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Pathogenicity of Entomopathogenic Fungus And Bacterium Against *Oryzaephilus surinamensis* L. (Coleoptera: Silvanidae) in Stored Dates

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Abstract – Saw toothed beetle, Oryzaephilus surinamensis (Coleoptera: Silvanidae) is regarded as the most devastating insect pest in stored dates. Adults and larvae feed on dates by making tunnels between the external fruit skin and flesh. Theentomopathogenic fungus and bacterium, Metarhizium anisopliae and Xenorhabdus nematophila was used as bio-control agents against this serious pest (O. surinamensis). Five concentrations of entomopathogenic fungus and bacterium (1×10^4, 1×10^5, 1×10^6, 1×10^7 and 1×10^8) were prepared; each replicated thrice for bioassays to determine their aptness against O. surinamensis. Haemocytometer was used for counting spores and spectrophotometer used for counting bacterial cells and colonies. To conduct insect bioassays, infested date fruits will be collected from different localities; O. surinamensis culture will be maintained in an incubator at 30-32°C temperature and 70-75% relative humidity in the 'Stored Product Entomology Laboratory'. Mortality of O. surinamensis was directly proportional to the concentration of M. anisopliae and X. nematophila. Highest mortality was recorded at concentration of 1×10^8 spores/ml all beetles were died after 6 days, on the other hand the highest mortality was observed at the concentration 1×10^8 cells/ ml all beetles were died after 5 days and vice versa. X. nematophila showed better results as compared to M. anisopliae. This effective control strategy has significant contribution towards development of commercial microbial formulations of M. anisopliae and X. nematophila and is recommended to be a part of integrated pest management of Saw toothed beetle.

Keywords – Oryzaephilus Surinamensis, Metarhizum Anisopliae, Xenorhabdus Nematophila, Mortality And Concentrations

I. INTRODUCTION

Date palm, *Phoenix dactylifera* is the most ancient tree cultivated since, 4000 B.C. and is a heavenly fruit; it has been described in many religious books. Pakistan is the 6th largest producer of the dates after Saudi Arabia, Algeria Egypt Iran, and Iraq with the production of 557279 tons per year.Date is the third most important fruit after citrus and mango in Pakistan.Oman face a serious problem in stored dates by saw toothed beetle (STB), and reduce the quantity and quality of date fruit being a nutritious and sweet 2500-3000 calories/kg supplying food [1] *O. Surinamensis* feeding on a broad range of stored grains and food products as well as nature wise it is a polyphagous and cosmopolitan insect pest, found in all areas

of the world typically in storage and packing stores of stored grains. *O. surinamensis* showed maximum infestation or damaged the dates with low moisture.

Insect pest of stored dates were controlled by using insecticides but the alternative control strategies are desirable due to pest resistance against insecticides and consumer desire for pesticide free dates [2]. There is need regarding awareness of farmers by growing date palm and the value of IPM is a selective approach to crop protection because it is eco-friendly [3]. STB has been primarily controlled by using fumigation of methyl bromide but entomopathogenic fungi are a safe term control as compared to chemical pesticide. The fungal and bacterial control agents are directly used in stored grains and other food commodities [4] [5]. Entomopathogenic fungus *M. anisopliae* was effective biological control agent against many insect pests of stored grains. Mostly, fungi infect their hosts and it produced hyphal bodies, when the fungus reaches the hemolymph because fungus germinate or enter through external insect cuticle although the digestive tract infection occurs with some species. So host mortality was observed due to invasion of organ, fungal toxin action and the reduction of nutrients [6] [7].

Entomopathogenic bacteria *X. nematophila* belongs to the family Enterobacteriaceae, which produce toxin protein for the wide collection of insect management. *Xenorhabdus* spp. produced 4-insecticidal proteins, Xpt A1, A2, B1 and C1 categorized as class A, B and C proteins respectively, which act as biocontrol agents without nematode, *Xenorhabdus, spp.* does not survive and has limited their use as biocontrol agent [8] [9]. *X. nematophila* is active, Gram-negative entomopathogenic bacteria [10] [11]. This bacteria supply important nutrients for nematode reproduction and growth because bacteria breakdown the host tissue by intracellular inclusions, hydrolytic enzymes and antimicrobial compounds and generate antibiotics which were diverse in quality and quantity relaying on species, strain, and growth media in calculation bacteria decrease microbial growth after secondary infection and producing antimicrobial compounds to keep a monogenic form which helps the nematodes grow and proliferate in the host haemoceal [12].

The specific objective of proposed research encompasses to determine the aptness of different concentrations of the entomopathogenic fungus, *Metarhizium anisopliae* and the bacterium, *Xenorhabdus nematophilla* against *Oryzaephilus surinamensis* in stored dates.

II. Materials and Method

Collection of *Oryzaephilus surinamensis* infested samples and maintenance of insect culture:

Infested samples of stored dates collected from different godowns/storages in different areas of the country. *O. surinamensis* culture sustained in the 'Stored Product Entomology Laboratory' of Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Pakistan in an incubator at 70 ± 5 % RH and 30 ± 2 °C temperature. Males and females identified based on Meta leg femur saw like projection in males.

Culture maintenance and inoculums preparation of *Metarhizium anisopliae* and *Xenorhabdus nematophilla*:

Entomopathogenic fungi *M. anisopliae* maintained in the 'Fungal Plant Pathology Laboratory' of the University.Initially the fungal culture grown in Potato Dextrose Agar (PDA) at 25°C for two weeks, and then multiplied in Potato Dextrose Broth medium to count the number of conidia/spores per unit volume. The conidia/spore grows on PDA medium.Later on spores/conidia counted by Haemocytometer at 24 hours interval. Different concentrations of spores/conidia established in distilled water in addition with Tween 80 (0.02%). For analysis diverse concentrations of *M. anisopliae* equipped. Similarly entomopathogenic bacteria, *X. nematophila,* arranged from CABI Bioscience, Rawalpindi. 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 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 was prepared to apply against *O. surinamensis*

Insect bioassays using different concentrations of *Metarhizium anisopliae and Xenorhabdus nematophila:*

In each plastic jar, 50g of dates put and enclosed with tightened muslin cloth and kept at 30°C temperature in incubator. Three pairs of *O. surinamensis* were released into each jar. Different concentrations (conidia/spores/ml) of fungi and bacteria were prepared for the experiment.The insecticidal aptness of different concentrations of fungi and bacteria against *O. surinamensis* was also.

Modeling aptness studies:

Regression model was used to study the relationship of treatment impacts on insect parameters. The model equation was

$y = a + bx$

Statistical Analysis:

The data recorded subjected to statistical analysis using appropriate statistical packages like SPSS for Windows program etc.

III. RESULTS

Effectiveness of *Metarhizum anisopliae* against adults of *Oryzaephilus surinamensis*

Days to 100% mortality of *O. surinamensis* adults treated with different concentrations of *M. anisopliae*

The highest 15.33 days to 100% mortality of F_1 emerged Saw toothed beetle was at 1×10^4 spores /ml of fungal concentration in comparison the minimum 6 days were noted through the concentration of 1×10^8 spores /ml. All the concentrations of *M. anisopliae* showed significantly different mortalities of Saw toothed beetle than the control concentrations (Fig 1).

To observe the effect of diverse concentrations on the mortality rate of newly emerged adults, linear regression model was used. When the fungal concentration were applied as evident from the regression equation (Y=-2.5046x + 20.266) the negative impact on the percent mortality of F1 adults were recorded. The intercept (a) value remained 20.26 but slope (b) was
-2.5. Hence as the concentration of *M. anisopliae* increased days to 100% mortality of F_1 was reduced @ -2.5. Coefficient of determination (R^2) was 0.98 which revealed that the fungal concentrations have 98% effect on the dependent variable (Figure 2).

Percent weight loss caused by *Oryzaephilus surinamensis* adults treated with different concentrations of *Metarhizum anisopliae*

According to figure 3 all the fungal concentrations were statistically dissimilar from other and resulted in effective virulence against STB except control as above 92% weight loss was reported. Among fungal concentrations maximum percent weight loss (81%) was caused by Saw toothed beetle at 1×10^4 spores/ml. As well as the minimum percent weight loss 12.33% was caused by *O. surinamensis* at 1×10^8 spore/ml.

As showed in Figure 4 percent weight loss caused by *O. surinamensis*in response to diverse fungal concentrations was experimented by linear regression model. Unfavorable impact of fungal concentrations on the feeding of STB was observed as presented by the modeled equation (Y=-15.824x + 113.4). The intercept (a) 113.4 and slope (b) -15.82 value was remained separately. So the concentration of *M. anisopliae* increased weight loss caused by STB decreased with the rate of -15.82. Determination coefficient (R^2) was 0.97 which showed that the fungal concentrations have 97% effects on the percent weight loss. The R^2 further confirmed the accuracy of model.

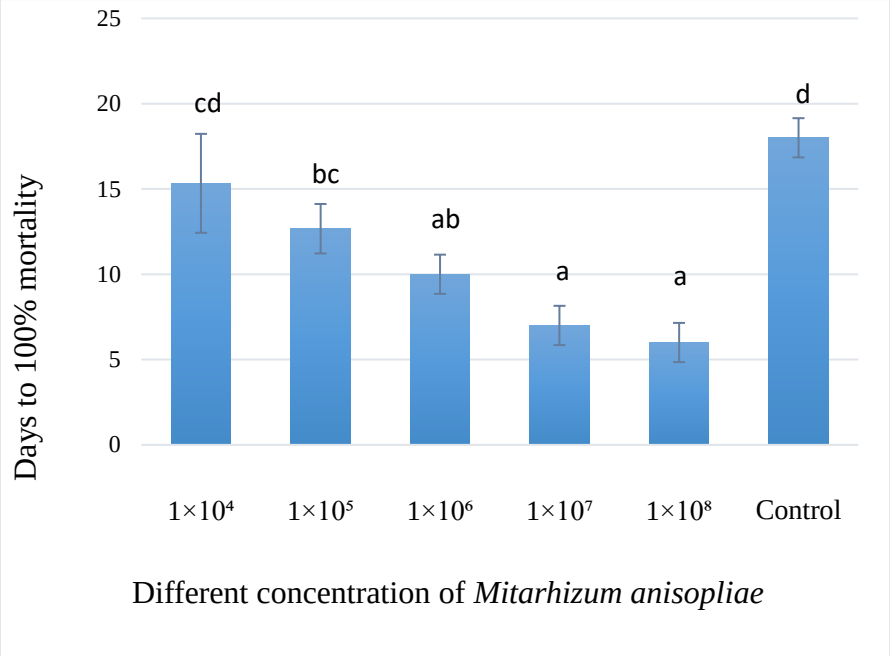

Fig 1: Days to 100% mortality of F_1 adults (Mean ± SE) of *O. Surinamensis* in stored dates treated with different concentrations of *M. anisopliae*

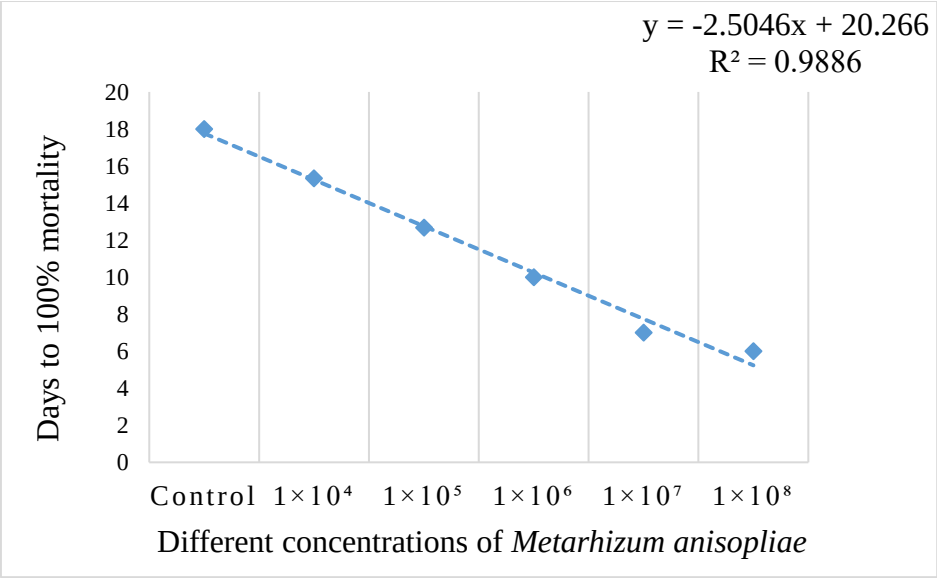

Fig 2: Modeling trend of 100% mortality of F_1 adults of *O. surinamensis* in response to different concentrations of *M. anisopliae*

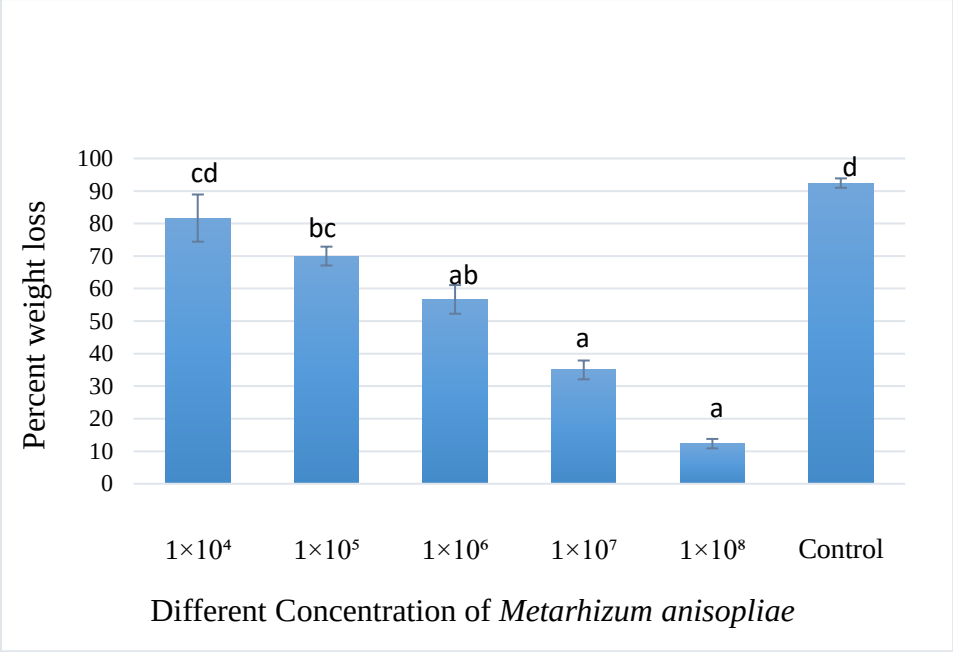

Fig 3: Percent weight loss (Mean ± SEM) caused by *O. surinamensis* in stored dates treated with different concentrations of *M. anisopliae*

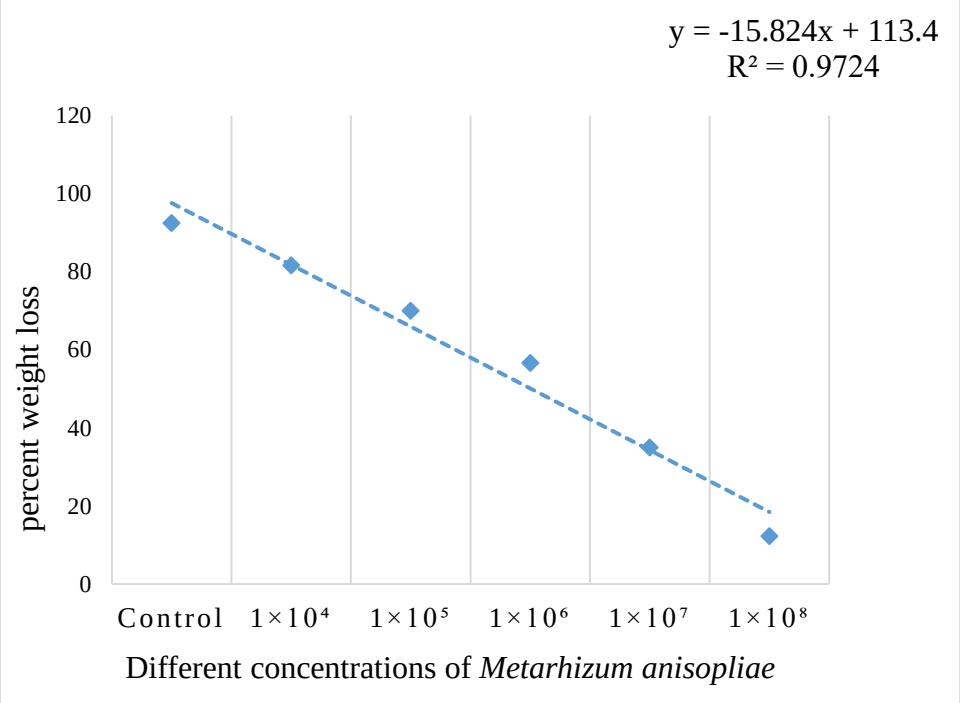

Fig 4: Modeling trend of percent weight loss caused by *O. surinamensis* in response to different concentrations of *M. anisopliae*

Effectiveness of *Xenorhabdus nematophila* against adults of *Oryzaephilus surinamensis*

Percent mortality of *Oryzaephilus surinamensis* adults treated with different concentrations of *Xenorhabdus nematophila*

In Fig 5 the 100% mortality of newly emerged Saw toothed beetle the least 5 days were required at 1×10^8 cells/ml of *X. nematophila*. The highest 13 days were reported for 100% mortality of F1 adults at 1×10^4 cells/ml. All treatments performed well other than the control.

To determine the effectiveness of fungal concentrations on the mortality of F_1 emerged STB, linear regression model was implemented. The model equation (Y= -2.6763x + 19.645) showed negative impact of bacterium concentrations on the percent mortality of F_1 adults. According to (figure 6) the intercept (a) value remained 19.64 but slope (b) was -2.67. Accordingly as the concentration of *X. nematophila* was increased days to 100% mortality of F_1 was reduced at the rate of -2.67. Coefficient of determination (R^2) was 0.92 which revealed that the fungal concentrations have 92% effect on the mortality of newly emerged adults meanwhile R^2 further confirms the accuracy of model.

Percent weight loss caused by *Oryzaephilus surinamensis* adults treated with different concentrations of *Xenorhabdus nematophila*

According to figure 7 all bacterial concentrations were statistically dissimilar from each other except 1×10^6 and 1×10^7. Among bacterial concentrations, the highest percent weight loss 56% was caused by Saw toothed beetle at concentration 1×10^4 cells/ml. whereas the concentration of 1×10^8 cells/ml was most effective with less than 6% weight loss and 80% weight loss occurred in control.

To determine the effectiveness of bacterial concentrations on the percent weight loss in treated dates STB, linear regression model was implemented. The modeled equation (Y= -11.057x + 87.2) showed that all the bacterial concentrations had negative impact on the feeding potential of STB. The intercept (a) and slope (b) value remained 87.2and -11.05, separately. So, percent weight loss decreased at the rate of -11.05 as the fungal concentration was increased. Concentrations of*X. anisopliae*have 83% effect on the feeding potential of STB as showed by the coefficient of determination (R^2) that was 0.83. The R^2 further confirms the precision of model to predict the effect of bacterial concentrations on weight loss (Figure 8).

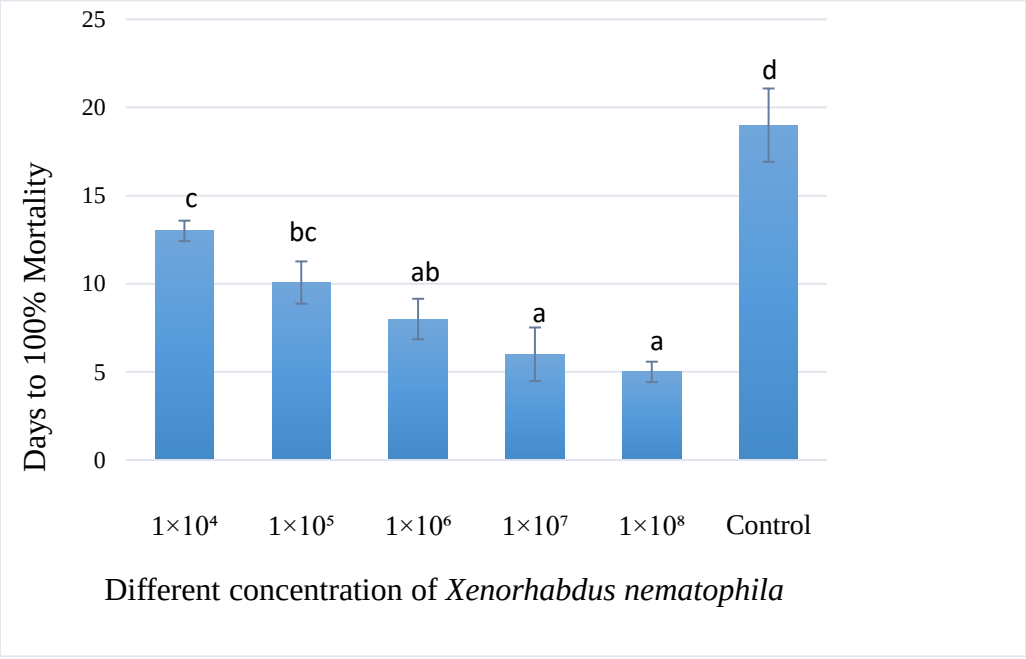

Fig 5: Days to 100% mortality of F1 adults (Mean ± SEM) of *O. surinamensis*in stored dates treated with different concentrations of *X. nematophila*

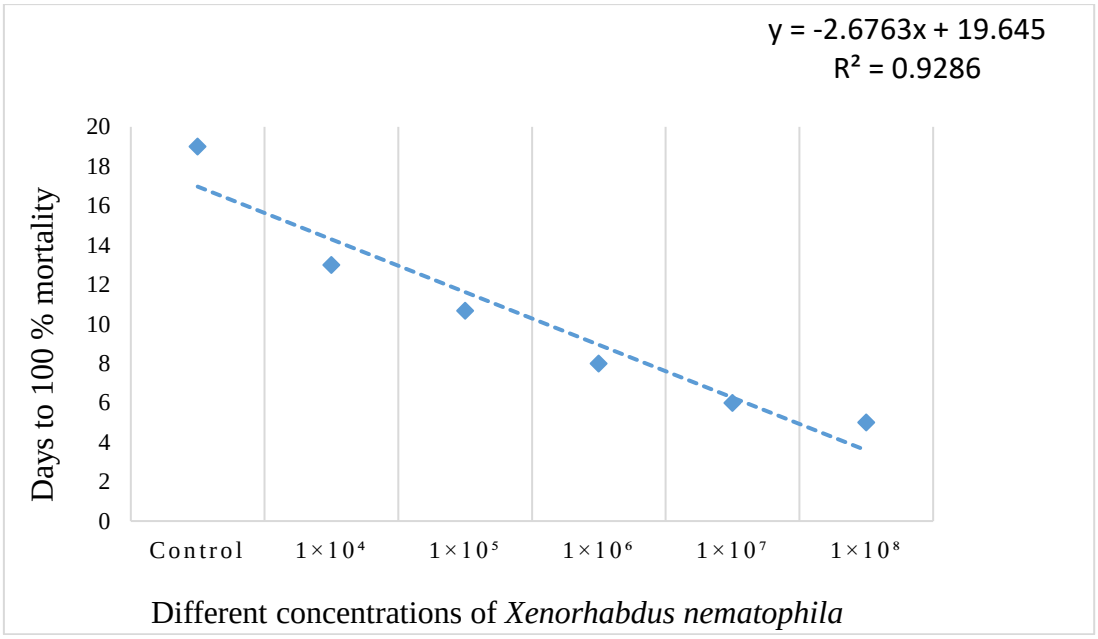

Fig 6: Modeling trend of 100% mortality of F1 adults of *O. surinamensis* in response to different concentrations of *X. nematophila*

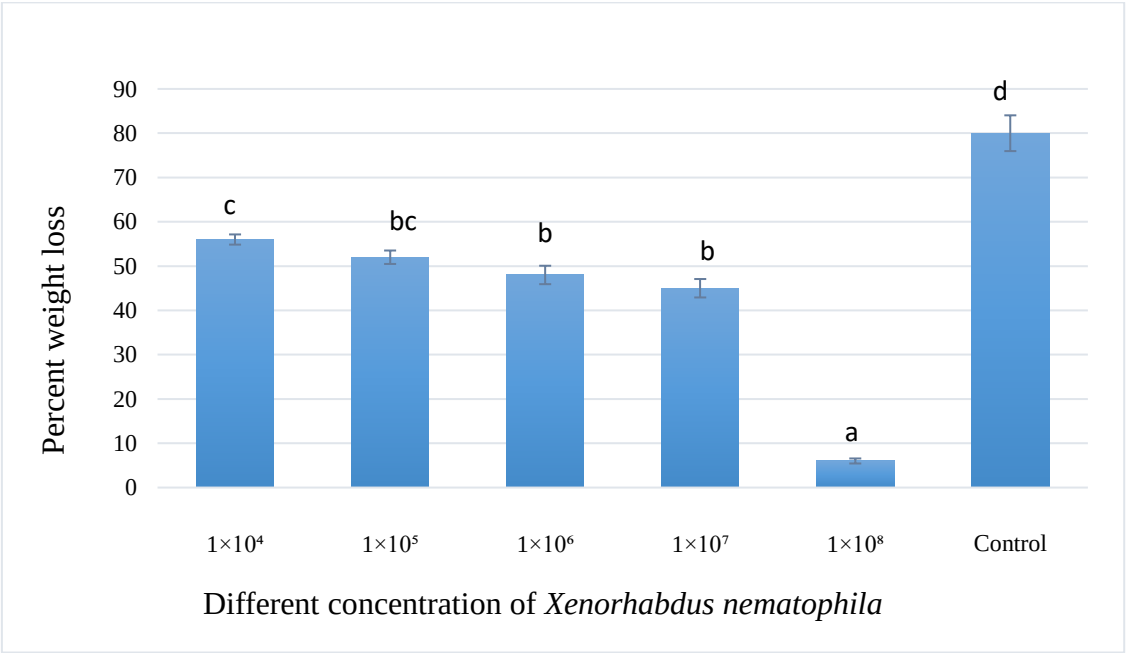

Fig 7: Percent weight loss (Mean ± SEM) caused by *Oryzaephilus surinamensis* in stored dates treated with different concentrations of *X. nematophila*

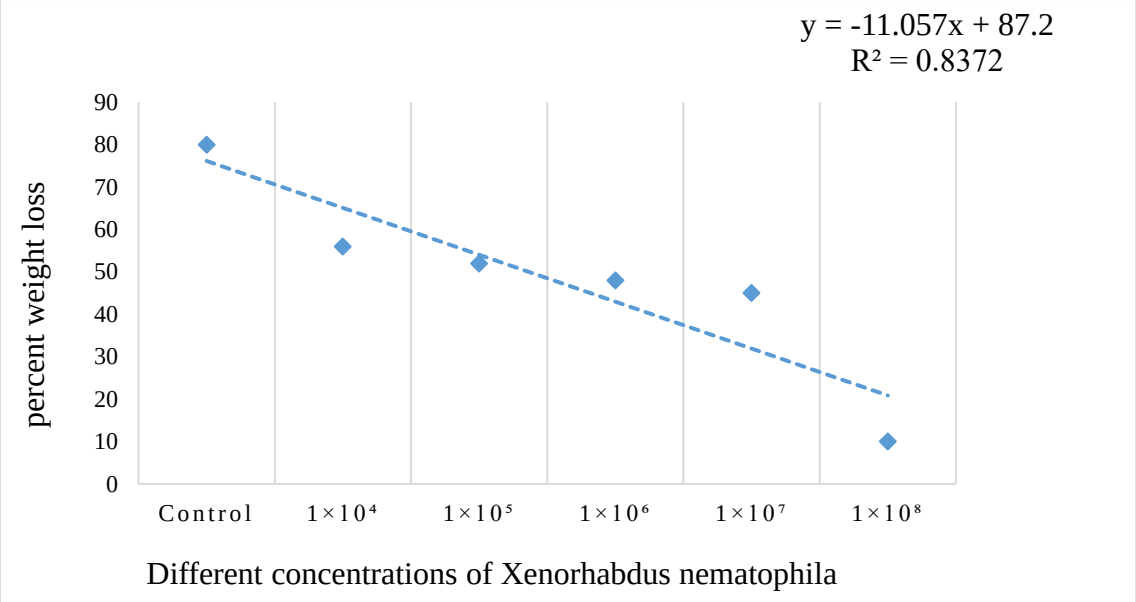

Fig 8: Modeling trend of percent weight loss caused by *O. surinamensis* in response to different concentrations of *X. nematophila*

IV. DISCUSSION

Results of this research were alike to [13] who experimented that the death rate was directly proportional to the fungal concentration as well as exposure. He further reported that STB was controlled by using the diverse fungal concentrations. Conducted experiment exposed that entomopathogenic fungus can cause outstanding mortality along with the reduction in fecundity rate at the high fungal concentration whereas, alike effects was reported by [5] using the entomopathogenic fungus *M. anisopliae* against *O. surinamensis.* [14] reported that the death rate of *O. surinamensis* was increased by using the highest concentration of *M. anisopliae*. Results displays that the adults of *O. surinamensis* in opposition to different concentrations of *M. anisopliae* showed similar results which observed in our research. According to [15], using of entomopathogenic fungus the adults of *O. surinamensis* was high susceptible. The results showed in this study that *O. surinamensis* pests could be controlled by using different concentration of fungus which is similar to our research.

Experiments conducted by [16] showed that entomopathogenic bacterium cause outstanding mortality along with the reduction in the birth rate at the maximum bacterium concentrations whereas, similar results showed by [17] who observed that *X. nematophila* was virulent pathogen that produce toxin protein against wide range of insects and reported that *X. nematophila* is an entomopathogenic bacterium which has broad-host-range insect pathogen which revealed high toxicity against insect pest whereas, similar effects were revealed in our research.Results exposed that the bacterial efficiency was directly proportional to the time exposure; higher concentrations were more valuable than the lower concentrations of *X. nematophila* against O. *surinamensis*. Conducted study showed that the entomopathogenic bacteria *X. nematophila* was more virulent against *O. surinamensis* in stored dates.

The consequences of the study provided a successful and safe biological control action and in future will guide to a helpful IPM programme for this economic pest.

V. CONCLUSION

The findings of this study have significant contribution towards development of commercial microbial formulations of *M. anisopliae and X. nematophila* and this effective control strategy is recommended to be a part of integrated pest management of Saw tooted beetle *O. surinamensis* for shorter and longer storage of dates.

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