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IMPACT OF THREE ENTOMO-PATHOGENIC BACTERIAL STRAINS AND ITS INFLUENCE ON AGRICULTURAL LEAF ROLLER PESTS OF *HARITALODES DEROGATA* (FAB.) (LEPIDOPTERA: NOCTUIIDAE)

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ABSTRACT

Insect pests are an important and probably the most challenging pest to control in agriculture, in particular when they feed on below ground parts of plants. The application of synthetic pesticides is problematic owing to side effects on the environment, concerns for public health and the rapid development of resistance. Entomopathogenic bacteria, notably Bacillus thuringiensis and bacillus species, are promising alternatives to chemical insecticides, for they are able to efficiently kill insects and are considered to be environmentally sound and harmless to mammals. Hence, the present study was highlighted to evaluate whether the experimental strains of these three human non pathogenic bacterial strains such as staphylococcus, actinomycetes and bacillus species. Amoungthe threestrains bacillus sp., showed more biocidal activity against the leaf roller pest of Haritalodes derogata (fab.). *Therefore the current research expressed the mortality rate of the fifth instar pests, sem and xrd studies also been depicted nearly 0.04 to 0.9nm range nano particles nanao particles were produced by the experimental entomopathogens of bacillus sp., hence the current research clearly indicates bacillus sp., possessed the potential biocidal activity against the leaf roller pest than the remaining two experimental entomopathogenic strains.*

Keywords: Haritalodes derogata, *Entomopathogens, experimental strains,*

Introduction

Bacteria are widespread in the environment and they have evolved a variety of interactions with insects including essential symbiosis (Feldhaar, 2011). While many bacterial species inhabit bodies of insects establishing different levels of mutualistic relationships, only a limited number of them behave as insect pathogens published by Vega and Kaya, (2012). Microbial control agents can be effective and used as alternatives to chemical insecticides. A microbial toxin can be defined as a biological toxin material derived from a microorganism, such as a bacterium or fungus and virus (Ignoffo and Couch, 1981). Pathogenic effect of those microorganisms on the target pests are so species specific (Lacey and Siegel, 2000). The latter have evolved a multiplicity of strategies to invade the host, to overcome its immune responses, to infect and to kill it (Pigott and Ellar, 2007). The mechanisms leading to these kinds of interactions are presumed to have ancient origin and to have developed throughout a long co-evolution process (Vilcinskas, 2010). In line with this concept, a variety of insecticidal toxins produced by certain spore forming entomopathogenic bacteria, have a similar structure and mode of action. This is the case for protein toxins produced by *Bacillus thuringiensis* Berliner (*Bt*) and localized in parasporal bodies (De Maagd *et al*., 2003). These toxins are normally very specific to a limited range of targets, while in other cases bacteria produce metabolites that show a broader insecticidal spectrum (Glare *et al*., 2012). Important information to understand the molecular mechanisms involved in diverse pathogen-host interactions are being produced as a result of modern “omic” studies. However, many aspects are still unrevealed and after few decades of microbial pest management dominated by *B. thuringiensis*, novel bacterial species with innovative modes of action have been discovered and formulated as new biopesticidal products (Ruiu *et al*., 2013).

The entomopathogenic bacteria domain has traditionally been well represented by members of the *Bacillaceae* family, such as *B. thuringiensis,* recently the discovery of Betaproteobacteria species that showed the broad-spectrum insecticidal properties. This group includes specific strains of *Burkholderia* spp. and *Chromobacterium* spp. lastly, certain Actinobacteria species have gained high scientific and commercial interest in relation to the production of a variety of metabolites acting as potent insecticides. This is the case for *Streptomyces* and *Saccharopolyspora* species. As a result of continuous industrial and academic screening activities, the discovery of new bacterial species and insecticidal metabolites is expected in the near future (Ruiu *et al*., 2015). This trend is also the result of modern legislative frameworks fostering the use of bioinsecticides in Integrated Pest Management

The effect by microbial entomopathogens occurs by invasion through the integument or gut of the insect, followed by multiplication of the pathogen resulting in the death of the host, e.g., insects. Studies have demonstrated that the pathogens produce insecticidal toxin important in pathogenesis (Burges, 1981). Most of the toxins produced by microbial pathogens which have been identified are peptides, but they vary greatly in terms of structure, toxicity and specificity (Hajeck and Leger, 1994). These microbial pesticides offer an alternative to chemical insecticides with increased target specificity and ecological safety so that they are used either unique or in combination with other pest management programmes (Shia and Feng, 2014). One definition for integrated pest management (IPM) which is most relevant to this practice comes from (Flint and van den Bosch, 1981): "It is an ecologically based pest control strategy that relies heavily on natural mortality factors and seeks out control tactics that disrupt these factors as little as possible (Gupta and Dikshit, 2010). Ideally, an integrated pest management program considers all available pest control actions, including no action, and evaluates the potential interaction among various control tactics, cultural practices, weather, other pests, and the crop to be protected" (Schnepf *et al*., 1998). These microbial as biocontrol agents present beneficiary. They have efficiency and safety for humans and other nontarget organisms. They are ecologically safe, so that other natural enemies are free of their threatening, leading to preservation of other natural enemies, and increased biodiversity in managed ecosystem. So, microbial agents are highly specific against target pests so they facilitate the survival of beneficial insects in treated crops. This may be the main reason that microbial insecticides are being developed as biological control agents during the last three decades.

MATERIALS AND METHODS

Bioassays were performed in the malankara catholic college, mariaagiri. Five experiments were carried out to determine the most appropriate methodology for toxicity bioassays of insecticides to *H.derogata*.The bioassay conditions were: temperature 25 ± 1° C, a photoperiod of 12 hour light and relative humidity 75 ± 5%. The hibiscus leaves used in the bioassays were collected from plants of the “*H. derogata*”. The experiments were carried out in a completely randomized design. Two recipients were used: glass Petridishes (9 cm diameter and 2 cm height) and two-liter transparent PET bottles. These recipients are standard containers used in bioassays of insecticide toxicity, For the PET bottle method, full hibiscus leaves from the plant apex were transferred to bottles. Four treatments with PET bottles were also carried out: 1) one hibiscus leaf inside the PET bottle, 2) one hibiscus leaf and one water- damped cotton, 3) one hibiscus leaf with its petiole wrapped by water-damped cotton in aluminum foil and 4) one hibiscus leaf with its petiole immersed in an ambar vial containing 120 ml of water. The same evaluations on leaf color and turgidity as well as statistical analysis were carried out of according to the results of the previous experiment.

SEM

SEM is the scanning electron microscope that creates various images by focusing a high energy beam of electrons onto the surface of a sample and detecting signals from the interaction of the incident electron with the sample's surface. SEM images have greater depth of field yielding a characteristic 3D appearance useful for understanding the morphology material. Magnification is of order 10,000 X and resolution 10 nm.

XRD

In XRD a large fraction of the X-rays that are not simply absorbed or transmitted by the object but are scattered. When an X-ray beam hits an atom, the electrons around the atom start to oscillate with the same fequency as the incoming beam creating an electric field. All directions have destructive interference, that is, the combining waves are out of phase and there is no resultant energy leaving the solid sample

GC-MS analysis of SMS_SU21

Identification of the chemical compounds present in the crude extract was carried out by GC-MS. Analysis was conducted on a Factor four™ capillary column (VF-5 ms, 30 m, 0.25 mm id, 0.25 μm film thickness; Varian, Middelburg, The Netherlands) with the following conditions: constant flow of Helium, 1.0 ml min^{-1}; the inlet temperature 285 °C remain the fixed throughout the analysis; injection volume, 2 μl (LVI) in the liner with an open purge valve (30:1 split ratio) initially and closed at 0.00 min, and open again (30:1) at 26.00 min and remain open till the end of the run; oven temperature program, 80°C for 2 min, then 18°C min^{-1} ramp to 260 °C and held for 6 min, again 4 °C min^{-1} ramp to 285 °C and held for 6 min. The MS instrument transfer line temperature was 280°C, with 220°C ion trap and 120 °C manifold temperatures. Full-scan (40–650 m/z) EI (auto) mode with20 μA filaments current was used for MS analysis from 5.00–28.00 min, which gave 0.78 s/scans (3μ scan). Target automatic gain control was 20,000, and the multiplier voltage was 1450 V. Baseline offset −5, peak find with S/N of the quantifier ion at least 3 and peak width 2 s was set as the parameters for processing the peaks in the chromatograms. Minimum similarity match with regards to the NIST library spectra was kept at 500 (reversed fit). Quantification was done on the basis of diagnostic ion and the peak assignments and integration were automatically done through software.

RESULTS

Contact toxicity of the experimental Pest

The pesticidal activities of Actinomycetes, *Streptococcus* and Bacillus subtilis microorganisms against to leaf roller pest were examined by direct contact application method (Table -1). Toxicity in with increasing concentration (dilution factor) experiment period and life stages of pest, it was does indicated that the 5^{th} instar. Pests were significantly susceptible to the three bacterial pesticides after 24 houses ($F=62.123$; $df = 4$; $p <0$) and 72 hours ($F = 36.231$; $df = 4$; $p < 0.001$) of treatment. *Bacillus subtilis* was showed compared with that of *Staphylococcus* sps and Actinomycetes bacterial Organisms.

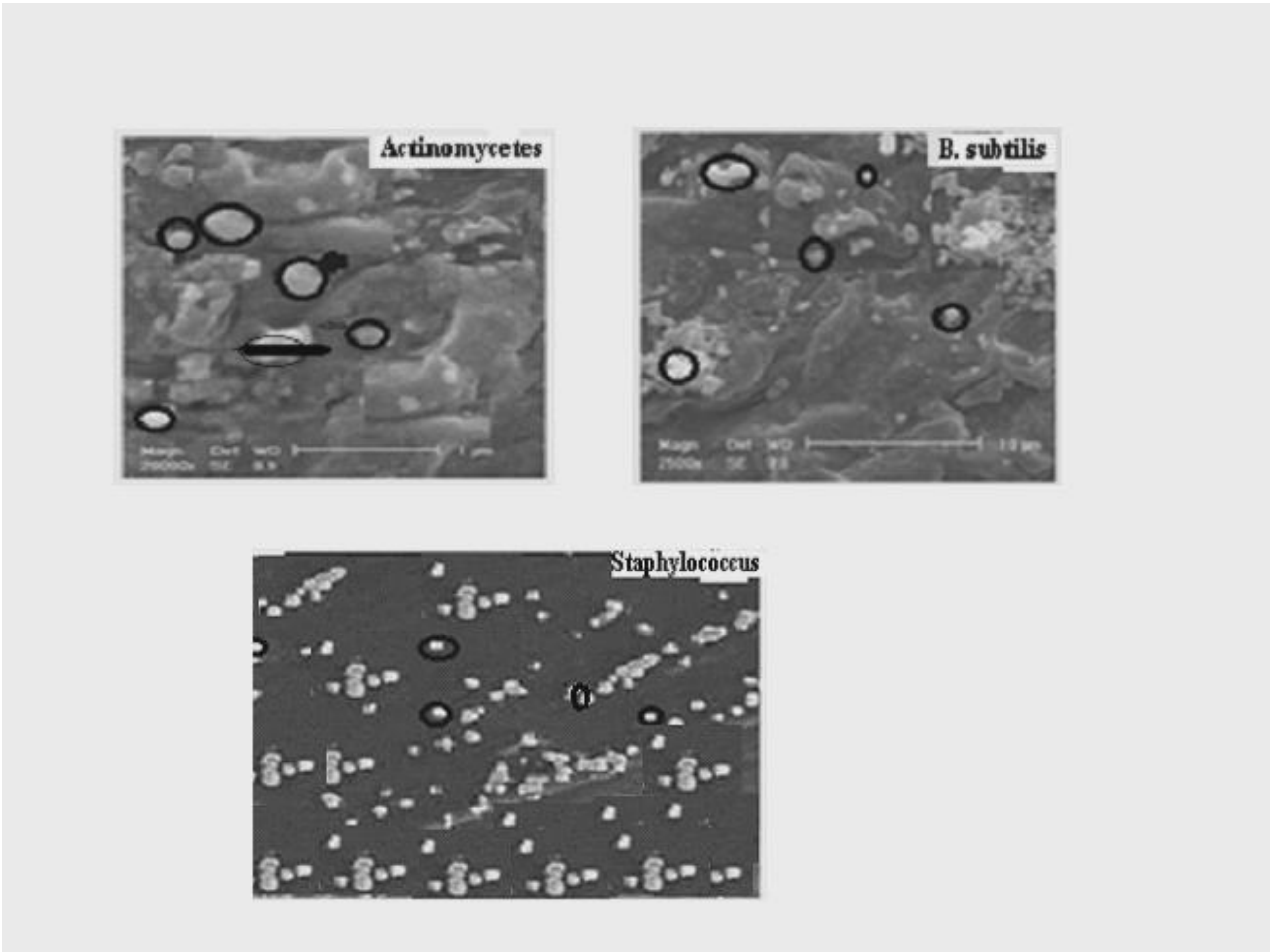
At the rate of $130mg/cm^2$, *Bacillus subtilis* cause 100% toxicity. Among the three different bacterial strains maximum concentration 107 *Bacillus* strains showed higher activity. On 89.11 ± 2.69, 93.17 ± 4.27, 98.56 ± 4.81 at 24, 48 and 72 hours respectively from the present result clearly showed whenever the concentration increased of the experimental pests mortality rate also been increased. Irrespectively depends upon the concentration of dilution factors as well as treatment periods. The overall results clearly depicted all the three experimental entomo-pathogenic bacteria strains. *Bacillus* revealed predominantly higher toxicity biopesticidal effect against the leaf roller pest. Followed by other maximum effect was noticed on Actinomycetes and *Streptomyces* sps., of bacterial biopesticides. This kind of similar result was also been published by Munnan and Wikadi, 1986 his findings were depicted the bacterial biopesticides of *Bacillus* sps., at the concentration of $8x10^7$ dilution factor per ml found to be effective in centrollince the population of leaf roller as well as hemiptean pests. Similarly Samson (1981) has been reported that the three different bacterial isolates were formulative against the majority of hemipteran pests. The results revealed that among the three different species *Bacillus* was found to be highly effective against leaf roller pests independently with it's life stages of leaf roller

Table-1: Effect of three entomopathogenic bacterial strains and its effect on the leaf roller pest of *Haritalodes derogata*

Name of the Organism	Concentration	Mean (%) Toxicity ± st			Lc80 95% mg/cm^2	X^2df	P-value
		24 hrs	48 hrs	72 hrs			
Actinomycetes	10^3	41.8±2.4	63.41±2.5	79.47±4.80	62.15	8.05	0.013
	10^5	55.6±3.5^{b}	75.83±1.4^{c}	86.01±3.93	79.43	1.27	0.017
	10^7	79.8±1.76^{c}	81.03±3.0^{a}	92.65±3.1	67.2	7.46	0.018
Staphylococcus	10^3	62.53±2.8^{a}	68.23±2.1^{a}	80.20±2.53^{a}	53.61	8.24	0.712
	10^5	74.35±2.0	77.11±2.0^{a}	85.47±2.8	69.08	3.41	0.545
	10^7	86.74±2.15	88.20±2.7	94.08±2.56^{b}	95.21	12.21	0.385
Bacillus subtilis	10^3	71.37±5.3^{c}	75.30±2.8	88.51±2.7^{a}	90.42	2.50	0.043
	10^5	80.52±3.7^{a}	85.53±4.0	93.11±3.5^{a}	67.32	3.28	0.776
	10^7	89.11±2.69	93.17±4.1	98.56±4.1	8.2	2.84	0.503

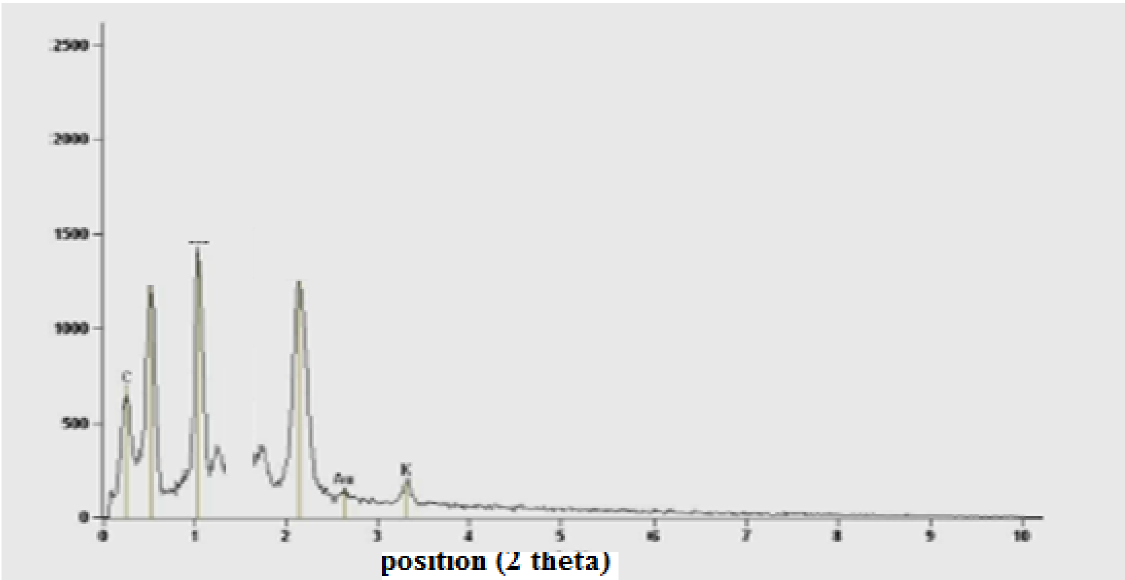
Numbers that share the same superscript letters, in the same row, are not statistically different at 95% level of confidence.

Fig. 1: Nano particle production of three experimental entomopathogenic bacteria by SEM analysis



From this figure showed that the very smallest nanorange particle was appeared in the range of 0.076nm to 0.84 for actinomycetes then 0.042 to 0.79nm and 0.22 to 0.031nm scale of nanoparticles produced by *Bacillus* and *Staphylococcus* sp., respectively. From the present result showed that the minimum to maximum pesticidal activity was expressed the order was *B*acillus, staphylococcus and actinomyctes. From the GCMS analysis conformed due to the *Bacilllus* strains metabolites possessed the suppressing or killing efficiency on the teated pests. Hence, this work proved these kind of entomopathogenic organism of *Bacillus* species was act as better biopesticidal entomopathogenic bacteria compared with other two strains are *Staphylococcus* sp., and Actinomycetes against the leaf roll pests of *H. derogate* (Fab.).

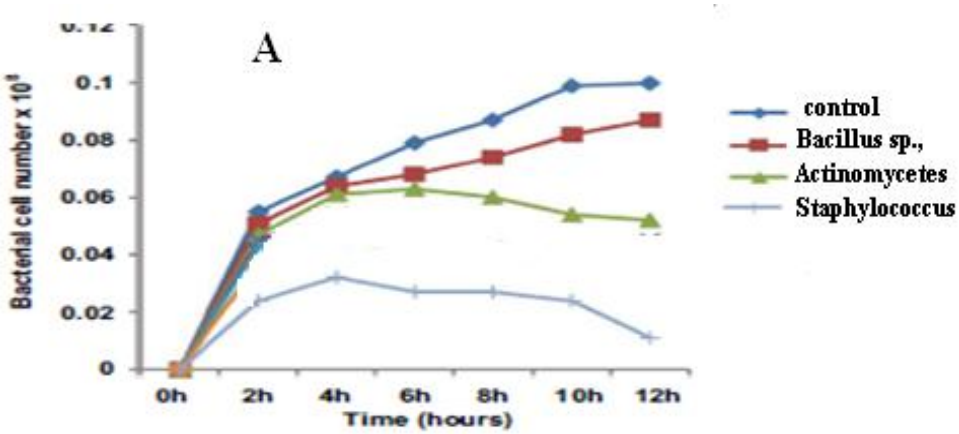
Fig-2: X-Ray diffraction pattern of prepared silver nanoparticles using experimental entomopathogenic bacteria of bacillus sp.,



X-RAY Diffraction analysis

XRD analysis is used to determine the phase distribution, crystallinity and purity of the synthesised nano particles particles. Fig shows the XRD patterns of bacillus sp., With reference to the JCPDS data file No. 04-0423 it was concluded that the nanoparticles were crystalline in nature having cubical shape with no such impurities. Hence the present research concluded the nearly 0.04 to 0.9nm range nano particles from the experimental entomopathogen of bacillus sp., were act as a better biocidalor biopesticidalagent against this leaf roller pest of *H. derogatta*.

Fig 3: Nanaoparticle production rate by the experimental organisms of three various entomopathogenic bacterial strains against the pest of *H. derogatta*



From the above mentioned figure clearly showed the bacterialcount based biopesticidal activity maximum observed in bacillus suspension followed by the actinomycetes and finally third bacterial strain of staphylococcus sp., Similar results alsobeen noticedonthe levelof nanoparticle based biocidalactivity treated on this experimental pest of *H. derogata* (fig-3)

Fig 4: Chromatogram view for haemolymph from the leaf roller pest of *Haritalodes derogata* **affected with** entomopathogenic bacterial strain of ***Bacillus*** **species.**

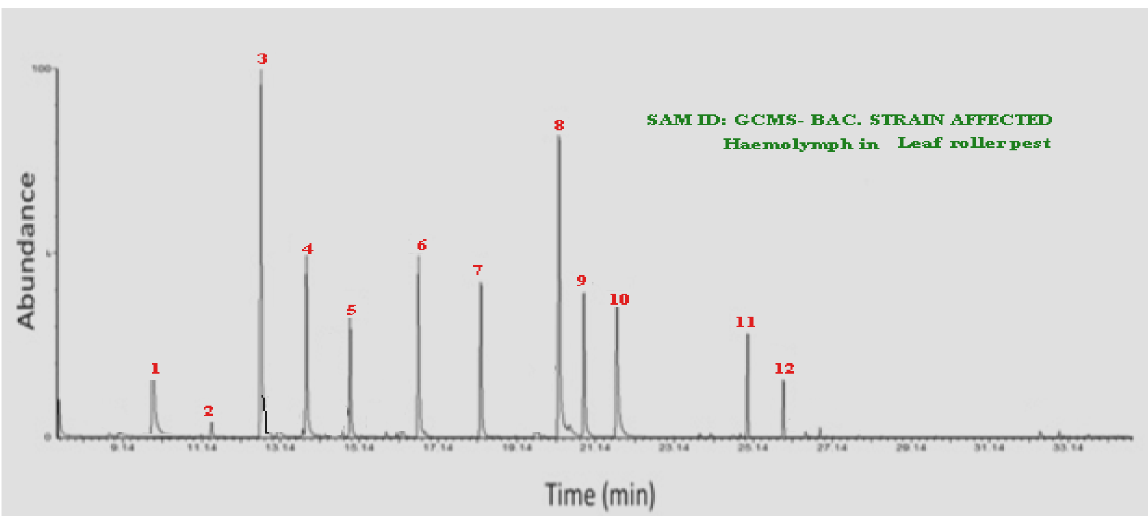


Table-2: Analytes of haemolymph from the leaf roller pest of *Haritalodes derogata* affected with entomopathogenic bacterial strain of *Bacillus* species by GCMS

S.No.	Retention time/RT	Compound(s) separated	Abundance (%)
1.	10.14	5- Dibutylcarbinol	**20**
2.	11.29	Trace	**09**
3.	13.12	2,3 Benzo Orthodiazine	**100**
4.	14.28	2,12-tetradecadiene	**52**
5.	15.14	Cyclo Propane Carboxylic acid	**39**
6.	17.14	Hexa deconoic acid	**28**
7.	18.25	**Beta Asarone**	**45**
8.	20.11	5-ethyl- phenyl-benzoic Pyrogallol	**40**
9.	21.26	2,5-Diphenyl Lignoceric acid	**85**
10.	22.28	unknown	**45**
11.	25.11	Trans- sesquilavandulyl acetate	**40**
12.	26.82	Unknown	**22**

From the bacterial pesticides treated died pest of *h.derogata* haemolymph showed the following secondary metabolites were identified through the gcms analysis. Apart fromthis results clearly showed that the totally twelve compounds were separated amoung the twelve compounds 2,3 Benzo Orthodiazine is act as a main peakcompound with 100% abundance and its retention time was 13.12 follwed by second level of peak compound named as 2,5-Diphenyl Lignoceric acid its abundance and retention time was 85%, 21.26mts respectively. Though, Beta Asarone compound was observed as a optimum peak level 45% of abundance. Interestingly, this compound is a secondary metabolite product of the treated entomopathogenic bacterial strain of bacillus, hence the current results were clearly proved while the majority of the fifth instar experimental pests were died only for the influence of this typical secondary metabolite compound present in the treated bacterial pathogens especially *bacillus* species. Hence the present study was showed that the bacillus bacterial strain was act as a best biocidal biopesticidal (microbial based) agent against leaf roller agriculturally important pest of *H. derogate* (Fab.).

Discussion

The rationale for the development and deployment of microbial insecticides for pest management is their environmental safety, specificity, and biodegradability (Nicholson, 2007). Some pathogens selected for commercial development, such as viruses and bacteria, may infect only a single or small number of closely related insect species. Others, such as fungi and nematodes, may affect a fairly wide range of insects and related arthropods (Piggot and Hilbert, 2004). However, the commercially available microbial pathogens are target specific and have not been shown to infect vertebrates or plants (De Maagd *et al*., 2003). The biodegradable nature of the microbial pesticides does not leave any harmful residues in the environment, and does not enter the food chain. The biodegradable nature of the microbial pesticides does not leave any harmful residues in the environment, and does not enter the food chain (Glare and Callaghan, 2000). Microorganism e.g., a bacterium, fungus, virus or protozoan as the active ingredient can control many different kinds of pests, although each separate active ingredient is relatively specific for its target pest (Hoch *et al*., 2003). For example, there are fungi that control certain weeds and other fungi that kill specific insects previously reported by several authors Ferron, (1971); Lacey and Siegel, (2000). Bacterial pathogens used for insect control are spore-forming, rod-shaped bacteria in the genus *Bacillus.* They occur commonly in soils, and most insecticidal strains have been isolated from soil samples (Hoffmann and Frodsham, 1993). The *Bacillus genus* encompasses a large genetic biodiversity. *Bacilli* are present in an extremely large area of environments ranging from sea water to soil, and are even found in extreme environments like hot springs (Glare *et al*., 2012; Ruiu *et al*., 2015). From, the present study clearly indicated amoung the three experimental pathogens bacillus strains was potenial biopesticidal agent than the other expeimental strains such as *staphylococcus* and actinomycetes this kind of similar findings were already been reported through several researchers (Glare and Callaghan, 2000; Ruiu *et al*., 2015; Monteiro et al.,2005) bacterium could be one of the major sources of potential microbial biopesticides because it retains several valuable traits (Bravo *et al*., 2007).

Novel *Pseudomonas* strains can also readily be isolated from various insect species. An obvious approach to discover strains with entomopathogenic potential could there fore be the iso-lation of pseudomonas from the respective target organism (De Maagd *et al*., 2010). During the selection of strains for a new plant protection product the efficacy of the bacterium as an insecticidal organism, the persistence and competition on plant roots, and the resistance during he formulation process should be considered (Walsh *et al*, 2001). Moreover, a detailed risk analysis needs to be performed to ensure that the bacterial strains have no deleterious effects on human health and on thee environment (Papendick *et al*., 1986; Harwood and Wipat, 1996). This requires amongst others more research on the molecular basis and regulation of insecticidal activity in the seroot-associated pseudomonads (Hoy and Myths, 1999), thus procuring a natural containment mechanism for biocontrol. The collaboration of the scientific community with commercial companies may then be the key to the development and commercialization of new biopesti-cides based on entomopathogenic, root-associated *Pseudomonas* strains, just like the development of products such as Proradix, Cedomon, and Cerall already has demonstrated (Johnsson *et al*., 1998; Buddrus-Schiemann *et al*., 2010). Previously the following research donewith various researchers along with the entomopathogenic bacterial species Notably, strains of *P.fluorescens* were reported to exhibit insecticidal activity toward agricultural pest insects such as aphids (Hashimoto, 2002), phytophagous lady bird beetles (Otsu *et al*., 2004), andtermites (Devi and Kothamasi, 2009). In the same vein, a bioformulation of a combination of two *P.fluorescens* strains was demonstrated to simultaneously reduce the incidence of a herbivorous insect (the riceleafroller *Cnaphalocrocis medinalis*) and a phytopathogenic fungus (*Rhizoctonia solani*) in rice undergreen house and field conditions (Commare *et al*., 2002; Karthiba *et al*., 2010).

The scientific community working in the field of insect pathology is experiencing an increasing academic and industrial interest in the discovery and development of new bioinsecticides as environmentally friendly pest control tools to be integrated, in combination or rotation, with chemicals in pest management programs. In this scientific context, market data report a significant growth of the biopesticide segment. Acquisition of new technologies by multinational Ag-tech companies is the center of the present industrial environment. This trend is in line with the requirements of new regulations on Integrated Pest Management (Mettenmeyer, 2002). After a few decades of research on microbial pest management dominated by *Bacillus thuringiensis* (*Bt*), novel bacterial species with innovative modes of action are being discovered and developed into new products Bravo *et al*., 2007. Also other entomopathogenic microbial organisms are *Photorhabdus* spp. and *Xenorhabdus* spp., *Serratia* species, *Yersinia entomophaga*, *Pseudomonas entomophila*, and the recently discovered Betaproteobacteria species *Burkholderia* spp. and *Chromobacterium* spp. finally; Actinobacteria species like *Streptomyces* spp. and *Saccharopolyspora* spp. have gained high commercial interest for the production of a variety of metabolites acting as potent insecticides (Meadows, 1993; Ongena and Jacques, 2008).

Conclusion

In recent years, several microbes with potential insecticidal properties have come to light. Viruses, bacteria, fungi and protozoa that are known to produce an array of metabolites or toxins, form the basis for microbial insecticides. Since these versatile organisms are amenable for genetic engineering, strains with good insecticidal properties can be identified, evaluated and utilized for pest control. Hence the present study was showed that the among the hree bacterial entomopathogenic strains *Bacillus* bacterial strain was act as a preeminent biocidal biopesticidal (microbial based) agent against the series nontarget leaf roller agriculturally important pest of *Haritalodes derogata* (Fab.). Based upon the GCMS study and nano particle synthesized studies are depicted that vibrant potential compounds are secondary metabolite named as Beta Asarone is a main biopesticidal compound it was synthesized by *Bacillus* Sp., in addition other two strains of actinomycetes species followed by very smallest range of nanoparticle production were seen in *Bacillus* strains (0.042 to 0.79nm) than the other two strains such as 0.076nm to 0.84 scale of actinomycetes and nanoparticles 0.22 to 0.031nm produced by *Staphylococcus* sp., respectively. From this overall result were clearly showed that the three experimental entomopathogens *Bacillus* strain was more actively potent against the nontarget economically important leaf roller pest of *H. derogata* (Fab) than the other two bacterial strains.

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