Indian Journal of Biochemistry & Biophysics
Vol. 58, October 2021, pp. 478-485

Silver nanoparticles loaded pyrrole based pesticidal metabolites (AgNps-PFM) nanoconjugate induced impact on the gut microbion and immune response against lepidopteron pest *Spodoptera litura* (Fab.)

S Karthick Raja Namasivayam* & RS Arvind Bharani

Centre for Bioresource & Development (C-BIRD), Department of Biotechnology, Sathyabama Institute of Science and Technology, Chennai-600 119, Tamil Nadu, India

Received 10 August 2019; *revised 21 August 2021*

Pest control measures using nanobiotechnology principles is now being extensively utilized in the various parts of the world. Understanding the molecular mechanism behind the pestiicdal activity of the nanomaterials is essential to exploit the principles of nanomaterials as an effective pest control strategy. In the present investigation, impact of the nanoconjugate prepared from biogenic silver nanoparticles-fungal based insecticidal metabolites (AgNps-FM) on the gut microbion and immune response against major lepidopteron pest *Spodoptera litura* (Fab.) was studied. Nanoconjugate was prepared from silver nanoparticles from Pomegranate (Punica granatum) peel extract with the fungal based pesticidal metabolites. Highly stable nano structural nano conjugate thus obtained was evaluated against gut microbion and immune response of *Spodoptera litura*. Gut microbion status was studied by determination of microbial count, extra cellular enzymes potential of the gut microbial isolates adopting culture dependent methods. Nanoconjugate induced effect on the immune response was done by recording total hemocyte count and reduced total haemocyte count, phenol oxidase activity and lectin expression pattern Synthesized nanoconjugate brought about notable impact on the gut microbion count and their extra cellular enzyme potential. Reduced haemocytes count, poor lectin expression and reduced phenol oxidase production reveals the immune suppressive activity. High efficacy of the Nanoconjugate thus obtained would suggest the possible utilization of Nanoconjugate as an effective, safe pesticidal agent against economic important insect pests.

Keywords: Enzyme, Gut microbion, Haemocytes, Nanoparticles

The utilization of nanotechnology in agriculture has exceeded expectations into new research contemplates, which delineates to nanofood frameworks, nanobiotechnology and nanoremediation[1,2]. Use of nanotechnology in agribusiness incorporates plant reproducing, accuracy horticulture, malady control, biotechnology hereditary qualities, manure innovation and unified fields. In the event that ranchers are given great comprehension of farming creation framework, the use of nanotechnology has brilliant prospects like nanoformulations of compost, reconnaissance and control of nuisances and illnesses, instrument of host parasite association at atomic dimension, improvement of new age pesticides and their professions, conservations and bundling of nourishment added substances, reinforcing of normal filaments, expulsion of contaminants from water and soil, expanding the timeframe of realistic usability of blossoms and vegetables, mud based nanosensors for accuracy water the executives, recovery of salt influenced soils, adjustment of disintegration inclined surfaces[3,4]. Accuracy cultivating, antimicrobial nanomaterials for plant pathogens and improvement of nanopesticides are present day approaches in agribusiness. Nano-cultivating, nano sustenance and nano packaging are the highlights of nanotechnology in nourishment industry. Nano-sensors are connected in keen quality conveyance framework and in location of pathogen. Creepy crawly vermin weed and parasites are to be overseen by the utilization of nanobiopesticides, herbicides and fungicides[5]. These create draft and irritation safe yields. Use of nanotechnology improved nourishment quality and sustenance wellbeing likewise improves handling and sustenance[6]. This extended nanotechnology as another premise to convalesce winning harvest the board rehearses. The employments of nanomaterials, especially, metallic nanoparticles are getting more consideration because of their improved explicitness, steadiness, work biocompatibility, phenomenal quality, progressively substance reactivity, and having

*Correspondence:
E-mail: biologiask@gmail.com

a high electrical conductivity which will be utilized to abuse the standards of nanotechnology as a standout amongst the most encouraging, viable, green methodologies for bug control in the ongoing years[7].

Various reports reveals the diverse nanomaterials and their role in pest management against economic important pests associated with crops[8]. Our late examinations demonstrates that unmistakable pesticidal action of silver nanoparticles, parasitic metabolites stacked chitosan nanocomposite against *Spodoptera litura* would propose the conceivable usage of nanoprinciples as a compelling pesticidal operator[9,10]. In this examination, impact of fungal metabolites loaded with silver nanoparticles on gut microbion and immune response of *Spodoptera litura* has been completed. Among the different metallic nanoparticles, silver has turned out to be most encouraging nanomaterial in light of its high potential enemy of microbial activity and biocompatibility[11]. Potential natural exercises of silver nanoparticles against microorganism (microbes, infections, parasites and different pathogens) have announced somewhere else. Nonetheless, few reports are accessible on the pesticidal or insecticidal action of silver nanoparticles against monetary imperative creepy crawlies bothers. With this target, the present research work is attempted to assess pesticidal action of silver nanoparticles orchestrated from strip concentrate of pomegranate (Punica granatum) stacked contagious metabolites against major polyphagous pest *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae). *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) is an important lepidopteron pest associated with diverse plants[12] and is thought about thriftily indispensable in various stretches, nations including Indian subcontinent and Asian nations. *S. litura* is a defoliating bug that upsets the gather of different developed money crops, vegetables, organic products, weeds and elaborate plants by sustaining agreeably on leaves and it incites outsized financial fatalities in harvest plants. The controlling of *Spodoptera litura* to protect the enduring and unprecedented efficiency of harvests is an enormous experience in agrarian turf and in this way; pesticide treatment is extensively capable for its control. There is broad misgiving over unfavorable impact of pesticides on ecological and humanoid condition inferable from accumulation of pesticide leftovers notwithstanding ascent of pesticide opposition in the creepy crawlies[13]. Biological control utilizing assorted organic operators assume an imperative job in concealment or control of a wide scope of bug bugs related with different agro biological community.

Microbial biocontrol operators can be efficacious and work as substitutes to wide scope of compound pesticides[14]. Their enlarged activity will require expanded pathogen destructiveness and accelerate pathogen execution under animating natural conditions, better viability in their creation, improvements in definition that helps less demanding application, enlarged biological determination and delayed timeframe of realistic usability, better comprehension of how they will sufficient into coordinated frameworks and their interface with the earth and other incorporated irritation the executives (IPM) factors, more noteworthy increment of their ecological additions and acknowledgment by cultivators and the general network[15]. Among the microbial agents, organisms are those which support ailment signs in creepy crawlies and furthermore grasp growths from quick executioners to finish parasites that convey illness side effects in the host[16]. Biocontrol of *Spodoptera litura* utilizing entomopathogenic contagious strains under research center and field trails unmistakably uncovers the parasitic biocontrol operators displayed particular pesticidal action by chronicle greatest mortality to the larval instars and trademark changes in the existence stages[17,18]. Growths are likewise known to create a wide scope of insecticidal metabolites like beauvericin, cyclodepsipeptide particle delivered by Beauveria and destruxins of *Metarhizium* species[19]. However, less report are accessible on the broad method of activity of insecticidal metabolites and their use design in horticulture division. In the present investigation, impact of the nanoconjugate prepared from biogenic silver nanoparticles-fungal based insecticidal metabolites (AgNps-FM) on the gut microbion and immune response against *Spodoptera litura* (Fab.) was studied. Gut microbion status was studied by determination of microbial count, extra cellular enzymes potential of the gut microbial isolates adopting culture dependent methods. Nanoconjugate induced effect on the immune response was done by recording total hemocyte count and reduced total haemocyte count, phenol oxidase activity and lectin expression pattern

Materials and Methods

Synthesis of silver nanoparticles-pesticidal metabolites (AgNps-PM) nanoconjugate

Nanoconjugate was prepared from highly stable silver nanoparticles that derived from peel extract broth of pomegranate (Punica granatum). Reduction

of precursor with the peel extract brought about highly stable, crystalline, nano structural silver nanoparticles as described by our previous studies[20]. Metabolites used for nano conjugate preparation was extracted from fungal strain *Nomuraea rileyi* (F) Samson as described in our previous study[21]. This study reveals the extra cellular metabolites of the fungal origin with pesticidal compounds like pyrrolol. Nanoconjugate was synthesized from the silver nanoparticles and the fungal metabolites as reported by our earlier studies via green science principles as reported by our very recent study[22]. Characterization of the synthesized nano conjugate was done by suitable analytical techniques like UV visible absorption spectroscopy, fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) to determine structural, functional properties of the nano conjugate. Simple dispersion method that we carried out brought about nano structural, well aggregated particles with 85-95 nm.

Nanoconjugate induced gut microbion stress

Bioassays

III instar larvae of *S. litura* were selected for this study. Freshly molted healthy larvae were allowed to feed castor leaves sprayed with different concentration of free silver nanoparticles, fungal metabolites and AgNps-PM separately. Three replicates and control wereg were maintained Respective treatment groups were incubated at 28°C.

Gut microbion status

Nanocomposite induced effect on the gut microbion status was studied by determination total count (bacteria) and extra cellular enzyme potential adopting culture dependent methods. Gut tissue was aseptically dissected out followed by homogenization with sterile saline. Homogenate was serially diluted in sterile saline under aseptic condition. Known volume of aliquot was plated on sterile nutrient agar, incubated at 37°C for 24-48 h. After the incubation period, viable bacterial colonies were counted and recorded. Pure culture of the respective bacterial strain was identified by morphological, cultural and biochemical characteristics[23]. Nanoconjugate induced effect was studied by recording the differences in generic composition and the results were represented as generic composition inhibition (%). Effect of nanoconjugate on the extra cellular enzyme production potential was also done. In this method, the respective pure culture was inoculated on the nutrient medium supplemented with starch (amylase), casein (protease) and carboxyl methyl cellulose (cellulose). From the data of enzyme producers in the respective treatment group, enzyme production inhibition (%) was determined.

Impact of nanoconjugate on the immune response

Total hemocyte count

Effect of AgNps-PM on the total haemocyte count of III instar larva was carried out by the modified method of Gupta[24]. Hemolymph was drawn from the respective treatment group of larvae into WBC pipette (0.5 mL – physiological saline) and was vigorously shaken for few minutes, poured on Neubauer haemocytometer. Observation of hemocytes was done under Olympus light microscope at 40X.

Effect of nano treatment on Phenoloxidase (PO) assay was done by the modified method of Cotter and Wilson[25]. The hemolymph was collected from respective groups of III instar larvae was transferred to the sterile eppendorf with 0.4 mL of phosphate buffered saline followed by the addition of 0.1 mL of 20 mM LDOPA. Spectrophotometric measurement of dopachrome as the end product reveals PO activity. The increase in absorbance was measured at 475 nm and the amount of PO in the sample was calculated where one unit is the amount of enzyme required to increase the absorbance by 0.001 per min.

Lectin analysis

Effect of nano conjugate on lectin activity from respective treatment groups was also studied. The rate of lectin formation was determined using haemoagglutination titration.

Results and Discussion

Fungi are the important groups of biopesticidal agents against an economically important pests and the effective management of fungal and fungal based, metabolites as pest control management are highly utilized[26]. Nanotechnology principles for the formulation of various agro active agents including fungal metabolites is an attractive, effective strategy of pest control due to the higher efficacy, less non target effect and best biocompatibility. In this study, enhanced pesticidal activity of fungal pesticidal agent *Nomuraea rileyi* (F.) Samson mediated pesticcidal metabolites were formulated with silver nanoparticles and the synthesized nanoconjugate was evaluated for the enhanced pesticidal activity against *S. litura.*

Synthesis of silver nanoparticles-pesticidal metabolites (AgNps-PM) nanoconjugate

Nanoconjugate was prepared from highly stable silver nanoparticles that derived from peel extract broth of pomegranate (Punica granatum). Reduction of precursor with the peel extract brought about highly stable, crystalline, nano structural silver nanoparticles as described by our previous studies[20]. Metabolites used for nano conjugate preparation was extracted from fungal strain *Nomuraea rileyi* (F) Samson as described in our revious study[21]. Pesticidal metabolites were extracted from the fungal strain was readily soluble in ethyl acetate reveals the presence of Pyrrolo based pesticidal metabolites.

Silver nanoparticles loaded fungal metabolites were characterized by various analytical techniques[22]. Characterization of the synthesized nano conjugate was done by suitable analytical techniques like UV visible absorption spectroscopy, fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) to determine structural, functional properties of the nano conjugate. Simple dispersion method that we carried out brought about nanostructural, well aggregated particles with 85-95 nm. High stability was confirmed by no sign of aggregates, color changes or turbidity in the prepared nano conjugate which exposed at different time periods (Table 1).

Pesticidal activity

Pesticidal activity was studied by determination of cumulative mortality against larval instars that reand developmental parameters of *S. litura* different life stages.Results are represented as activity index percentage calculated from mortality rate data obtained from free nanoparticles and metabolites against respective larval instars that reported in our studies[22]. Our finding reveals that nanoconjugate brought about notable effect on the all the tested developmental parameters.

Table 1 — Effect of storage (days) on the stability of nano conjugate

Incubation time (Days)	Colour change / turbidity	Aggregates
10	-	-
20	-	-
30	-	-
40	-	-
50	-	-

-Absent

Effect on gut microbion status

Determination of gut microbion status also reveals the notable pesticidal activity. The bacterial flora resides in the gut of the insects is considered as a notable metabolic resource to the nutrition of the insects by various means[27]. Disruption of gut microbiota causing a severe effect to the insect physiology this would be effective in controlling the pests. In this study, nanoconjugate induced effect was studied by recording the differences in generic composition and the results were represented as generic composition inhibition (%). A total of 105 isolates belong to 4 genera like *Bacillus*, *Pseudomonas*, *Alcaligenes* and *Micrococcus* were isolated from the gut and the occurrence or frequency of distribution was found to be changed in the nanoconjugate treatment. Results indicates that all the tested bacterial generic composition was found to be inhibited significantly in the nanoconjugate treatment (Fig. 1). It can be seen that synergistic activity of silver nanoparticles and the active principles of bioactive metabolites which formulated with silver nanoparticles brought about diverse molecular mechanism which in turn inhibits the bacterial growth. Followed by bacterial generic composition, marked effect on the extra cellular enzymes production potential was also reduced in the nanoconjugate treatment (Fig. 2), Inhibitory potential of the active principles of nano conjugate also exhibits the marked effect on the enzyme production potential.

Effect on the cell mediated immune response

Pesticidal activity was further confirmed by studying cell mediated immune response of *S. litura*. Effect on the immune response was determined by changes in total hemocytescount, phenoloxidase activity and lectin analysis. Insects have well developed protection system against invading external materials like production of antimicrobial peptides, various intermediates of nitrogen, oxygen, cytokines, prophenoloxidase (PO), phagocytosis, nodulation, encapsulation. Previous studies on pest control measures have focused on larvicidal activity of nanomaterials. However, to the best of our knowledge, there have been no reports on the silver nanoparticles, silver nanoparticles loaded metabolites based immune suppression which shot down the immune system and in turn cause mortality against *Spodoptera litura*. In the present study, AgNps-FM nanoconjugate and its immune suppression studies has

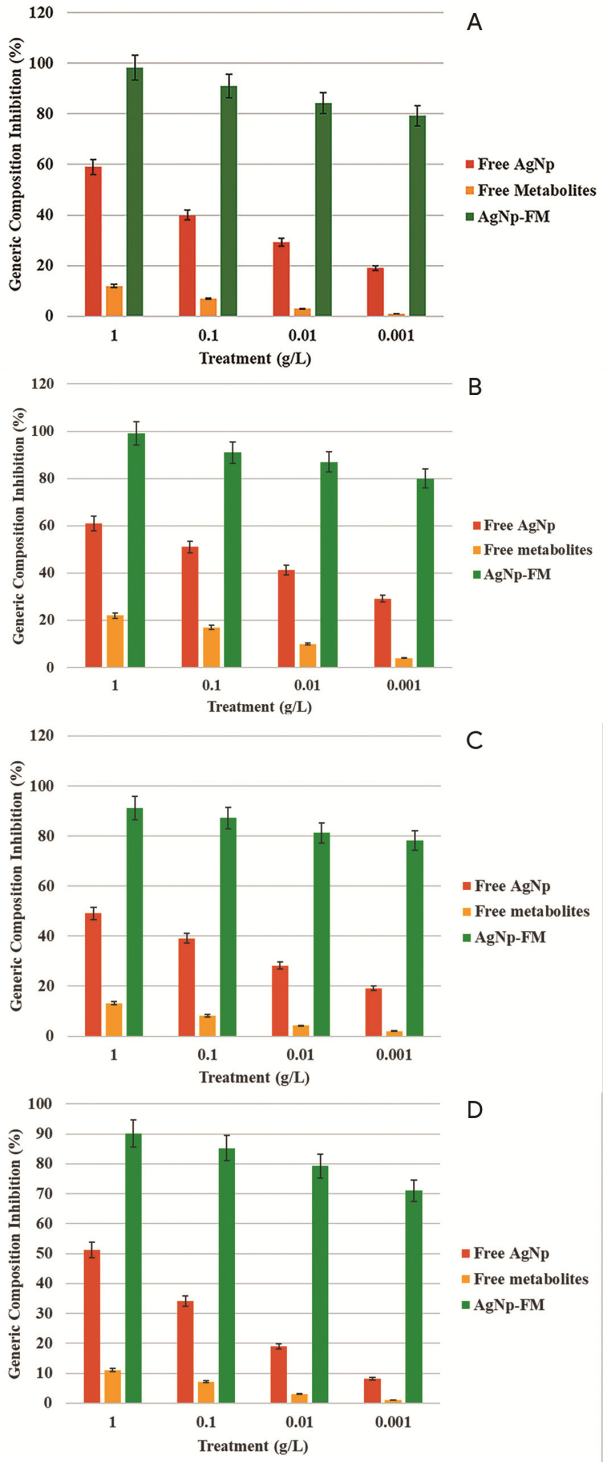

Fig 1 — Generic composition inhibition of gut microbion (A) *Bacillus*; (B) *Pseudomonas*; (C) *Alcaligenes*; and (D) *Micrococcus*

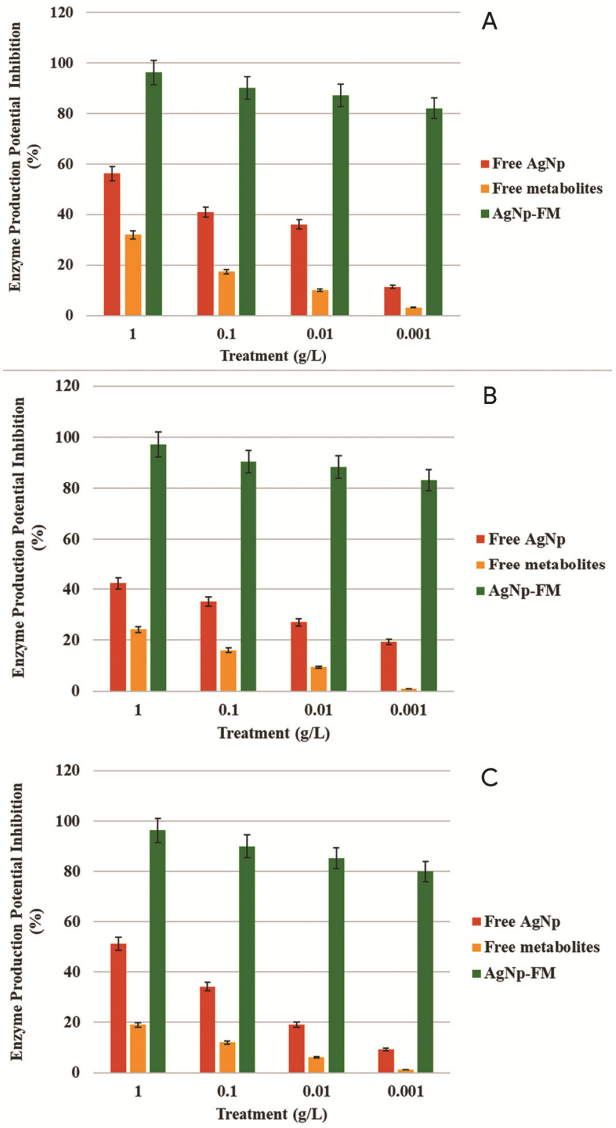

Fig 2 — (A) Amylase; (B) Protease; and (C) Cellulase production potential inhibition (%) of gut microbion isolates

studied. Total hemocyte count plays a major role in immune system by phagocytosis, nodule formation, encapsulation and hence the total number of haemocytes reflect the involvement of immune system to deal with pathogens or chemical molecules[28]. The results clearly indicates that nano conjugate recorded distinct effect on the total hemocytes count. Exposure to the nano conjugate treatment, significant reduction of different hemocytes like plasmatocytes, pro hemocyte granular, oenocytoid, spherulle was recorded (Fig. 3). Among the various types of hemocytes, plasmatocytes were found to dominant followed by prohemocytes. Significant decrease of all the hemocytes was recorded in 1.0 g/L of nano conjugate followed by 0.1 g/L. However, no changes in all the hemocytes was inferred from free fungal metabolites (FM). Metabolites and free silver nanoparticles with all the dosages did not increase or reduce total hemocytes count (THC). Previous studies

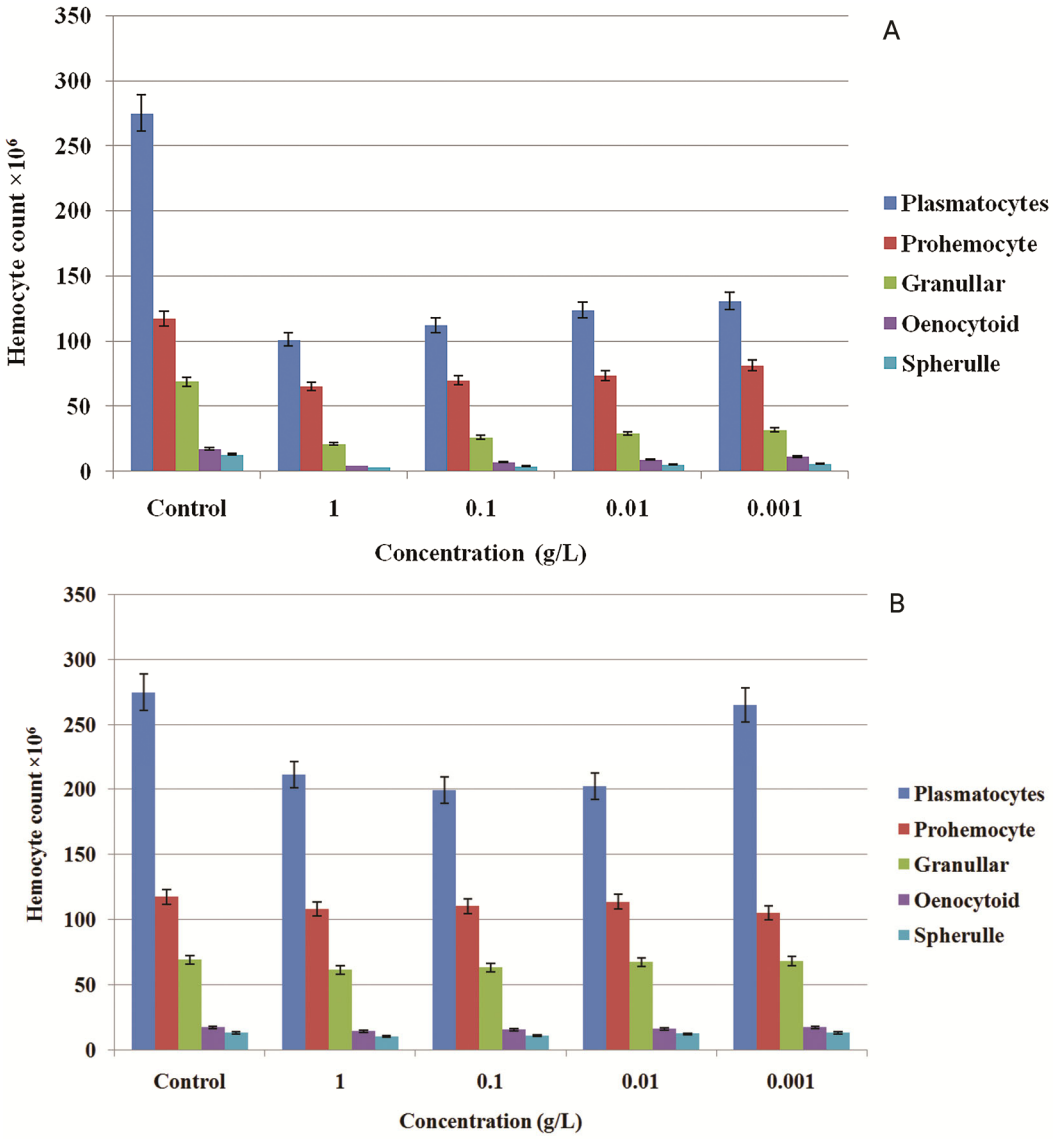

Fig 3 — (A) Effect of free Silver nanoparticles; and (B) Nanoconjugate on total hemoytes count of *Spodoptera litura*

using pathogens[26,27] and plant extracts mediated effect on the total hemocyte count of economic important insect pests have reported[28]. This is the first report of nanoparticles or nanoparticles loaded metabolites mediated immune suppression studies on *S. litura* and the nanoprinciples induced suppressed immune response was measured by studying changes in the total hemocyte count (THC-0, phenol oxidase (PO) activity and lectin expression studies. Further, nano conjugate mediated influence on phenol oxidase (PO) activity was studied. Results presented in Figure 4 showed that there was dose dependent decrease in enzyme activity. After the application of 1.0 and 0.1 g/L dosages of nano conjugate treatment, significant reduction of PO activity was recorded (P =0.5%). However, all the dosages of fungal metabolites (FM) treatment, PO activity was not found to be significantly differed from that of control. Phenol oxidase enzyme plays an important role in recognition of exogenous chemicals, parasites, parasitoids and pathogens in various arthropods[29]. Increase in phenol oxidase activity has long been correlated with increased resistance to pathogen[30] and decrease of phenol oxidase activity is attributed to weakening of immune system[31]. In the present investigation, AgNp-FM treatment brought about drastic reduction of PO activity which clearly indicates weakening of immune system of *S. litura*. As a result, AgNp-FM was found to be highly virulent which suppressed the immune response of the host and known to cause mortality.

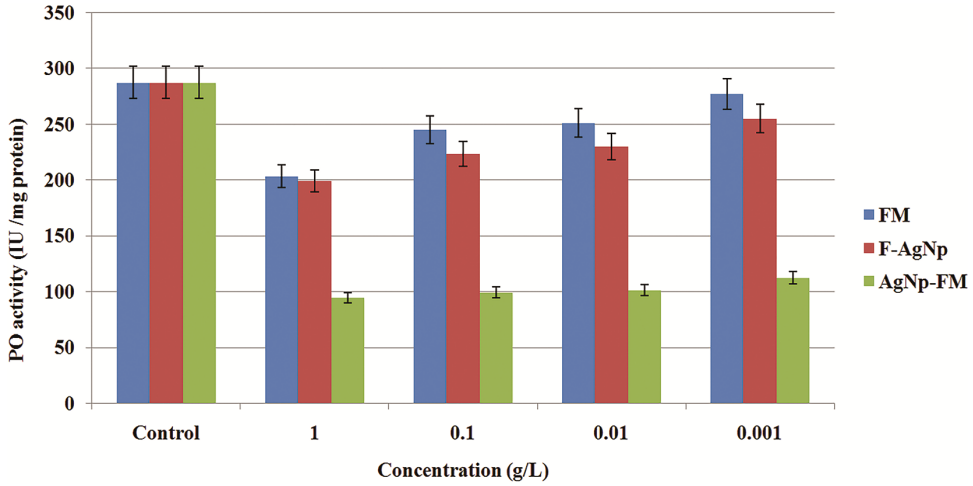

Fig 4 — Effect of silver nanoparticles, fungal metabolites and Nanoconjugate on total hemoytes count of *Spodoptera litura*

Table 2 — Effect of free AgNp, fungal metabolites (FM) and AgNps-FM on the formation of hemoagglutination at different time periods

Treatment	Concentration (g/L)	Hemoagglutination (HA) at different time (min)
		30 45 60 75 90 105 120
Free silver nanoparticles (F-AgNps)	1.0	- - + + + + +
	0.1	- - + + + + +
	0.01	- - + + + + +
Free fungal metabolites	1.0	- - + + + + +
	0.1	- - + + + + +
	0.01	- - + + + + +
Nano conjugate (AgNps-FM)	1.0	- - - - - + +
	0.1	- - - - - + +
	0.01	- + + + + + +
Control		+

+Presence of hemoagglutination, -Absence of hemoagglutination

Further, nano conjugate mediated stress on the immune system was studied by lectin expression studies. Our results showed that lectin was expressed in *S. litura* when it was exposed to AgNps-FM, AgNp and fungal metabolites which was determined by formation of hemoagglutination at different time periods Lectin profile adopting hemoagglutination (HA) assay indicates that maximum dosages of nano conjugate recorded delayed HA and 0.01 g/L resulted in faster lectin formation. Free fungal metabolites, free silver nanoparticles at all the dosages showed HA at different time interval (Table 2). Recent reports reveals the potential application of silver nanoparticles[32]. Fungal based biocontrol agents and their role in agriculture has also extensively reported[33]. Incorporation of nanotechnology principles in fungal biocontrol agents is a novel strategy to enhance the biocontrol potential.

Conclusion

Nanotechnology principles in agriculture sector is now being extensively utilized as pest control agent, smart or controlled nutrient delivery agent, plant pathogens or disease detection sensors which are recolonized the agriculture. impact of the nanoconjugate prepared from biogenic silver nanoparticles-fungal based insecticidal metabolites (AgNps-FM) on the gut microbion and immune response against major lepidopteron pest *Spodoptera litura* (Fab.) was studied. These observations would suggest the possible utilization of silver nanoparticles loaded fungal metabolites as an effective pesticidal agent associated with economic important crops. Further study under field trail will be helpful to formulate and develop AgNp-FM as a green pesticide against *Spodoptera litura*.

Acknowledgement

We Acknowledge Department of Science & Technology (DST), Ministry of Science & Technology, Government of India for the financial support (Reference Number SR/FT/LS-111/2009(G).

Conflict of interest

All authors declare no conflict of interest.

References

1 Huiqing Z, Nian H, Geliang Y, Qing L &Yonghua S, Bufalin-loaded bovine serum albumin nanoparticles demonstrated improved anti-tumor activity against hepatocellular carcinoma: preparation, characterization, pharmacokinetics and tissue distribution. *Oncotarget*, 8 (2017) 63323.

2 Subramanian KS & Tarafdar JC, Prospects of nanotechnology in Indian farming. *Indian J Agric Sci*, 81 (2011) 887.

3 Jha Z, Behar N, Sharma SN, Chandel G, Sharma DK & Pandey MP, Nanotechnology: prospects of agricultural advancement. *Nano Vision*, 1 (2011) 88.

4 Namasivayam SKR, Kumar EG & Reepika R, Synthesis of silver nanoparticles from *Lactobacillus acidophilus* SK 01 stain and evaluation of genomic toxicity. *Nanomicro Lett*, 6 (2010) 112.

5 Gul HT., Saeed S & Khan FZ, Entomopathogenic fungi as effective insect pest management tactic: A review. *Appl Sci Bus Econ*, 1 (2014) 18

6 Prasad R, Kumar V & Prasad, KS, Nanotechnology in sustainable agriculture: present concerns and future aspects. *Afr J Biotechnol*, 13 (2014) 705.

7 Khot LR, Sankaran S, Maja JM, Ehsani R & Schuster EW, Applications of nanomaterials in agricultural production and crop protection: a review. *Crop Prot*, 35 (2012) 64.

8 Xing K, Zhu X, Peng X & Qin S, Chitosan antimicrobial and eliciting properties for pest control in agriculture: a review. *Agron Sustain Dev*, 35 (2015) 569.

9 Rao CUM, C, Devi KU & Khan PAA, Effect of combination treatment with entomopathogenic fungi *Beauveria bassiana* and *Nomuraea rileyi* (Hypocreales) on *Spodoptera litura* (Lepidoptera: Noctuidaeae). *Biocontrol Sci Technol*, 16 (2006) 221.

10 Jow GM, Chou CJ, Chen BF & Tsai JH, Beauvericin induces cytotoxic effects in human acute lymphoblastic leukemia cells through cytochrome c release, caspase 3 activation: the causative role of calcium. *Cancer Lett*, 216 (2004) 165.

11 Shelby KS &Popham HJ, Plasma phenoloxidase of the larval tobacco budworm, *Heliothis virescens*, is virucidal. *J Insect Sci*, 6 (2006) 1.

12 Raja N, Biopesticides and biofertilizers: ecofriendly sources for sustainable agriculture. *J Biofertil Biopestic*, 4 (2013) 1.

13 Ramanujam B, Rangeshwaran R, Sivakmar G, Mohan M & Yandigeri MS, Management of insect pests by microorganisms. *Proc Indian Nat Sci Acad*, 80 (2014) 455.

14 Kirkland BH, Eisa A & Keyhani NO, Oxalic acid as a fungal acaracidal virulence factor. *J Med Entomol*, 42 (2005) 346.

15 Lacey LA, Frutos R, Kaya HK & Vail P, Insect pathogens as biological control agents: do they have a future? *Biol Control*, 21 (2001) 230.

16 Khashaveh A, Ghosta Y, Safaralizadeh MH &Ziaee M, The use of entomopathogenic fungus, *Beauveria bassiana* (Bals.) Vuill. in assays with storage grain beetles. *J Agric Sci Technol*, 13 (2011) 35.

17 Sahayaraj K & Namasivayam SKR, Field evaluation of three entomopathogenic fungi on groundnut pests. *Tropicultura*, 29 (2011) 143.

18 Karthikeyan A & Selvanarayanan V, *In vitro* Efficacy of *Beauveria bassiana* (Bals.) Vuill. and *Verticillium lecanii* (Zimm.) viegas against selected insect pests of cotton. *Recent Res Sci Technol*, 3 (2011) 142.

19 Onofre SB, Gonzalez RR, Messias CL, Azevedo JL & Barros NMD, LC_{50} of the peptide produced by the entomopathogenic fungus *Nomuraea rileyi* (Farlow) Samson active against third instar larvae of *Anticarsia gemmatalis* (Lep.: Noctuidae). *Braz Arch Biol Technol*, 45 (2002) 269.

20 Bharani RSA & Namasivayam SKR, Biogenic silver nanoparticles mediated stress on developmental period and gut physiology of major lepidopteran pest *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae)—An eco-friendly approach of insect pest control. *J Environ Chem Eng*, 5 (2017) 453.

21 Namasivayam SKR, Bharani RSA & Karunamoorthy K, Insecticidal fungal metabolites fabricated chitosan nanocomposite (IM-CNC) preparation for the enhanced larvicidal activity-An effective strategy for green pesticide against economic important insect pests. *Int J Biol Macromol*, 120 (2018) 921.

22 Namasivayam SKR & Bharani, RSA, Biocompatible silver nanoparticles-loaded fungal metabolites nanoconjugate (agnp–fm) preparation for the noteworthy pesticidal activity. *Natl Acad Sci Lett*, 2 (2021) 221.

23 Buchanan RE & Gibbons NE, Bergey's Manual of Determinative Bacteriology, (5th Ed. Williams and Wilkins, Baltimore, Maryland) 1974, 234.

24 Gupta AP, Insect immunocytes and other hemocytes: Roles in celluar and humoral immunity. (*Immunology of insects and other arthropods*), 19 (1991) 118.

25 Cotter SC & Wilson K, Heritability of immune function in the caterpillar *Spodoptera littoralis*. *Heredity*, 88 (2002) 229.

26 Khanikor B & Bora D, Effect of Plant Based Essential Oil on Immune Response of Silkworm, *Antheraea assama* Westwood (Lepidoptera: Saturniidae). *Int J Indust Entomol*, 25 (2012) 139.

27 Saelim S & Zwiazek JJ, Preservation of thermal stability of cell membranes and gas exchange in high temperature acclimated *Xylia xylocarpa* seedlings. *J Plant Physiol*, 156 (2000) 380.

28 Hseu YC, Chang WC, Hseu YT, Lee CY, Yech YJ, Chen PC & Yang HL, Protection of oxidative damage by aqueous extract from *Antrodia camphorata* mycelia in normal human erythrocytes. *Life Sci*, 71 (2002) 469.

29 Salvemini D & Cuzzocrea S, Superoxide, superoxide dismutase and ischemic injury. *Curr Opin Investig Drugs*, 3 (2000) 886.

30 Kirkland BH, Westwood GS & Keyhani NO, Pathogenicity of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* to Ixodidae tick species *Dermacentor variabilis*, *Rhipicephalus sanguineus*, and *Ixode sscapularis*. *J Med Entomol*, 41 (2004) 705.

31 Yu XQ, Tracy ME, Ling E, Scholz FR & Trenczek T, A novel C-type immulectin-3 from *Manducasexta* is translocated from hemolymph into the cytoplasm of hemocytes. *Insect Biochem Mol Biol*, 35 (2005) 285.

32 Bagyaraj S, Karthick SA, Gomathi S, Sandini S, Sowmiya R, Devi B & Vaithiyanathan D, Preparation and Characterization of Silver Nanoparticle/Aloe Vera Incorporated PCL/PEO matrix for wound dressing application. *Indian J Biochem Biophys*, 58 (2021) 35.

33 Bashyal BM, Zaidi NW, Singh US & Aggarwal R, Effect of fungal biocontrol agents on enhancement of drought stress tolerance in rice (*Oryza sativa* L.). *Indian J Biochem Biophys*, 57 (2020)101.