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Research Article

ENTOMOPATHOGENICITY OF PHOTORHABDUS BACTERIA AGAINST PULSE BEETLE, *CALLOSOBRUCHUS CHINENSIS* L. (BRUCHIDAE: COLEOPTERA) IN CHICKPEA GRAINS

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Abstract **–** Pulse beetle, being cosmopolitan is responsible for severe losses in lentil, maize, cowpea, mung bean and sorghum. In chickpea, 50 to 60 percent losses in seed weight and 46 to 66 percent protein losses have been recorded due to severe attack of *Callosobruchus chinensis*. Indiscriminate use of pesticides and fumigants to control pulse beetle has resulted in insecticide resistance, environmental hazards, residual toxicity and pest resurgence so the control trends have been changed and particularly biological control measures have attained high significance like entomopathogenic bacteria were used as alternate bio-control agents against this serious pest. During this research the pathogenicity of entomopathogenic bacteria *Photorhabdus temperate* was observed against pulse beetle at different concentrations. Bacterial culture was grown in Luria broth agar at 25°C in incubator, and then it was multiplied in neutralized distilled water medium to get single colonies of bacteria on this medium. Initially, concentrations 8×10^7 and 5.6×10^7 cells/ml of *P. temperate* were obtained by counting no. of cells using spectrophotometer. After that we diluted these bacterial concentrations into required concentrations like 1×10^6, 1×10^7 and 1×10^8 cells/ml. The concentration 1×10^8 cells/ml was reported as the highly efficient in controlling the fecundity with the minimum 4.82 eggs/grain. The least number of F_1adults (12.1) was observed at this bacterial concentration. The same concentration showed minimum damage in terms of holes development in grains. Results revealed that the higher concentrations were more effective than the lower concentrations of entomopathogenic bacteria. The findings of the research provided an effective and safe biological control measures and in future will lead to an effective IPM programme for this economic pest.

Key words: Callosobruchus chinensis, Photorhabdus temperate, Management, Pathogenicity, Chickpea

INTRODUCTION

Chickpea due to its high protein content has become an important component of human diet in the developing countries. It comprises starch, protein, sugar, crude fiber, fat, ash, calcium and other minerals

and vitamins [1]. It is severely damaged in storages. The extreme post-harvest losses in storages are one of the main restrictions in cultivation of chickpea with maximum yield. Due to heavy infestation the chickpea grains lose their germination capacity and become unfit for human consumption. The bruchids have been observed to be the most important species in chickpea during storage. Once damaged chickpea grains by bruchids are not suitable for planting due to poor germination and not acceptable for food or feed due to spoilage, bad smell and toxin is produced [2].

Callosobruchus chinensis (Bruchidae: Coleoptera) is known as the most devastating insect pest of stored chickpea. It is cosmopolitan in distribution and having a proficiency to damage stored grains also along with a serious threat to field cultivated host plants. It is reported that infestation caused by this beetle results in severe loss in seed weight and protein content of pulses that are 55-60% and 46-66% individually [3]. Synthetic pesticides and fumigants are commonly used to control this pest. But chemical control measures cause serious health risks on consumption and deteriorate the quality of grains. It also results in environmental pollution and persistent toxicity as well as creates pesticides resistance among bruchids [4].

Entomopathogenic microorganisms like bacteria and fungi have more potential to cause mortality in insects. Entomopathogenic bacteria produce mainly three kinds of toxins viz., digestive toxins, cyto-toxins and neurotoxins. *Photorhabdus temperate* and *Xenorhabdus nematophila* and other members of Enterobacteriaceae produce toxins that are similar to Bt toxins. The entomopathogenic bacteria of the genera *Xenorhabdus* and *Photorhabdus* live symbiotically within infective juvenile entomopathogenic nematode species of *Steinernema* and *Heterorhabditis,* respectively [5]. One of the pathogenic mechanisms of these bacteria includes host immunodepression, which leads to lethal septicemia. It has been known that *X. nematophila* inhibits phospholipase A2 (PLA2) to induce host immunodepression. Here, we tested the hypothesis of PLA2 inhibition using another bacterial species involved in other genera. *P. temperate* subsp. *temperate* is the intestinal symbiont of an entomopathogenic nematode, *H. megidis*. The bacteria caused potent pathogenicity in a dose-dependent manner against the fifth instar larvae of a test target insect, *Spodoptera exigua*, as early as 24 h after the intra-hemocoelic injection. In response to the live bacterial injection, hemocyte nodulation (a cellular immune response) and prophenoloxidase (pPO) activation were inhibited, while the injection of heat-killed bacteria significantly induced both immune reactions. To prove this generalized hypothesis, we used other bacterial species (*X. bovienni*, *X. poinarii*, and *P.luminescens*) involved in these two genera. All our experiments clearly showed that these other bacteria also share their inhibitory action against PLA2 to induce host immunodepression [6].

These bacteria upon inoculation have shown virulence against several insect pests including *Plutella xylostella* and *Galleria mellonella*, however oral toxicity in sweet potato whitefly, mosquitoes and Colorado potato beetle has been detected. They are attributed towards the pathogenicity of the entomopathogenic nematodes by suppressing the immune response of the insect [7].

The objective of proposed investigation encompassed to find out the aptness of different concentrations of entomopathogenic bacteria *Photorhabdus temperate* against *Callosobruchus chinensis* in stored chickpea grains.

MATERIALS AND METHODS

Maintenance of pulse beetle culture:

Infested samples of stored chickpea grains were collected from National Agriculture Research Centre (NARC), Islamabad and different flour mills/godowns/storages in different areas of the Potowar region in the Province Punjab, Pakistan. *C. chinensis* culture was sustained in the 'Stored Product Entomology Laboratory' of the university in an incubator at 70±5 % RH and 30±2 °C temperature.

Bacterial source and maintenance of their culture:

Entomopathogenic bacteria, *Photorhabdus temperate* were arranged from CABI Bioscience, Rawalpindi, Pakistan. Initially the culture on Nutrient Agar (NA) plates was streaked at 25ºC for 4-6 days. Culture was purified by re-streaking single colony on NA. Purified cultures were multiplied in

nutrient broth at 200 rpm for two to three days. To count/optimize the colony forming units (cfu's) per unit volume, serial dilution- plate count method was employed for drawing dilution curve between optical density (OD) and cfu's. Different concentrations of bacteria were prepared to apply against *C. chinensis*.

Insect bioassays:

In each plastic jar, 50g of chickpea grains was put. Each jar was covered with muslin cloth squeezed and then placed in an incubator at 30ºC. Ten pairs of *C. chinensis* were released into each jar. Five different concentrations of bacteria were prepared for insect bioassay. Each treatment had three replications.

The insecticidal potency of bacteria against *C. chinensis* was studied according to the following parameters:

i. Eggs per grain:

Mean number of eggs per chickpea grain laid by PB was computed to conclude the impact of treatment on egg laying capacity (fecundity). Ten chickpea grains were arbitrarily chosen from every replication and eggs laid on those chickpea grains were counted.

ii. Holes per grain:

Mean number of holes per chickpea grain was ascertained through checking the quantity of holes on ten chickpea grains chose arbitrarily from every jar.

iii. F_1 adults emerged:

Number of F_1 (newly hatched) grown-ups in every container was checked to see restraint of PB rise.

iv. Insect inhibition rate:

Rate diminishment in rise of F_1 grown-ups or restraint rate was computed by utilizing the accompanying formula.

$\%IR = \{(C_n - T_n) / C_n\} \times 100$

Where

C_n = Number of newly emerged adults in untreated jar (control)

T_n = Number of newly emerged adults in treated jar

Statistical analysis:

The data recorded was subjected to statistical analysis using appropriate statistical packages like SPSS 22.0 for Windows program etc. Moreover the graphical work was done using Microsoft excel programme.

RESULTS AND DISCUSSION

Number of eggs (Mean ± SEM) laid by *Callosobruchus chinensis* in chickpea grains treated with different concentrations of *Photorhabdus temperate*

By viewing the Table 1, it is clear that all used concentrations of *Photorhabdus temperate* like, 1×10^6, 1×10^7 and 1×10^8 gives a good results by reducing the no. of eggs of pulse beetle as compared to the control. The least no. of eggs laid by the concentrations 1×10^8 and 1×10^7 in which the values were 4.82 and 4.92 no. of eggs per grain. The concentration 1×10^6 gives 5.14 no. of eggs which is much lower than the control in which the no. of eggs were 15.16 which is much higher as compared to all above used concentrations. So according to the above data it is clearly visible that the no. of eggs decreases with the increase in concentrations of entomopathogenic bacteria *P. temperate*.

Number of holes (Mean ± SEM) made by *Callosobruchus chinensis* in chickpea grains treated with various concentrations of *P. temperate*.

According to the Table 2, it is cleared that no. of holes made by *C. chinensis* in chickpea grains are reducing with the increase in concentrations of entomopathogenic bacteria. The maximum no. of holes was seen in control 4.1, which was significantly different and much higher as compared to the other used concentrations. The least no. of holes was recorded in concentrations 1×10^8 and 1×10^7 and value

given by $1 \times 10^6$was 3.08 which is lower than the control but higher as compared to other used concentrations.

Table 1 Number of eggs (Mean ± SEM) per grain laid by *C. chinensis* in chickpea grains treated with different concentrations of *P. temperate*

Sr.No.	Concentrations of *Photorhabdus temperate* (cells/ml)	No. of eggs per grain (Mean ± SEM)
1	1×10^6	5.14 ± 0.02 b
2	1×10^7	4.92 ± 0.01 a
3	1×10^8	4.82 ± 0.02 a
4	Control	15.16 ± 0.12 c

Table 2 Number of holes (Mean ± SEM) per grain made by *C. chinensis* in chickpea grains treated with different concentrations of *P. temperate*

Sr.No.	Concentrations of *Photorhabdus temperate* (cells/ml)	No. of holes per grain (Mean ± SEM)
1	1×10^6	3.08 ± 0.008 b
2	1×10^7	2.96 ± 0.035 a
3	1×10^8	2.91 ± 0.011 a
4	Control	4.1 ± 0.043 c

Number of F1 adults (Mean ± SEM) emerged in chickpea grains by using different concentrations of entomopathogenic bacteria *Photorhabdus temperate*

As we can see in Table 3, all bacterial concentrations of *P. temperate* (1×10^6, 1×10^7 and 1×10^8 cells/ml) were statistically different and showed an excellent results by reducing the emergence of F1 adults in chickpea grains as compared to the control. The concentration 1×10^8gives the minimum no. of adults 12.1 in chickpea grains, while in concentrations 1×10^6, 1×10^7 the no. of adults recorded were 18.1 and 15.1 respectively. All these concentrations showed better inhibiting rate of F1 adults in chickpea grains as compared to control.

Percent inhibition rate (Mean ± SEM) of *Callosobruchus chinensis* in chickpea grains treated with different concentrations of *Photorhabdus temperate*

Figure 1 showed that with the increased bacterial concentration the inhibition rate of newly emerged pulse beetle was significantly increased. Bacterial concentration 1×10^8 cells/ml showed highest inhibition rate with more than 74% IR. On the other hand, least inhibition of newly emerged was observed in 1×10^6 concentration with near about 60% IR. Rather than control all the treatments proven better in the inhibition of newly emerged pulse beetle adults.

Table 3 Number of F_1 adults emerged (Mean ± SEM) of *C. chinensis* in chickpea grains treated with different concentrations of *P. temperate*

Sr.No.	Concentrations of *Photorhabdus temperate* (cells/ml)	No. of F1 Adults emerged (Mean ± SEM)
1	1×10^6	18.11 ± 0.008 c
2	1×10^7	15.1 ± 0.023 b
3	1×10^8	12.1 ± 0.86 a
4	Control	45.1 ± 0.05 d

The findings are in conformity with [8] who reported that *Photorhabdus temperate* and *xenorhabdus nematophila* which are entomopathogenic bacteria are very useful in minimizing the insect immune response by suppressing catalytic activity of phospholipase (PLA2) which gives prevention of biosynthesis immune mediating eicosanoids. From this study it is proved that (PLA2) inhibitors can only obtained by the culture broth of above given two bacteria's. This application or experiment showed us a new pathway to control these two bacteria's by using (PLA2) as an insecticide novel. [9] concluded that microscopic organisms connected with entomopathogenic nematodes produce mixes with antibacterial and antifungal action. In the present study, *Xenorhabdus* spp. also, *Photorhabdus* spp. were developed in society medium (in vitro) and in *Galleria mellonella* (L.) contaminated with entomopathogenic nematodes (in vivo), and the impact of their filtrates on the mortality of Meloidogyne incognita second stage adolescents (J2s) was explored. The microorganisms filtrates got by media society and hemolymph were connected with various types of entomopathogenic nematodes and put in direct contact with J2s of M. incognita. The mortality of the adolescents was evaluated after 24 h. After weakening of the hemolymph from tainted *G. mellonella*, the filtrates got from the in vivo society and in vitro society delivered an 80% and 20% death rate, separately. Along these lines, the nature of substances with nematicidal movement was influenced by the system used to develop the microbes, mirroring the distinction between development in vivo and in vitro and the significance of the insects.

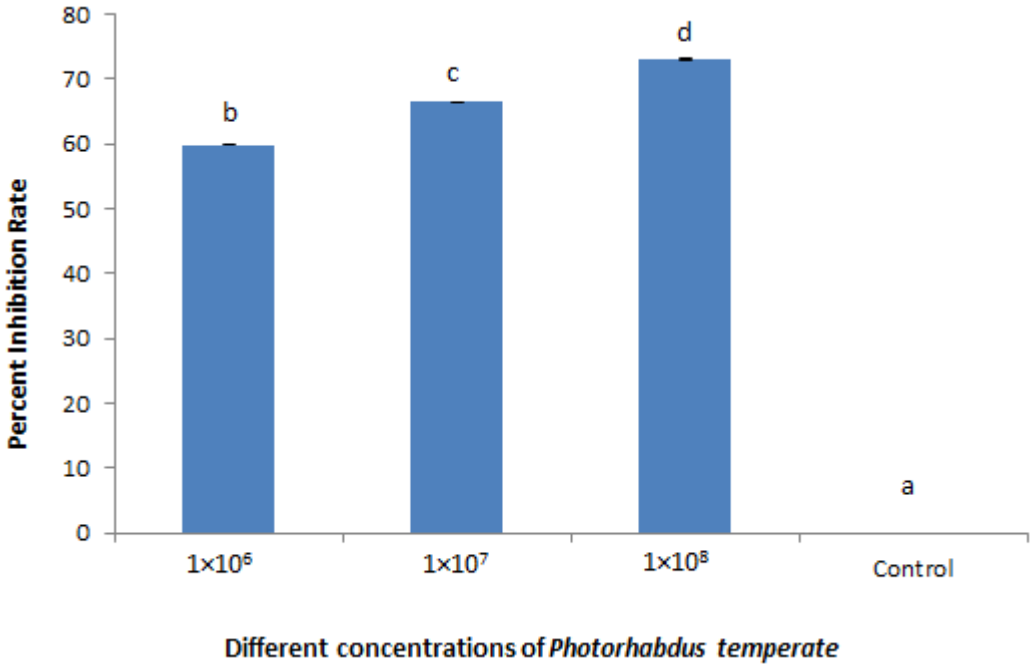

Figure 1 Percent inhibition rate (Mean ± SEM) of *C. chinensis* in chickpea grains treated with different concentrations of *P. temperate*

According to [10], *photorhabdus luminescens* create a mutualistic link between nematode which is a pathogen for insects. Variants of *P. luminescens* excessively come up and used frequently because they can change phenotypic character which are very useful forcollaboration of host. VAR has colonial character and showed up phenotypically changed displayed by delayed pathogenicity when directly inject in *Spodoptera litura*. In this experiment we get to know the importance of transcriptomic variation in finding out the external change & late pathogenicity due to VAR found to be modified. In the end we concluded that some phenotypic character of VAR can be changed transcriptionally indicated the multi-factorial ability of pathogenicity in insects. [11] Experiments showed that *Xenorhabdus* is entomopathogenic bacteria that linked with nematodes. The nematode-bacteria couplepoisons and destroys insects, with together partners donating to insect diseases and by getting nutrients from insects bacteria gave it to nematode. Both *Photorhabdus* and *xenorhabdus* gave same services to different nematode hosts by the means of distinctive physiological and metabolic tools. For their specific genomes such differences are responsible. In spite of the resemblances in lifestyle between *Xenorhabdus* and*Photorhabdus* bacteria, a relative analysis of the *Xenorhabdus, P. luminescens*, and *Photorhabdus asymbiotica* genomes advises genomic difference. These conclusions point out that evolutionary variations shaped via symbiotic relations can follow various routes to get similar end points [12].

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