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Nano particles influence on the production and multiplication of Bio pesticide, *Beauveria bassiana* (Balsamo) Vuillemin

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Abstract
Nanoparticles with greater strength, excellent electrical conductivity and improved chemical reactivity, the utilisation of nanoparticles exhibits particular targeted properties. From an agricultural standpoint, nanotechnology holds great promise for the use of Nano capsules to manage insect infestations. Biopesticides are one of these less harmful strategies for overcoming the insecticide resistance of the *Spodoptera litura* population. One of the most powerful biopesticides against this bug is *Beauveria bassiana*. In contrast, the current investigations examined the interaction between the most promising biopesticides for *S. litura* on groundnut and certain growth nutrients, including Ca, Mg, Fe, and Zn at nanoscale. With several diseases, nanoparticles have been proven to have antibacterial and antifungal activities at particular dosages. Studies on the use of nanoparticles in other fields, such as biomedicine and pharmaceuticals, where they act as growth and development catalysts, have been presented under various headings here when appropriate, despite the paucity of literature on nanoparticle-based biopesticides and nanoparticle-based insecticides.

Keywords: Nanoparticles, biopesticides, production, multiplication, *Beauveria bassiana*

Introduction
The use of nanoparticles demonstrates distinctive targeted properties with increased strength, high electrical conductivity, and additional chemical reactivity. 10-9 m-diameter nanoparticles have unusual chemical, physical, and biological characteristics. From the perspective of agriculture, nanotechnology holds enormous promise for the management of insect pests using Nano capsules. One of these less dangerous methods to combat the *Spodoptera litura* population's insecticide resistance is biopesticides. *Beauveria bassiana* has emerged as one of the most effective biopesticides against this insect. Contrary to this, the current studies were conducted to determine the synergistic effect of specific growth nutrients, namely Ca, Mg, Fe, and Zn at nano size, with the most promising biopesticides against *S. Litura* on groundnut. Nanoparticles have been shown to have antibacterial and antifungal properties with many pathogens at specific doses. Although there is little literature on nanoparticle-based biopesticides and nanoparticle-based insecticides, studies on how nanoparticles are used in other fields, such as biomedicine and pharmaceuticals, where they act as growth and development catalysts, have been presented under various headings here when appropriate.

Dosage standardization and evaluation of different concentrations of test nano material on the growth and multiplication of the biopesticides: According to Alves *et al.* (2002) [3], *B. bassiana* isolate 447 (ATCC 20872) displayed a yeast-like phase on MacConkey agar and was virulent to *Tetranychus urticae* and *Diatraea saccharalis*. After a 24-hour incubation period, *B. bassiana*'s yeast-like cells formed via budding from germinating conidia. Fungal colonies started out as circular and mucoid cells with an average cell size of 5 to 10 m, but with time they developed mycelia and conidia. Different *B. bassiana* isolates were generally capable of producing yeast-like cells on MacConkey medium, but the growth rates and time of this process differed. When 107 cells/ml were employed, *B. bassiana* 447 yeast-like cells were more virulent than conidia against *D. saccharalis*. The estimated mean survival period at 108 cells/ml was 7.7 days for the conidial suspension and 5.4 days for the yeast suspension, possibly reflecting faster germination. The findings of the bioassay showed that *B. bassiana*'s yeast-like structures generated on MacConkey agar are useful as inoculum for applications against arthropod pests and may even be superior to conidia against some species.

The two culture media evaluated by Senthamizhlselvan *et al.* (2010) [36], Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA), exhibited the highest mycelial growth in *Beauveria bassiana* isolates. In PDA media, isolates of VpNlKKL 2121 (65.22mm), BbMtKKL 2107 (65.16mm), BbMdKKL 2106 (63.44mm), and FmNvKKL 2121 (62.78mm) showed the highest growth, while VpPmKKL 2120 (37.33mm) showed the lowest growth. BbMtKKL 2107 and BbMdKKL 2106 both experienced the fastest growth in SDA media (68.02mm and 66.91mm, respectively). The growth rate that was the slowest was found in VpPmKKL 2120 (32.18 mm). The *B. bassiana* isolates BbMtKKL 2107 (8.90 X 108 spores/ml) and BbMdKKL 2106 (8.77 X 108 spores/ml) had the highest spore counts and were comparable to FmNvKKL 2124 and VpPmKKL 2120.with PDA media.

The growth of *B. thuringiensis* was examined by Valicente *et al.* (2010) [41] on three different mediums. The seed culture (strain 344 of B. thuringiensis tolworthi, from the Embrapa Maize and Sorghum Microorganism Bank) was created using shake flasks and cultivated in LB medium plus salts for 18 hours before being incubated on a rotary shaker at 200 revolutions per minute (rpm) at 30° C for 96 hours. The first media contained Luria Bertani (LB) along with salts (FeSO4, ZnSO4, MnSO4, and MgSO4) and 0.2% glucose. The second medium contained 1.5% glucose and 0.5% soybean flour along with salts, while the third medium contained liquid swine manure at 4% along with 0.2% glucose. *B. thuringiensis* tolwothi (seed culture) was injected into each of the three medium after they had been cleaned and sterilised at a stirrer speed of 200rpm, for 96 hours at 30ºC. Regular pH readings were taken, along with measurements of cell mass expressed in lyophilized grammes per litre and viable spore counts per millilitre of media. During the fermentation phase, there were pH variations in all three media. Within 96 hours of incubation, the media 1 and 2 produced the most viable spores, 2.0 x 108 CFU/mL, whereas medium 2 also produced the most biomass, 1.18 g/L. The medium with the highest spore concentration, medium 1, had 1.4 x 109 spores/mL after 96 hours of fermentation. All *Bt* generated in all three medium killed above 60% of *S. frugiperda* first instar larvae, according to tests on efficacy against those larvae.

The antifungal properties of zinc oxide nanoparticles (ZnO NPs) and their mode of action against two post-harvest pathogenic fungi (Botrytis cinerea and Penicillium expansum) were studied in this work, according to YangLiu *et al.* (2011) [45]. We employed ZnO NPs of 70 15 nm diameters and concentrations of 0, 3, and 12 mmol l1. The antifungal properties of ZnO NPs were investigated, and the morphology and cellular compositions of fungal hyphae treated with ZnO NPs were characterised using conventional micro biological plating, scanning electron microscopy (SEM), and Raman spectroscopy. The findings demonstrated that *B. cinerea* and *P. expansum* growth can be considerably inhibited by ZnO NPs at concentrations greater than 3 mmol l1. ZnO NPs showed two distinct antifungal activity against B. cinerea and *P. expansum*, according to SEM pictures and Raman spectra. ZnO NPs altered cellular processes, resulting in fungal hyphae distortion, which prevented B. cinerea from growing.

According to Chakravarthy *et al.* (2012) [7] assessment of the bio-efficacy of inorganic nanoparticles against the cutworm *S. litura* (Lepidoptera: Noctuidae), nanoparticles can be used as a substitute management strategy to investigate the possibility that CdS, Nano-Ag, and Nano-TiO2 particles may have negative effects on *S. litura*. The second instar *S. litura* larvae dose-response data revealed the pathogenicity of nanoparticles, with the LC50 of CdS being 508.84, and the LC50 of Nano-TiO2 and Nano-Ag being 791.10 and 1403.14 ppm, respectively. The three nanoparticles that were tested showed promise against *S. litura* larvae and can therefore be employed specifically to reduce the pest.

Justin and Thomas (2012) [14], iron oxide nanoparticles in a chain-like structure with a length of 100-200 nm were found to reduce S. aureus viability as demonstrated by a decline in the ratio of live to dead cells. These iron oxide nanoparticles were found to inhibit bacteria activity (mean average diameter = 9 nm, zeta potential under experimental conditions = 19.09 mV). At concentrations of 3 mg/mL, iron oxide nanoparticles were shown to reduce cell viability at 4, 12, and 24 hours compared to bacteria culture controls without nanoparticles and compared to lower concentrations of nanoparticles.

Patil (2012) [27] studied on potential WP formulations of *N. rileyi* and *M.anisopliae*. The results revealed that out of nine test media, Sabouraud's dextrose broth with yeast extract (SDYE) emerged as best medium for mass production of *N.rileyi* as well as *M.anisopliae* which yielded highest biomass of 6.10g and 7.20g/40ml medium with viability of $8.33x10^8$ and $12.33x10^8$ cfu/ml, respectively. The biomass productivity and virulence of both the fungi increased with the increase in concentration of the inoculums and the standardized optimum concentration of bioactive ingredient was 30% fungal culture at 10 DAI (v/v) for the formulations considering the biomass, viability and bioefficacy.

Sehroon Khan *et al.* (2012) [35] studied entomopathogenic fungal isolates, three each of *Beauveria bassiana* and *Verticillium lecanii*, were screened for pathogenicity test against the *M. persicae* to select high virulent isolate with the most suitable application and to determine the role of individual enzyme in its virulence. Two treatments that is, conidial shower (190±23 conidia/mm^2) and filtrate (3 ml filtrate per treatment from six days liquid broth culture of $1.0x10^8$ conidia ml^{-1}) were conducted for virulence or toxicity test and a comparison was made shower (190±23 conidia/ mm2) and filtrate (3 ml filtrate per treatment from six days liquid broth culture of $1.0x10^8$ conidia ml^{-1}) were conducted for virulence or toxicity test and a comparison was made between treatments and among fungal isolates against the target pest. The *B. bassiana* 70 and *B. bassiana* 76 showed high toxicity (77.14 and 80.86%, respectively) in filtrate application at 6^{th} day of incubation. The pathogenicity test revealed the selection, effective application of most virulent isolate and the role of individual enzyme to develop an alternative control agent against *M. persicae*.

Namasivayam *et al.*, (2013) [21] studied natural occurrence of major fungal biopesticide *Nomuraea rileyi* (Farlow) Samson, associated with agricultural field soil in an area around Tamil Nadu. Effect of metallic nanoparticles such as silver, copper and the respective nanoparticles coated with chitosan on the post treatment persistance of *N. rileyi* was also studied and found the distinct effect on the growth of *N. rileyi* in copper nano particles with high concentration.

Priyanka and Arun (2013) [29] studied nano-sized particles of ZnO have been claimed to have pronounced antimicrobial activities than large particles. Antimicrobial/antifungal potentiality of ZnO on five pathogens (*Escherichia coli* MTCC 443, *Staphylococcus aureus* MTCC 3160, *Bacillus subtilis* MTCC 441, *Aspergillus Niger* MTCC 281, *Candida*

albicans MTCC 227) and the influence of particles size of these inorganic powder on its antimicrobial /antifungal efficacy was considered in the present study. Results indicated that zinc oxide nanoparticles do have strong antibacterial and good antifungal activity against selected strains of bacteria and fungus as compared to that of conventional zinc oxide particles.

Yehia and Ahmad (2013) was investigated the antifungal efficiency of zinc oxide nanoparticles (ZnO NPs) against two pathogenic fungal species, *F. oxysporum* and *P. expansum*. The antifungal activity of ZnO NPs was found to be concentration dependent. Hence, maximal inhibition of mycelial growth corresponded to the highest experimental concentration (12 mg L−1), where 77 and 100% growth inhibition was observed for *F. oxysporum* and *P. expansum*, respectively. The effect of ZnO NPs on the mycotoxins fusaric acid and patulin production by *F. oxysporum* and *P. expansum*, respectively, was investigated using HPLC quantification. It was observed that ZnO NPs prevented both mycotoxins synthesis in a concentration dependent manner. The scanning electron microscopy (SEM) revealed obvious deformation in the growing mycelia treated with ZnO NPs in *F. oxysporum* which may be the cause of growth inhibition.

Ingle, (2014) studied on *Nomuraea rileyi* (Farlow) Samson which is a potential biological control agent against several lepidopterist pests. Different synthetic and semi synthetic solid, liquid media, carbon and nitrogen source and grains were evaluated for large scale production of fungus *N. rileyi*. Among the solid and liquid media SMAY medium was found to support for maximum growth and abundant sporulation within short time followed by SDA and BCY. In respect to carbon and nitrogen source maltose and sodium nitrate were found to be the best for spore germination followed by dextrose and potassium nitrate. Seven food grains were evaluated for their suitability and results revealed that crushed sorghum and maize with 1% yeast extract proved as favourable for the higher conidial production.

Esteban-Tejeda *et al.*, (2015) [10] tested non-toxic biocides based on low melting point (1250°C) transparent glasses with high content of ZnO (15–40wt%). These glasses have shown an excellent biocide activity (logarithmic reduction >3) against Gram negative (*E. coli*), Gram positive (*S. aureus*) and yeast (*C. krusei*); they are chemically stable in different media (distilled water, sea-like water, LB and DMEN media) as well as biocompatible. The cytotoxicity was evaluated by the Neutral Red Uptake using NIH-3T3 (mouse embryonic fibroblast cells) and the cell viability was > 80%. These new glasses can be considered in several and important applications in the field of inorganic non-toxic biocide agents such as medical implants, surgical equipment, protective apparels in hospitals, water purifications systems, food packaging, food storages or textiles.

Rose sawfly *Arge rosae* (Hym; Argidae) is the most serious pest of flowers of Rosaceae family. In order to evaluate the potentiality of entomopathogenic fungi for controlling this pest, fourth instar larvae were maintained under controlled conditions and exposed to entomopathogenic fungi, *Beauveria bassiana*. The bioassay tests were carried out by immersion method with concentrations 2 x10^4, 2x 10^5, 2 x10^6, 2 x10^7 and 2x10^8 conidia/ml of Tween-80 solution (0.03%) in distilled water. Each concentration was replicated four times with ten insects in each replicate. The results showed that relatively high efficacy of these isolates on this insect pest. In case of IRAN 403C isolate, values of LC50 and LT50 were obtained as 5.54 x10^5 conidia/ml and 3.92 days at 2x10^8 conidia/ml, respectively. Results showed that IRAN 403C isolate caused the highest mortality in larvae in comparison with other isolates with a mean of 70% mortality using 10^7 conidia/ml (Roya Khosravi *et al.*, 2015) [31].

Thomas *et al.*, (2015) [39] conducted a simple technique for bacterial and yeast CFU estimations from diverse samples with no prior idea of viable counts, designated as single plate-serial dilution spotting (SP-SDS) with the prime recommendation of sample anchoring (10^0 stocks). For pure cultures, serial dilutions were prepared from 0.1 OD (10^0) stock solution and 20 µl aliquots of six dilutions (10^1–10^6) were applied as 10–15 micro-drops in six sectors over agar-gelled medium in 9-cm plates. For liquid samples 10^0–10^5 dilutions, and for colloidal suspensions and solid samples (10% w/v), 10^1–10^6 dilutions were used. Following incubation, at least one dilution level yielded 6–60 cfu per sector comparable to the standard method involving 100 µl samples. These cultures were tested on diverse bacteria, composite samples and *Saccharomyces cerevisiae*, SP-SDS offered wider applicability over alternative methods like drop-plating and track-dilution for cfu estimation, single colony isolation and culture purity testing, particularly suiting low resource settings.

Arshad *et al.*, (2016) [4] synthesized ZnO-SiO2 Nano composite via deposition- precipitation method by using acetonitrile as solvent and characterized by using different analytical techniques like Fournier Transformation Infrared Spectroscopy (FT-IR), Thermo-Gravimetric Analysis (TGA), Powder X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), and Dynamic Light Scattering (DLS). Biological potential such as antibacterial and antifungal activities were also studied by using Disc Diffusion method and Agar Well Diffusion Assay respectively. The particle size of ZnO-SiO2 Nano composite calculated by TEM was found to be 6.2 nm. The Nano composite showed better antibacterial activity than antifungal activity.

Neha Sharma *et al.*, (2016) [23] studied the antimicrobial activity of pure and doped ZnO Nano composites. Polyvinyl pyrrolidone capped Mn- and Fe-doped ZnO Nano composites were synthesised using simple chemical co-precipitation technique and were characterised using transmission electron microscope (TEM), X-ray powder diffraction (XRD), energy dispersive X-ray fluorescence (EDXRF), Fourier transform infrared (FTIR) spectroscopy and ultraviolet (UV) visible spectroscopy. The antimicrobial activities of nanoparticles (NPs) was observed against fungi, gram positive and gram-negative bacteria by using the standard disc diffusion method. The experimental results demonstrated that ZnO NPs doped with 10% of Mn and Fe ions showed maximum antimicrobial and photo degradation efficiency due to the synergistic effects of Mn and Fe loading.

Evaluation of *Beauveria bassiana* against *S. Litura* under laboratory conditions for their relative efficacy

Hicks *et al.*, (2001) [12] conducted laboratory bioassays of the entomopathogenic fungus *Beauveria bassiana* against different life stages of the pine beauty moth *Panolis flammea*, showed that this fungus has high potentiality to be used as a biological control agent. High mortality was recorded on fifth instar larvae treated with conidia at doses that are comparable

to the doses used against other serious pests. The sensitivity of the egg stage also showed some deformities and that eggs may also be a targeted stage while pupae were not severely affected by the fungus. Several species of Carabidae were tested with high doses of conidia and these potential predators suffered no adverse effects.

Ahmed and Katatny (2007) [1] were carried out a preliminary virulence test on three concentrations (1×10^6, 1×10^7 and 1×10^8 conidial spore ml^{-1}) of the aqueous conidial suspension of the four entomopathogenic fungi isolates *viz*., *Beauveria bassiana* IMI 382302, *Beauveria bassiana* IMI 386701, *Trichoderma harzianum* T24 and *Aspergillus flavus* Link was carried out against both larval and pupal stages of *Spodoptera littoralis* within 5 days of post-treatment. *B. bassiana* IMI 382302 showed relatively high dose dependant larval and pupal mortalities. While, strain IMI 386701 of *B. bassiana* showed a very weak mortality against pupae at its higher concentrations but no virulence against larvae was recorded. Injection of larvae with conidial suspension (5×10^3 conidia/larva) from each of fungal isolates showed humoral antifungal activity against *Beauveria bassiana* IMI 386701and *Aspergillus flavus* only. Thus, this study concluded that larvae of *S. littoralis* showed immune-dependant sensitivity to *T. harzianum* T24 and *B. bassiana* IMI 382302.

Hawary *et al.*, (2009) [9] evaluated the efficacy of entomopathogenic fungi products (i.e. Bio- Power (*Beauveria bassiana*), Bio-Catch (*Lecanicillium lecanii*) and priority (*Paecilomyces fumosoroseus*) against *Spodoptera littoralis* and *Agrotis ipsilon* larvae under laboratory conditions. Four different concentrations i.e., $0.125x10^9$, $0.25x10^9$, $0.5x10^9$ and $1x10^9$ spores/ 1000ml D.W of each formulation were used against each pest under investigation and compared with control insects. The results obtained show that Bio- Power was the most effective product followed by Bio-Catch and Priority against *S. littoralis* 3rd instar larvae, whereas the LC50, LC90 values were 0.2x109 and 1.5x109, 0.22x109 and 4.6x109, 0.44x109 and 4.7x109, respectively.

Qin *et al.*, (2010) [30] evaluated the entomopathogenic fungus *Beauveria bassiana* which acts slowly on insect pests through cuticle infection. A transgenic *B. bassiana* strain (BbV28) expressing Vip3Aa1 (a Vip3A toxin) was thus created to infect the larvae of the oriental leafworm moth *Spodoptera litura* through conidial ingestion and cuticle adhesion. The median lethal concentration (LC_{50}) of BbV28 against the second-instar larvae feeding on cabbage leaves sprayed with conidial suspensions was 26.2-fold lower than that of the wild-type strain on day 3 and 1.1-fold lower on day 7. The same sprays applied to both larvae and leaves for their feeding reduced the LC_{50} of the trans formant 17.2- and 1.3-fold on days 3 and 7, respectively. Median lethal times (LT_{50}s) of BbV28 were shortened by 23 to 35%, declining with conidial concentrations. The larvae infected by ingestion of BbV28 conidia showed typical symptoms of Vip3A action, i.e., shrinkage and palsy. However, neither LC_{50} nor LT_{50} trends differed between BbV28 and its parental strain if the infection occurred through the cuticle only. Their findings indicated that fungal conidia can be used as vectors for spreading the highly insecticidal Vip3A protein for control of foliage feeders such as *S. litura*.

Malarvannan *et al.*, (2010) [32] conducted an experiment against tobacco caterpillar, *Spodoptera litura* by using entomo-pathogenic fungi *Beauveria bassiana* was sub-cultured on Potato Dextrose Agar (PDA). Spore suspensions of four different concentrations (2.4×10^7, 2.4×10^6, 2.4×10^5, 2.4×10^4 conidia/ml) were prepared from the 15 day old culture of the fungi. A preliminary study on *B. bassiana* against *S. litura* larvae was done. Further treatment of the resultant pupae caused mortality and adult malformation. The healthy moth emergence was least in (2.4×10^4) spore concentration of the treatment, while the fecundity was completely arrested in the highest concentration.

Petlamul and Prasertsan (2012) [28] evaluated ten strains of the entomopathogenic fungi of *Metarhizium anisopliae* and *Beauveria bassiana* to find the most effective strain for optimization studies. The first criterion tested for strain selection was recorded the mortality of > 50% of *Spodoptera litura* larvae after inoculation of the fungus for 4 days. Results on several bioassays revealed that *B. bassiana* BNBCRC showed the most virulence on mortality *S. litura* larvae (80% mortality). *B. bassiana* BNBCRC also showed the highest germination rate (72.22%). However, its conidia yield (7.2×10^8 conidia/mL) was lower than those of *B. bassiana* B 14841 (8.3×10^8 conidia/mL) and *M. anisopliae* M 6 (8.2×10^8 conidia/mL). The highest accumulative radial growth was obtained from the strain B14841 (37.10 mm/day) while the strain BNBCRC showed moderate radial growth (24.40 mm/day). Amongest these criteria, selection based on virulence and germination rate lead to the selection of *B. bassiana* BNBCRC. *B. bassiana* B14841 would be selected if based on growth rate while *M. anisopliae* M6 and M8 possessed the highest enzyme activities.

Susceptibility of different biological stages of *Spodoptera litura* to various strains of entomopathogenic fungi was evaluated under laboratory conditions at Department of Entomology University of Agriculture, Faisalabad using the insect immersion method. Virulence potential of the entomopathogenic fungi varied with different biological stages of the insect pest. Eggs and larvae were comparatively more susceptible to infections by entomopathogenic fungi, while pupae were less susceptible. The susceptibility of the insect to entomopathogenic fungi decreased with the advancement in age of larvae of the insect. The LC50 values for eggs were 1.13×10^6, 4.82×10^6 and 2.45×10^7conidia ml^{-1} in *M. anisopliae* L6, *I. fumosorosea* 32 and *B. bassiana* 25, respectively. The median lethal concentration (LC50) for 3rd instar larvae was 1.11×10^7conidia ml^{-1} in *B. bassiana* 25 and 2.17×10^7conidia ml^{-1} in *I. fumosorosea*. Mortality of the larvae increased with increase in conidial concentrations and time elapsed after treatment (Asi *et al*., 2013).

Indriynti *et al.*, (2017) applied *Beauveria bassiana* is a parasitic mold for insect, it is commonly used as a control agent obtained from Estate Crop Protection Board (BPTBUN) in Salatiga, Central Java as dust formulation against *Spodoptera litura* attacked tobacco plants in Salatiga. The *S. litura* larvae were obtained from tobacco farm, then adapted to laboratory environment for two days before used for Bioassay. There were five different doses of treatments *viz*., 1g 100 mL^{-1}, 2g 100 mL^{-1}, 4g 100 mL^{-1}, 8g 100 mL^{-1} and 0 g 100 mL^{-1} (as control) and in each treatment 10 larvae used and repeated five times. The result showed that *B. bassiana* with 8 g100 mL-1 concentration was more effective to kill *S.litura* larvae than others doses. The important finding of this research is that *B. bassiana* can be used to control *S. litura* larvae safely which was not pollute the environment.

Nurhayati *et al.*, (2017) [24] found that the entire body of *S.*

litura is almost obscured by the white-colored mycelia. With a 10^9/mL density to shut down up to 50% of the population takes 5 days and 95% of death takes 10 days. *B. bassiana* infects the insects through the cuticle and multiplies in the body of the insect, while producing beauverisin toxins which can damage the structure of the insect cell membrane to death. Kaur and Padmaja (2008) [16] conducted the laboratory studies on *B. bassiana* (1.3×10^6 spores/ml) and three new chemistry insecticides [emamectin benzoate (Timer ®), flubendiamide (Belt ®) and chlorantraniliprole (Coragen ®)] alone and in mixtures at dose less than the 2 folds of recommended field dose were tested against 2 nd and 4 th instar larvae of *S. litura*, under laboratory conditions by using larval immersion and leaf dip method. The interaction of *B. bassiana* and new chemistries showed the higher percentage of mortalities in both tested instars larvae as compared to alone treatment. Bb* Coragen ® (91.43%, 86.14%) interaction showed the highest larval mortality followed by Bb*Belt ® (84.60%, 76.88%) and Bb*Timer ® (74.60%, 70.86%) for 2 nd and 4 th instar larvae respectively. Similarly decrease in pupation rate and adult emergence was observed in alone and combined treated applications. *B. bassiana* and sub-lethal doses of new chemistries significantly increased the larval and pupal duration while decrease in adult longevity was observed.

Kaur *et al.*, (2011) tested the virulence of *B. bassiana* against second, third and fourth instar larvae of *S. litura* by using 4 concentrations i.e. $2.03x10^8$, $4.03x10^6$ and $1.47x10^5$ spores/ml. All the treatments showed in significantly higher virulence compare to the control. Besides the larval mortality, the eggs descended from treated larvae showed significant decrease in hatchability, pupal and adult deformities.

Moorthi *et al.*, (2011) evaluated the isolates of entomopathogenic mitosporic ascomycete, *Beauveria bassiana* against *Spodoptera litura* using leaf topical spray method. The results indicated that Bb_{02}, Bb_{09} and Bb_{10} isolates were recorded the high mortality of 66.67, 73.33 and 80.0% respectively was obtained four days after treatment and LC_{50} value of the isolates was $2.1x10^6$, $3.6x10^7$ and $1.2x10^7$ conidia ml^{-1} for Bb_{02}, Bb_{09} and Bb_{10}, respectively. The LT_{50} value for $Bb_{02}$and Bb_{09} was 4.8 days, whereas it was 4.0 days in Bb_{10}@10^8 spore mL^{-1}. The germination percentage of fungi was 79.83, 88.33 and 95.53% observed in Bb_{02}, Bb_{09} and Bb_{10}, respectively. However among these isoates Bb_{10} I was most virulent with potential for the management of *S.litura*.

Kanani *et al.*, (2015) [15] evaluated nine biopesticides against leaf eating caterpillar, *Spodoptera litura* Fabricius infesting castor during 2012-2013. Among these biopesticides SLNPV @250 LE/ha and *Beauveria bassiana* (0.4%) protected the crop from pest and produced higher yield. The highest (28.33q/ha) seed yield of castor was obtained from the plots treated with SNPV and *Beauveria bassiana* found effective and economical treatments than the other treatments occupied first rank.

Sweta and Sobita (2017) [38] studied the efficacy of *B.bassiana* on different larval stages of *Spodoptera litura* using 1%, 2%, 3%, 4% and 5% in $2.3x\ 10^6$ conidia/ml under laboratory conditions. The results revealed that percent mortality was quiet high in early instars of larvae as compared to late instars. The highest dose of 5% contain $2.3x10^6$ conidia/ml brought 91.66. 90.00, 88.33, 78.77, 66.11 and 49.99 percent mortality in 1st, 2nd, 3rd, 4th, 5th and 6th instar larvae respectively. Thus the higher dose of *B. bassiana* higher will be the mortality.

To assess the field efficacy of the nanomatrial formulated biopesticides produced which are proved effective in the laboratory against *S.litura* on groundnut.

Broza *et al.*, (1984) [6] were tested the efficacy of liquid formulations of *Bt* var. *entomocidus* sprayed on commercial cotton fields of 4 and 6 ha in 1982 by ground application and in 1983 by aerial application. The microbial insecticide successfully controlled first and second instars of the Egyptian cotton leafworm, *Spodoptera littoralis*.

Umeda and Fredman (1995) [40] evaluated the several commercial formulations of *Bacillus thuringiensis* (Al) insecticides were applied on cabbage and lepidopterous pests including *Tricoplusia* it (cabbage looper, CL), *Spodoptera exigua* (beet armyworm, BAW), and *Plutella xvlostella* (diamondback moth, DBM) and results indicated that all the treatments were effectively reduced the population. Among them ten commercial products did not appear to vary significantly in controlling cabbage looper which was dominant species present in the cabbage.

Wraight and Ramos (2005) [43] were applied commercial biopesticides based on the fungal pathogen *Beauveria bassiana* strain GHA and the bacterial pathogen *Bacillus thuringiensis tenebrionis* alone and in combination (tank mixed) against larval populations of the Colorado potato beetle, *Leptinotarsa decemlineata*, in small plots of potatoes in three seasons under field conditions. Interactions between the two products were evaluated in terms of pest-control efficacy. The *B. bassiana* (formulated as Mycotrol) was applied at low and medium concentration of 1.25 and 2.5×10^{13} conidia/ha, while *B. thuringienis* (formulated as Novodor) was applied at low and high label rates of 40.3 and 120.8×10^6 Leptinotarsa units/ha. Biopesticides applied thrice at weekly intervals and observed that bacterial pesticide alone provided 50–85% control of beetle larvae within 14 days after the initial application, while applications of the mycopesticide alone produced less than 25% control. However the combination of treatments produced a statistically significant 6–35% greater reduction in larval populations. These results indicated that *B. thuringiensis* and *B. bassiana* have strong potential for integrated biologically based management of Colorado potato beetle.

Ali *et al.*, (2010) reviewed, a number of approaches to pest control via genetic engineering have been developed and genetically engineered crops expressed insecticidal characteristics under cultivation for the last 15 years. The use of *Bacillus thuringiensis* genes encoding endotoxins with insecticidal characteristics is the major approach and a number of such *B. thuringiensis* genes have been expressed in crops with variable level of efficiency.

Sahayaraj and Namachivayam (2011) [33] were tested entomopathogenic fungi *Beauveria bassiana* (Balsamo) *Vuillemin* (Deuteromycotina: Hyphomycetes), *Paecilomyces fumosoroseus* (Wize) (Deuteromycotina: Hyphomycetes), *Verticillium lecani* Viegas (Deuteromycotina: Hyphomycetes) against groundnut pests, *Aphis craccivora* (Koch) (Homoptera: Aphididae), *Aproarema modicella* (Deventer) (Lepidoptera: Gelechiidae), *Mylabris pustulata* Faust (Coleoptera: Meloidae) and *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) in field conditions. Among the tested fungi, *V. lecanii* suppressed 62% of *A. craccivora* population at 39 Days after Seedling Emergence (DASE) while *B. bassiana* reduced 72% of *S. litura* larval population

(0.73 larvae). The infestation of *S. litura* and *A. modicella* were greatly reduced after the treatment of *B. bassiana* and highest pod yield (1721.31 kg/ha-1) and cost benefit ratio (1: 1.93) was recorded.

Salam *et al.*, (2011) [34] evaluated the biological activities of two species of bacteria isolated from soil of cotton fields identified as *Bacillus subtilis* strain NRC313 (*BS* NRC313) and *Bacillus thuringiensis* strain NRC335 (*BT* NRC335) against the third larval instar of the cotton leafworm, *Spodoptera littoralis* (Boisd.). The different entomo-pathogenic bacteria of *BS*NRC313 and *BT* NRC335 contained 10×10^8 cell/ml, and caused mortality of 100 and 97.3% for the above mentioned strains, respectively.. The results indicated that *Bacillus subtilis* was more potent than *Bacillus thuringiensis*. Field applications of *B. thuringiensis*, *B. subtilis* and Reldan achieved 55.6, 67.4 and 89.4% reduction of the cotton leafworm *Spodoptera littoralis* in clover plants under field conditions.

Lalitha *et al.*, (2012) [18] conducted a field experiment in a randomized block design to evaluate 28 *Bacillus thuringiensis* isolates along with a reference strain HD1 and untreated control against *Spodoptera litura* in groundnut. The larval population of *S. litura* per meter row at 3 days after spray (DAS) was lowest (9.0) in treated plot with *Bt* strain 341. Similarly the per cent reduction of larval population over pretreatment was maximum (56.83%) in HD1 reference strain and it was followed by the *Bt* strain 375 (51.45%). Mean per cent reduction of larvae over pre-treatment was maximum (68.32%) in HD1 reference strain followed by *Bt* strain 21 (57.27%). The minimum larval population of *S. litura* (5.0) was recorded at 7DAS in treated plot with *Bt* strains HD1, 375 and 416. Per cent leaf damage due to *S. litura* was minimum (12.83%) in plots treated with HD1 reference strain followed by strain 375 (14.06%). Highest pod yield (3900 kg/ha) was recorded in the plots treated with HD1 reference strain followed by *Bt* strain 375 (3870.0kg/ha).

Hemasree *et al.*, (2013) [11] studied the effect of entomopathogenic fungi *Nomuraea rileyi* against the larvae of II, III and IV instars of *Achaea janata* indicated that larval mortalities to be positively correlated with the concentrations of all three cultures. First subculture and insect culture were almost equally efficacious in causing the disease while themortality was slightly lowered in subculture II. Reduction in larval mortality was noticed with advancement of the age in *A. janata* larvae. Almost 100 per cent larval mortality was observed in IInd instar larvae when treated with $1x10^8$ spores ml^{-1} concentration and it was 5 – 15 per cent when treated to III and IV instar larvae.

Suganthy and Sakthivel (2013) [37] were carried out field experiments from August, 2011 to December, 2011 to study the field efficacy of bio-pesticides against *Spodoptera litura* on *Gloriosa superba*. The results of experiments revealed that efficacy of *Bacillus thuringiensis* (*Bt*) @ 2 ml/lit was realized only at seven days after treatment and persisted even after 14 days of second spray. Fourteen days after treatment, data indicated that *Bt* was next in the order of efficacy after chemical pesticides and flavonoids.

Zohn *et al.*, (2013) [47] evaluated *Beauveria bassiana* (Balsamo) for control of Citrus thrips, *Scirtothrips citri* (Moulton), is one of the major sucking pest most widely recognized for causing damage to citrus and mango fruits. Citrus thrips in blueberries grown under two watering regimes (drip irrigation with and without overhead sprinklers) and used two fungal formulations (commercially available spores in suspension vs. colonized seed). The observations are recorded at two different periods that is, for 2 to 3 days after treatment and found significant differences in thrips densities in water regime treatment and fungal formulation. Thrips population were reduced significantly with both fungal treatments at 3 days after treatment, but at 6 days only results with colonized seed differed from the control treatment.

Patel *et al.*, (2014) [25] were conducted a field trial on soybean in *kharif* seasons of 2011-2012 to study the efficacy of certain entomopathogens *viz*., *Beauveria bassiana*, *Metarhizium anisopliae*, *Verticilium lecanii*, *Bacillus thuringiensis var. kurstaki* @ 5 g/l along with standard check - Quinalphos 25 EC @ 1.5 g/L and Spinosad 45%SC @ 73 g a.i. /ha against lepidopteran defoliators. *Bacillus thuringiensis* @ 10^{13} spores/ha followed by *B. bassiana* @ 10^{13} spores/ha were the most effective treatments when applied as foliar sprays at 38, 41 and 45 days old crop. These treatments were effective in reducing the foliage feeder larval population. The highest grain yield was obtained in the treatment, *B. thuringiensis* var. *kurstaki* (474.77 kg/ha). The lowest yield was recorded in the control (215.23 kg/ha) which was significantly inferior to the rest of the treatments.

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