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**Research Article****Effectiveness of entomopathogenic bacteria, *Xenorhabdus nematophila* and *Photorhabdus temperate* against sawtoothed grain beetle, *Oryzaephilus surinamensis***Talha Habib¹, Farid Asif Shaheen¹, Usama Afzal¹, Farah Naz²¹Department of Entomology, PMAS-Arid Agriculture University Rawalpindi, Pakistan.²Department of Plant Pathology, PMAS-Arid Agriculture University Rawalpindi, Pakistan.**ABSTRACT**

Oryzaephilus surinamensis (Coleoptera: Silavinidae) is considered as one of the major insect pests under stored product conditions all over the globe. It causes severe damage such as weight loss and the stored products are reducing their quantity and quality. The effectiveness of *Xenorhabdus nematophila* and *Photorhabdus temperate* was studied against *Oryzaephilus surinamensis* in the current study. Total eggs laid by *O. surinamensis* are significantly reduced by all concentrations of *X. nematophila* and *P. temperate* and adults' number as compared to the control. The higher concentrations gave the higher reduction and vice versa. In the same way, total number of bacteria regarding inhibition of *O. surinamensis* F₁ adult's emergence gave statistically significant results. The highest bacterial concentration of 1×10^8 cells per ml showed maximum rate of inhibition and minimum rate of inhibition was noted in 1×10^4 cells per ml. There was a direct relation between inhibition rate and concentration. On the other hand, the days to 100% mortality of F₁ adults of *O. surinamensis* that were fed on dates treated with the highest concentration of *P. temperate* and *X. nematophila* were nearly equal. On another side, the highest damage was observed by the insects in the dates treated with 1×10^4 cells per ml. In the same way, concentrations of 1×10^5 , 1×10^6 and 1×10^7 cells per ml resulted in higher loss of weight to the stored dates. Similarly, all concentrations of *X. nematophila* and *P. temperate* have caused mortality of *O. surinamensis* significantly as compared to the control. There is a direct relation between insects' mortality and concentration. The maximum mortality was observed at 1×10^8 cells per ml concentration and the minimum at 1×10^4 cells per ml. The current study will have significant effect on the formulation of microbial development of *P. temperate* and *X. nematophila* to control *O. surinamensis* in stored dates.

Keywords: *Phonix dactylifera*; *Oryzaephilus surinamensis*; *Xenorhabdus nematophila*; entomopathogenic bacteria; *Photorhabdus temperate*.

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INTRODUCTION

The date palm (*Phonix dactylifera* L.) belongs to the family Arecaceae and order Arecales. In Pakistan date palm is at 3rd number in commercial fruits after Mango and Citrus that is why date palm is one of most important fruit because at world level Pakistan is 4th largest producer (Noonari et al., 2015). The annual production of dates in Pakistan from 550,000 to 650,000 metric tons (MT) (Fatima et al., 2016). Due to their high carbohydrate content (70–80%), dates are rich in protein and a good source of quick energy (Ghnimi et al., 2017). The Sawtoothed Grain Beetle (STGB) (*Oryzaephilus surinamensis*) (Coleoptera: Silavinidae) is regarded as a significant pest of stored products worldwide. STGB results in serious harm, including weight loss and a reduction in the amount and quality of post-harvest stored dates (Ahmed et al., 2012). These insects have strong chewing type mouth parts, they are attracted to the stored products due to their aroma and odor (Moawad and Al-Ghamdi, 2013).

STGB is not only a pest of dates but also damages other stored products like dried meats, rice, tobacco, barely, pasta, milo, sugar, seed, nutmeat and candies (Campbell et al., 2004; Sissons, 2016). Due to loss in stored grain, Because of their effective ability to control pests, a lot of pesticides have been utilized as protectants worldwide due to losses in stored grain. However, the negative consequences could also include the toxicity of grains, which could have fatal consequences for organisms that are not intended targets as well as sanitary and phytosanitary problems (Phillips and Throne, 2010). Due to a lack of knowledge about the costs of pesticides, their harmful effects on human health, and the emergence of genetic resistance to these chemicals, the adoption of less expensive control measures has become necessary for the protection of the environment and human health (Thind et al., 2016). Over the past few decades, biological control has drawn a lot of interest as an integrated pest management (IPM) component or as an alternative to chemical pesticides (Subramanyam and Hagstrum, 2012). They serve as efficient substitutes for chemicals while posing no negative environmental consequences. Gram-negative, motile, rod-shaped entomopathogenic bacteria, *P. temperate* and *X. nematophila*, are members of the Enterobacteriaceae family (Akhurst and Boemare, 1988; Mourant et al., 1996). Because of this symbiotic relationship, bacteria in nematode guts seek refuge from both antagonists like telluric bacterial consortia and bacteria found inside insect guts, as well as from soil stressors. For this reason, it is impossible to isolate soil samples from nematode hosts alone. This harmful symbiosis can parasitize and kill host larvae belonging to the orders Orthoptera, Diptera, Hymenoptera, Lepidoptera, Coleoptera, and Isoptera. *P. temperate* and *X. nematophila* and other members of Enterobacteriaceae produce toxins that are similar to Bt toxins (Boemare, 2002; Belien, 2018). The indiscriminate use of chemicals in storages has led to issues like resistance and toxic residues in food products moreover, the environmental and residue problems are the important risks to human health. Therefore, the present study was executed to evaluate different concentrations of these entomopathogenic bacteria against lesser grain borer attacking stored wheat grains.

MATERIALS AND METHODS

The culture of *Oryzaephilus surinamensis* was raised on dates, and its robust culture was preserved in plastic jars sealed with an elastic rubber band and covered in muslin fabric. These jars were put in the SANYO (MIR-553 BOD) incubator in the Department of Entomology's Stored Product Entomology Laboratory at PMAS-Arid Agriculture University Rawalpindi. The incubator was adjusted to $27 \pm 0^{\circ}\text{C}$ and $70 \pm$ percent relative humidity.

Bacterial Cultural Maintenance

Entomopathogenic bacteria's cultures were attained from Agriculture Collection Centre of Korea. While maintaining the temperature 25°C for 2 to 4 days, bacterial culture was streaked on agar plates for more purified samples. For the purification of individual colony, maintained culture was streaked on nutrient agar (NA) media and for the multiplication of obtained refined cultures, replication was done in nutrient broth media for 24 hours at 200 revolutions per minute (rpm). By using the serial dilution plate count method, it was possible to determine the optical thickness and cells/ml needed to calculate and modify the colony shaping units per unit volume dilution curvature. Additionally, unique bacterial concentrations were generated for use against STGB (Reynolds, 2005).

Insect Bioassays

In each jar, 50g of dates were placed. The jars were secured with muslin cloth, fixed with elastic band and then set in hatchery at temperature and humidity mentioned earlier. STGB ten pairs were shifted into each jar and various concentrations of these bacteria were prepared for the bioassay. The effectiveness of entomopathogenic bacteria against STGB was studied according to the following parameters.

Eggs Number

The average STGB egg count was calculated to assess the impact of various treatments on egg laying capability. Verify how many eggs there are for every date in each jar; the average was determined.

Young Ones of STGB

To measure the suppression of *O. surinamensis* emergence, young adults (F_1) were estimated in individual jars using varying concentrations of entomopathogenic bacteria.

Inhibiting Effects of Entomopathogens

Inhibiting effect of entomopathogens on emergence of *O. surinamensis* F_1 was measured by following formula (Iqbal et al. 2018)

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

Where C_n = number of newly emerged adults in untreated jar n (control) and T_n = Number of newly emerged adults in treated jar.

Number Of Days To 100% Mortality of F₁ Adults

The number of days to 100% mortality of *O. surinamensis* F₁ was calculated through this application against new appeared progeny.

Weight Loss (%)

After the experiment, this equation was used to calculate the percentage of dates that had lost weight.

$$\text{Weight loss (\%)} = \frac{(\text{Initial weight} - \text{Final weight}) \times 100}{\text{Initial weight}}$$

Statistical Analysis

The recorded data was analyzed for results using an appropriate statistical package like SPSS 22.0 software. Moreover, the graphical representation was done in the Microsoft Excel program.

Modeling Aptness Studies

Regression model was used to study the impact relationship of treatment on insect parameter. The equation of model was

$$y = a + bx$$

Where y is the insects' parameter and x represent treatments of bacterial concentrations

Firstly, simple linear regression was used to check the distinct effect of each treatment on insect parameters. Afterwards the impact of all treatments on insect parameters was modeled by using multiple linear regression approach and supported with forward stepwise regression. The model performance was evaluated by R².

RESULTS AND DISCUSSION

Eggs Number Laid by STGB in Stored Dates Treated with Different Concentrations of *Photorhabdus temperate* And *Xenorhabdus nematophila*

All the concentration of *P. temperate* significantly reduced number of eggs laid by STGB. The highest number of eggs per date (10) was counted in control. In contrast, 4.33 eggs, the fewest number, were counted in 1×10⁸, the highest concentration. Maximum reductions were seen at the highest concentration, while the lowest concentration showed the least amount of decreases (Table 1). The highest number of eggs (7) per date was counted when the dates were treated with *X. nematophila* bacterial concentration of 1×10⁴cell/ml. while the lowest number of eggs per date (4) was count when the bacterial concentration was 1×10⁸ cells/ml. All the concentration gives better results as compared to control. Along with the increase in the bacterial concentration the number of eggs laid per date was reduced (Table 1). To check the results of different entomopathogenic bacterial concentrations of *P. temperate* on the number of eggs laid, linear regression model was used. The equation of model was (Y= -1.1143x + 10.844) show that bacterial treatments have negative effect on the fecundity. The value of intercept (a) remained the 10.844 while the value of slope (b) was -1.1143. The determination of coefficient (R²) was 0.98 which exposed that bacterial treatment in which independent variable has 98% effect on dependent variable. Additionally, (R²) forecast how treatments with entomopathogenic bacteria will affect the STGB oviposition rate (Figure 1). To check the pathogenicity effect of different entomopathogenic bacterial concentrations of *X. nematophila*, the equation of model was (Y= -1.0286x + 9.933) that indicated bacterial concentrations had negative effect on STGB oviposition rate. The slope (b) was -1.0286, but the intercept (a) stayed at 9.933. The calculated coefficient of determination (R²) was 0.92, indicating that the concentration of bacteria in which the independent variable has a 92% effect on the dependent variable. Additionally, (R²) forecast how treatment with entomopathogenic bacteria will affect STGB fecundity rate (Figure 2).

Number Of Adult F₁ STGB Emerged in Stored Dates Treated with Various Concentrations of *Photorhabdus temperate* And *Xenorhabdus nematophila*

As compared to other treatments, in highest concentration (1×10⁸) of *P. temperate*, F₁ progeny count of *O. surinamensis* (9) was lowest but in control F₁ progeny (30) count was highest. In the concentrations 1×10⁵ and 1×10⁶ of *P. temperate*, F₁ progeny of *O. surinamensis* (13) and (12.66) produced number was same as well as in lowest concentration 1×10⁴ a greater number of F₁ progeny of *O. surinamensis* (15.33) was produced as compared with all other treatments (Table 2). In highest concentration 1×10⁸ of *X. nematophila*, F₁ progeny of *O. surinamensis* (12) count was lowest which was notably contrasting as compared to other treatments. There was a remarkable difference in progeny of F₁ count (16.33) and (15) in concentration of 1×10⁶ and 1×10⁷. Only in control treatment greater number of F₁ progeny (30.33) was observed. Using various entomopathogenic bacterial concentrations, the number of F₁ emerged adults of STGB was counted, and the results were displayed using a linear regression model (Figure 3). According to the model's equation (Y= -3.3524x + 26.956), the number of newly emerging bacteria treated with

entomopathogenic bacteria showed negative results. The slope (b) was -3.3524, while the intercept (a) was still 26.956. Hence the concentration of *P. temperate* increased then the number of F₁ emerged adults decreased @ -3.3524. The determination of coefficient R² was 0.69 which indicated that different bacterial treatments in which independent variable have 69% influences on dependent variable. The linear regression model (R²) further confirms the accuracy to expect results of entomopathogenic bacterial treatments on the number F₁ emerged adults of STGB.

Similarly, STGB adults were tested by using different entomopathogenic bacterial concentration then result revealed that number of adult F₁ emerged by applying linear regression model. The equation of modeled was (Y= -3.1524x + 29.756). The slope (b) value was -3.1524, and the intercept (a) value stayed at 29.756. On the other hand, the number of F₁ freshly emerging adults of STGB dropped at -3.1524 when the concentration of *X. nematophila* rose. A distinct bacterial concentration in which the independent variable has an 85% impact on the dependent variable was indicated by the determination coefficient of (R²), which was 0.85. The accuracy of the linear regression model R² in predicting the impact of entomopathogenic bacterial concentration on the quantity of F₁ newly emerged adults of STGB is further confirmed (Figure 4).

Percent Inhibition Rate of STGB in Stored Dates with Different Concentrations of *Photorhabdus temperate* and *Xenorhabdus nematophila*

All the concentrations of *P. temperate* gave statistically significant results regarding the inhibition of STGB F₁ emergence of adults. The inhibition rate (70%) of STGB was high when the when the bacterial concentration was 1×10⁸cells/ml and the percent inhibition rate was recorded minimum in control. Likewise, the bacterial concentration of 1×10⁸ cells/ml *X. nematophila* showed the highest percent inhibition rate (60%). While the minimum percentage inhibition rate (40%) was observed with 1×10⁴cells/ml. There was a direct relation between concentration and percent inhibition rate (Figure 5). The impact of various *P. temperate* bacterial concentrations on the percent inhibition rate was determined using a linear regression model (Figure 6). The modeled equation (Y= 11.366x + 9.91124) illustrates the observed positive effects of varied bacterial treatments on the inhibition of newly emerging adults. While the value of the slope (b) was 11.366, the value of the intercept (a) stayed at 9.1124. In order for the inhibition rate to rise up with the bacterial concentration, it did so at 11.366. The distinct bacterial concentrations in which the independent variable had a 71% effect on the defendant variable's percent inhibition rate were revealed by the coefficient of determination (R²), which was 0.71. The accuracy of the model to depict the impact of bacterial therapy on the percent inhibition rate of newly young adults of STGB is further confirmed by the linear regression model R².

The linear regression model was then used to investigate the impact of various entomopathogenic bacterial treatments of *X. nematophila* on the percent inhibition rate. As indicated by the modeled equation (Y= 10.348x + 1.5667), the hopeful effect of varying bacterial concentration on the inhibition of newly emerging adults was seen. The slope (b) value was 10.348 while the intercept (a) value stayed at 1.5667. Therefore, the different bacterial concentration increased inhibition rate also increased @ 10.348. The coefficient of determination R² was 0.85 which showed that bacterial concentration in which independent variable has 85% effect on dependent variable. The linear regression model R² further confirms accuracy of applied model to show the effect of different bacterial treatment on percent inhibition rate of newly adults emerged of STGB (Figure 7).

Days Until All F₁ Adults of STGB Die in Stored Dates Treated with Various Entomopathogenic Bacterial Concentrations

In control 100% mortality rate was recorded for maximum 22 days, which was different from other treatments but after 10 and 12 days of *P. temperate* at concentration of 1×10⁵ and 1×10⁶ no remarkable difference was noted. The 100% mortality in STGB was observed at highest concentration of *P. temperate* 1×10⁸ within minimum 5.3 days and 100% mortality was observed within 15.3 days at lowest concentration 1×10⁴ of *P. temperate*. All results were noticeably different with all other treatments except for control. At highest concentration 1×10⁸ of *X. nematophila*, a minimum of 5 days were required to record 100% mortality of STGB's F₁ progeny, while a maximum of 22 days were required to summarize 100% STGB mortality in the control group. However, 100% mortality of F₁ of STGB was seen at concentrations of *X. nematophila* at 1×10⁵ and 1×10⁶, with a small difference in days (12.33 and 9), as illustrated in Figure (8). A linear regression model was used to assess the impact of various *P. temperate* concentrations on the mortality rate of recently emerging individuals. The regression equation (Y = -3.128x + 22.93) clearly shows that applying the bacterial concentrations had a negative effect on the percent mortality of F₁, which was decreased to -3.1238. The dilution of bacteria had a 94% effect on the death of newly emerged adults, according to the coefficient R² determination of 0.94. The R² further validates the model's accuracy in illustrating how bacterial treatment affects the number of days required to achieve 100% mortality (Figure 9). A linear regression model was employed to

determine the impact of *X. nematophila* bacterial concentration on the mortality of F1 emerging STGB. The model equation ($Y = -3.1524x + 22.644$) illustrates how the concentration of bacteria has a negative effect on the % mortality of F1 adults. While the value of the slope (b) was -3.1524, the value of the intercept (a) was 22.644. Days to 100% mortality of F1 was reduced at a rate of -3.1524 as the concentration of *X. nematophila* was raised. The calculated coefficient R^2 was 0.92, indicating that the mortality of freshly emerged individuals was 92% influenced by the bacterial concentration. Additionally, R^2 validates the accuracy of model Figure 10.

Percent Weight Loss of STGB in Stored Dates Treated with Various Entomopathogenic Bacterial Concentrations

The lowest weight loss, measured by STGB in dates, was 8% for the maximum concentration of *P. temperate* 1×10^8 ; this result was notably different from all other treatments, while the control group saw the largest weight loss, which was 35%. *P. temperate* concentration 1×10^5 showed more than 50% weight loss. *X. nematophila* at concentration of 1×10^5 showed more than 50% weight loss in contrast to other treatments while in control 39.33% maximum weight loss by STGB was recorded. The highest concentration of *X. nematophila* showed the least weight loss 9.33% by STGB and result was significantly different from other treatments as shown in Figure 11. Figure (12) demonstrated how a linear regression model was used to determine the percentage of weight loss that STGB caused in response to various entomopathogenic bacterial treatments. The model's equation ($Y = -5.0857x + 35.577$) was used to assess the detrimental effect of bacterial treatment on STGB feeding. The value of intercept (a) was 35.577 and the value of intercept (b) was -5.0857. So that the bacterial treatment of *P. temperate* increased then the weight loss caused by STGB @ of -5.857. The determination of coefficient R^2 was 0.90 which exposed that the bacterial treatment has 90% effects on the percent weight loss. However, the regression model R^2 further confirmed the correctness to expect result of entomopathogenic bacterial treatment on the number of percent weight loss of STGB. To check the effect of different entomopathogenic bacterial treatment on the percent weight loss in stored dates caused by STGB then linear regression model was applied. The linear regression equation ($Y = -5.6389x + 42.399$) revealed that different bacterial treatment had negative effect on feeding potential of STGB. The value of intercept (a) 42.399 and the value of intercept (b) were -5.6389. The bacterial treatment of *X. nematophila* was increased then weight loss caused by STGB decreased @ of -5.6389. The determination of coefficient R^2 was 0.97 which showed that different bacterial treatment of *X. nematophila* had 97% effect on the percent weight loss. R^2 confirmed the accuracy of different bacterial treatment on number of percent weight loss by STGB as shown in the figure (13).

Table 1. The mean \pm standard error of number of eggs laid by STGB in stored dates treated with varying concentrations of *Xenorhabdus nematophila* and *Photorhabdus temperate*.

Sr. No	Different Concentrations	Number of Eggs	
		<i>Photorhabdus temperate</i>	<i>Xenorhabdus nematophila</i>
1	1×10^4	8.66 ± 1.5 cd	7 ± 1.00 c
2	1×10^5	7.00 ± 1.00 bc	6.66 ± 0.57 c
3	1×10^6	6.33 ± 1.52 ab	6 ± 1.00 bc
4	1×10^7	5.33 ± 1.52 ab	4.66 ± 0.57 ab
5	1×10^8	4.33 ± 0.57 a	4 ± 1.00 a
6	Control	10 ± 1.00 d	9.66 ± 0.57 d

Means within columns and rows followed by the same letters are not significantly different ($P \leq 0.05$); DMRT, Duncan, 1951.

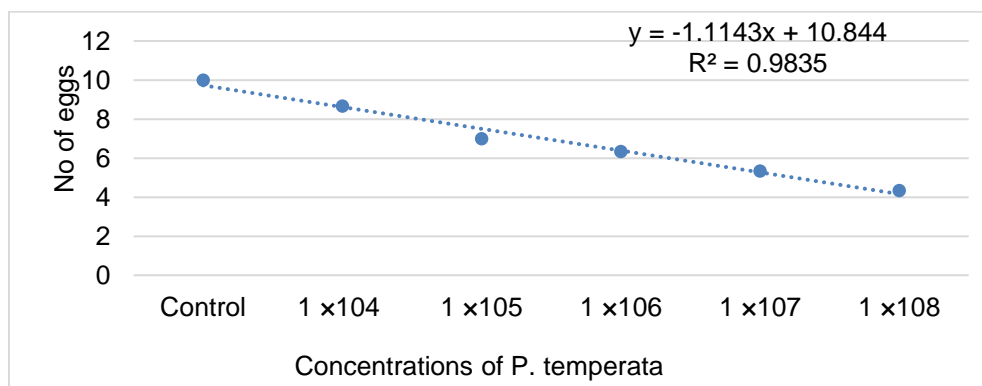


Figure 1. Aptness of modeling trend of mean number of the eggs per gain laid by STGB in response to different bacterial concentrations of *P. temperate*.

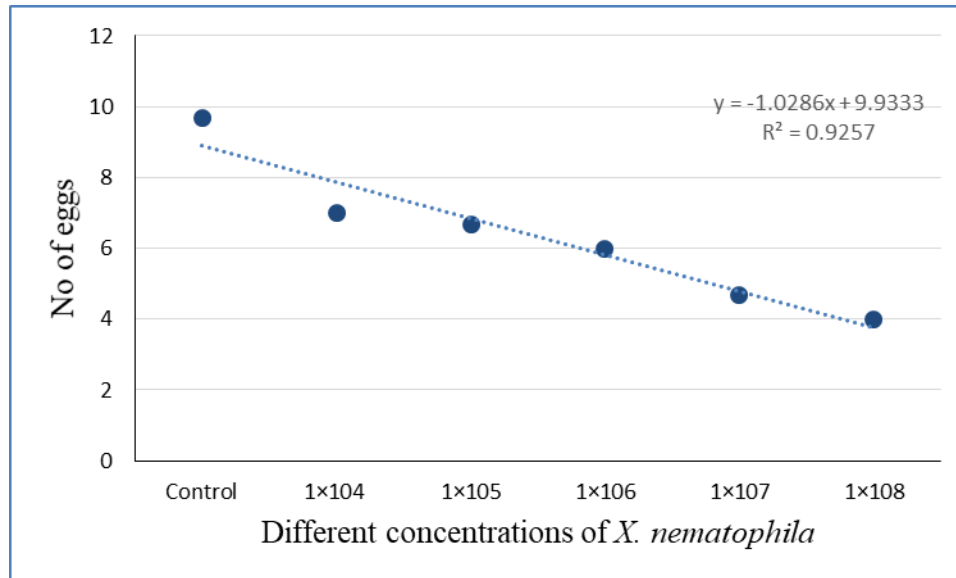


Figure 2. Aptness of modeling trend of mean number of the eggs per gain laid by STGB in response to different bacterial concentrations of *X. nematophila*.

Table 2. Number of F₁ emerged adults (Mean ± SEM) STGB in stored dates treated with different concentrations of *Photorhabdus temperate* and *Xenorhabdus nematophila*.

Sr. No.	Different concentrations	F ₁ emerged STGB of F ₁ Emerged STGB	
		<i>Photorhabdus temperate</i>	<i>Xenorhabdus nematophila</i>
1	1x10 ⁴	15.33± 1.52 c	20.66± 0.57 d
2	1x10 ⁵	13.00± 2.00 bc	18.00±1.00 c
3	1x10 ⁶	12.66± 1.52 bc	16.33± 0.577 bc
4	1x10 ⁷	11.33± 0.50 ab	15.00±1.00 bc
5	1x10 ⁸	9.00±1.00 a	12.00± 1.00a
6	Control	30.00± 2.00 d	30.33±2.00 e

Means within columns and rows followed by the same letters are not significantly different ($P \leq 0.05$); DMRT, Duncan, 1951.

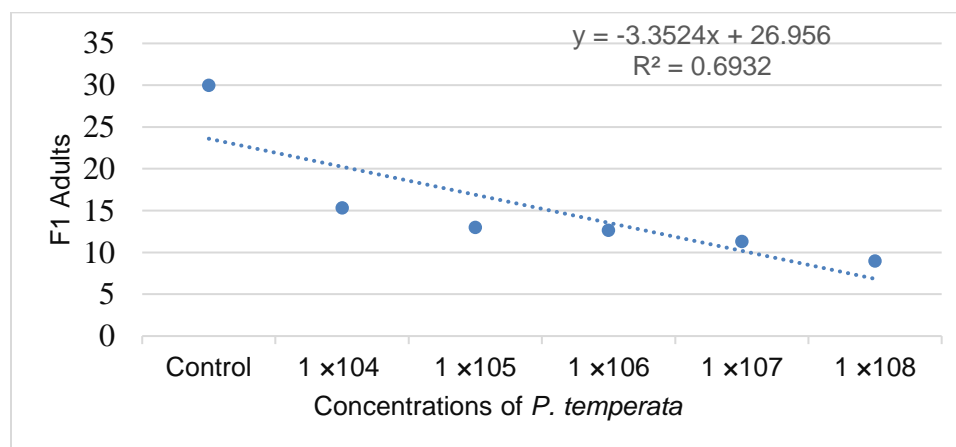


Figure 3. Aptness of modeling trend of F₁ new emerged adults of the STGB after application of different concentrations of *P. temperate*.

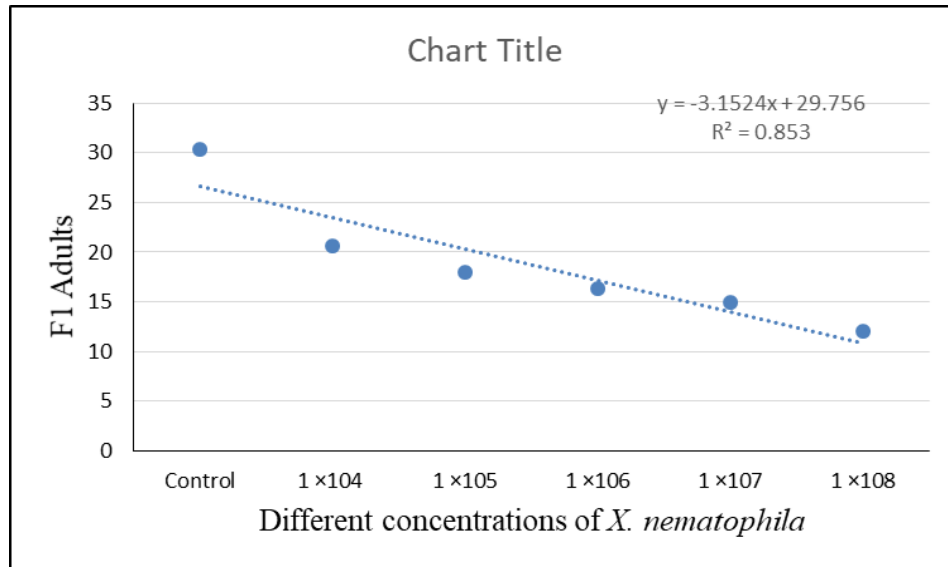


Figure 4. Suitability of the F1 newly developed adults' modeling trend of the STGB in response to various *X. nematophila* bacterial concentrations.

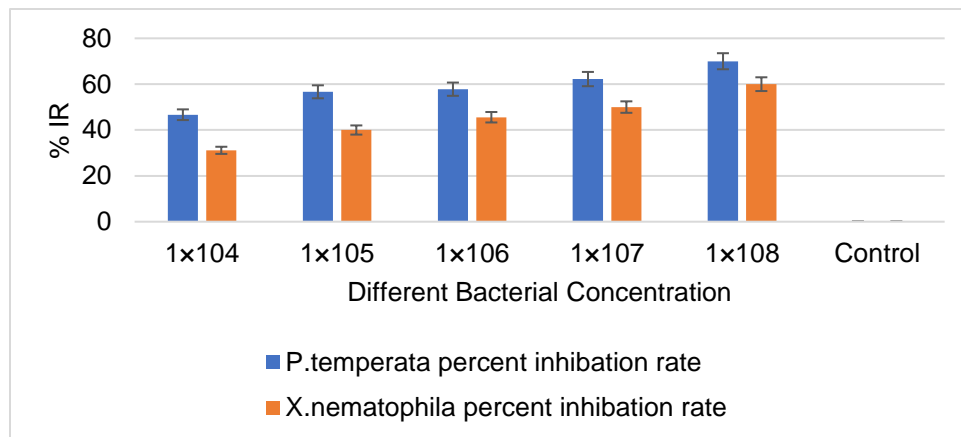


Figure 5. Percent inhibition rate (Mean ± SEM) of STGB in response to different entomopathogenic bacterial concentrations.

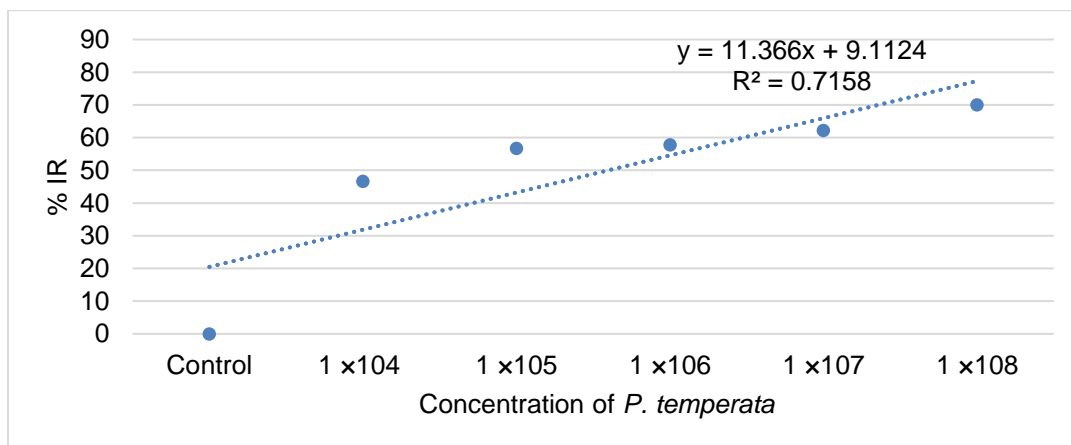


Figure 6. Suitability of modeling trend of STGB percent inhibition rate in response to various *P. temperate* bacterial concentrations.

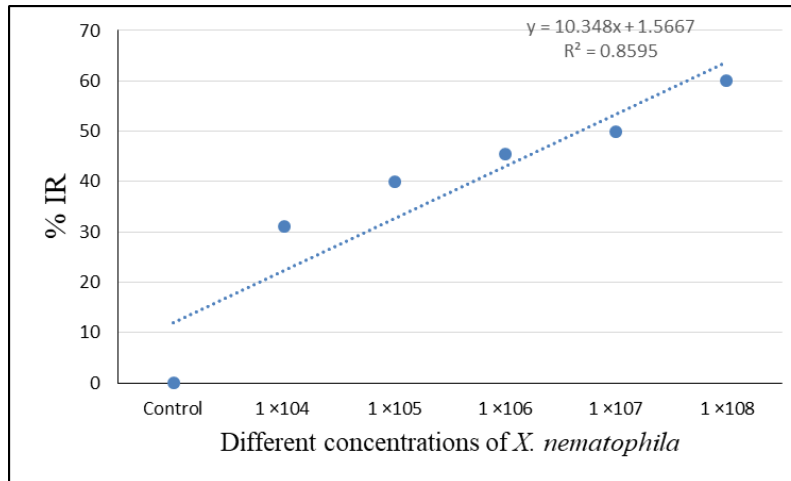


Figure 7. Aptness of modeling trend of percent inhibition rate of STGB after application of different concentrations of *X. nematophila*.

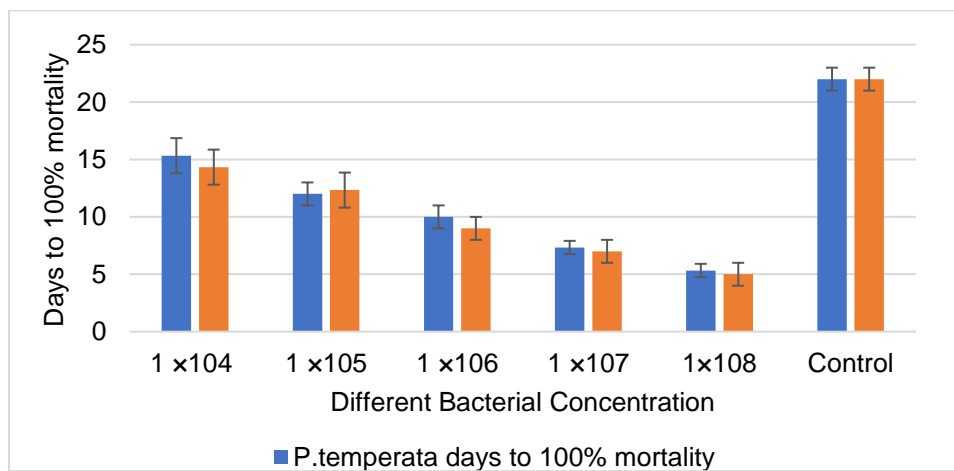


Figure 8. Days taken to 100% mortality (Mean ± SEM) due to STGB in response to various *P. temperate* and *X. nematophila* concentrations.

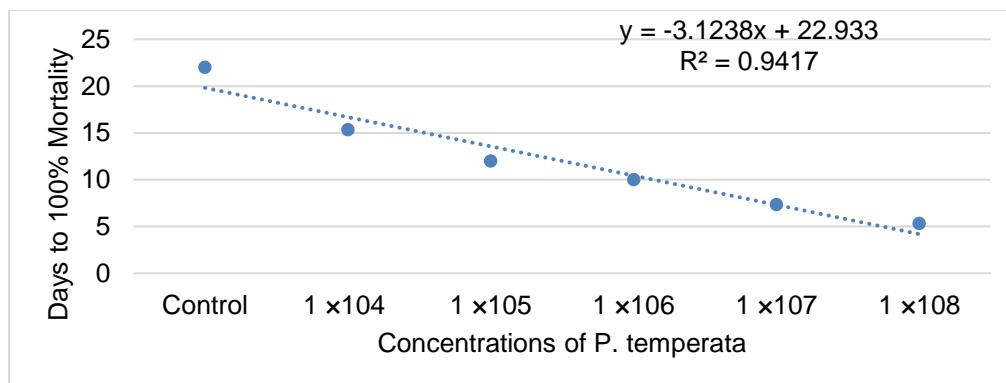


Figure 9. Aptness of modeling trend of Day to 100% mortality of F₁ of STGB after using different bacterial concentrations of *P. temperate*.

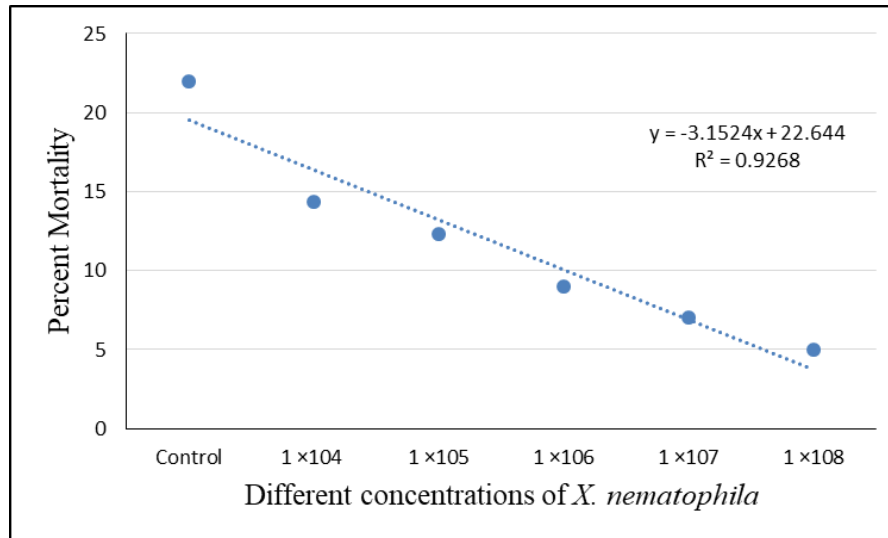


Figure 10. Aptness of modeling trend of Day to 100% mortality of F₁ of STGB in response to different bacterial concentrations of *X. nematophila*.

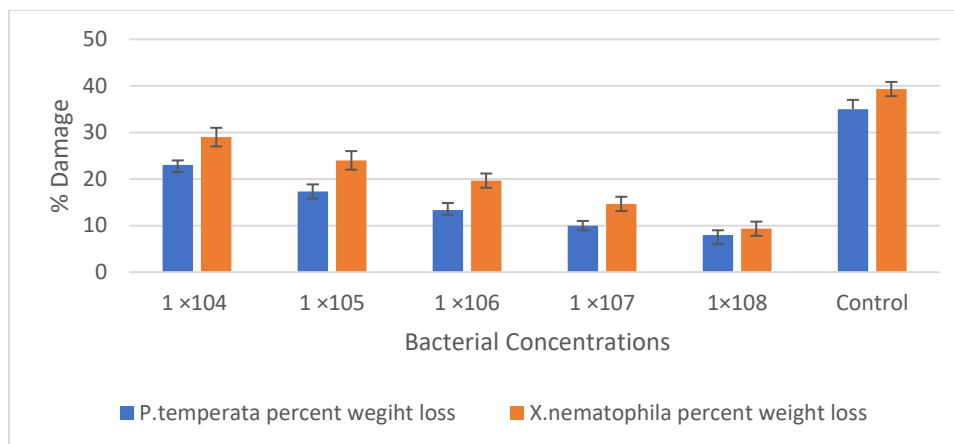


Figure 11. Weight loss % caused by STGB in response to different concentrations of *P. temperate* and *X. nematophila*.

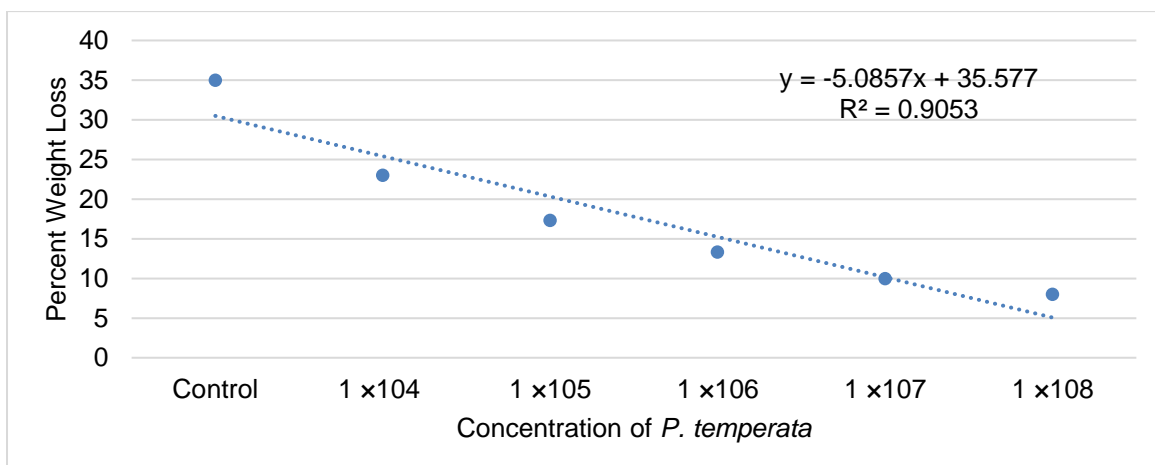


Figure 12. Aptness of modeling trend of weight loss % caused by the STGB after using different concentrations of *P. temperate*.

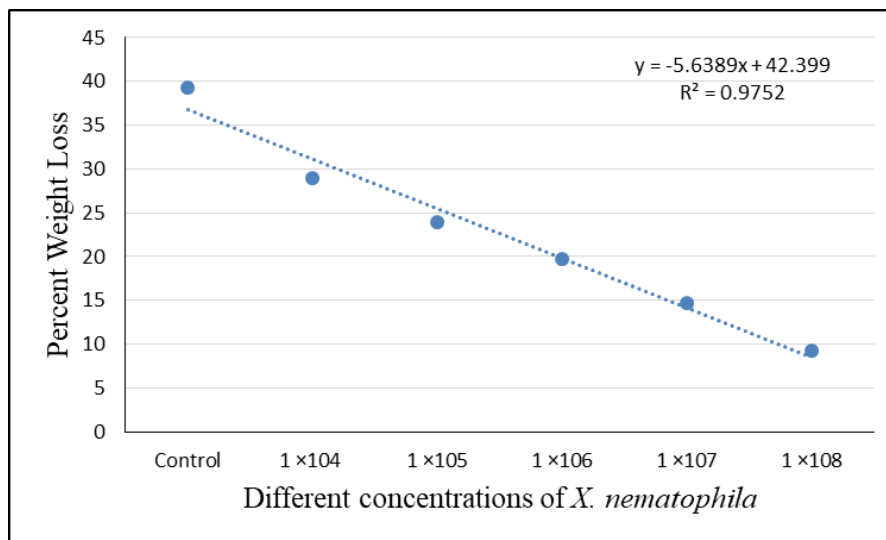


Figure 13. Adequacy of the modeling trend for percentage of weight loss resulting from STGB in response to different bacterial concentrations of *X. nematophila*.

DISCUSSION

The current findings have similar results of according to Shrestha and Kim (2010), *X. nematophila* and *P. temperate* share a symbiotic relationship with *S. carpocapsae* and *H. megidis*, respectively. Less information was known about the typical host range of nematodes, yet two bacteria that are employed to treat *T. castaneum* showed a significant variance in pathogenic viability. Compared to *X. nematophila*, *P. temperate* had six times greater pathogenicity. The infections' repressive effects on phospholipase A2 actions, which are necessary for the emergence of *T. castaneum* resistant reaction, were not the origin of the variation. Cultures were converted into natural and liquid byproducts; however, the liquid byproducts showed greater efficacy. Similar research on mosquitoes conducted by Park (2015) suggested that bacterial immunosuppressants may make mosquitoes more susceptible to *Bacillus thuringiensis*. At sixteen hours following treatment, *B. thuringiensis* had an LC₅₀ value of 2.9×10⁵ and 2.2×10⁵, respectively, making it extremely lethal to the target insect pest. The bacterium culture increased the toxicity of *B. thuringiensis* to mosquito larvae. The LC₅₀ values of *B. thuringiensis* to larvae of *Albopictus* decreased to 1.5×10⁵ when combined with *X. nematophila*, 1.7×10⁵ when combined with Xh, and 1.9×10⁵ when combined with *P. temperate*. The LC₅₀ values of *B. thuringiensis* to *Cx. pipiens* larvae were less than 1.2×10⁵ in the combination of *X. nematophila*, 1.3×10⁵ in the combination of Xh, and 1.5×10⁵ in the combination of *P. temperate*. The addition of benzylideneacetone or oxindole, which is derived from *X. nematophila* and *P. temperate*, also boosted the toxicities of *B. thuringiensis israelensis* to mosquito larvae.

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