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Research Article

EFFICACY OF BIOGENIC SYNTHESISED SILVER NANOPARTICLES ON BEHAVIOURAL CHANGES OF *Helicoverpa armigera*

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Abstract: The grounds of this study were to utilize the biogenic synthesized silver nanoparticles from *Leucas aspera, Cyperus rotundus* and *Cynodon dactylon* for the control of polyphagous pest *Helicoverpa armigera*. Synthesized silver nanoparticles were subjected for different characterization like XRD, EDX, FTIR and Raman spectroscopy. The bioassays (*i.e.*, larval weight and survival rate) and also its interaction with gut protease activity (trypsin and chymotrypsin) was evaluated employing LA, CR, and CD AgNPs, as well as crude plant extracts against the 4th instar larvae. The stretching vibration of distinct functional groups of the samples was demonstrated by the existence of FTIR measurements. XRD graphs confirm the AgNPs crystallize in centered cubic phase and the change in peak intensity with different plant extracts was noticed. The body mass, length of the larvae was dramatically lowered by the synthesized sample of silver nanoparticles. They also suppressed the activity of gut proteases (trypsin and chymotrypsin). Our studies could aid in the development of new approaches to address insecticidal resistance in *H. armigera*.

Keywords: *Helicoverpa armigera, Biosynthesized silver nanoparticles, Leucas aspera, Cynodon dactylon, Cyperus rotundus, Antifeedant, Larvicidal, Gut protease*

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Introduction

Agriculture is by far the most basic aspect of every country and also an integral aspect in progressing toward an immobile human lifestyle. To compensate for crop losses and financial constraints caused by numerous biological and chemical variables, the farming community have undergone substantial changes. Chemical insecticides and fertilisers were used extensively to boost yields. Regrettably, unrestrained use of chemical insecticides and fertilisers has caused a spike in pathogen and pest resistance, reduced soil species diversity, reduction in nitrogen fixation contributes to pesticide bioaccumulation, pollinator decline, destruction of bird habitat, toxic and non-biodegradable, negatively affecting human health [1-3]. Despite the fact that assessing the chemistry of bio pesticides offers a reasonable framework based on evolutionary selection for chemicals that are more specific to an insect, have faster decomposition, lower environmental persistence, and don't often increase in concentration in the environment, it has been the least preferred by farmers because their efficacy is poor at different geographical conditions and laggard in pest control [4-6].

Nanoparticles could be an excellent and viable choice for achieving long-term agricultural success. The dexterity of nanotechnology has the potential to precisely form matter of 10-100nm size *i.e.*, small size can increase the surface area of active compound in turn increase solubility, improving bioavailability (*i.e.*, increased contact with the target cell) [7-9], safeguard the active component from degradation so that it can be released for a long time and with precision, efficacy, and specificity [10]. Plant-based silver nanoparticles production is a one-step biosynthesis approach that is speedy, low-cost, and ecologically benign. [11]. A green synthesised sliver nanoparticles approach's main objective is larvicidal, insecticidal, antibacterial, viral and inflammatory properties/application. Various experts from all over the world have cited multiple studies demonstrating the insecticidal activity of AgNPs. The physiological effect of AgNPs derived from *Cassia fistula* L aqueous extracts was investigated using *A. albopictus* and *Culex pipiens* fourth instar larvae and also enzyme assays supported the study [12].

Cotton, pigeon pea, chickpea, tomato, okra, black gram, corn, and a range of other crops are all affected by *Helicoverpa armigera*. The pest's larvae feed on the reproductive systems of plants, causing a loss of around a one third of the yield. *H. armigera* has the ability to adapt to a variety of cropping systems due its high polyphagous fecundity, and pesticide resistance. Serine proteases such as trypsin and chymotrypsin are prevalent in the digestive tract of *H. armigera* [13]. Insect gut proteases are crucial for the energy and growth of insects by aiding in the digestion of proteinaceous foods. One of the most important techniques for reducing the insect pest is to inhibit gut proteases. For sustenance, Lepidopteran insect pests depend mainly on trypsin and chymotrypsin-like digestive proteases [14, 15]. The identification of gut protease inhibitors from plants that are effective against *H. armigera* has indeed been described by several experts. Using biosynthesized gold nanoparticles from *Jatropha curcas L. latex*, serine protease restriction can be seen in a range of insects, including Aedes aegypti, beetles, and Pseudococcidae pests. [16]. AgNPs were observed to kill juvenile *Hematophagous flies, Hippobosca maculate*, and bovine ticks, *Rhipicephalus* (*Boophilus*) *microplus*, across both high and low quantities. [17]. *Culex quinquefasciatus* and *Anopheles subpictus* 4th instar larvae were successfully controlled using AgNPs synthesised from *Eclipta prostrata* leaf extract [18].

The present study aims to utilise *Leucas aspera, Cyperus rotundus,* and *Cynodon dactylon* which are exquisite plant species (*i.e.*, most ecological, locally available, reproducible, economically priced). They compete with most crops for water, sunlight, nutrients, and space, making it an excellent plant for fabrication of silver nanoparticles. XRD, EDX, FTIR and Raman spectroscopy were used to characterise the synthesised silver nanoparticles. The behavioural (*i.e.*, larval weight and survival rate) alterations in *H. armigera* were detected with AgNPs and leaf extract and also evaluating the alter efficacy of gut protease activity in *H. armigera* (trypsin and chymotrypsin).

Materials and Procedures

The silver nitrate used in this study was purchased from Sigma-Aldrich. Plant species such as *Leucas aspera, Cyperus rotundus,* and *Cynodon dactylon* were randomly picked from the University of Mysore in Mysuru, India.

Preparation of plant extracts and biogenic synthesis of silver nanoparticles

All three plants leaves were cleansed by washing them in the double distilled water. Thereafter, the material was dried in the shade. In a magnetic stirrer, the powdered leaves were blended with 100 mL distilled water and incubated/heated for 30 minutes. The extract was refrigerated and decanted before being filtered and kept at -4°C using Whatman filter paper. LA, CR, and CD were assigned to crude leaf extracts from *L. aspera, C. rotundus,* and *C. dactylon*, respectively.

Mixing 50 mL of leaves extract in 100 mL of 1 mM $AgNO_3$ solution at 70°C while stirring constantly yield silver nanoparticles. The colour variations in the reaction mixture from pale yellow to reddish brown indicate the production of AgNPs. Followed by next step, AgNPs formed were washed 5-6 times with distilled water and ethanol before being centrifuged for 10 minutes at 10000 RPM, pellet obtained was freeze, dried and stored at −4°C until use. The synthesised AgNPs were subjected to UV-vis, for characterization. AgNPs produced with *L. aspera, C. rotundus,* and *C. dactylon* leaf extract were designated as LA-AgNPs, CR- AgNPs, and CD-AgNPs, respectively.

Characterization Techniques

The morphological and structural examination of synthesised AgNPs was performed using Fourier Transform infrared spectroscopy, X-ray diffraction, Energy Dispersive X-Ray Spectroscopy and Raman spectroscopy. An X-ray diffractometer was used to determine the structural characterisation and crystalline structure of metal nanoparticles (Rigaku, BD63000074). FTIR spectrophotometer is used to examine the chemical compositions of silver nanoparticles and also the existence of functional groups (Nicolet-6900, Model: 912A0637). HORIBA's Xplora Plus-42308 was used to analyse the Raman spectra of silver nanoparticles.

Tested insects

The culture of the tested insect *H. armigera* (4th instar larvae) was received from an insect breeding lab, ICAR-NBAIR, Hebbal Bengaluru 560024. They were raised in a lab at a temperature of 26°C, a relative humidity of 65.5°RH and 12 hours of photoperiod on a chickpea-based artificial diet. Chickpea (100g), methyl PHB (2g), sorbic acid (1g), yeast (10g), agar (12.75g), ascorbic acid (3.25g), multivitamin tab (1 capsule), vitamin E (2 capsule), streptomycin (0.25g), 10% formalin (5ml), and water made up the artificial diet. The larvae of the 4th instar were collected and tested in the lab in a bioassay.

Bioassay of *Helicoverpa armigera* using sliver nanoparticles

Antifeedant assay and Larvicidal activity: The diet choice tested with required modification was used to determine the antifeedant and larvicidal activity in *Helicoverpa armigera* 4th instar larvae. In brief, the larvae were divided into 3groups of 6 larvae in each group and the selection was complete randomized design. All larvae were weighed. The each artificial diet cube was cut 5cm on all sides; each pre weighed was placed in a petri dish. Because the *Helicoverpa armiger* species exhibit high rates of regnant cannibalism, the studies were conducted using newly moulted and per weighed 4th instar larvae that had been deprived for 3 hours were allowed in each petri dish and permitted to feed on all of the test materials (*i.e*., plant extract and silver nanoparticles), containing 100 µM/g concentrations and a control was maintained on artificial diet. The experiment was carried out in triplets. After 24 hours, the weights of diet cubes were recorded to track the larvae's diet consumption. Mortality and morphological abnormalities linked with growth disrupting effects were identified in the larvae.

Extraction of midgut

After 24 hours of treatment, the midgut of 4th instar larvae was dissected under a microscope. The midgut was excised and rinsed in cold 50mM Tris-HCl. pH -7.4, then pulverized in a pestle and mortar in pH 7.4 Tris-HCl buffer at 4 °C. The samples were centrifuged at 10,000g for 10 minutes in a 1.5ml Eppendorf tube containing Tris-HCl buffer. The supernatant was precipitated with ammonium sulphate to achieve 70 per cent protein saturation. The precipitate was recovered by centrifugation at 10,000g for 10 minutes, diluted in 50 mM Tris-HCl buffer (pH 7.4), and kept at -20°C until used in further investigations.

RNA isolation

After the excavation of worms, they were stored in the nuclease free water and frozen in the -4°C until further process. On the day of RNA extraction, the stored samples were washed mildly with the PBS to remove excess preserving solution and 200µl TRIZOl reagent (Invitrogen) was added for RNA extraction. Total RNA was isolated by homogenizing the samples using motorized pestle performing sonication for five strokes with 20secs "ON" and 15secs "OFF". The solution was combined with an equivalent volume of chloroform (200 µl) and centrifuged at 10,000 rpm for 10 minutes to separate it. The aqueous layer was collected and precipitated with isopropanol, and the particulate was reconstituted in nuclease-free water and quantified with Nanodrop after centrifugation (Denovix). Electrophoresis on a 1 per cent denatured agarose gel was used to assess the purity of the RNA. The quality of rRNA (28S and 18S) bands at a 2:1 ratio was used to assess RNA integrity.

cDNA Synthesis and real time PCR

Isolated RNA was used for cDNA synthesis using High capacity kit (Invitrogen). Briefly, the total reaction mixture was made up of buffer, dNTP's, random primers, reverse transcriptase enzyme with 1 µg of RNA and total reaction volume was twenty microliters. The programme for cDNA synthesis was followed according the manufacturers manual. Upon completion of the synthesis the volume was made up to 100 µl for the further analysis. The cDNA was amplified for trypsin and chymotrypsin (were coded as target 1 and 2 respectively) by real time PCR using Qiagen detection system with SYBR green as a detection dye. The specific primers for the target 1, 2 and beta actin were as follows,

Target 1 forward 5' GCGTAAAGGATGCGGTTGG 3'
Reverse 5' CAGGATGGCAACCATCCATG 3'
Target 2 forward 5' CACCATCTTCATCTTCCAATCCGTGTGC 3'
Reverse 5' GTGTTGATACGAGTACCACCGAAGAAC 3'
β-actin forward 5' GATCGTGCGCGACATCAAG 3'
Reverse 5' GCCATCTCCTGCTCGAAGTC 3'.

The SYBR green master mix containing cDNA products was amplified using the following protocol: denaturation at 95°C for 5 minutes, annealing at 60°C for 2 minutes, and extension at 72°C for 2 minutes. These conditions were repeated for 35 cycles and melting curve was obtained by heating the samples from 60°C to 95°C in 1°C increment in every cycle. The expression of target genes was normalized to the internal control β-actin and levels were calculated using delta Ct ($2^{-\Delta\Delta Ct}$) method. The values were expressed as the relative fold gene expression. Here the trypsin and chymotrypsin are named target 1 and target 2 respectively.

Results & Discussion

Characterization

XRD analysis

X-ray diffraction was used to determine the crystalline nature of biosynthesized AgNPs, and the results are shown in [Fig-1(a-c)]. The peaks at 38.31°, 44.17°, 64.10°, and 77.56° in the LA sample correspond to planes (111), (200), (220), and (311) of the cubic structure of silver nanoparticles, as shown in [Fig-1a]. Particles in the nanoscale range are supported by the width and intensity of peaks. The (111), (200), (220), and (311) planes of cubic phase of silver nanoparticles, respectively, correspond to the 38.29°, 44.50°, 64.61°, and 77.43° diffraction peaks 2 for the CR sample [Fig-1b]. The maxima of the CD sample at 38.43°, 44.64°, 64.81°, and 77.61° correspond to the (111), (200), (220), and (311) planes of face centred cubic AgNp's, as shown in graph 1(c). The diffraction angles of all samples are well matches with JCPDS data: 04-0783 [19, 20]. The Scherrer equation, $D=K\lambda/\beta Cos\theta$, was used to determine the particle size of the samples. D is the crystallite size, and λ is the X-ray beam wavelength β is the FWHM value.

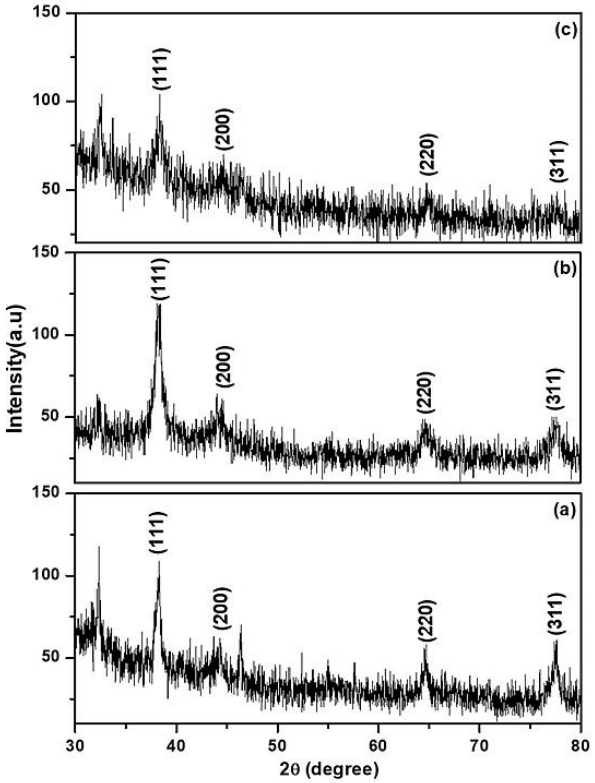

Fig-1 XRD spectra of Ag nanoparticles Graph (a-c) respectively, represent the XRD spectrum for LA, CR and CD samples

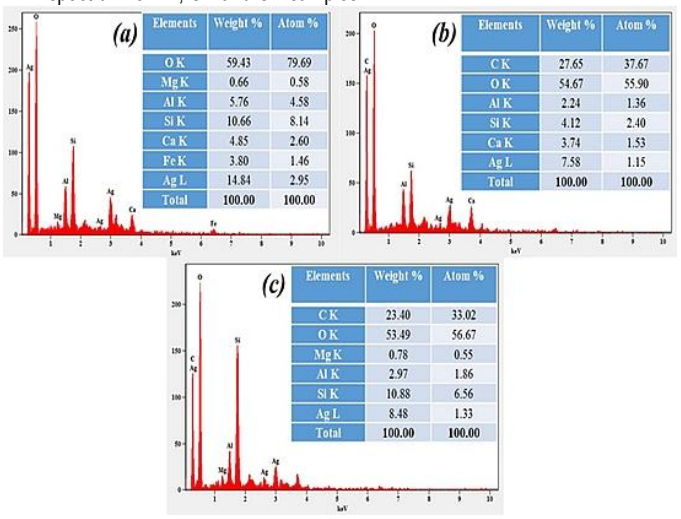

Fig-2 EDX spectra of AgNPs (a-c) respectively

The calculated value of average crystallite size of LA, CR and CD sample is found to be 2.49nm, 2.37nm and 2.25 nm, respectively. As compared to LA and CD samples, the XRD spectrum of CR samples reveals a high crystallinity and a modest change in diffraction angle due to higher rate of reduction of $AgNO_3$.

EDX

The EDX spectra of samples LA, CR, and CD are shown in [Fig-2a-c]. All samples have a prominent peak around 3 keV in their EDX spectra, indicating the existence of silver and justifying the formation of silver nanoparticles. The weight percentage and atomic percentage of silver varies with different plant extracts due to the removal of unreacted organic and inorganic contaminants during product washing.

FTIR Analysis

FTIR spectroscopy was used to identify the different functional groups contained in samples and the results are displayed in [Fig-3a-c].

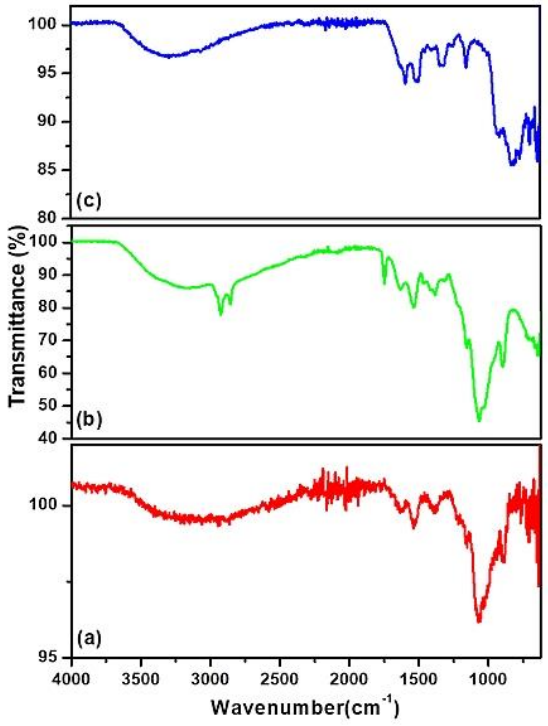

Fig-3 FTIR spectra of sliver nanoparticles. Graph (a-c) represent FTIR spectrum of LA, CR and CD samples

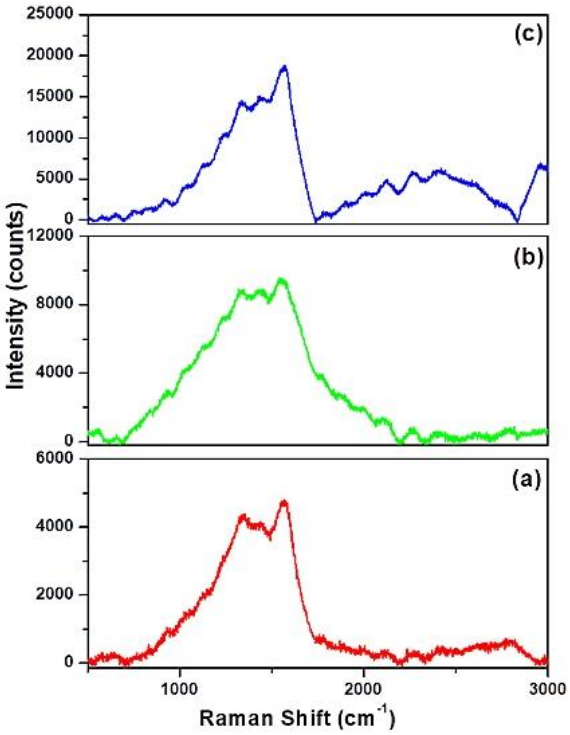

Fig-4 Raman spectrometric analysis of AgNP's, Graph (a-c) represents the Raman spectra of LA, CR and CD samples

The availability of flavonoids and terpenoids in the all plant extracts may allow for efficient silver nanoparticles capping and stability, resulting in the formation of all of these peaks. The peaks at 1611 cm^{-1}, 1271 cm^{-1}, and 1065 cm^{-1} are attributed to various squeezing vibrations of the OH, CH, C=C, and C-N groups, respectively [21]. The highest point 3078 cm^{-1}, 3327 cm^{-1} due to chemisorbed/physisorbed H_2O and CO_2 molecules [22].

Table-1 Relative expression of traget1 (trypsin) and target 2 (chymotrypsin) against Beta actin of leaf extracts and silver nanoparticles

	NOR	LA	CD	CR	LA AgNPs	CD AgNPs	CR AgNPs
TARGET 1 Ct	21.12	19.58	19.8	19.26	19.57	19.75	19.65
TARGET 2 Ct	16.21	15.01	14.7	14.8	15.01	15.04	15.01
Beta-actin Ct	15.12	15.42	15.86	15.06	14.09	15.04	14.04
T1 dcT (gene-ctrl)	6	4.16	3.94	4.2	5.48	4.71	5.61
T2 dcT (gene-ctrl)	1.09	-0.41	-1.16	-0.26	0.92	0	0.97
T1 2^-dct without	1	3.5801	4.16986	3.5801	1.43396	2.44528	1.31039
T2 2^-dct without	1	2.82843	4.75683	2.54912	1.12506	2.12874	1.08674

The merging of peaks related to aldehydes (1692 cm^{-1}), diketones (1566 cm^{-1}), alkanes (1469 cm^{-1}), amines (1334 cm^{-1}), and ethers (1185 and 1051 cm^{-1}). Peaks at 1593 cm^{-1}, 1509 cm^{-1}, and 1152 cm^{-1} are attributed to the stretching of diketones, polysaccharides, and aromatic compounds, as seen in graph 3 (c). Peaks emerged in the mid all 3 FTIR region of the spectrum at 2355.96 and 1358.68 cm^{-1} due to atmospheric CO_2 adsorption [23]. Weak absorption peaks associated with aromatic compounds (824.56 and 749.83 cm^{-1}), along with halogen compounds (538.93 cm^{-1}), were observed. The stretching vibration of aromatic chemicals included in extract causes a shift of strong peak at 825cm^{-1} of CD sample towards lower wavenumber side as compared to spectra of LA and CR samples.

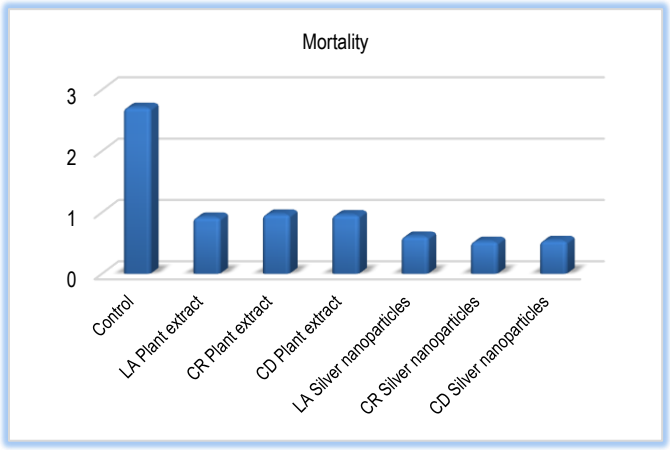

Fig-5 Effects of a diet containing LA, CR, and CD leaf extracts and LA, CR, CD AgNPs on the mortality rate of *H. armigera* larvae

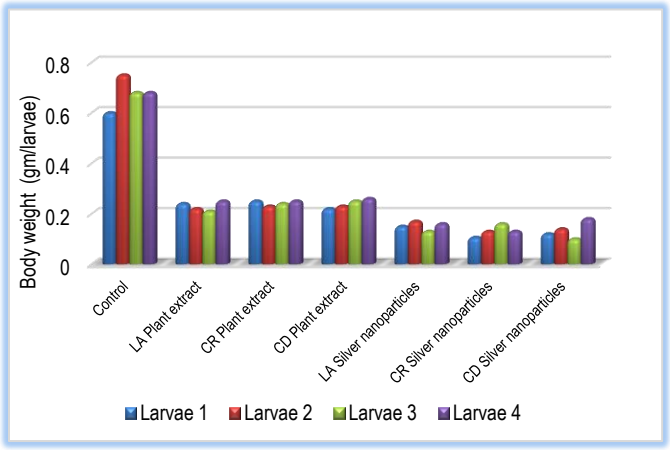

Fig-6 Effects Larvae body weight fed on diet containing LA, CR, CD crude leaf extracts and LA, CR, CD AgNPs

Raman spectroscopy

To probe the molecular structure and composition of samples we used the Raman spectroscopy and results are presented in [Fig-4a-c. Raman spectra of all samples show the slight change in pattern due to presence of aromatic and halogen compounds present in samples and their strong scattering properties. The sharp peaks around at 1348cm^{-1} and 1550 cm^{-1} due to the symmetric and antisymmetric vibration of C=O of carboxylic group [24, 25]. The small peaks noticed around at 1253cm^{-1}, 1145 cm^{-1},1064 cm^{-1} and 932 cm^{-1} are ascribed due to the in/out plane bending of C-C, C-N and C-H groups respectively [26]. The peak at 1456 cm^{-1} is characteristic of asymmetric vibration of C-N group. Thus, the change in these Raman bands, it can be concluded that aromatic and halogen present in plant extract are encapsulated on surface of silver nanoparticles. The broad peak around at 2500cm^{-1} for CD sample [Fig-4c] due to vibration of aromatic and alkanes of plants extract absorbed on surface of nanoparticles. These results are well matches with biosynthesis of AgNP's by other researchers [27].

Role of AgNPs on *H.armigera* growth and development

Pesticide infiltration, often due to inadequate application, had long been a concern in the control of a variety of pests and weeds [28] which seems to be particularly true for *H. armigera*, which is resistant to the majority of pesticides [29]. Nanoparticles insecticidal properties are connected to physical qualities that drive physiological changes [30].

Under laboratory circumstances, the larvicidal effectiveness of LA, CR and CD crude leaf extract and LA, CR, CD-Ag NPs was evaluated. After 24 hours of exposure with respective treatments, *Helicoverpa armigera* 3rd instar larvae were assessed. The all 3 leaf extracts showed moderate Antifeedant activity and larval mortality, whereas no mortality was recorded for non-treated control. [Fig-5] revealed silver nanoparticles exhibited maximum death rate against larvae for larvae while leaf extracts moderate mortality rate. The above signifies that as compared to plant extract and control, larvae treated with AgNPs ingested the least amount of treated feed and died at a higher rate. The Antifeedant activity data shown in [Fig-6] revealed that all examined components, save the control, had feeding deterrent activity, with the exception of leaf extract (75 per cent) and AgNPs (98 per cent). [Fig-7a-c] shows the physiological changes undergone by the larvae treated with silver nanoparticles *i.e.*, decreased in larval body weight decreased body length and reduced movement. [Fig-7d-f] shows the physiological changes undergone by the larvae treated with crude leaf extract *i.e.*, body weight increased. Larvae fed AgNPs synthesised showed a substantial reduction in growth and development rate when compared to larvae fed plain leaf extract and an experimental diet. Lepidoptera larvae have been observed to grow more slowly when fed artificial diets supplemented with additional metals such as zinc, cadmium, copper, and lead [31, 32]. The methanol has been proven to be an excellent solvent for extracting bioactive compounds from plants with good larvicidal activity [33]. The detrimental impact of the plant extract might be likely to result in significant metabolites found in this plant [34, 35]. Synthesized AgNPs from *E. prostrata* have been shown to be efficient against the insect pest *S. oryzae* [36]. AgNPs produced from *Nelumbo nucifera* leaves extract demonstrate larvicidal action versus *Culex quinquefasciatus* and *Anopheles subpictus* [37]. Within 14 days of treatment, at a dosage of 20 ppm, the insecticidal effect of Nano silver colloid and ethanol-based colloid sulphur Nano silver revealed nearly 100% mortality against case-making clothes moth, *T. pellionella* (L.) larvae, damaging wool fibres [38].

Midgut protease (trypsin and chymotrypsin) activity

Furthermore, our findings on gut proteases (trypsin and chymotrypsin) support the produced silver nanoparticles from LA, CR and CD has an effective entomotoxicology potential on *Helicoverpa armigera* larvae. The LA and CR crude leaf extract showed threefold increase in the activity while there was twofold decease in respective synthesised silver nanoparticles. The CD extract showed fourfold increased and decrease in two folds in CD AgNPs. The consumption and survival rate of larvae in diets containing silver nanoparticles were significantly reduced.

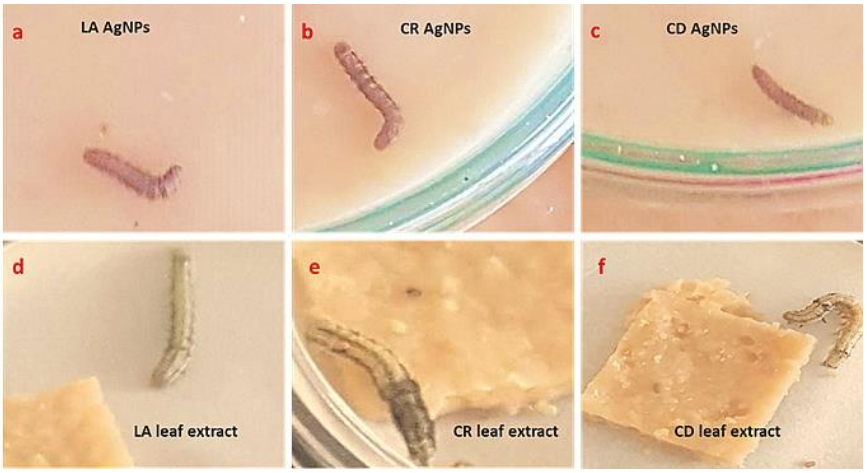

Fig-7 Influence of silver nanoparticles on larval weight (a-c) and crude leaf extract on larval weight (d-f)

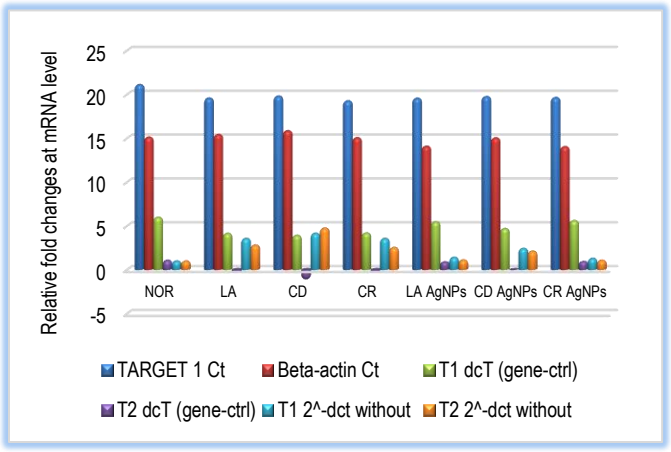

Fig-8 Expression of target 1 (trypsin) in the LA, CR, CD crude leaf extract and LA AgNPs, CR AgNPs, CD AgNPs

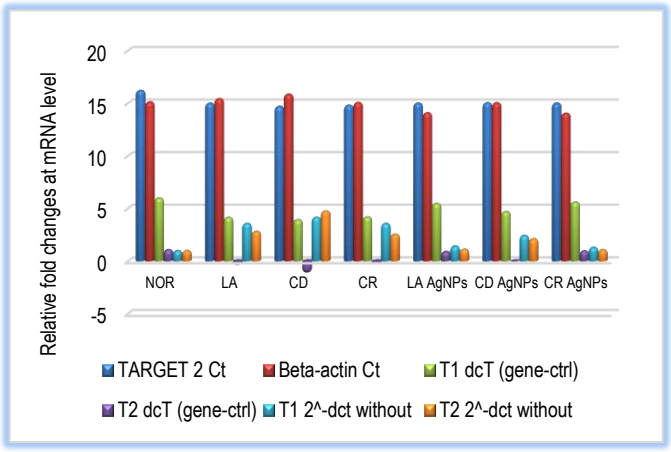

Fig-9 Expression of target 2 (chymotrypsin) in the LA, CR, CD crude leaf extract and LA AgNPs, CR AgNPs, CD AgNPs

The AgNPs adhering to the target binding site resulted in a reduction in protease activity (trypsin and chymotrypsin) and also suggest a link between both the protein and the nanoparticles because when trypsin change occurs. Insect digestive proteases accelerate the release of free amino acids from ingested protein, providing vital nutrients for growth and development. Many studies have been reported by researchers the activity of phytochemicals on the regions that control gastrointestinal motility and metabolism was identified as the cause of feeding restraint [39]. The results of the quantitative real-time PCR studies revealed that all the crude leaf extracts and sliver nanoparticles stimulated the protease activity. From [Fig-8] and [Fig-9], it's observed the gut protease (trypsin and chymotrypsin) activity slightly increased by four-fold in the larvae treated with cured extract when compared to control. The larvae treated with sliver nanoparticles showed three folds decreased activity when compared to crude extract. It was observed that amylase activity in *H. armigera* is modulated by biosynthesized silver nanoparticles [40]. The impact of rapid starch hydrolysis destruction was explored by studying the interaction of AgNPs with α-amylase [41]. As pepsin and trypsin are so similar, it's logical to assume that AuNPs can improve trypsin stability as well. The pepsin anchored on AuNPs surface was more stable than just the free enzyme [42].

Conclusion

We focused on environmentally friendly silver nanoparticle fabrication from LA, CR, and CD plant extracts in this study. Various approaches were used to characterise the physical properties of produced silver nanoparticles *i.e.*, XRD spectra confirm the formation of AgNP's in phase centered cubic, FTIR results verify the formation and composition of components, and the findings of Raman spectroscopy are highly correlated. These silver nanoparticles were employed in control of pest *Helicoverpa armigera*.

Despite crude leaf extracts of LA, CR, and CD had no effect on larvae, nanoparticles synthesised from crude leaf extract caused considerable cytotoxicity in the form of lower body weight and development in larvae. Hence silver nanoparticles synthesised from LA, CR and CD plant can effectively use as an agent to control *Helicoverpa armigera* pest (*i.e.*, act as Antifeedant agent, which subsequently deplete the growth of larvae and increase the mortality rate). Mimicking particles nature technique by enabling AgNPs as insecticidal chemicals could be a cost-effective way to manage insect pests while still minimizing the environmental impact. The above findings would also offer as a conceptual framework for the implementation of novel nanoparticles to combat pesticide resistance.

Application of research: The biosynthesized silver nanoparticles could be used as alternative for chemical pesticides as it is eco-friendly, cost-effective. The study provides information for the control of *Helicoverpa armigera*.

Research Category: Biogenic nanoparticles and Pest control

Abbreviations:

H. armigera- Helicoverpa armigera
FTIR- Fourier Transform infrared spectroscopy
XRD- X-ray diffraction
EDX- Energy Dispersive X-Ray Spectroscopy
LA- *L. aspera- Leucas aspera*
CR - *C. rotundus-Cyperus rotundus*
CD - *C. dactylon- Cynodon dactylon*
AgNPs- silver nanoparticles
LA-AgNPs- *Leucas aspera* silver nanoparticles

CR-AgNPs - *Cyperus rotundus* silver nanoparticles
CD-AgNPs - *Cynodon dactylon* silver nanoparticles
NBAIR - National Bureau of Agriculture Insect Resources
Target 1- Trypsin
Target 2- Chymotrypsin

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****Research Guide or Chairperson of research: Dr S. Lokesh**
University: University of Mysore, Mysuru, 570 005, Karnataka, India
Research project name or number: PhD Thesis

Author Contributions: All authors equally contributed

Author statement: All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area / Sample Collection: NBAIR (larvae purchased)

Cultivar / Variety / Breed name: Nil

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

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