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EFFECTIVENESS OF *PHOTORHABDUS TEMPERATA* AND *XENORHABDUS NEMATOPHILA* AGAINST *CALLOSOBRUCHUS CHINENSIS* ATTACKING STORED CHICKPEA GRAINS

Mohsin Iqbal[1*], Farid Asif Shaheen[1], Abdul Rauf Bhatti[2], Ahmed Zia[2], Imran Bodlah[1], Farah Naz[3] and Muhammad Fiaz[4]

[1]Department of Entomology, PMAS-Arid Agriculture University Rawalpindi, Pakistan, 46300.
[2]National Insect Museum NARC, Islamabad, Pakistan, 46000.
[3]Department of Plant Pathology, PMAS-Arid Agriculture University Rawalpindi, Pakistan, 46300.
[4]*Faculty of Veterinary and Animal Sciences, PMAS, Arid Agriculture University Rawalpindi, Pakistan, 46300.*

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***Corresponding Author**:
Mohsin Iqbal

E-mail: mohsin@uaar.edu.pk

ABSTRACT

Callosobruchus chinensis (L.) (Coleoptera: Bruchidae) is one of the most destructive insect pests of *Cicer arietinum* L. (Fabaceae) in storages and renders grains unfit for human consumption. In the present study, entomopathogenic bacteria (*Photorhabdus temperata* (Heterorhabdtidiae) and *Xenorhabdus nematophila* (Steinermitidae) were used as effective bio-control agents against this pest and proven good alternatives to chemicals. More number of eggs was counted in grains treated with lowest concentration (1×10^4) of *P. temperata* as compared to *X. nematophila.* Less number of holes was oberved, in grains treated with highest concentration (1×10^8) of *X. nematophila* as compared to *P. temperata.* More number of F_1 adults emerged in chickpea grains was found when treated with highest concentration of *X. nematophila* as compared to *P. temperata.* Lower inhibition rate of *C. chinensis* adults was found , fed on chickpea grains treated with lowest concentration of *P. temperata* as compared to *X. nematophila.* Where as, days to 100% mortality of F_1 of *C. chinensis* adults was almost equal, fed on chickpea grains treated with highest concentration of *P. temperata* and *X. nematophila.* Weight loss (11.1%) was higher in chickpea grains treated with lowest concentration of *P. temperata* as compared *to X. nematophila.* The damage was caused by *C. chinensis* adults was lower than caused by *P. temperata*.

Keywords: *Cicer arietinum*, *Callosobruchus chinensis*, Entomopathogenic bacteria, *Photorhabdus temperata* and *Xenorhabdus nematophila*.

INTRODUCTION

Chickpea ranks 2nd in area under cultivation and 3rd in production among pulses in the world (CGIAR, 2017). In Pakistan, annual production of 0.5m tonnes of dry seed is obtained from an area of around 1m hectares (FAO, 2016). In storages, it has severe post harvest losses due to *Callosobruchus chinensis* (Zia *et al*., 2011), which causes about 10% damage and renders grains unfit for human consumption (Aslam, 2004). For the control of stored grain insect pests, grain protectants and fumigants have been used for long earlier. However, effectiveness of these chemicals have been decreased due to the ability of insects to develop resistance (Rajendran and Sriranjini, 2008). Environmental awareness and residue problems are the important risks to human health. Botanicals have also been used as safe grain protectants against stored grain insect pests but also resulted in resistance issues. Therefore, another alternative approaches is to entomopathogens such as bacteria and fungi to manage insect pests (Bakkali, *et al*., 2008). Entomopathogenic bacteria (EPB), namely *P. temperata* and *X. nematophila* are symbiotic in nature. Significant pathogenicity has been observed between these two bacteria against stored insect pests. Main target insect pests of these bacteria are stored grain insect pests namely red flour beetle and other insects like *Spodoptera litura*, diamond back moth (Jung and Kim, 2006; Seongchae and Yonggyun, 2006).

The aim of this study was to manage the pulse beetle *Callosobruchus chinensis* in stored chickpea using entomopathogenic bacteria, *Photorhabdus temperata* and *Xenorhabdus nematophila*. A little work has been done regarding entomopathogenic bacteria in Pakistan. The main target insect pest of these bacteria are the pulse beetles. Following materials and methods were used to conduct the experiments.

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MATERIALS AND METHODS

Infested samples of stored chickpea grains were collected from different research stations including National Agriculture Research Council (NARC) Islamabad, Pakistan. *C. chinensis* culture was maintained in an incubator at temperature of 302 °C and 705 % relative humidity in the Department of Entomology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan.

For bioassays, chickpea cultivar NOOR-2009 was obtained from NARC, Islamabad. Agtoxin was applied for fumigation to kill already existing insect pests (Shaheen *et al*., 2006). Entomopathogenic bacteria *P. temperata* isolate (ANU101) and *X. nematophila* isolate (K1) were obtained from Korean Agricultural Culture Collection (KACC), NAC, RDA, Suwon, 441-707, Korea. Initially the culture on Nutrient Agar (NA) plates was streaked at 25°C 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 the optical density (OD) and cfu's. Different concentrations of bacteria viz., 1×10^4, 1×10^5, 1×10^6, 1×10^7 and 1×10^8 cells/ml were prepared.

Insect bioassays

In each jar, 50g of chickpea grains was shifted in plastic jars. The jars were enclosed with muslin fabric made tighter and then shifted to incubator at 28±2°C. Few numbers of the target insects (10) were shifted into each jar. Different concentrations viz., 1×10^4, 1×10^5, 1×10^6, 1×10^7 and 1×10^8 cells/ml of both bacteria were prepared for the experiment. Parameters of study included number of eggs, number of holes, number of F_1 progeny adults emerged, percent inhibition rate (% IR), days to 100% mortality of F_1 emerged, weight loss(%), and percent damage. The data was recorded in day time upto sixty days.

The formulae used were;

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

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

$$\text{Weight loss (\%)} = \frac{\text{(initial weight – weight of damaged grains)}}{\text{initial weight}} \times 100$$

$$\text{Percent damage} = \frac{\text{(No. of damaged grains)}}{\text{No. of total grains}} \times 100$$

The experiment was repeated thrice. The statistical software SPSS 20.0 for Windows program was used for one factor analysis. Duncan's Multiple Range Test (DMRT) was applied to all the means. Moreover the graphical work was done using Microsoft Excel programme. Regression models were used to learn the relationship of treatments impact regarding insect's parameters. Following equation was used;

$y = a + bx$

Whereas

y = Insects parameter

x = Treatments of bacterial concentrations

Initially linear regressions were used to check separate effects of every treatment regarding parameters of insects. After that, impact of treatment regarding parameters of insects was modeled with the help of multiples linear regressions. The performance models were checked by R2.

RESULTS AND DISCUSSION

Number of eggs per grain

Table 1 shows that highest number of eggs (16) was counted in control. Average nine and eight eggs were recorded in concentrations of *P. temperata* i.e. 1×10^4 and 1×10^5 which were not significantly different with each other. Lowest number of eggs (1.3) was observed in highest concentration of *P. temperata* i.e. 1×10^8 which was significantly different from all other dilutions. Average number of eggs (8.6), (8) and (7) were found in concentrations of 1×10^4, 1×10^5 and 1×10^6 of *X. nematophila* which were not so much significant with each other. Highest number of eggs (18) was produced in control. Lowest number of eggs (2) was recorded in highest concentration i.e. 1×10^8 which was significantly different with the rest of the concentrations.

The pathogenic effects of different dilutions of entomopathogenic bacteria *P. temperata* were noted using linear regression model. The model equation (Y= -2.6314x + 16.46) indicated that treatments had negative effects on the oviposition rate of pulse beetle. The intercept (a) value remained 16.46 whereas slope (b) was -2.63. Equation exposed that dilutions of *P. temperata* indicated opposite relations with eggs numbers. Co-efficient of determination (R^2) was 0.92 that depicted that treatments (Independent variable) had 92% effect on the dependent variable. The R^2 furthermore verified correctness of model to anticipate effects of bacteria treatments on oviposition rate (Figure 1).

Likewise, pathogenicity effect of diverse concentrations of entomopathogenic bacterium *X. nematophila* was observed with the help of linear regression model. The model equation (Y= -2.7086x + 17.413) depicted that treatments had negative effect on over oviposition rate of pulse beetle. The intercept (a) value remained 17.41 where as slope (b) was -2.70. Equation revealed that concentrations of *X. nematophila* showed opposite relation with the number of eggs. Coefficient of determination (R^2) was 0.83 which showed that treatments (Independent variable) had 83% effect on the dependent variable. The R^2 further confirmed the accuracy of model to predict effect of bacterial treatments on oviposition rate (Figure 2).

Number of holes per grain

Table 2 shows that maximum number of holes (7) was counted in control where no concentration of *P. temperata*

was used which was significantly different with all other concentrations. Minimum number of holes (1) was seen in highest concentration of *P. temperata* (1×10^8) where the maximum deterrence towards the formation of holes was observed. There was 3.6 and 2.6 number of holes recorded in lower dilutions of 1×10^5 and 1×10^6 which were not significantly different with each other.

Whereas concentrations of 1×10^5 and 1×10^6 of *X. nematophila* allowed *C. chinensis* to make 3.6 and 3 number of holes per grain which were not considerably variant with everyone. Lowest concentration of *Xenorhabdus nematophila* i.e. 1×10^4 was found to be less effective as compared to other treatments because more number of holes per grain (4) were produced. Maximum number of holes by *C. chinensis* (7) was produced in control.

The pathogenic effects of different dilutions of entomopathogenic bacteria *P. temperata* were noted using linear regression model. The model equation (Y= -1.2029x + 7.7267) indicated that treatments have negative effects on the oviposition rate of pulse beetle. The intercept (a) value remained 7.72 where as slope (b) was -1.20. Equation exposed that dilutions of *P. temperata* indicated opposite relations with holes numbers. Co-efficient of determination (R^2) was 0.96 that depicted that treatments (Independent variable) had 96% effect on the dependent variable. The R^2 furthermore verified correctness of model to anticipate effects of bacteria treatments on oviposition rate (Figure 3).

Likewise, pathogenicity effect of diverse concentrations of entomopathogenic bacterium *X. nematophila* was observed with the help of linear regression model. The model equation (Y= -1.0943x + 7.2067) depicted that treatments had negative effect on the oviposition rate of pulse beetle. The intercept (a) value remained 7.20 where as slope (b) was -1.09. Equation revealed that concentrations of *X. nematophila* showed opposite relation with the number of holes. Coefficient of determination (R^2) was 0.91 which showed that treatments (Independent variable) had 91% effect on the dependent variable. The R^2 further confirmed the accuracy of model to predict effect of bacterial treatments on oviposition rate (Figure 4).

Number of F_1 adults per jar

The highest number of F_1 progeny was seen (43) in control. Lower number of F_1 progeny of *C. chinensis* (10.66) was counted in highest concentration (1×10^8) of *P. temperata* which was significantly different as compared with other treatments. Almost same number of F_1 of *C. chinensis* (14.66) and (14) was produced in concentration of *P. temperata* i.e. 1×10^5 and 1×10^6 respectively. More number of F_1 of *C. chinensis* (15) was produced in lowest concentration i.e. 1×10^4 as compared with all other treatments (Table 3).

The lowest number of F_1 progeny of *C. chinensis* (11.33) was counted in highest concentration i.e. 1×10^8 of *X. nematophila* which was significantly different as compared to all other treatments. No significant difference in population of F_1 of *C. chinensis* (14) and (12.66) was seen in concentration of 1×10^6 and 1×10^7, respectively. Highest number of F_1 of *C. chinensis* (41.33) was recorded in control (Table 3).

The linear regression model was used to evaluate effectiveness of diverse dilutions (Figure 5). As obvious from modeled equation (Y= -4.8677x + 35.312), dangerous effects of dilutions of bacteria regarding F_1 appeared adults were observed. The intercept (a) remained 35.31 where as slope (b) was -4.86. So, as dilutions of bacteria (*P. temperata*) enhanced, number of new appeared pulse beetle lowered @ -4.86. Coefficient of determination (R^2) was 0.55 that indicated that treatments had 55% impact on the dependent variable. The R^2 furthermore verified accuracy of model.

Similarly linear regression model was applied to check success of diverse concentrations (Figure 6). As evident from modeled equation (Y= -4.61x + 34.577) indicated adverse effects of bacterial concentrations on the F_1 emerged adults was noted. The intercept (a) remained 34.57 where as slope (b) was -4.61. So, as the bacterial concentration (*X. nematophila*) increased, number of newly emerged pulse beetle decreased at the rate of -4.61. Coefficient of determination (R^2) was 0.57 which showed that the treatment had 57% impact on the dependent variable. The R^2 further confirmed the correctness of model.

Percent inhibition rate of F_1 adults

Percent inhibition rate in different treatments of *P. temperata* of (1×10^5) and (1×10^6) was observed as (62.69%) and (65.72%) respectively which was significantly different with each other as well as with all other treatments. Maximum percent inhibition rate of *C. chinensis* (72.41%) was noted in highest concentration of *P. temperata* (1×10^8) which was also significantly different with all other treatments. Minimum percent inhibition rate was recorded in control. The highest percent inhibition rate (75.28%) was enumerated in highest concentration of *X. nematophila*. No significant difference in percent inhibition rate (65.35%) and (65.82%) was recorded in lowest concentration of 1×10^4 and 1×10^5. Further, lowest percent damage was exhibited in control (Figure 7).

The linear regression model was used to evaluate effectiveness of diverse dilutions (Figure 8). The efficient effects of diverse dilutions of bacteria regarding inhibition of new appeared were noted as obvious from modeled equation (Y= 11.182x + 16.001). The intercept (a) value remained 16.00 where as slope (b) was 11.18. Consequently, as dilutions of bacteria enhanced, inhibition rate was also enhanced @ 11.18. Coefficient of determination (R^2) was 0.58 that discovered that dilutions (independent variable) had 58% effects on the dependent variable (inhibition rates). The R^2 furthermore verified the correctness of model to anticipate effects of bacterial treatments on inhibition of new appeared insects.

Likewise, linear regression model was applied to check efficacy of different concentrations. Encouraging effect of different bacterial concentrations on the inhibition of newly emerged was detected as evident from the modeled equation (Y= 11.319x + 17.931). The intercept (a) value remained 17.93 where as slope (b) was 11.31. Accordingly, as the bacteria concentration increased, inhibition rate was also increased at the rate of 11.31. Coefficient of determination (R^2) was 0.55 which exposed that the concentrations (independent variable) had 55% effect on the dependent variable (inhibition rate). The R^2 further confirmed the accuracy of model to predict effect of bacterium treatments on the inhibition of newly emerged *C. chinensis* (Figure 9).

Days to 100 % mortality of *C. chinensis*

Maximum number of days (17.6) to 100% mortality was

counted in control which was significantly different with all other treatments. No significant difference of days 10.66 and 10.3 was observed in *P. temperata* concentration of 1×10^5 and 1×10^6, respectively. Minimum number of days 5.3 to 100% mortality in *C. chinensis* was measured in highest concentration of *P. temperata* (1×10^8). More number of days (13.3) to 100% mortality was noticed in lowest concentration of *P. temperata* (1×10^4), which was significantly different with all other treatments too except control.

The minimum number of days (5.6) to 100% mortality of F_1 of *C. chinensis* was recorded in highest concentration of *X. nematophila* (1×10^8) which was significantly different with rest of all treatments. Maximum number of days (18.33) to 100% mortality of *C. chinensis* was enumerated in control. A slight difference was of days (9.6 and 0.88) to 100% mortality of F_1 of *C. chinensis* in *X. nematophila* concentration of 1×10^5 and 1×10^6 respectively (Figure 10).

To evaluate efficiency of dilutions of bacteria (*P. temperata*) regarding deadF_1 emerged pulse beetle, linear regression model was used. The model equation (Y= -2.3057x + 18.753) indicated negative impacts of bacteria dilutions regarding percent mortality of F_1 adults. According to Figure 11, the intercept (a) value remained 18.75 but slope (b) was -2.30. Consequently, as quantity of *P. temperata* was enhanced, days to 100% mortality of F_1 was lowered @ -2.30. Coefficient of determination (R^2) was 0.95 that exposed that dilutions of bacteria had 95% effects regarding deaths of new appeared adults meanwhile R^2 furthermore verified correctness of model(Figure11).

Likewise, to determine the effectiveness of bacteria concentrations on mortality of F_1 emerged pulse beetle, linear regression model was implemented. The model equation (Y= -2.2329x + 18.753) showed negative impact of bacterium concentrations on the percent mortality of F_1 adults. According to figure 12, the intercept (a) value remained 18.75 but slope (b) was -2.23. Accordingly, as the concentration of *X. nematophila* was increased, days to 100% mortality of F_1 was reduced at the rate of -2.23. Coefficient of determination (R^2) was 0.86 which revealed that bacterial concentrations had 86% effect on the mortality of newly emerged adults meanwhile R^2 further confirmed the accuracy of model (Figure 12).

Percent weight loss in chickpea grains

Lowest percent weight loss (11.1%) by *C. chinensis* in chickpeas was observed in highest concentration of *P. temperata* (1×10^8) which was significantly different from all other treatments. The highest percent weight loss (83.43%) was calculated in control. More than fifty percent weight loss was observed in *P. temperata* concentration of 1×10^5.

More than fifty percent weight loss was observed in *X. nematophila* concentration of 1×10^4 as compared to all other treatments. The maximum percent weight loss (84.10%) by *C. chinensis* was measured in control. The minimum percent weight loss (5.7%) by *C. chinensis* was recorded in highest concentration of *P. temperata* which was significantly different from all other treatments (Figure 13).

To check efficiency of bacteria dilutions (*P. temperata*) regarding weight loss in treated chickpea, linear regression models were used. The modeled equation (Y= -12.207x + 97.79) indicated that whole bacteria dilutions had negative impacts regarding feeding worth of pulse beetle. The intercept (a) and slope (b) values retained at 97.79 and -12.20, separately. Dilutions of *P. temperata* had 85% effect on the feeding worth of pulse beetle as exposed by coefficient of determination (R^2) that was 0.85. The R^2 furthermore verified correctness of models to anticipate effects of bacterial dilutions on weight loss (Figure 14).

To determine the effectiveness of bacterial concentrations on the percent weight loss in treated chickpea, linear regression model was implemented. The modeled equation (Y= -11.97x + 93.174) showed that all the bacterial concentrations had negative impact on the feeding potential of pulse beetle. The intercept (a) and slope (b) value remained 93.17 and -11.97, separately. Concentrations of *X. nematophila* have 77% effect on the feeding potential of pulse beetle as showed by the coefficient of determination (R^2) that was 0.77. The R^2 further confirmed the precision of model to predict the effect of bacterial concentrations on weight loss (Figure 15).

Percent damage of chickpea grains

The minimum percent damage by *C. chinensis* was counted in highest concentration of *P. temperata* as compared to all other treatments which was also significantly different. More than fifty percent damage was exhibited in concentration of 1×10^6 as compared to concentration of 1×10^7 and 1×10^8. The maximum damage (96.1%) was recorded in control.

More than fifty percent damage was observed in concentration of *X. nematophila* (1×10^5) which was significantly different with rest of the treatments. The minimum percent damage (20.16%) was recorded in highest concentration of *X. nematophila* (1×10^8). Maximum damage (91.58%) was observed similarly in control (Figure 16). To check efficiency of bacterial dilutions (*P. temperata*) regarding % damage in treated chickpea, linear regression models were used. The modeled equation (Y= -11.14x + 102.2) indicated that whole bacterial dilutions had negative impacts regarding feeding worth of pulse beetle. The intercept (a) and slope (b) values retained at 102.2 and -11.14, separately. Dilutions of *P. temperata* had 87% effect on the feeding worth of pulse beetle as exposed by coefficient of determination (R^2) that was 0.87. The R^2 furthermore verified correctness of models to anticipate effects of bacterial dilutions on % damage (Figure 17).

To determine the effectiveness of bacterial concentrations on the percent damage in treated chickpea, linear regression model was implemented. The modeled equation (Y= -11.963x + 101.76) showed that all the bacterial concentrations had negative impact on the feeding potential of pulse beetle. The intercept (a) and slope (b) value remained 101.76 and -11.96, separately. Concentrations of *X. nematophila* have 87% effect on the feeding potential of pulse beetle as showed by the coefficient of determination (R^2) that was 0.87. The R^2 further confirmed the precision of model to predict the effect of bacterial concentrations on % damage (Figure 18).

Results of the present study were compared with some previous works. Similar results were found by Shrestha and Kim (2009) who described *Xenorhabdus nematophila* and *Photorhabdus temperata* as symbiotically related to nematodes, *Steinernema carpocapsae* and *Heterorhabditis megidis*, correspondingly. There was less detail available on ordinary host range of nematodes, but a considerable

variation in pathogenic viability was recorded in two bacteria used to manage *Tribolium castaneum*. *Photorhabdus temperata* showed 6-times higher pathogenicity than *Xenorhabdus nematophila*. The pathogens variation was not caused with respect to their repressive effects on phospholipase A_2 actions that were requisite for appearance of resistant reaction of *Tribolium castaneum*. Cultures were transformed into liquid and natural byproducts, whereas more effective action was observed in the liquid byproducts.

In another study, Park (2015) while working over mosquitoes revealed that bacterial immune suppratants might increase vulnerability of mosquitoes to *Bacillus thuringensis*. *B. thuringiensis* is deadly to the target insect pest with LC_{50} value of 2.9×10^5 and 2.2×10^5 at sixteen hours after treatment, respectively. When culture of bacterium was added, it enhanced *B. thuringensis* toxicity to the larvae of mosquitoes. The LC_{50} values of *B. thuringiensis* to larvae of *albopictus* were lessened to 1.5×10^5 in *X. nematophila* combination, 1.7×10^5 in *Xh* combination and 1.9×10^5 in *P. temperata* combination. The LC_{50} values of *B. thuringensis* to larvae of *Cx. pipiens* were lessend i.e. 1.2×10^5 in *X. nematophila* combination, 1.3×10^5 in *Xh* combination, and 1.5×10^5 in *P. temperata* combination. When benzylideneacetone or oxindole was added which was created from *X. nematophila* and *P. temperata* also increased *B. thurengiensis Israelensis* toxicities to the larvae of mosquitoes.

In another research conducted by Seonghae and Yonggyun (2006) wherein they described that the bacteria caused higher deaths of 3rd stage of larva of *S. exigua*, but did not exhibit similar higher pathogenic viability to the 5th stage of larva, when ingested. Higher deaths in 3rd stage of larva were happened by anti-biotic action in opposition to *B. cereus*, requisite for maximum growth of *S. exigua*. Enhancing the pathogenic viability in 5th stage of larva, haemocoel must have bacteria. *B. thuringiensis* was applied in helping bacteria to enter from lumen to heomocoel of *S. exigua.* Combinations of bacteria application was found highly pathogenic against 5th stage of larva of *S. exigua*.

Table 1.
Number of eggs (Mean ± SE) per grain in chickpeas applied with different concentrations of *P. temperata and X. nematophila*..

S. No.	Concentrations (Cells/ml)	Number of eggs per grain	
		P. temperata	*X. nematophila*
1	1×10^4	9±0.57d	8.6±0.66b
2	1×10^5	8±0.57d	8±0.57b
3	1×10^6	5.6±0.33c	7±0.57a
4	1×10^7	3.6±0.33b	4±0.57a
5	1×10^8	1.3±0.33a	2±0.57a
6	Control	16±1.15e	18±1.15c

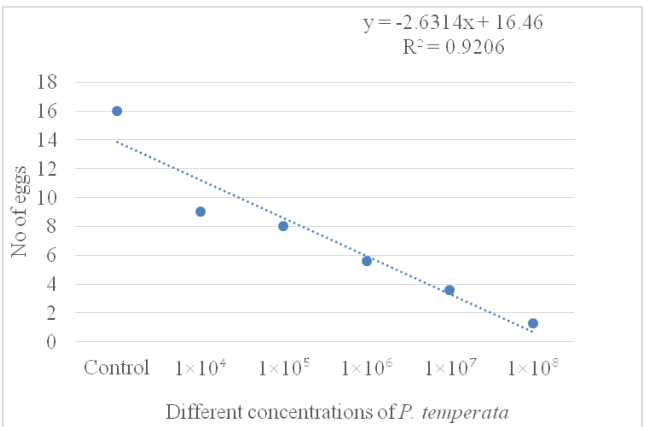

Fig. 1
Modeling trend for mean number of eggs by pulse beetle in response to different concentrations of *P. temperate.*

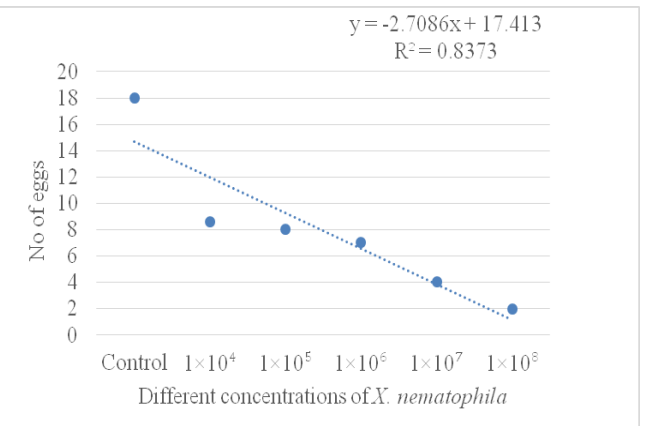

Fig. 2
Modeling trend for mean number of eggs by pulse beetle in response to different concentrations of *X. nematophila*

Table 2.
Number of holes (Mean ± SE) per grain in chickpeas treated with different concentrations of *P. temperata and X. nematophila.*

S. No.	Concentrations (Cells/ml)	Number of holes per grain	
		P. temperata	*X. nematophila*
1	1×10^4	5.3±0.33d	4±0.57c
2	1×10^5	3.6±0.33bc	3.6±0.33bc
3	1×10^6	2.6±0.33bc	3±0.57bc
4	1×10^7	1.6±0.33ab	2±0.57ab
5	1×10^8	1±0.33a	0.66±0.66a
6	Control	7±0.57e	7±0.57d

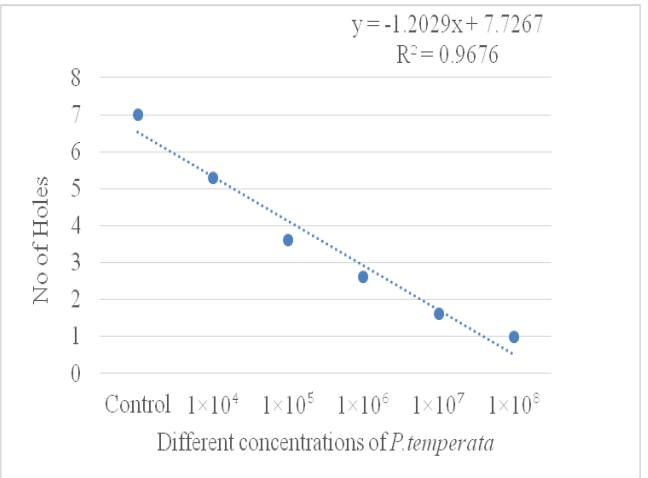

Fig. 3
Modeling trend for mean number of holes in response to different concentrations of *P. temperata*

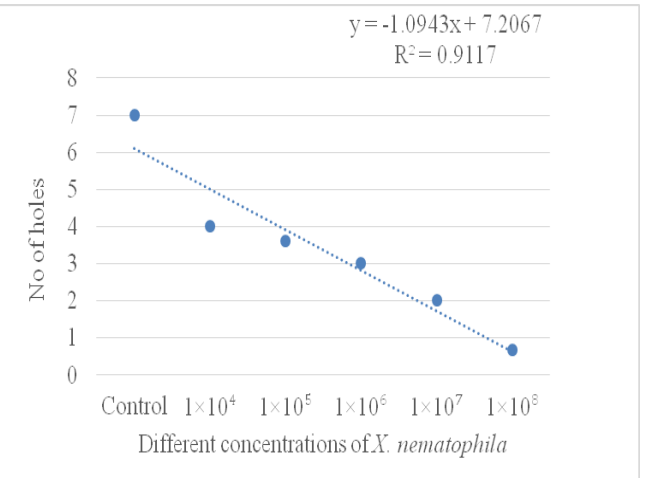

Fig.4
Modeling trend for mean number of holes in response to different concentrations of *X. nematophila*

Table 3.
Number of F_1 adults emerged (Mean ± SE) in grains treated with different concentrations of *P. temperata and X. nematophila.*

S. No.	Concentrations (Cells/ml)	Number of F_1 adults per jar	
		P. temperata	*X. nematophila*
1	1×10^4	15±0.57c	16±0.57b
2	1×10^5	14.66±0.33c	15.33±0.33b
3	1×10^6	14±0.57c	14±0.57ab
4	1×10^7	12.33±0.33b	12.66±0.33ab
5	1×10^8	10.66±0.66a	11.33±0.33a
6	Control	43±0.57d	41.33±2.40c

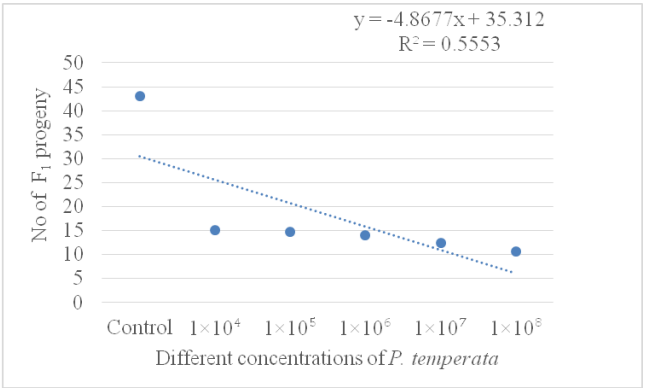

Fig. 5
Modeling trend for emergence of F_1 adults of pulse beetle in response to different concentrations of *P. temperata*

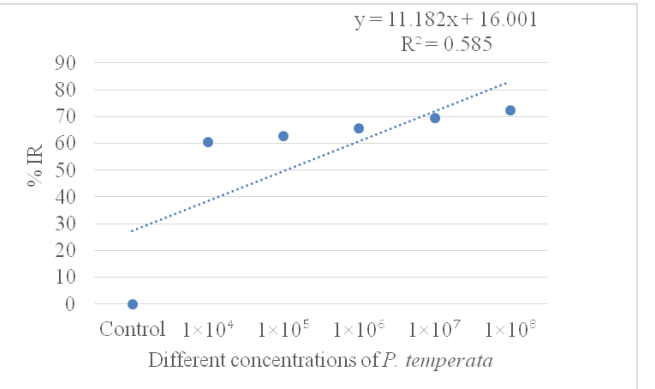

Fig. 8
Modeling trend for percent inhibition rate (Mean ± SE) of pulse beetle in stored chickpea treated with different concentrations of *P. temperata*

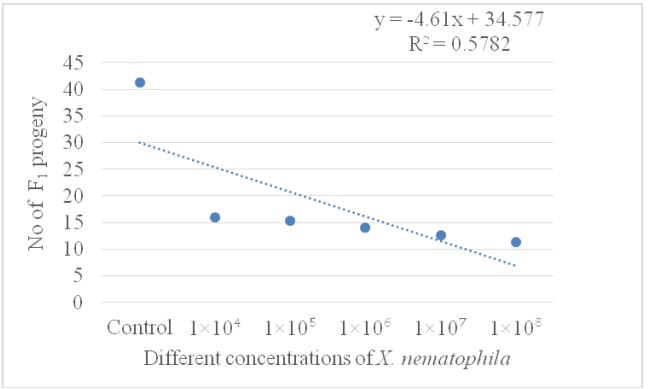

Fig. 6
Modeling trend for emergence of F_1 adults of pulse beetle in response to different concentrations of *X. nematophila.*

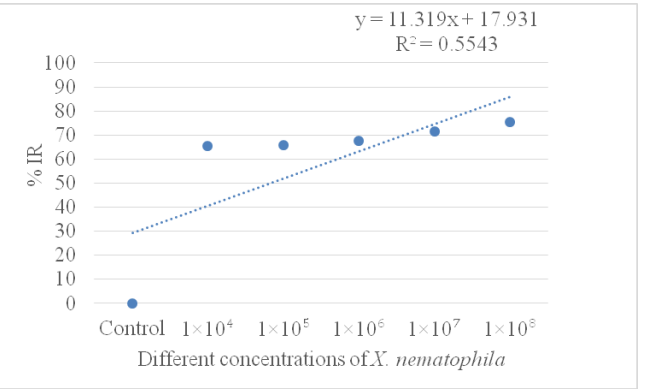

Fig. 9
Modeling trend for percent inhibition rate (Mean ± SE) of pulse beetle in stored chickpea treated with different concentrations of *X. nematophila*

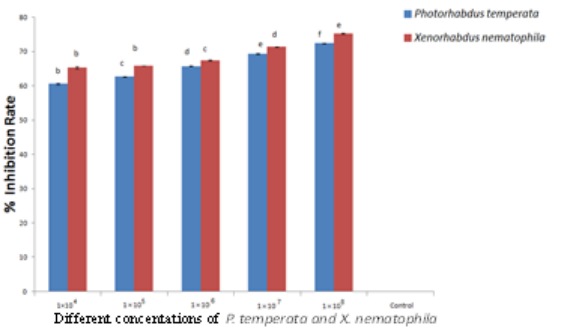

Fig. 7
Percent inhibition rate (Mean± SE) of *C. chinensis* adults fed on chickpea grains treated with different concentrations of *P. temperata and X. nematophila*

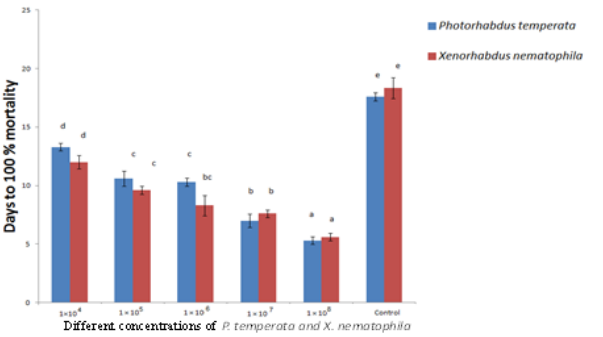

Fig. 10
Days to 100% mortality of F_1 (Mean± SE) of *C. chinensis* adults fed on chickpea grains treated with different concentrations of *P. temperata and X. nematophila*

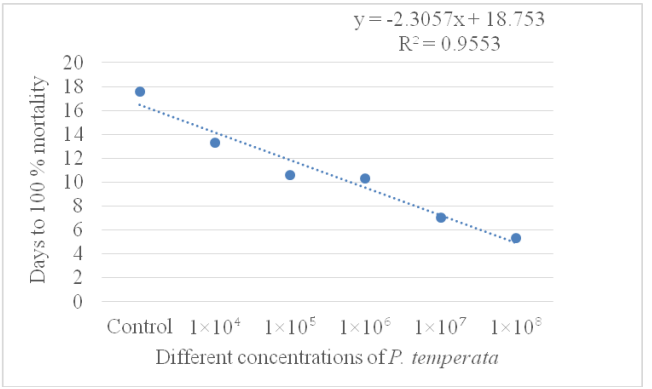

Fig. 11
Modeling trend for days to 100% mortality of F_1 adults (Mean ± SE) in stored chickpeas treated with different concentrations of *P. temperata*

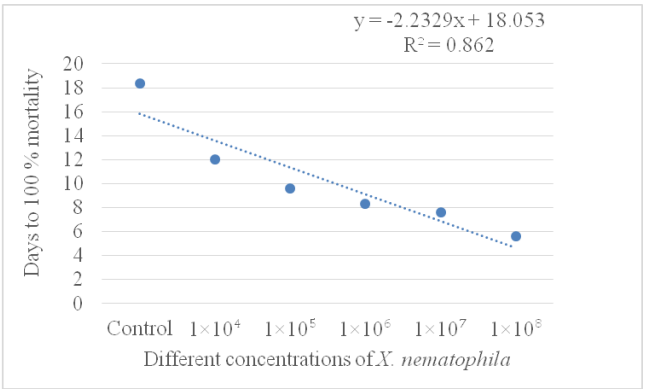

Fig. 12
Modeling trend for days to 100% mortality of F_1 adults (Mean ± SE) in stored chickpeas treated with different concentrations of *X. nematophila*

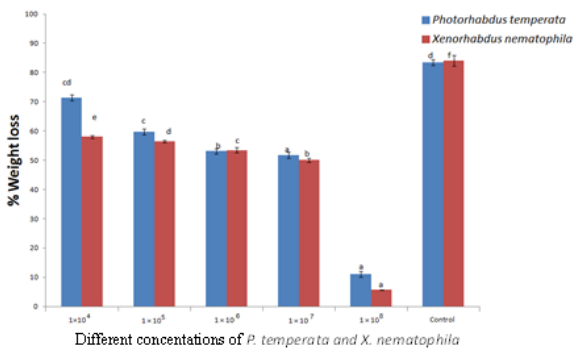

Fig. 13
Percent weight loss (Mean± SE) in chickpea grains treated with different concentrations of *P. temperata and X. nematophila*

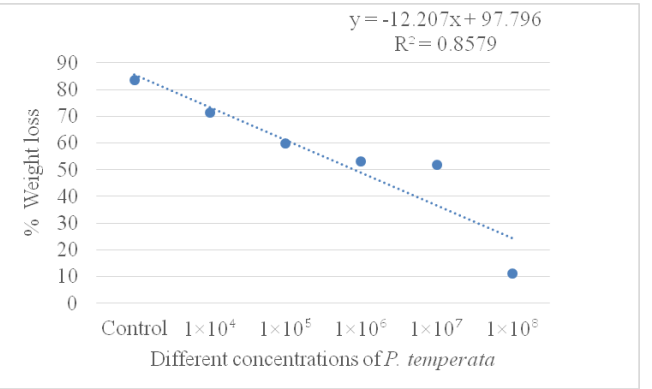

Fig. 14
Modeling trend for percent weight loss (Mean ± SE) in stored chickpeas treated with different concentrations of *P. temperata*

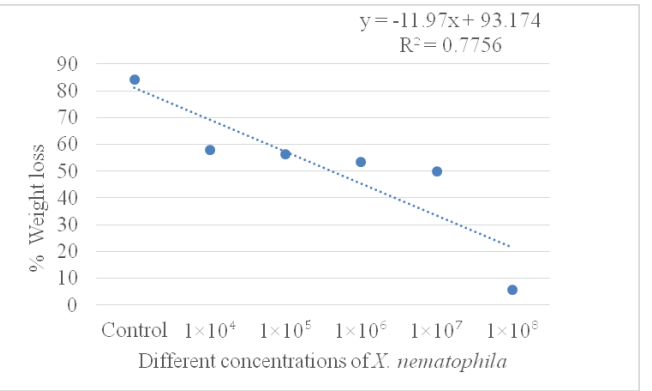

Fig. 15
Modeling trend for percent weight loss (Mean ± SE) in stored chickpeas treated with different concentrations of *X. nematophila*

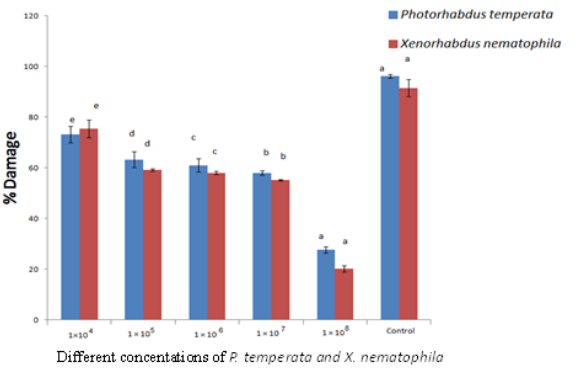

Fig.16
Percent damage (Mean± SE) in chickpea grains treated with different concentrations of *P. temperata and X. nematophila*

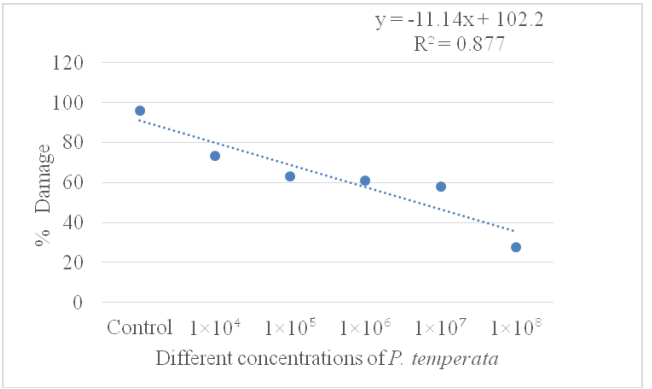

Fig. 17:
Modeling trend for percent damage (Mean ± SE) in stored chickpeas treated with different concentrations of *P. temperata*

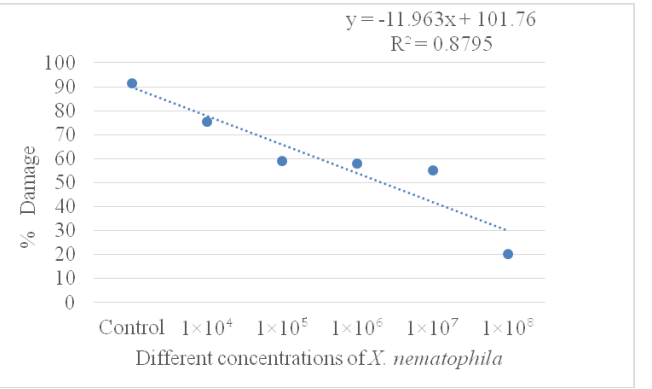

Fig. 18
Modeling trend for percent damage (Mean ± SE) in stored chickpeas treated with different concentrations of *X. nematophila*

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