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Biosynthesis, characterization and evaluation of green synthesized zinc nanoparticles against *Helicoverpa armigera* (Hub.) (Noctuidae: Lepidoptera)

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Abstract
Nanoparticles are environmentally friendly novel technology that provide a platform for a cost- effective, long-term solution to the management of economic insect pests. *Helicoverpa armigera* (H.) (Lepidoptera: Noctuidae) is a one of the most prominent and persistent polyphagous pests that inflicts significant damage to all main phenological phases of different crops. The major goal of this investigation was to provide an alternative management strategy by biosynthesising, characterizing, evaluating, and demonstrating the bio effectiveness of *Adhathoda vasica* and *Asafoetida* based zinc nanoparticles against 2nd instar larvae of *H. armigera* under laboratory conditions. In this experiment, zinc green nanoparticles were synthesised biologically from leaf extracts of *A. vasica* and *Asafoetida* using a magnetic heating method, and their bioefficacy was tested on *H. armigera*. UV-Visible spectroscopy was used to validate the creation of silver nanoparticles, and a Particle Size Analyzer (PSA) was used to determine particle size and distribution. The results revealed that silver nanoparticles biosynthesized from *A. vasica* and *Asafoetida* had average diameters of 94.60 nm and 75.80 nm, respectively. Scanning Electron Microscopy (SEM) scans revealed that the majority of the nanoparticles were irregular in shape. In comparison to *A. vasica* based nanoparticles, *Asafoetida* based zinc nanoparticles were found to be more effective and had stronger insecticidal efficacy. At 2000 ppm, *Asafoetida* based ZnNPs registered larval mortality of 86.67 per cent against 2nd instar larvae, followed by *A. vasica* based ZnNPs, with 81.67 per cent mortality. The mean larval mortality increased when the concentration and exposure intervals were gradually increased. Leakage of bodily fluids, sluggishness, inactivity, brittleness, and other symptoms were produced by zinc nanoparticles.

Keywords: Zinc nanoparticles (ZnNPs), particle size analyzer. *A. vasica, Asafoetida, H. armigera*

Introduction
India is primarily an agrarian country, with agriculture being the primary source of income for the majority of the population. Cotton is one of the most significant staple crops farmed throughout the country for its fibre content, brittleness, and other qualities. Because pest intrusion is one of the most significant impediments to cotton cultivation, several management strategies, such as cultural, biological, and chemical tools, have been identified and demonstrated to be effective in pest management. Chemical control is mostly available and commonly exploited as a last resort to manage the pest population once the pest has surpassed the threshold level, despite the fact that cultural, biological, and mechanical management tools, among others, have been used for successful pest management to some extent. Pesticidal residues, degradation of soil structure, toxicity to non-targets, resistance development (Ahmad *et al*., 1999) [2], pest resurgence, and other consequences of lavish and indiscriminate chemical use have all resulted in environmental and public harm (Subramanyam and Hagstrum, 1995; Okonkwo and Okoye, 1996) [11, 7].

Nanotechnology has recently been discovered and proven to be an alternative to chemical pesticides. It also provided current and simple techniques for fabricating and designing unique nanoparticles (1–100 nm) from a variety of low-cost nanomaterials (Silver, Iron, Copper, and so on) (Benelli and Lukehart, 2017; Ortigosa *et al*., 2012) [4, 8]. Nano-pesticides are nano-sized particles that have the potential to eliminate the shortcomings of present pest management strategies such as ecological degradation, resistance development, expensive insecticidal formulations, and non-target toxicity of insecticides (Smith *et al*., 2008; Benelli and Lukehart, 2017) [10, 4]. Plants, fungi, bacteria, and other biological sources are commonly used to make nanoparticles.

Even though synthesis of different metallic and non-metallic nanoparticles from plant extracts has been widely synthesized and evaluated for its bioefficacy, investigation biosynthesis and insecticidal activity of zinc nanoparticles against insects are scanty. Hence, the present study was aimed to biosynthesize and evaluate the insecticidal activity of green zinc nanoparticles against cotton bollworm, *H. armigera* (Instar 2). The cotton boll worm, *Helicoverpa armigera* (Hub.) (Lepidoptera: Noctuidae), is a polyphagous pest that feeds on a variety of staple agricultural crops such as cotton, tomato, maize, chickpeas, tobacco, and others, and has been reported to cause significant economic harm. Affected squares in cotton fields open prematurely and become fruitless. Some of the injured bolls may fall off, while others will either not produce lint or generate low-quality lint. The damage also makes the crop vulnerable to secondary fungus and bacterial infections, which can lead to fruit rot. Plant damage to the growing meristems can stifle development, delay maturity, and cause bolls to drop (Basu *et al*., 1985; Manjunath *et al*., 1985) [3, 6].

Materials and Methods

Insect rearing

During the months of August and September of 2019-20, laboratory rearing of *H. armigera* larvae was started from chick pea field obtained larvae. In little plastic pots, these larvae were fed sensitive cotton plant leaves (4.5 cm x 3.5 cm). From the first through the second instar, these larvae were reared in groups, and then individually from the third instar until they reached the pre-pupal stage. For successful pupation, the pre-pupating larval population was carefully removed from individual containers and put to a medium containing a mixture of sand and soil. The newly emerged adults were then placed in glass chimneys with clean muslin cloth coverings to provide a safe environment for mating and oviposition. The moths were fed a sugary syrup or 10.00 per cent sucrose solution before settling on a clean muslin cloth to lay their eggs. The fabric was then placed in a 500 mL glass container with moist cotton inside, and the opening of the glass container was tightly covered with polythene strip and a strong rubber band. After the eggs hatched, the phototrophic larval population was placed to the top of the container and gathered with a camel hair brush without contaminating anything. For continued population rearing, these larvae were fed sensitive cotton leaves. In addition, to minimise inbreeding depression, the laboratory population was maintained by mixing adults collected at night into the larval culture.

Preparation of plant extracts

Fresh leaves of both *A. vasica* were procured from trees found in the UAS Dharwad campus and *Asafoetida* powder was procured from the local market. To remove all contaminants, the leaves were properly rinsed with running tap water and then distilled water. Using a mortar and pestle, five grammes of *A. vasica* leaves and ten grammes of *Asafoetida* powder were weighed and ground into a paste. 100 mL distilled water was added to the crushed leaves and filtered with filter paper (Whatman no. 1). After that, it was boiled for 60 minutes at 80 °C. This extract was used to make silver nanoparticles and then stored in the refrigerator (4 °C) for later use.

Green synthesis of zinc nanoparticles from plant extracts.

For synthesis of green nanoparticles, magnetic heating method was used. 10 ml of plant extract were added to 90 ml aqueous solution of zinc acetate (1 mM) and kept at room temperature for further processes. The colour change was observed from pale yellow to dark yellow/ light brownish colour when heated along with magnetic stirring at 80 °C for 60 minutes, which indicated that the green silver nanoparticles were synthesized due to the interaction between plant extract and zinc metal ions. A test control was maintained without adding zinc acetate, which didn't show any colour changes.

Characterization of zinc nanoparticles

The UV-visible Spectrophotometer was used to characterise green nanoparticles with wavelengths spanning from 200 to 700 nm. PSA was used to determine the average size and distribution pattern of green nanoparticles (Particle Size Analyzer). The morphology or surface topology of green produced silver nanoparticles was also evaluated using SEM (Scanning Electron Microscopy).

Bioassay of zinc nanoparticles

The susceptibility of *H. armigera* larvae to various doses of zinc nanoparticles was investigated. Each nanoparticle was treated at five different concentrations ranging from 500 to 2000 ppm to achieve mortality rates of 10.00 to 100.00 per cent. For around 60 seconds, excised cotton leaf discs with an average diameter of 60 mm were dipped in varying concentrations of each experiment. After 60 minutes of drying in the shade, the leaf discs were placed on Petri dishes (above cotton tissue papers). Before beginning the experiments for the stated treatment, preliminary dosages were set in order to establish death rates ranging from 10.00 to 100.00 per cent. Leaf discs treated with distilled water served as controls for each treatment. As an insecticidal check, 0.30 g/l of Emamaectin benzoate was used. In each replication of each treatment, ten *H. armigera* caterpillars were supplied. For each treatment, a minimum of three replications of all concentrations were used. The *H. armigera* death rate was observed after 24, 48, 72 and 96 hours after treatment intervals to further evaluate experimental treatments. Any larvae that were unable to move when disturbed on their backs were deemed dead. Formula was used to calculate the percentage reduction in *H. armigera* population compared to the test control.

$$\text{Per mortality} = \frac{\text{Number of dead insects}}{\text{Total number of insects}} \times 100$$

$$\text{Corrected per cent mortality} = \frac{\text{T - C}}{\text{100 -C}} \text{ 100 (Abbott's formulae)}$$

T- Per cent mortality in nanoparticle treatment C- Per cent mortality in test control

Statistical analysis

The results obtained were subjected for statistical analysis (ANOVA) using a completely randomized block design. The mean values of treatments were then subjected to Duncan's Multiple Range Test (DMRT).

Results and Discussion

Biosynthesis and characterization of zinc nanoparticles from *A. vasica* and *Asafoetida*

The colour shift in the nanoparticle solution after incubation

A. vasica and *Asafoetida* extract with zinc nitrate solution showed that zinc was converted into zinc ions in the green produced nanoparticle combination. Fresh zinc acetate and plant extract suspension was pale greenish/yellow in colour. It turned pale brownish after 60 minutes of continuous magnetic heating at 80 °C to complete the green synthesis process. (Fig. 1 A and 1 B).

The formation of zinc nanoparticles by *A. vasica* and *Asafoetida* was confirmed using a UV Spectrophotometer, which revealed absorption peaks at 350 nm (Fig. 2 A and 2 B). The size of the nanoparticles was determined using a Particle Size Analyzer after the nanoparticle formation was confirmed using a UV Spectrophotometer. The diameter of ZnNPs made from *A. vasica* and *Asafoetida* was 94.60 nm and 75.80 nm, respectively. Surface Electron Microscopy was used to further characterise the nanoparticles. The majority of the nanoparticles in both nanoparticles were irregular in shape, as revealed by SEM images. (Fig. 4 A and 4 B).

Bio-efficacy of zinc nanoparticles against *H. armigera* (Instar 2)

Biological efficacy of *A. vasica* and *Asafoetida* encapsulated zinc nanoparticles were evaluated against 2nd instar larvae of *H. armigera*, divulging the significant impact of different concentrations from 500 to 2000 ppm along with an insecticidal check (table 1 and 2).

Fig 1: A) Colour change of *A. vasica*-zinc acetate solution due to zinc nanoparticle formation

Fig 1: B). Colour change of *Asafoetida*-zinc acetate solution due to zinc nanoparticle formation

Effect of zinc nanoparticles against larval mortality of *H. armigera* (Instar 2)

The physiology of the treated larva was disrupted as soon as it started feeding on the treated leaves. This resulted in bodily rupture, fluid leaking, and sluggishness, among other things. This occurred as a result of nanoparticles acting in a variety of ways on different parts of the insect's body. When nanoparticles came into touch with insect cuticle, they physically absorbed wax and lipids, causing cuticular rupture. Disruption in food intake and altered metabolic activity causes deformities such as shrinkage and brittleness in the treated larvae.

Zinc nanoparticles could attach to Sulphur and Phosphorous in proteins and nucleic acids, respectively, resulting in reduced membrane permeability, organelle denaturation, enzyme denaturation, and cell death. Ultimately mortality of larva was confirmed when seen unconscious and immobile when disturbed on its back. The treated larva was found to be devoid of any bodily fluids, blackish in colour, and rigid but prone to breaking quickly after mortality. (Fig 5 A and 5 B).

Emmamectin benzoate @ 0.25 g/l, which was utilised as an insecticidal control, was shown to be significantly superior to all nanoparticle concentrations and showed the maximum larvae mortality of 100.00 per cent at 72 and 96 hours after treatment. However, no larval death was seen in the presence of the precursor, plant extract, or distilled water (control).

The bioefficacy of varied doses (500-2000 ppm) of zinc nanoparticles against 2nd instar larvae is given in tables 1 and 2. After 24 hours of treatment, *A. vasica* and *Asafoetida* based zinc nanoparticles recorded 10.00 to 23.33 percent larvae mortality and 10.0 to 20.00 percent larval mortality, respectively, at concentrations of 500 to 2000 ppm.

A similar trend was followed after 48 hours of treatment with a larval mortality of 53.33 and 48.33 per cent reported at 2000 ppm of *A. vasica* and *Asafeotida* based ZnNPs, respectively. Meanwhile A. *vasica and Asafoetida* based ZnNPs recorded larval mortality in the range of 33.33 to 40.00 and 30.00 to 40.00 per cent at 500 to 1500 ppm, respectively. Insecticidal check, Emamectin benzoate was found to be significantly superior to all the dosages of nanoparticles. No larval mortality was recorded by *A. vasica* extract, *Asafoetida* extract and precursor solution.

At 72 hours following treatment, the maximum mortality rate of 80.00 per cent was recorded by *Asafoetida* based ZnNPs at 2000 ppm, followed by *A. vasica* based ZnNPs with larval mortality of 70.00 per cent. Both *A. vasica* and *Asafoetida* based ZnNPs at 1500 ppm resulted in 58.33 per cent mortality. 1500 ppm of both *A. vasica* and *Asafoetida* based ZnNPs recorded 58.33 per cent larval mortality. Similarly, larval mortality of 53.33 and 55.00 was registered by *A. vasica* and *Asafoetida* based ZnNPs, respectively at 1000 ppm. Lowest larval mortality of 51.67 and 48.33 per cent was recorded at 500 ppm of *A. vasica* and *Asafoetida* based ZnNPs, respectively.

Highest larval mortality of 86.67 per cent was recorded at 2000 ppm of *Asafoetida* based ZnNPs, followed by *A. vasica* based AgNPs with 81.67 per cent larval mortality after 96 hours of treatment. 1500 ppm of *A. vasica* and *Asafoetida* based ZnNPs recorded 78.33 per cent larval mortality, followed by 1000 ppm of *A. vasica* based ZnNPs with 68.33 per cent larval mortality. Larval mortality of 63.33 per cent was registered at 500 ppm of *A. vasica* based ZnNPs, which was found to be on par with 1000 ppm of *Asafoetida* based ZnNPs. Lowest larval mortality of 60.00 per cent was registered at 500 ppm of *Asafoetida* based ZnNPs

All the findings of the present study on implication of green ZnNPs is confirmed and strengthened by the report of Kadakarai *et al*., (2018) [5] conducted an investigation of synthesis of green zinc nanoparticles from *Sargassum wightii* and concluded that ZnNPs were effective against *Helicoverpa armigera*. with considerable larval mortality against different instars. ZnO nanoparticles exhibited highest mortality rate on first (100%), second (100%) and third (92.34%), instar larvae of *H. armigera* at lower concentrations.

In addition, Sarayut *et al*., (2021) [9] also confirmed that zinc nanoparticles at concentrations 100 to 500 ppm, were effective against all instars of *Spodoptera frugiperda* in baby corn by causing effective ovicidal activity, larval mortality, adult fecundity etc.,

Since the investigation on biosynthesis and evaluation of ZnNPs on insect pests are rarely conducted, information regarding the evaluation of zinc nanoparticles against lepidopteran pests, especially against cotton bollworms are scanty.

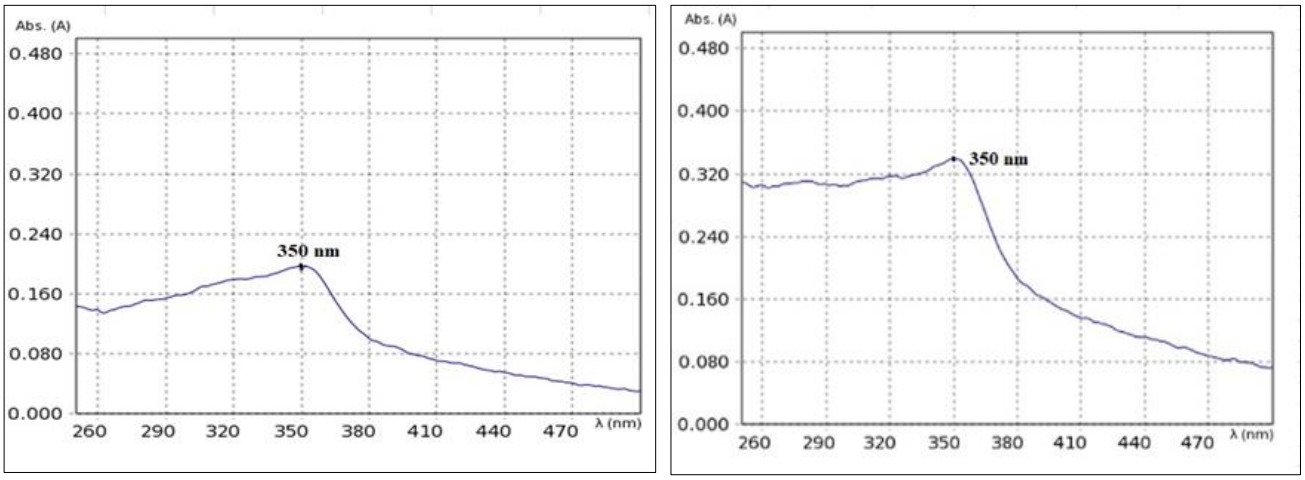

Fig 2. A) UV visible spectroscopy of *Adathoda vasica* based ZnNPs. B) UV visible spectroscopy of *Asafoetida* based ZnNPs

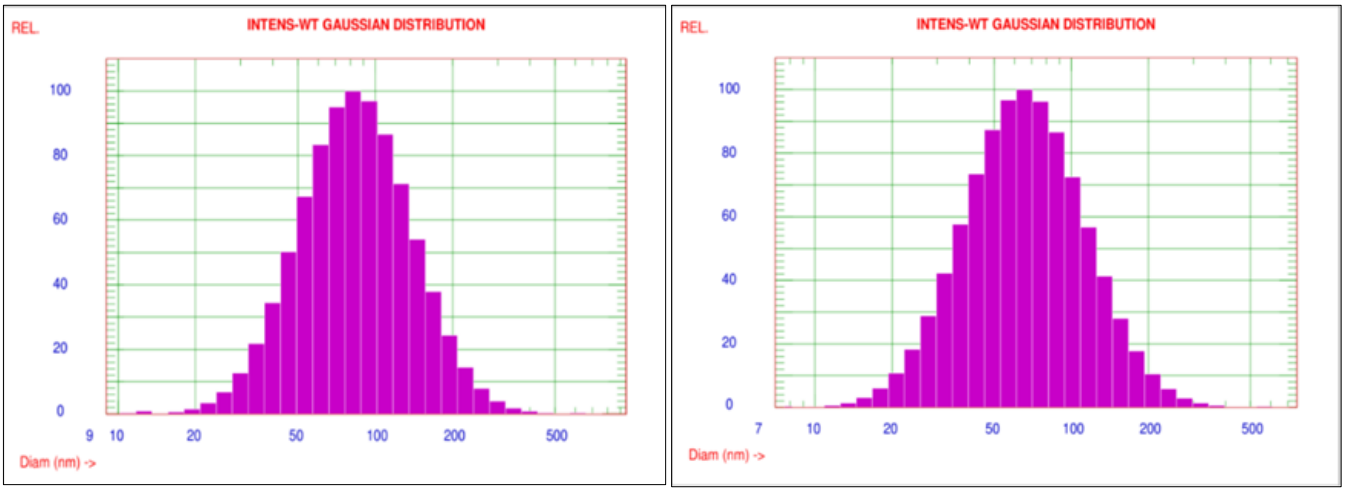

Fig 3: A) Particle Size Analyzer image of *Adathoda vasica* based ZnNPs at 94.60 nm. b) Particle Size Analyzer image of *Asafoetida* based ZnNPs at 75.80 nm.

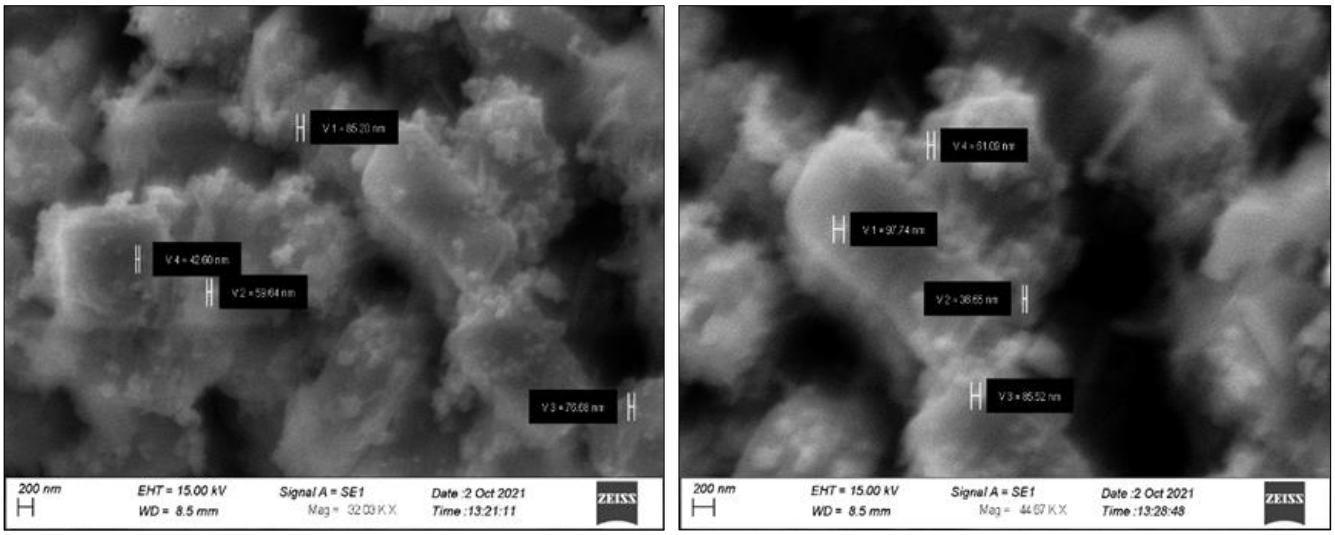

Fig 4: A) Scanning electron microscopic image of *Adathoda vasica* based ZnNPs at 94.60 nm. b) Scanning electron microscopic image of *Asafoetida* based ZnNPs at 75.80 nm.

Table 1: Effect of green ZnNPs synthesized from leaves of *Adathoda vasica* on larval mortality of *H. armigera* (Instar 2)

Concentrations	Hours After Treatment (HAT)			
	24	48	72	96
ZnNPs 500 ppm	10.00	33.33	51.67	63.33
	(21.41)e	(35.26)e	(45.95)e	(52.73)e
ZnNPs 1000 ppm	15.00	38.33	53.33	68.33
	(22.78)d	(38.25)d	(46.90)d	(55.75)d
ZnNPs 1500 ppm	20.00	40.00	58.33	78.33
	(26.56)c	(39.23)c	(49.79)c	(62.25)c
ZnNPs 2000 ppm	23.33	53.33	70.00	81.67
	(28.88)b	(46.90)b	(56.78)b	(64.65)b
Zinc acetate (1mM)	0.00	0.00	0.00	0.00
	(0.25)f	(0.25)f	(0.25)f	(0.25)f
A. vasica extract-5. 00%	0.00	0.00	0.00	0.00
	(0.25)f	(0.25)f	(0.25)f	(0.25)f
Emamectin benzoate @ 0.25g/l	35.00	70.00	100.00	100.00
	(36.27)a	(56.78)a	(95.00)a	(95.00)a
Untreated control	0.00	0.00	0.00	0.00
	(0.25)f	(0.25)f	(0.25)f	(0.25)f
S.Em.±	0.78	0.98	0.68	0.70
CV	8.12	6.26	3.24	3.01
CD @ 1%	3.23	4.05	2.80	2.93

Figures in the parentheses are angular transformed values.
In columns, means followed by same letter do not differ significantly by DMRT (P=0.05)
* HAT-Hours After Treatment
*ZnNPs-Zinc nanoparticles

Table 2: Effect of green ZnNPs synthesized from leaves of *Asafoetida* on larval mortality of *H. armigera* (Instar 2)

Concentrations	Hours After Treatment (HAT)			
	24	48	72	96
ZnNPs 500 ppm	10.00	30.00	48.83	60.00
	(18.43)d	(33.21)e	(44.32)e	(50.76)e
ZnNPs 1000 ppm	18.33	38.83	55.00	63.33
	(25.34)c	(38.25)d	(47.86)d	(52.73)d
ZnNPs 1500 ppm	20.00	40.00	58.33	78.33
	(26.56)b	(41.16)c	(49.79)c	(62.25)c
ZnNPs 2000 ppm	20.00	48.33	80.00	86.67
	(26.56)b	(44.32)b	(63.43)b	(68.58)b
Zinc acetate (1mM)	0.00	0.00	0.00	0.00
	(0.25)e	(0.25)f	(0.25)g	(0.25)g
Asafoetida extract-10. 00%	0.00	0.00	10.00	25.00
	(0.25)e	(0.25)f	(18.43)f	(30.00)f
Emamectin benzoate @ 0.25g/l	35.00	85.00	100.00	100.00
	(36.27)a	(67.21)a	(95.00)a	(95.00)a
Untreated control	0.00	0.00	0.00	0.00
	(0.25)f	(0.25)f	(0.25)g	(0.25)g
S.Em.±	0.78	1.23	1.51	1.77
CV	7.97	7.48	6.65	6.81
CD @ 1%	3.23	4.97	6.24	7.32

Figures in the parentheses are angular transformed values.
In vertical columns, means followed by same letter do not differ significantly by DMRT (P=0.05)
* HAT-Hours After Treatment
*ZnNPs-Zinc nanoparticles

Fig 5: A) Effect of *Adathoda vasica* based ZnNPs against *Helicoverpa armigera* (2nd instar)

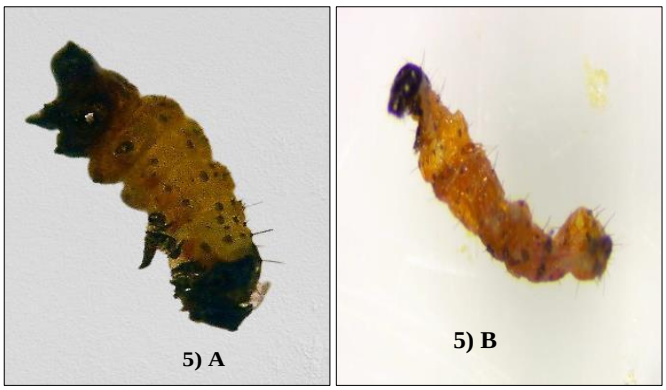

Fig 5: B) Effect of *Asafoetida* based ZnNPs against *Helicoverpa armigera* (2nd instar)

Conclusion

A. vasica and *Asafoetida* leaf extracts worked as both educing and stabilising agents for the biological synthesis of ZnNPs to a larger extent. Plant extracts served as reducing agents, transforming metallic forms to their ionic counterparts. Plant extracts also aided in the stabilisation of ionic forms by acting as a carrier around them and avoiding aggregation. It's also obvious that at 2000 ppm, both AgNPs were proven to cause 100.00 per cent larval mortality after 96 hours of treatment. *Asafoetida* based ZnNPs was superior as compared to *A. vasica* based ZnNPs, since it is observed to cause increased larval mortality at early intervals following treatment. This is due to the higher penetration of Asafoetida based ZnNPs to the larval cuticle. The lower nano size of *Asafoetida* based ZnNPs compared to *A. vasica* based ZnNPs contributed to this.

References

1. Abbott WS. A method for computing the effectiveness of an insecticide. Journal of Economic Entomology. 1925;18:265-267.
2. Ahmad M, Arif MI, Ahmad Z. Detection of resistance to pyrethroids in field populations of cotton Jassid (Homoptera: Cicadellidae) from Pakistan Journal of Economic Entomology. 1999;92(6):1246-1250.
3. Basu AK, Sundarmurthy VT. Management of cotton insect pests in polycrop system in India. Outlook Agriculture. 1985;14:79-82.
4. Benelli G, Lukehart CN. Application of green synthesized nanoparticles in pharmacology, parasitology and entomology. J Clust. Sci. 2017;28:1-2.
5. Kadarkarai M, Mathath R, Chellaswamy P, Ali TA, Udaiyan S, Rajapandia R, *et al*. *Sargassum wightii*-synthesized ZnO nanoparticles reduce the fitness and reproduction of the malaria vector *Anopheles stephensi* and cotton bollworm, *Helicoverpa armigera*. Phys. Mol. Pl. Path. 2018;101:202-213.
6. Manjunath TM, Bhattnagar VS, Pawer CS, Sidhanantham S. Economic importance of *Heliothis armigera* (Hubner) in India an assessment of their natural enemies and host plants. In: Proceedings of International Workshop on Biological Control of *Heliothis armigera*. New Delhi, India, 1985, 105-119.
7. Okonkwo EU, Okoye WI. The efficacy of four seed powders and the essential oils as protectants of cowpea

and maize grains against infestation by *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae) and *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) in Nigeria. International Journal of Pest Management. 1996;42:143-146.
8. Ortigosa MS, Valenstein JS, Lin VSY, Trewyn BG, Wang K. Gold functionalized mesoporous silica nanoparticles mediated protein and DNA codelivery to plant cells via the holistic method. Adv. Funct. Mater. 2012;22:3576-3582.
9. Sarayut P, Rajula J, Rahman A, Vivekanandhan P, Thungrabeab M, Mekchay S, *et al*. Insecticidal Effect of Zinc Oxide Nanoparticles against *Spodoptera frugiperda* under laboratory conditions in Chiang Mai, Thailand. Insects. 2021;12:2017.
10. Smith K, Evans DA, Hiti GAE. Role of modern chemistry in sustainable arable crop protection. Philos. Trans. R. Soci. B. 2008;363:623-637.
11. Subramanyam B, Hagstrum DW. Resistance measurement and management, Marcel Dekker. 1996;331-397.