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BIOSYNTHESIS OF BIOACTIVE NANOPARTICLES FROM SELECTED BACTERIA ON LEGUMINOUS PLANT PEST APHIS CRACCIVORA AND HELICOVERPA ARMIGERA

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ABSTRACT

The present study nonpathogenic bacterial flora of *Bacillus., Pseudomonas* and *E. coli* was living one of the vital bacterial biopesticides, as it is eco-friendly has less residual activity, compatible with many chemical pesticides, self perpetually nature. This study expressed that three treated experimental bacterial strains of *Bacillus* bacterial organisms make higher concentration of 10^7 dilution factor depicted maximum mortality rate of adult *A. craccivora*. Moreover, other subsequent studies of biochemical compounds and SDS-Protein profile also denoted as clearly visible to drastically reduction at the level of appearance of the polypeptides of the protein band. Meanwhile, quantitative biochemical estimation of carbohydrate and protein content also been fluctuated based upon the effect of the bacterial pesticide. Though, the qualitative mode of result showed the number of polypeptide protein bands are faded also denatured when treated with *Bacillus* sp. compared with other two experimental bacterial isolates of *pseudomonas* and *E. coli* also control. Hence, the current research project obtained peak highlighted pesticidal effect given by nonpathogenic flora *Bacillus* bacterial organism compared than other two organism of *E. coli, pseudomonas* organism. Therefore *Bacillus* sp., can be implemented as one of the major entomopathogenic bacterial biopesticidal component in IPM programme as well as agriculture eco-friendly manner of usage for suppress or resist the growth of the Hemipteran and Lepidopteran economically important pests on agricultural field especially affect the legume plants during the time of vegetative production.

INTRODUCTION

In the past, efforts focused on reducing the detrimental effects of pesticides on human and environmental health, while continuing to mainly rely on chemical control. Agriculture has had to face the destructive activities of numerous pests like fungi, weeds and insects from time immemorial, leading to radical decrease in yields (Salma, 2011). Therefore, an ecofriendly alternative to chemical pesticides is biopesticides (Suman and Dikshit, 2010)

Biopesticides may be derived from animals, plants and microorganisms and include living organisms, their products or byproducts which can be used for the management of pests injurious (Kumar, 2013).

Biopesticides fall into three types according to the active substances: (i) microorganisms; (ii) biochemical; and (iii) semiochemicals (David *et al.*, 2015). Hence the Biopesticides mean the use of fungi, bacteria, viruses, protozoa and nematodes through inundative or inoculative release for the biological control of insect pest, diseases, weeds and nematodes in agriculture, medicinal, veterinary, forestry and horticultural eco-systems concept profounded by Erayya *et al*., (2013).

Cowpea (*Vigna unguicualata* L. Walp.) is an annual legume that is adapted to warm conditions and sensitive to low temperatures. Cowpea aphid, *Aphis craccivora* (Koch) is a threat to cowpea growers in all over the country. Both nymphs and adults suck plant sap and cause serious damage right from the seedling to pod bearing stage. Due to heavy infestation, young seedlings succumb to death, whereas the older plants show symptoms such as stunting, crinkling and curling of leaves, delayed flowering, shriveling of pods and finally resulting in yield reduction (Saranya *et al.*, 2010).

The use of insecticides in controlling aphids generally, leads to many problems, not only increasing resistant strains of aphids to these chemical substances, but also in induction of pollution to man and beneficial insects such as bees and other pollinators, insect parasitoids and predators (Maghraby, 2012). Bacterial biopesticides are the most common and cheaper form of microbial pesticides (CananUsta, 2013). A nanomaterial may be defined as any material (insulators conductor (or) semiconductors) synthesized on the size range of roughly 1 to 100 nm. At this size and dimensional range, especially any material will exhibit different properties from those it would as an atomic cluster (or) as the larger bulk materials reported by (Rajesh *et al*., 2012).

MATERIALS AND METHODS

Collection of experimental pest

The experimental pests of *Aphis craccivora* (Koch) (adult stage) and *Helicoverpa armigera* (Hubner) (4^{th} and 5^{th} instars) were collected from the leaves of leguminous plants.

Biochemical modulations

Carbohydrate and protein estimation was performed using anthrone method and Lowry's method respectively.

Synthesis of silver nanoparticle from *Escherichia coli, Bacillus* and *Pseudomonas*

Prepare 10 mL silver nitrate solution. Add 750µL silver nitrate solution into a test tube and 250 µL bacterial cultures of *Escheichia coli*, *Bacillus* and *Pseudomonas*. Observe the colour change.

SEM Analysis and XRD Analysis

The morphological features of synthesized silver nanoparticles from bacteria were studied by Scanning Electron Microscope (JSM-6480 LV). After 24 hours of the addition of $AgNO_3$ the SEM slides were prepared by making a smear of the solutions on slides. A thin layer of platinum was coated to make the samples conductive. Then the samples were characterized in the SEM at an accelerating voltage of 20 KV.

Statistical Analysis

Statistically significant differences between bacterial pesticide treatments were determined using one-way ANOVA followed by Tukey's multiple comparison carried out in the percentage mortality of Adult *A. craccivora* (Koch.) The antifeedent activity data were statistically analyzed using one-way analysis of variance (ANOVA) ($P \leq 0.05$) and comparisons were made based on Duncan's multiple range test using Microstat v. 2.5, 1991 of fifth instar of *H. armigera* (Hub.).

RESULT

Synthesis of Nanoparticle by the three various experimental Bacterial strains

Nanoparticle production and its colour changing observation with duration of nanoparticle formation were clearly presented the table 8-10. Remarkably various colour formations appeared for production of the nanoparticle with Silver nitrate solution accompanied with the respective experimental organisms. From the result indicated extremely experimental pests were react with silver nitrate solution quietly change the original colour of the resulted product changed light colour to light pruple, golden colour, dark purple colour see a plate-2.

Pupal Deformities of Bacterial consortium treated *H. armigera*

Various kinds of deformities were observed on pupae from the moulted fifth instar larvae of the *H. armigera* due to influence of three experimental bacterial biopesticides. When the fifth instar larval stages subjected with three different nonpathogenic bacterial pesticides most of the fifth instars were remarkably affected during the time of moulting, as a result of this the moulted larvae into affected deformed pupae because the treated larvae failed to detach completely from exuvium of the moulted skin. Some of the pupae didn't have fully formed cuticle especially when *Bacillus* treated pupae severely affected at the level of internal torsion of the pupae formation. However, other two organisms treated fifth instar larvae moulted pupae was less remarkable changes appeared when compared with *Bacillus* treated larva of the *H. armigera*. Though, no other deformities appeared on control developed fifth instar to pupae of this experimental pest.

Transmission Electron micrography Analysis

TEM analysis showed that the silver to be nanosized and well dispersed denoted plate -1. When the three bacterial pesticides react with Silver nitrate solution they could be produced measurable size of nanoparticles ranges between minimum to maximum such as 13.67 to 38.09nm, 10.58 to 47. 39nm and 19.35 to 34.67nm for *Pseudomonas* sp., *Bacillus* and *E. coli.* Among the three experimental nonpathogenic bacterial pesticides, Bacillus sp., organisms produced nano particle sizes were significantly maximum measurable size compared with other two experimental bacterial organisms. From the overall present results showed that amoung the three bacterial pesticides *Bacillus* organisms could be produced more biocidal potential activity against the non-target pest population of the Agriculture field (Plate-4).

SEM and XRD Pattern Analysis of three Experimental Bacterial bio-pesticides

From the experimental nonpathogenic as well as entomopathogenic bacterial strains produced nanoparticles react with 1 silver nitrate solution such a colour changed (purple colour) resulted solutions were used for the SEM and XRD analysis. The differentiated intensities were recorded from 35 to 80 at 2 theta angles for *Pseudomonas*, *Bacillus* and *Escherichia coli* organisms respectively (Plate-5-7). The diffraction pattern from SEM (Plate-5) corresponds to pure silver nitrate metal powder. The XRD pattern indicates that the nanoparticles had ovoidal (*Pseudomonas*), spherical (*Bacillus*) and cubic spherical (*Escherichia coli*). Structure of the Nano sized (ranging from 0.498, 0.568 and 0.512) bioactive (or) biocidal molecules were observed. Three peaks with adjacently appeared from the XRD pattern of Ag_2O showed in P_1 40, 45 and 65 theta angles them remaining three smallest peak lines also been observed with various theta angles such as 50 to 55, 70-75 and 75-80.

Similarly other too experimental entomopathogenic bacterial species expressed the SEM with XRD pattern analysis similarly three various intensities of peaks such as 40, 45 and 53 of 2 theta degrees were observed. Followed by three other lowest peaks such as 65, 74 and 75.5, 2 theta degrees accompanied with the minimum intensities also been noticed from Bacillus sp., of bacterial organism.

Furthermore, is the third organism of *Escherichia coli* denoted XRD pattern clearly showed single peak (36.58) theta angles followed by other continuous adjacent peaks are sequentially lowered with its unique intensities. For this experimental organism showed one lower peak such as theta angle is 77.85. Apart from the above mentioned nanoparticle study stated that the obtained silver nanoparticles had a partially purified. Though the observed various intensities as well as different theta angles depicted peaks were clearly showed broadening and experimentally showed, hence the three organism especially *Bacillus* sp. probably related to the effect of nanosized particles ranges from 1m-P1, 200nm-B1 and 150nm- E1 respectively. Moreover, these derived particles possessed the various crystalline biological particularly biocidal in the form of macromolecules present in the experimental three organisms.

From the present results among clearly indicates the three bacterial strain of *Bacillus* showed definite also appreciate pesticidal effect rather than other two organisms of *Pseudomonas* and *E. coli*. The obtained result illustrate that silver ions had indeed been reduced to Ag_2O by the bacterial suspensions under biopesticicidal reaction conditions.

Table 1: Effect of Biochemical molecules of three various bacterial biopesticide treated fifth Adult stage of *A. craccivora* (Koch.)

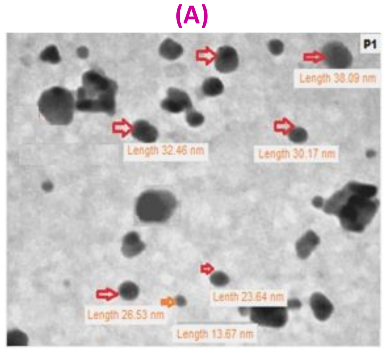
S.No.	Sample	Biochemical moietie(s)	
		Carbohydrate (µg/mg)	Protein (µg/mg)
1	*Bacillus* sp.	1.59±0.07*	0.65±0.02**
2	*Pseudomonas* sp.	1.25±0.05	1.67±0.07*
3	*Escherichia coli*	1.10±0.031^{NS}	1.32±0.10*
4	Control	1.69±0.00	2.96±0.08

*- indicates statistically significant at p= 5% level by Unpaired sample t-test

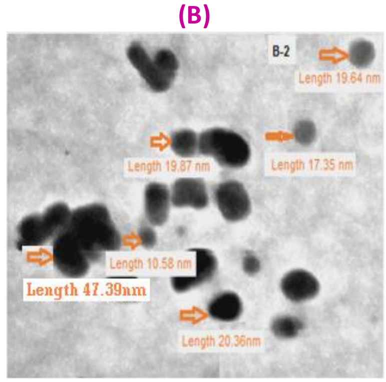
**- indicates highly significant with P= 0.05% level

Plate 4: Transmission Electron Microscopy of silver nanoparticles .All micrographs A) 13-38.09nm *Pseudomonas* B). 10 - 47.39nm bar scale *Bacillus* (C) 19.35-34.67nm *E. coli* bar scale.

(A)



(B)



(C)

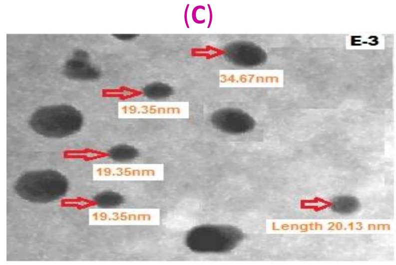


Plate 5: SEM micrographs of silver nanoparticles produced by the reaction of 1 mM $AgNO_3$ solution with (0.498nm particle size) Entomopathogenic bacteria (P1)-*Pseudomonas*

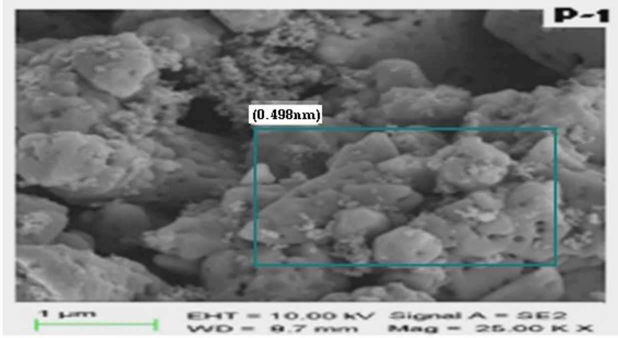


Plate 5a: XRD - Pattern of Silver nanoparticle Synthesised By*Pseudomonas sp.,* of entomopathogenic bacteria

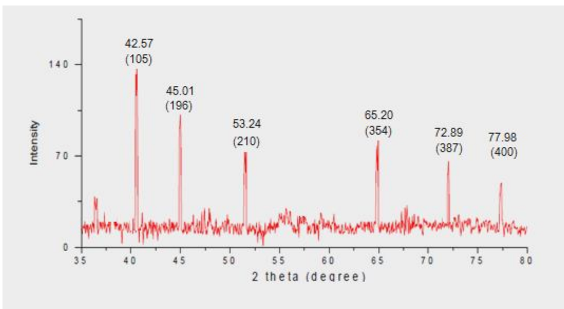


Plate 6: SEM micrographs of silver nanoparticles produced by the reaction of 1 mM $AgNO_3$ solution with (0.568nm particle size) (B2) – *Bacillus*

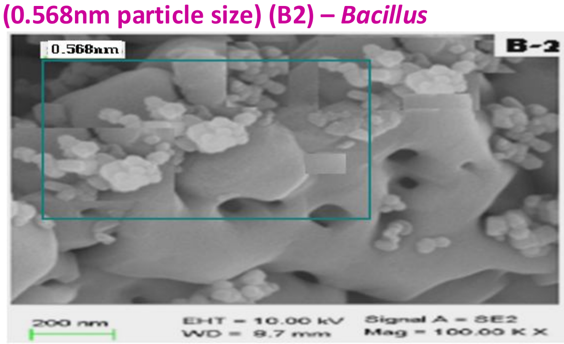


Plate 6a: XRD -Pattern of Silvernano Particle Synthesised by Bacillus sp., of entomopathogenic bacteria

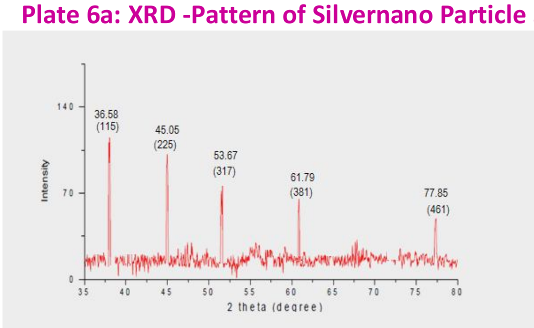


Plate 7: SEM micrographs of silver nanoparticles produced by the reaction of 1 mM $AgNO_3$ solution with (0.473nm particle size) (E-3) – *E. coli*

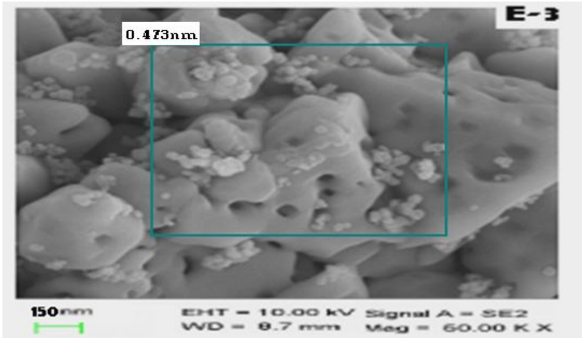
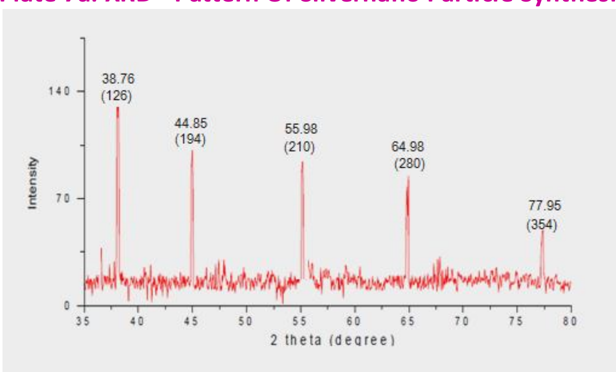


Plate 7a: XRD - Pattern Of Silvernano Particle Synthesised By *E. coli* of entomopathogenic bacteria



DISCUSSION

Chemical pest control agents are extensively used in all countries of the world but they are regarded as ecologically unacceptable. Therefore, there is an increased social pressure to replace them gradually with biopesticides which are safe to humans and non-target organisms. The harmful environmental implications of the synthetic chemicals have compelled to search for some alternative methods.

Srinivasan, 2012 discussed microbial pesticides based on the soil-borne bacterium *Bacillus thuringiensis* (Bt) are among the most widely used group of biopesticides. Formulations based on various Bt subsp., *kurstaki* and Bt. Aizawai have been found to be effective against several other lepidopteran pests either alone (or) in combination with other. This kind of similar results also been agreed by serial either research such as James *et al*., 2007. However, the present study also conformed with the other kind of concept that was the entomopathogenic potential, controversy has arisen regarding the pathogenic lifestyle of *B. thuringiensis.* Recent reports claim that *B. thuringiensis* requires the co-operation of commensal bacteria within the insect gut to be fully pathogenic (Roh *et al*., 2007 and Kumar *et al*., 2008)

Several reports have been confirmed the effectiveness of entomopathogenic microbes especially nonpathogenic bacterial of *Bacillus, Pseudomonas* and *Escherichia coli* (Ooi, 1980) organisms against various pests affected on subjected vegetables such as cowpea, legume etc. (Shelton *et al*., 2008; Sanehdeep *et al*., 2011) for instances, some of the entomopathogenic bacterial isolates were known to possess Oiscidan and conicidal effect against legume ped bores (Srinivasan *et al*., 2007). It was somewhat accepted result, when compared with this present research work.

Nanosized inorganic particles, of either simple or composite nature, display unique physical and chemical properties and represent an increasingly important material in the development of novel nanodevices which can be used in numerous physical, biological, biomedical, and pesticidal applications identified from microbial consortium especially nonpathogenic to opputunistic bacterial isolates, this present study denoted concept opined by the following researchers (Richards *et al*., 2000; Herrera *et al*., 2001). It is well known that silver ions and silver-based compounds are highly toxic to microorganisms (Brigger *et al*., 2002) showing strong biocidal effects on as many as 16 species of bacteria including *E. coli* (Chan *et al*., 2002). Because of the biocidal activities confirmed the incorporation of silver nanoparticles into the membrane structure. This observation is crucial for explaining the antibacterial mode of these particles. It is clear that treated bacteria also show significant changes in and damage to membranes, which are recognized by the formation of “pits” on their surfaces consequences with similar view, was reported by Slawson *et al*., (1992); Hamouda and Baker, (2000).

Further research and development of biological pest control methods must be given priority and people in general and agriculturists in particular must be educated about the handling and use of such control measures. All this will lead to a general understanding about the benefits of biopesticides as green alternative. However, the need in the present day context is on IPM, INM, ICM and GAP and by practicing these non-pathogenic in human but pathogenic in agricultural pest such as *Bacillus* sp., *Pseudomonas* and *E .coli* like bacterial flora quality of life and health will be assured for the microbial pesticides especially bacterial biopesticide in the agricultural field.

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