Avena sativa*), a biennial herbaceous plant that belong to the Poaceae family. In the extraction process parameters like temperature, time and mass of materials used were optimized in order to achieve a higher antioxidant capacity. The nanoparticles were characterized using a UV visible spectro-photometer, X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), Particle size analyzer (PSA) and scanning electron microscopy (SEM). The catalytic activity of both $NaBH_4$ (BFeNPs) and *A. sativa* (DFeNPs) mediated nanoparticles were tested through the decolourization efficiency of methyl orange (MO) by UV irradiation and antioxidant capacity by 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging assay. The effect of initial MO concentration and FeNPs loading on decolourization was also investigated (Harshiny *et al*., 2015)

Mosquitoes are vectors of many different disease agents around the world. Control of mosquito – borne diseases is becoming increasingly difficult because of increasing resistance of mosquitoes to pesticides (Ranson, 2001). However, the discovery of the insecticide Dichloro – diphenyl – trichloroethane (DDT) in the 1940s was a major breakthrough in the control of vector borne diseases. The application of these chemical insecticides, which has been in use for decades, has met with tremendous setbacks in the light of the development of vector resistance and some attend environmental hazards. In addition, Francisco (2021) reported other deleterious effects of insecticides on non – target organisms. With these problems in focus, it becomes increasingly necessary to search for an alternative in the development of environmentally safe; biodegradable, low cost, indigenous methods for mosquito control which can be used with minimum care by individuals and communities in specific situations (Mgbemena and Tochi, 2012). Plants are a valuable source of repellents, and practises based on plant materials as repellents are still widely used in developing nations. Phytochemicals of plant origin with mosquito control potential are being used as alternative sources in integrated vector management due to the limitations of chemical pesticides. The aim of

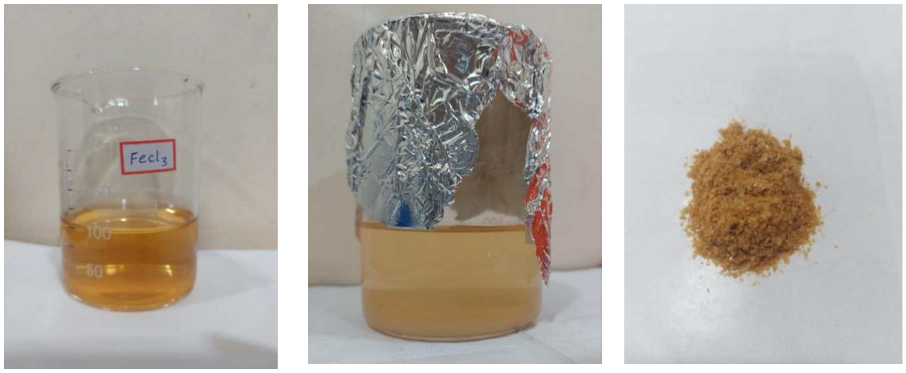


Fig. 1: Synthesis of Iron nanoparticles.

the present study was to evaluate the larvicidal activity of iron nanoparticles, green synthesized using seed extract of *Avena sativa* and also to determine the efficacy of biosynthesized iron nanoparticles for anti-mosquito larvicidal activity.

Materials and Methods

The extract of the seeds of *Avena sativa* was prepared by the decoction method and filtered. The iron nanoparticles were synthesized, separated and concentrated by repeated washing and centrifugation. The final suspension was dried in hot air oven and nanoparticles obtained were used for further experimental studies (Barabadi *et al*., 2021). The synthesis of nanoparticles was confirmed and monitored with the help of the analytical methods like UV- visible Spectra analysis, SEM and XRD which help to investigate distinctive properties of iron nanoparticles that were synthesized biologically (Balanmurugan *et al*., 2014). The bioactive constitutes present in seed extract of *Avena sativa* was determined by Gas Chromatography (Agilent 6890 series) equipped with HP-5MS column mass spectrometer.

The larvae of *Culex quinquefasciatus* were collected from stagnant water in open containers and were maintained under laboratory conditions with relative humidity (70-80%) and temperature (27±2°C) as per the reported protocol (Selvan *et al*., 2018). The larvicidal activity was evaluated by keeping the larvae in separate test tubes containing 0.025, 0.05, 0.1, 0.25, 0.5 µg/ml iron nanoparticle with 5 ml de-ionized water, respectively and the mortality of the larvae was recorded after 24 h exposure.

The body of the larvae was fixed by keeping in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 µm and stained with hematoxylin and eosin for histological study.

Results

The green synthesis of iron nanoparticles was carried out by adding 1 mm $FeCl_3$ solution into the aqueous seed extract of *Avena sativa* which results a color change from yellowish brown to pale yellow due to the reduction of $FeCl_3$ solution to Fe^{3+} ions which indicate the formation of iron nanoparticles (Fig. 1). The biologically synthesized iron nanoparticles were analyzed using UV-Vis spectroscopy, Scanning electron microscopy (SEM), and X - ray diffraction analysis.

The UV-vis spectra of iron nanoparticles synthesized displayed a peak around 400- 500 nm due to the formation of FeNPs (Fig. 2). This peak corresponded to the surface Plasmon resonance of the synthesized FeNPs (Kumar *et al*., 2018).

SEM technique was employed to characterize the size, shape and morphology of iron nanoparticles and SEM images obtained for the iron nanoparticles of seed extract of *Avena sativa*

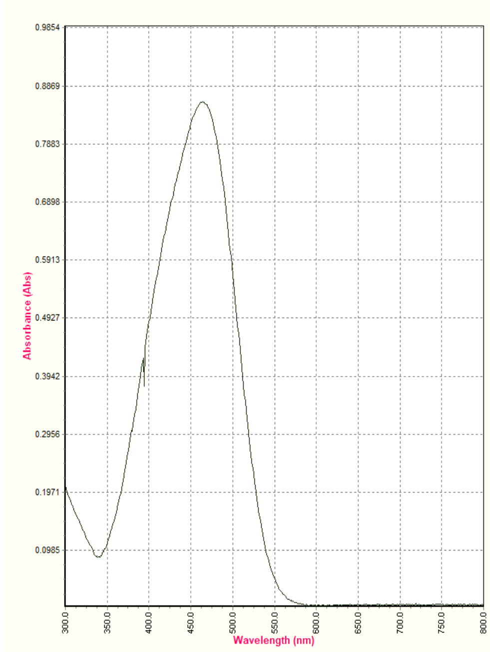


Fig. 2: UV Spectrum of FeNPs.

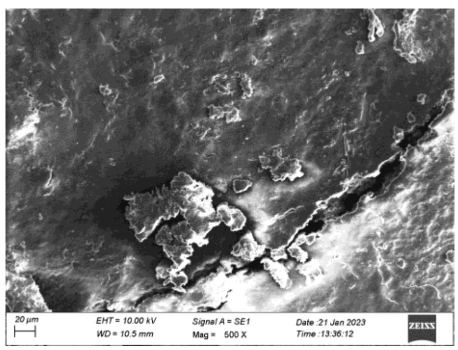


Fig. 3: SEM Analysis 500X.

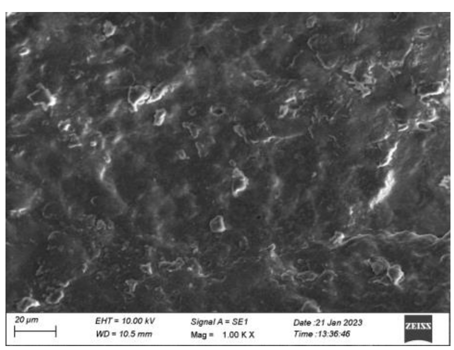


Fig. 4: SEM Analysis 1.00 KX.

were as shown in Figures 3 to 7. The SEM micrograph revealed that the synthesized iron nanoparticle were aggregated as irregular shapes and ranges from 100-200 nm with inter particle distance. The larger size of particle is due to capping agent binding the FeNPs.

The crystalline nature of biologically synthesized FeNPs has been investigated by monitoring typical X-Ray Diffraction (XRD) pattern (Fig. 8), which indicates the formation of

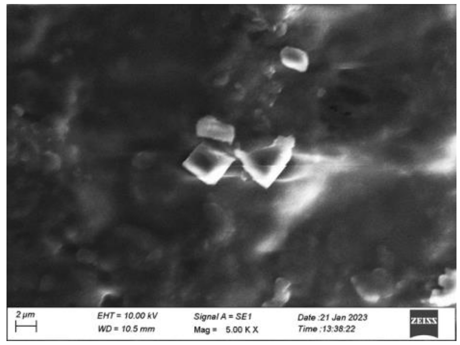


Fig. 5: SEM Analysis 5.00 K X.

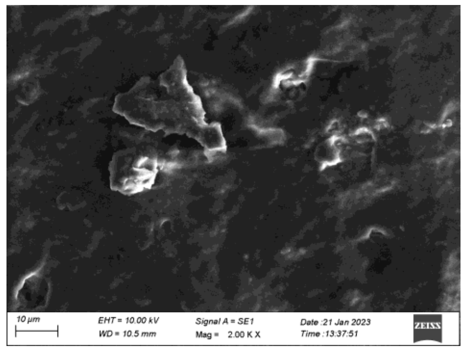


Fig. 6: SEM Analysis 2.00 KX.

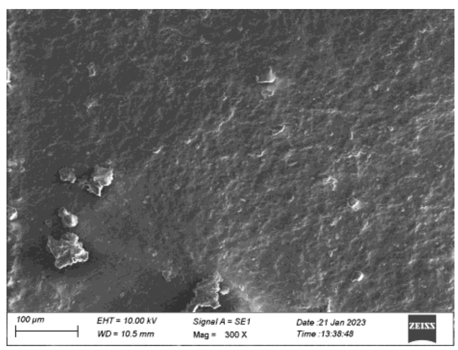


Fig. 7: SEM Analysis 300X.

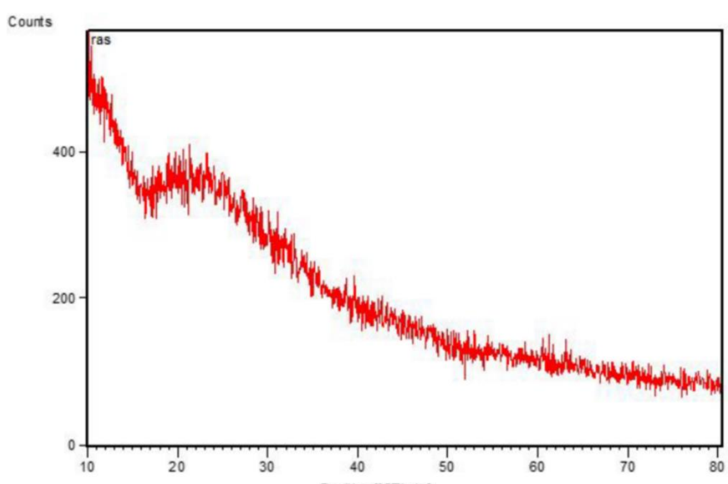


Fig. 8: XRD Analysis of FeNPs.

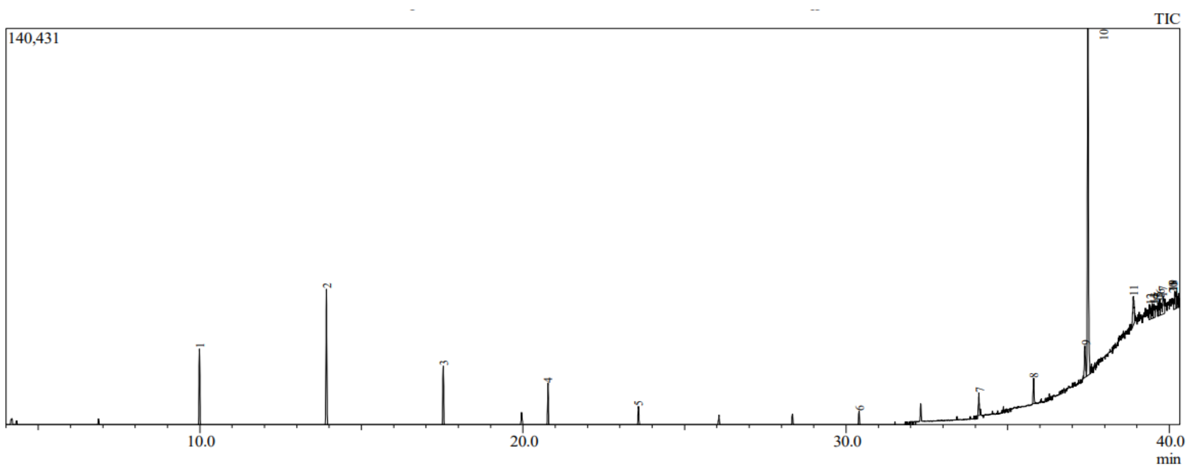


Fig. 9: GCMS analysis of *Avena sativa* seed.

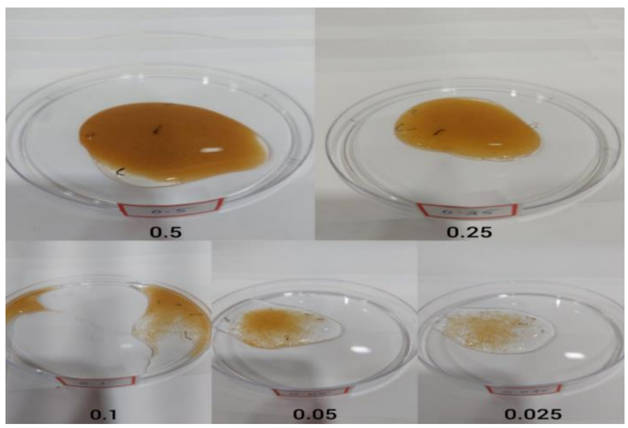


Fig. 10: Mosquito larvicidal activity of iron nanoparticles at different concentrations.

iron nanoparticles. The experimentally obtained and standard diffraction angles and peaks were compared and the results are in agreement with the standard patterns of XRD of FeNPs.

Characterization of bioactive fragment of Avena sativa seed extract using GC-MS analysis

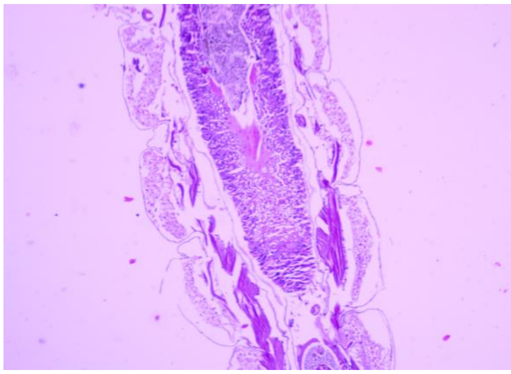
GC-MS analysis was carried out to determine the possible chemical compounds in it. From the chromatogram (Fig. 9), it was observed that there were about 20 compounds present in the bioactive fraction however, the major component was 1,4-Benzenedicarboxylic acid with molecular formula $C_8H_6O_4$ and molecular weight of 166.13, an organic compound which is used for synthesis of polymer. The peak was found in the retention time 390 min. The identification was done by the employment of NIST library. From the GCMS data it was evident that the bioactive fragment composed 13 compounds as per the NIST Library. Overall, these phytoconstituents have been shown to possess different biological activities and industrial applications such as antibacterial, antioxidant, etc. Hence, the present study is expected to help in identifying the larvicidal activity of aqueous seed extract of *Avena sativa*.

Mosquito larvicidal activity of iron nanoparticles

Mosquitoe larvae kept in ferric chloride solution were found to be alive whereas in the tubes containing iron nanoparticlewith a dose of 0.5 µg, the larvae were dead within 24 h. The mortality rate of the larvae were more depending on the concentration of FeNPs (Fig. 10).

Histological studies

A small portion of the body of mosquito larvae kept in the experimental set up were selected at


(A) Control

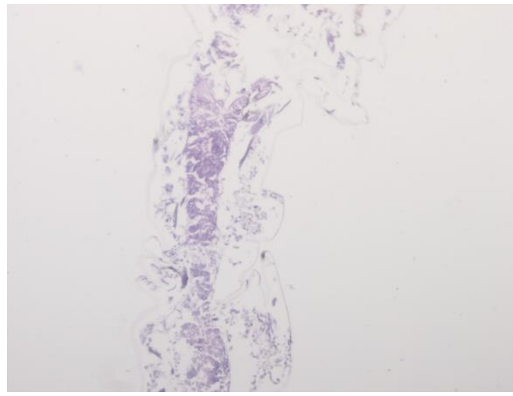

(B) Test

Fig. 11: Hisotogical studies.

Table 1: Probit Analysis

Dose µg/ml	Actual percentage	Probit Per cent	N	R	E(R)	Difference	Chi-Square
0.025	25.00	18.13	4	1.00	0.73	0.27	0.13
0.05	25.00	34.54	4	1.00	1.38	-0.38	0.16
0.1	50.00	54.57	4	2.00	2.18	-0.18	0.03
0.25	87.75	78.59	4	3.51	3.14	0.37	0.20
0.5	87.75	90.41	4	3.51	3.63	-0.11	0.03

Overall Model Test
Total chi-square 0.55
D.F. 3
Prob Level 0.91
Dose 50: 0.08562621 (Estimate); Std.Error = 0.03662341

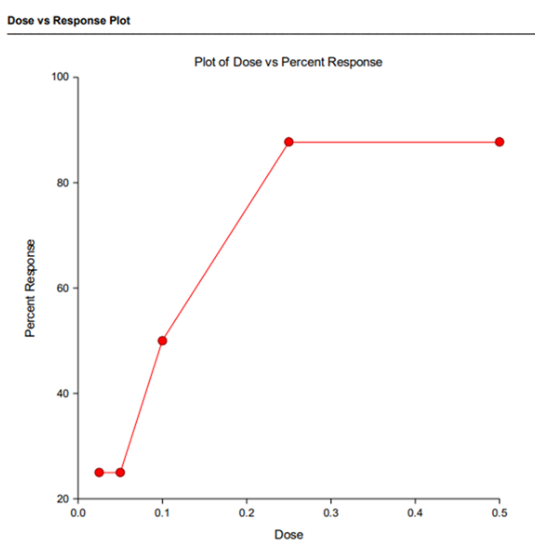


Fig. 12: Plot of Dose vs % response.

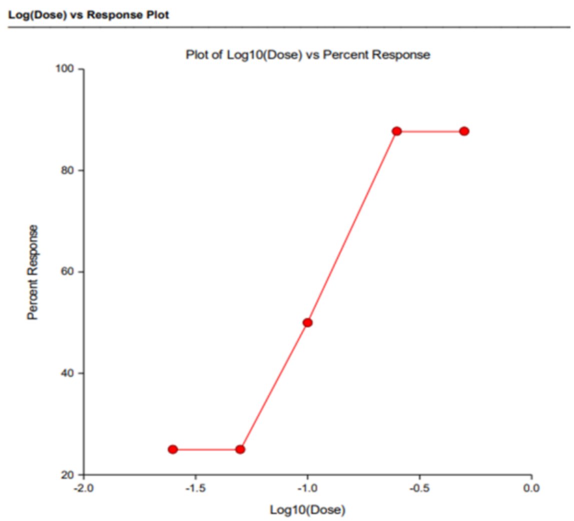


Fig. 13: Plot of Log10(Dose) vs % Response.

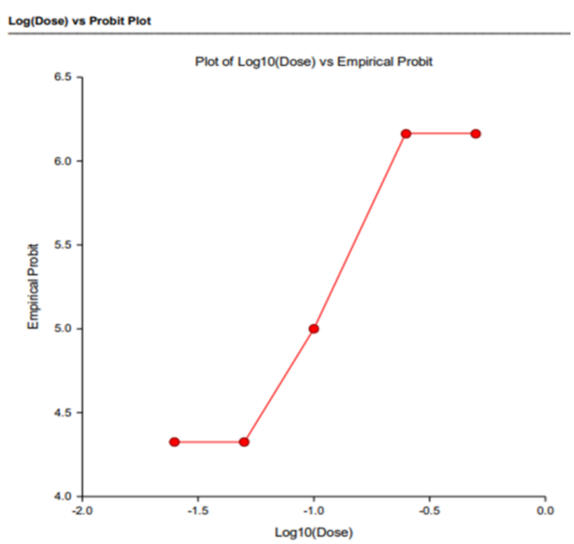


Fig. 14: Plot of Log10(Dose) vs % Empirical Probit.

random for histological analysis. A control sample was also kept. It was examined for changes that could have resulted from mycotoxin damage or mycotic infection. The histological analysis Test (Fig. 11B) showed great variations as compared with control (Fig. 11A). Gastrointestinal epithelial cells of larvae in control were normal with compactly stained cytoplasm, spherical nuclei, clearly defined chromatin, and visible peritrophic membrane. It was evident that the metamorphosis of larvae was lacking in test (Fig. 11B). The reason for this may be explained form the Acetylcholin-esterase activity assay. Drug had an immense Acetylcholinesterase activity inhibition, this would probably has a great impact on neurotransmission and thereby metamorphosis. A similar kind of action was previously noted in apiaceae plant essential oils and their constituents against *Aedes albopictus*. In the test samples the larvae exhibited necrotic, shrunken, and diffuse gastrointestinal epithelial cells with karyopyknotic nuclei, necrotic epithelial cells remained attached to basement membrane, necrotic microvilli and peritrophic membranes appeared diffuse, and epithelial cells appeared disorganized. While in control, the gastrointestinal epithelial cells of larvae were normal with compactly stained cytoplasm, spherical nuclei, clearly defined chromatin, and visible peritrophic membrane (Fig. 11).

Probit Analysis

Probit analysis revealed the value of Dose50 of synthesized iron nanoparticles as 0.08562 µg/ml which indicates its toxicity (Table 1; Figs. 12 to 14)

Discussion

Nanotechnology has significant applications in various fields including agriculture, medicine, energy, climate, chemistry, heavy industry, and consumer goods. They have a high surface area to volume ratio, which is the most important aspect that explains why these nanomaterials are so commonly used. Nano-particles (1-100 nm) offer distinctive structural and physico-chemical properties as compared to their bulk counterparts, owing to the surface to volume ratios. The most recent advancement in nanotechnology has led to the expansion in the synthesis of nano-particles by different chemical and physical methods. However, these methods have a negative impact on the environment and living organisms, since unreacted chemicals are discharged into the environment. Therefore, there is a need to fabricate the nano- particles using environmentally benign techniques (Barabadi *et al*., 2021). Plants are nature's "chemical factories" and vast repertoires of secondary metabolites that can be utilized as redox mediator and stabilizer for the nano-particles (Priya *et al*., 2021).

Mosquito vector borne diseases are a major human health problem in all countries. There is no cure to many and these transmitted diseases and regulating disease transmission relies mainly on vector management to control this vector. Immature stages must be considered a preliminary threat which can be analysed by using chemical compounds which prevent development of adult mosquitoes in aquatic environments without the damaging other organisms (Mgbemena and Tochi, 2012). Many insecticides are used against various mosquito population but they have become resistant to many of them. In the present work, we have reported the green synthesis of iron nanoparticle using with *Avena sativa* seed extract in which the protein constituent, polyphenols, alkaloids act as a reducing as well as capping and stabilizing agent of nanoparticles. Thus the synthesis of iron nanoparticle from aqueous extract of *Avenasativa* was rapid and effective. The synthesised nano-particles were characterised using XRD, SEM and GC-MS. When histological and morphological studied done, it was found that the largest of clarity of segmentation damaged cuticles and thinning of body. At higher concentration the larvae showed swollen and transparent bodies with damaged interior tissues and cuticles.

These results suggest that the green synthesis of FeNPs from aqueous extract of *Avena sativa* can be conveniently used for synthesis of iron nanoparticle which could be useful in various potential application including drug formulation and biomedical application in the future. Mosquito larvicidal bio-assay revealed that the green synthesized FeNPs using the seed extract of *Avena sativa* have a significant larvicidal effect against mosquito larvae. It is found to exhibit potent larvicidal activity by killing the larvae within 24 h exposure. The highest concentration (0.5 µg/ml) used in this work caused 100% mortality and probit analysis shows Dose50 of 0.08562 µg/ml for the green synthesized iron nanoparticles. Thus, the green-synthesized FeNPs have the potential to act as biocontrol agents.

Conclusion

The mosquito larvicidal bio-assay revealed that the green synthesized FeNPs using the seed extract of *Avena sativa* have a significant larvicidal effect against mosquito larvae. It is found to exhibit potent larvicidal activity by causing 100%

mortality of the larvae within 24 h with the highest concentrationof 0.5 µg/ml.The method of synthesis of FeNPs is eco-friendly, rapid, cost-effective and could serve as an alternative to chemical methods and can be used after biosafety tests.

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Conflict of Interest

The authors declare no conflicts of interest.

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