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PATHOGENICITY OF *PHOTORHABDUS LUMINESCENS* AND *XENORHABDUS NEMATOPHILA* AGAINST *RHYZOPERTHA DOMINICA* (BOSTRICHIDAE: COLEOPTERA) IN STORED WHEAT GRAINS

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ABSTRACT

Wheat grains are attacked by more than 23 insect species in Pakistan during storage. Lesser grain borer, *Rhyzopertha dominica* Fabricius (Bostrichidae: Coleoptera) is the most common grain feeding species of all the cereal grains in many parts of the world. Both adults and grubs can damage the stored grains. In this study, entomopathogenic bacteria, *Xenorhabdus nematophila* and *Photorhabdus luminescens* were evaluated for the management this serious pest. All the concentrations of *P. luminescens* and *X. nematophila* significantly reduced number of eggs laid by *R. dominica*, number of holes and number of adults as compared to the control. The highest concentrations gave the maximum reductions while the minimum reduction was observed with the lowest concentrations. Similarly, all the concentrations of the bacteria gave statistically significant results regarding the inhibition of *R. dominica* F_1 emergence of adults. The bacterial concentration of 4×10^8 cells/ml showed the highest percent inhibition rate while the least percent inhibition rate was observed with 4×10^4 cells/ml. There was a direct relation between concentration and percent inhibition rate. Regarding percentage weight loss, all the concentrations were statistically different from the control. The concentration of 4×10^8 cells/ml was the most effective with the least percent weight loss. On the other hand, the maximum damage was done by the insect in the grains treated with the concentration 4×10^4 cells/ml. Similarly, the concentrations of 4×10^5, 4×10^6 and 4×10^7 cells/ml resulted in greater weight loss to stored wheat grains. Likewise, all the concentrations of *P. luminescens* and *X. nematophila* caused significant mortality of *R. dominica*. The mortality of the insect increased with an increase in the concentration and the time interval. The highest mortality was obtained with the bacterial concentration of 4×10^8 cells/ml and the lowest mortality was recorded with concentration of 4×10^4 cells/ml. The present research will have significant contribution towards development of microbial formulations of *X. nematophila* and *P. luminescens* to manage *R. dominica* in stored wheat.

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INTRODUCTION

Wheat (*Triticum aestivum* L.) belonging to the family Gramineae is the 2nd most important crop in the world which is frequently used for the production of noodles, biscuits, flour, alcoholic beverages, cookies, biofuel and other various products. Worldwide Pakistan is the 10th largest country in wheat production. In Pakistan, wheat is the diet of 60% of the population (Mutwali et al., 2015). The Lesser grain borer (LGB) (*Rhyzopertha dominica*) Fabricus, (Bostrichidae: Coleoptera) is one of the most damaging insect pests of stored grains which infests the grains at different pre- and the post-harvest levels (Edde, 2012). The LGB is an insect pest which is distributed worldwide. It is present in different storage structures like godowns and in field during harvesting time of wheat (Nadeem et al., 2011). *R. dominica* is not only a key pest of corn and wheat but also damages other stored commodities like tobacco, peanuts, beans, biscuits, cocoa beans (Edde, 2012). *R. dominica* and other stored grain pests cause enormous financial damages to agricultural commodities in the form of grain damage and product weight loss. By producing frass and unfriendly odor, the grains become unhealthy and unsuitable for consumption and human digestibility (Arthur et al., 2012).

Worldwide damage of stored grains has been estimated by 5-10%. In certain tropical states, the damage of stored grains reaches up to 30% (Ali et al., 2009). Insect pests of stored commodities can cause post-harvest damage up to 20% or more in developing countries and 9% in developed countries (Phillips and Throne, 2010). *R. dominica* is the most challenging pest as compared to other stored grain pests and its control with grain protectants is of great significance. To control *R. dominica*, phosphine fumigation has been the most effective method (Ruiu et al., 2013). However, this pest has developed resistance to grain protectants (Lorini and Galley, 1999), numerous pyrethroid based insecticides (Collins, 2006) and all the accepted insecticides like organophosphorus (Edde, 2012).

Photorhabdus luminescens and *Xenorhabdus nematophila* belong to the Enterobacteriaceae family and are motile, rod shaped and gram negative bacteria. These bacteria are passed out in intestines of infective juveniles that invade insect haemocoel through the mouth, spiracles, and anus where they are released from infective juveniles into the hemolymph of intestines (Wang and Gaugler, 1998). Subsequently, the insect body shows black or red color and slowly decomposition takes place. After utilization of food, the infective juveniles emerge from the insect body to search out new hosts. Except Antarctica, *Xenorhabdus nematophila* and *Photorhabdus luminescens* are present in all the continents of the world and have approximately 30 species (Ferreira et al., 2013; Hominick, 2002; Tailliez et al., 2010). Almost 100 species of *Steinernema* and 26 species of *Heterorhabditis* have been reported worldwide (Adams et al., 2006; CImen et al., 2014; Malan et al., 2012; Phan et al., 2014; Xing-Yue et al., 2012). *X. nematophila* and *P. luminescens* produce numerous secondary metabolites which include antimicrobial and insecticidal compounds such as (phenethylamines, benzylideneacetone, xenocoumacins, 3,5-dihydroxy-4-isopropylstilbene and indole) (Eleftherianos et al., 2007).

The objective of present study was to evaluate different concentrations of entomopathogenic bacteria *Photorhabdus luminescens* and *Xenorhabdus nematophila* against lesser grain borer attacking stored wheat grains.

MATERIALS AND METHODS

Collection of *Rhyzopertha dominica* infected samples and maintenance of insect culture: Stored wheat grains infested with *R. dominica* were collected from different research stations, godowns and storages in different areas of the Punjab. *R. dominica* culture was preserved in the Department of Entomology in an incubator at the 68 ± 5% relative humidity and 32 ± 3 ℃ temperature. Grains of wheat cv. NARC-11 were obtained from the Notational Agriculture Research Center, Islamabad for the bioassay.

Bacterial source and maintenance of their culture: Entomopathogenic bacteria, *Photorhabdus luminescens* and *Xenorhabdus nematophila*, were obtained from the CABI Bioscience, Rawalpindi. A single colony was selected from the pure culture on the basis of morphological and phenotypic characteristics to produce large quantities of bacterial cells. The bacterial colony was inoculated into the autoclaved nutrient broth in the flask. In the shaking incubator, the flask was placed at 150 rpm for 24 hours at 25 ± 3 ℃. The concentration of bacterial cells in the suspension was measured by optical density in the spectrophotometer at 600 nm wavelength. For the bioassay experiment, the concentration of

entomopathogenic bacteria in the suspension was adjusted to the 4 x 10^7 cells/ml, 3 percent Tween 80 was added as an emulsifier. Subsequent concentrations were prepared by the addition of requisite quantity of broth and were adjusted to 4 x 10^4, 4 x 10^5, 4 x 10^6, 4 x 10^7 and 4 x 10^8 cells/ml.

Insect Bioassay

Experiment No.1

Stored wheat grain samples were placed in each plastic jar and closed with muslin cloth and kept in an incubator at a temperature of 32 ± 3 ℃. Five pairs of *R. dominica* were released into each plastic jar containing 50 g of treated and untreated stored wheat grains. Five different concentrations of entomopathogenic bacteria *P. luminescens* and *X. nematophila* viz. 4 x 10^4, 4 x 10^5, 4 x 10^6, 4 x 10^7 and 4 x 10^8 cells/ml were prepared for experimental trial. Each treatment had three replications. Insecticidal potency of entomopathogenic bacteria against *R. dominica* was studied according to the following parameters

Number of eggs per wheat grain: To check the efficacy of the bacterial treatments on the fecundity of *R. dominica*, twenty grains were randomly selected from each plastic jar to count eggs and number of average eggs per wheat grain were calculated.

Number of holes per wheat grain: The average number of holes per wheat grain was calculated by randomly selecting twenty grains and the total number of holes were counted.

Number of the emerged adults of F_1: To calculate the inhibition of *R. dominica* emergence, number of F_1 (newly hatched) adults in each plastic jar were counted.

Experiment No.2

Mortality of *R. dominica* was observed in the treated and un-treated 50 g of stored wheat grains. Petri plates of 7 cm (38.5 cm^2) diameter containing Whatman No. 1 filter paper were used to check the effectiveness of treatments against adults' life span after 24, 48 and 72 hours. Each treatment had three replications. Five pairs of *R. dominica* were released in each Petri plate and the plates were placed in an incubator at 32 ± 3 ℃.

Percentage inhibition rate of insect (% IR): The percentage inhibition rate of F_1 adults of *R. dominica* was determined by using the following formula:

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

Where,

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

T_n = Number of the newly emerged adults in the treated plastic jars.

Percent weight loss (damage) of wheat grains: The percent weight loss of the grains was calculated by the following formula:

$$\text{Percent Weight loss} = \frac{\text{Initial weight} - \text{damaged and sound grains weight}}{\text{Initial weight}} \times 100$$

Statistical analysis: The recorded data were subjected to statistical analysis using statistical package SPSS 16.0. The graphical work was done in the Microsoft Excel program.

RESULTS AND DISCUSSION

Experiment No.1

Effect of *P. luminescens* and *X. nematophila* on number of the eggs: All the concentrations of *P. luminescens* and *X. nematophila* significantly reduced number of eggs laid by *R. dominica* as compared to the control. The highest concentrations gave the maximum reductions while the minimum reduction was observed with the lowest concentrations (Table 1).

Table 1: Number of eggs (Mean ± SEM) per grain laid by *Rhyzopertha dominica* treated with different concentrations of *P. luminescens* and *X. nematophila.*

Concentration	No. of eggs laid by *R. dominica* per grain (Mean ± SEM)	
	P. luminescens	*X. nematophila*
4 ×10^4	8.00 ± 0.58 b	7.00 ± 0.58 b
4 ×10^5	5.00 ± 1.15 a	6.00 ± 0.58 ab
4 ×10^6	5.00 ± 0.58 a	5.00 ± 0.58 ab
4×10^7	4.00 ± 1.15 a	4.00 ± 0.58 a
4×10^8	4.00 ± 0.58 a	4.00 ± 1.15 a
Control	10.00 ± 1.15 b	10.00 ± 0.58 c

Effect of *P. luminescens* and *X. nematophila* on number of holes: Both the bacteria had significant effects on the

number of holes made by the insect pest on wheat grains. All the bacterial applications statistically showed better results than the control. With the increasing concentration of entomopathogenic bacteria, the number of holes per grain decreased. The least number of holes per grain was recorded in bacterial concentration 4×10^8 cells/ml. However, the concentration 4×10^6 and 4×10^7 cell/ml were statistically similar to one another. On the other hand, the concentration 4×10^4 cells/ml was the least effective (Table 2).

Table 2: Number of holes (Mean ± SEM) per grain made by *Rhyzopertha dominica* treated with different concentrations of *P. luminescens* and *X. nematophila.*

Concentration	No. of holes by *R. dominica* per grain (Mean ± SEM)	
	P. luminescens	*X. nematophila*
4×10^4	5.00 ± 0.58 b	5.00 ± 1.15 ab
4×10^5	4.00 ± 0.58 ab	4.00 ± 1.15 a
4×10^6	3.00 ± 0.58 ab	3.00 ± 1.15 a
4×10^7	3.00 ± 1.15 ab	3.00 ± 0.58 a
4×10^8	2.00 ± 0.58 a	2.00 ± 0.58 a
Control	8.00 ± 1.15 c	7.00 ± 0.58 b

Effect of *P. luminescens* and *X. nematophila* on number of adults: All the bacterial concentrations of *P. luminescens* and *X. nematophila* were statistically better in the F_1 adults' emergence when compared to the control. The bacterial concentration 4×10^8 cells/ml gave the minimum number of the F_1 adults while the concentrations of 4×10^4 cells/ml showed maximum number of adults. The number of the emerged adults was inversely proportional to the applied concentration. The concentration 4×10^5, 4×10^6 and 4×10^7 cells/ml were showed statistically different results with one another (Table 3).

Table 3: Number of F_1 adults of *Rhyzopertha dominica* emerged (Mean ± SEM) treated with different concentrations of *P. luminescens* and *X. nematophila.*

Concentration	No. of emerged F_1 adults of *R. dominica* (Mean ± SEM)	
	P. luminescens	*X. nematophila*
4×10^4	18.00 ± 0.68 d	16.00 ± 0.58 d
4×10^5	15.00 ± 0.58 c	14.00 ± 0.58 cd
4×10^6	13.00 ± 1.15 bc	12.00 ± 0.58 bc
4×10^7	11.00 ± 1.15 ab	10.00 ± 0.58 ab
4×10^8	9.00 ± 1.15 a	9.00 ± 0.58 a
Control	30.00 ± 0.58 e	32.00 ± 1.15 e

Effect of *P. luminescens* and *X. nematophila* on percent inhibition rate of *Rhyzopertha dominica* in wheat grains: All the concentrations of the bacteria gave statistically significant results regarding the inhibition of *R. dominica* F_1 emergence of adults The bacterial concentration 4×10^8 cells/ml showed the highest percent inhibition rate while the least percent inhibition rate was observed with 4×10^4 cells/ml. There was a direct relation between concentration and percent inhibition rate (Figure 1 and 2).

Effect of *P. luminescens* and *X. nematophila* on percent weight loss of *Rhyzopertha dominica* in wheat grains: All the concentrations were different statistically from the control. It is clear from figures 3 and 4 that the higher concentration resulted in less damage and vice versa. The concentration of 4×10^8 cells/ml was the most effective with the least percent weight loss. On the other hand, the maximum damage was done by the insect in the grains treated with the concentration 4×10^4 cells/ml. Similarly, the concentrations of 4×10^5, 4×10^6 and 4×10^7 cells/ml resulted in greater weight loss to stored wheat grains (Figure 3 and 4).

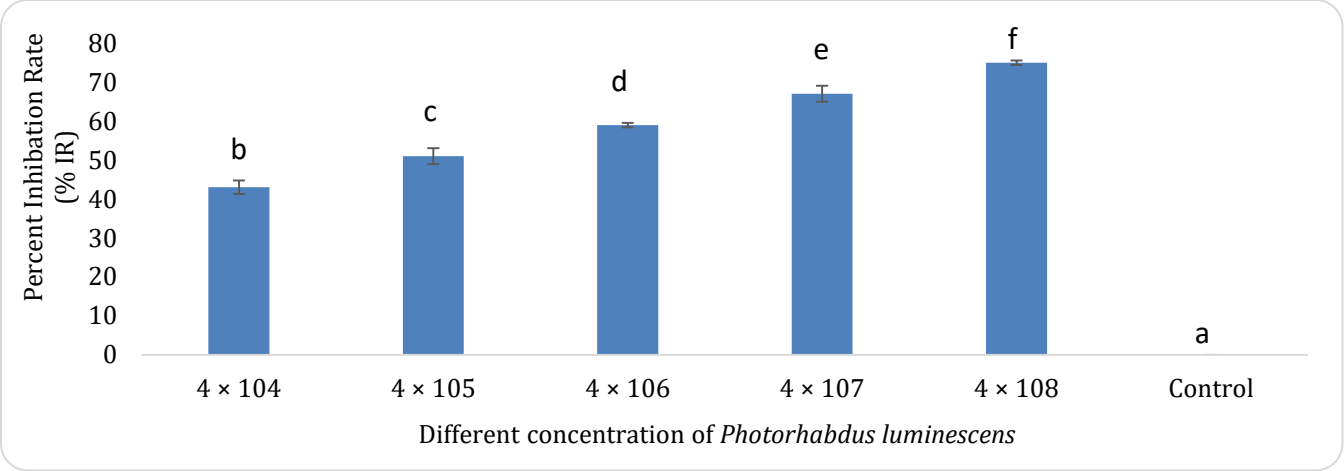

Figure 1: Percent inhibition rate (Mean ± SEM) of *Rhyzopertha dominica* adults fed on the wheat grains treated with the different concentrations of *Photorhabdus luminescens.*

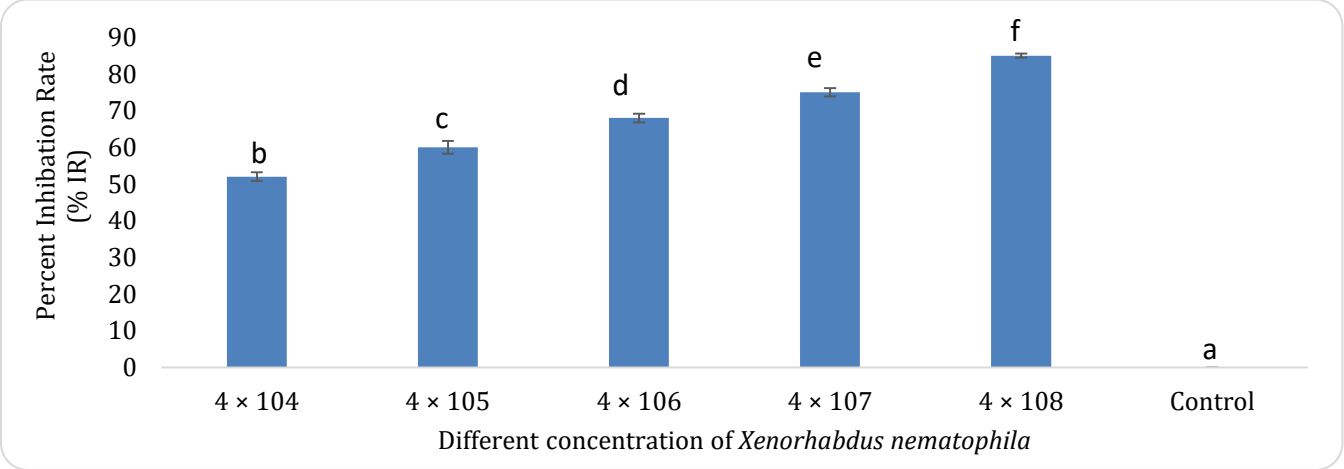

Figure 2: Percent inhibition rate (Mean ± SEM) of *Rhyzopertha dominica* adults fed on stored wheat grains treated with different concentrations of *Xenorhabdus nematophila.*

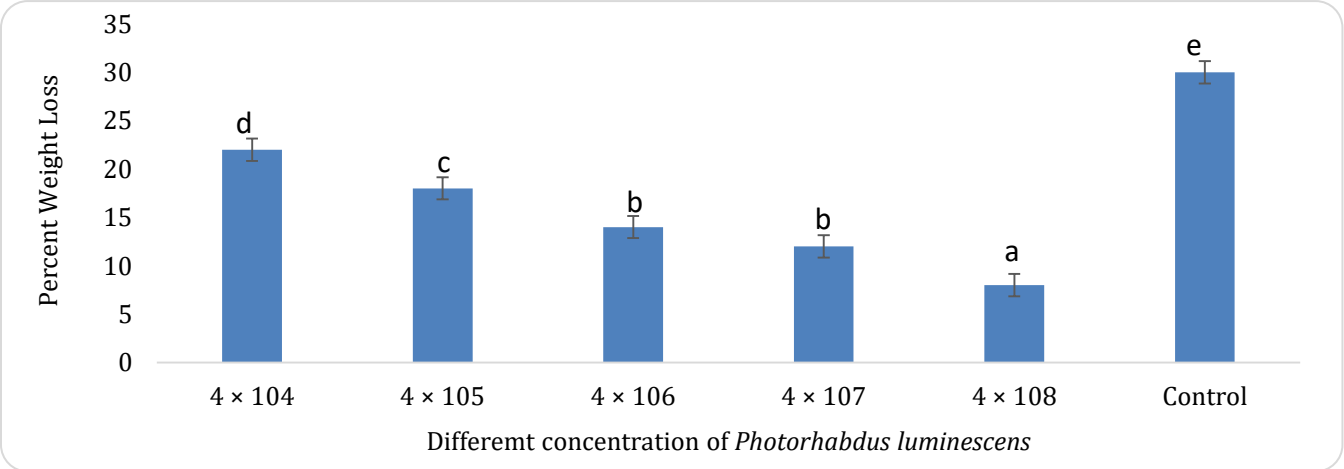

Figure 3: Percent weight loss (Mean ± SEM) of the wheat grains infested with *Rhyzopertha dominica* treated with different concentrations of *Photorhabdus luminescens.*

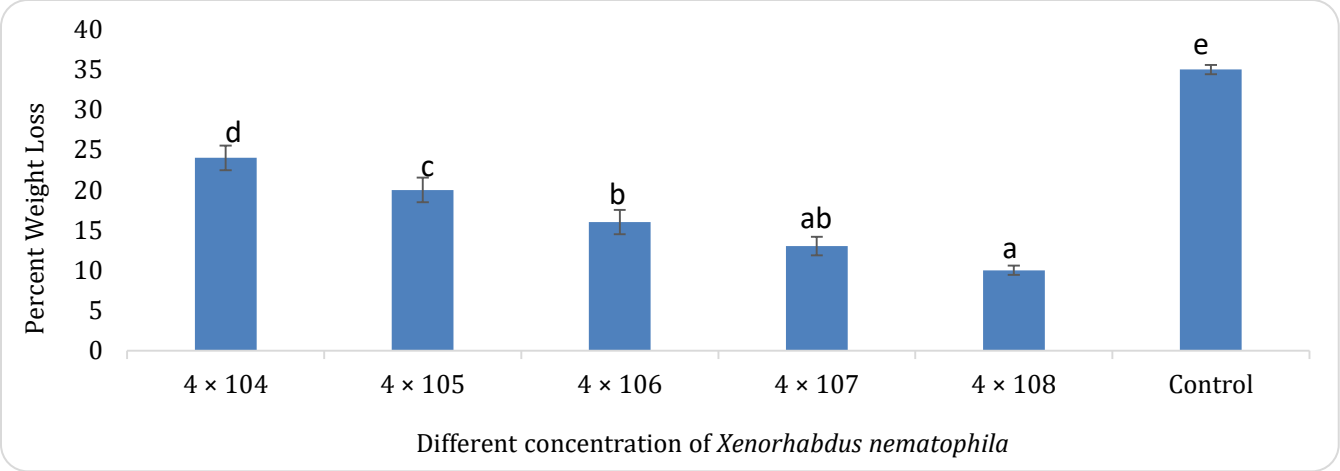

Figure 4: Percent weight loss (Mean ± SEM) of stored wheat grains infested with *Rhyzopertha dominica* treated with different concentrations of *Xenorhabdus nematophila.*

Experiment No. 2

Effect of *P. luminescens* and *X. nematophila* on mortality of *Rhyzopertha dominica*: All the concentrations of *P. luminescens* and *X. nematophila* caused significant mortality of *R. dominica*. The mortality of the insect increased with an increase in the concentration and the time interval. The highest mortality was obtained with the bacterial concentration of 4 × 10^8 cells/ml and the lowest mortality was recorded with concentration of 4 × 10^4 cells/ml (Figure 5 and 6).

The present findings are in agreement with those of Rahoo et al. (2011) who observed that the highest entomopathogenic bacterial concentrations were the most effective than the lowest concentration. Similarly, Rahoo et al. (2019a) and Rahoo et al. (2019b) used entomopathogenic bacteria *X. nematophila* against different stored grains insect pests which further confirmed our findings. Rahoo et al. (2017) proved that *X. nematophila* was toxic to different insect larvae which corroborated the present findings. (Abdel-Razek, 2003) also reported that entomopathogenic bacteria *Photorhabdus luminescens* and *Xenorhabdus nematophila* have shown virulence against the diamond back moth. Likewise, Rahoo et al. (2018a) and Rahoo et al. (2018b) found that with the increase in time duration, the mortality was also increased and these results are in line with those reported in the present study.

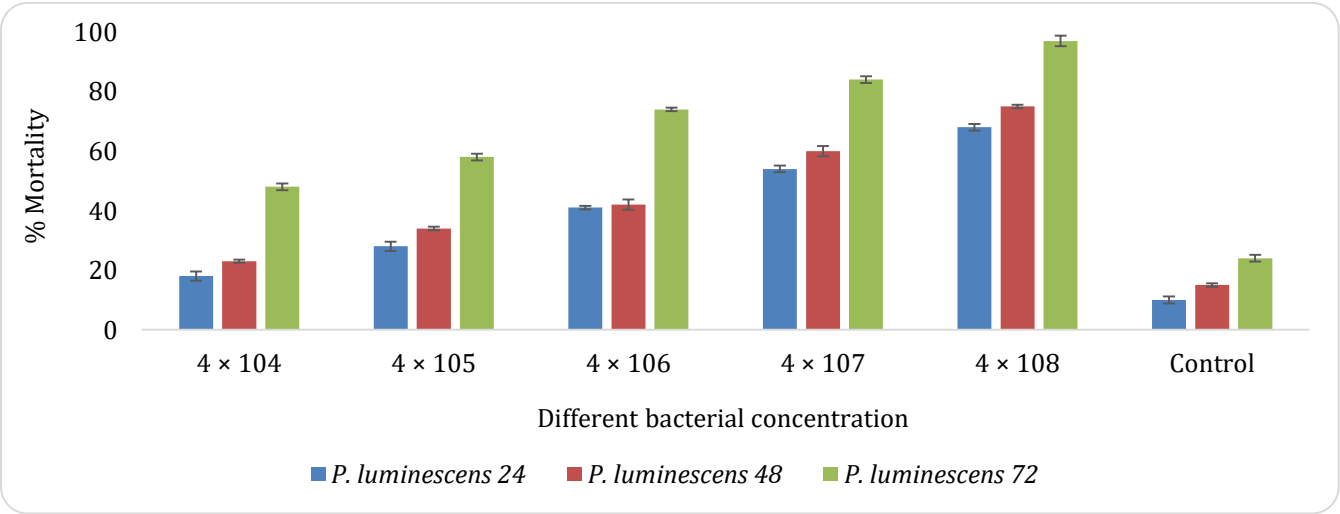

Figure 5: Percent mortality of *Rhyzopertha dominica* after different time intervals by the application of *P. luminescens.*

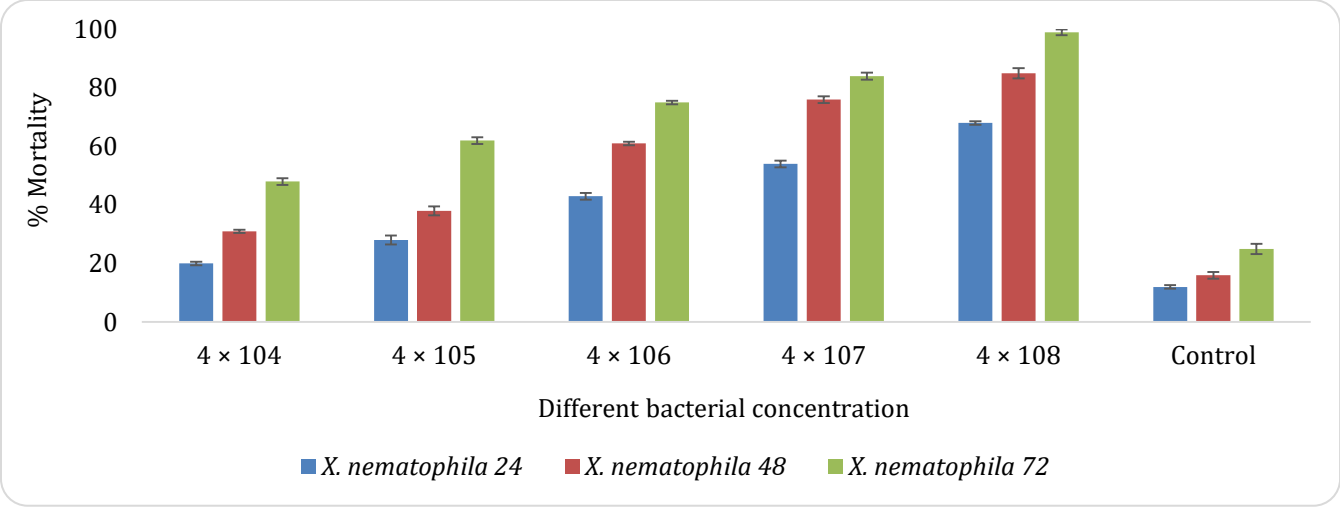

Figure 6: Percent mortality of *Rhyzopertha dominica* after different time intervals by the application of *X. nematophila.*

The present research will have significant contribution towards development of microbial formulations of *X. nematophila* and *P. luminescens* to manage *R. dominica.* in stored wheat.

Authors' contribution

MIB, FAS and MFN designed the study, MIB, MR and MR executed the experimental work, MUR and MA analyzed the data. FAS, MFN and MUR supervised the work and helped in preparation of the manuscript. All the authors edited and approved the write-up.

Conflict of interest

The authors declare no conflict of interest.

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