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The aptness of entompathogenic bacteria against sawtoothed grain beetle (Oryzaephilus surinamensis [L.]) (Coleoptera: Silvanidae) in dates

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Abstract

Objective

The sawtoothed grain beetle, Oryzaephilus surinamensis (L).[8] is known as one of the most devastating insect pests in stored dates, infrequently found in newly harvested dates and raisins but developed a high plenty in dry fruits particularly after long storage. [0]Indiscriminate use of pesticides and fumigants to control insects have resulted in insecticide resistance, environmental hazards, residual toxicity, and pest resurgence so the control trends have been changed and particularly biological control agents like entomopathogenic bacteria Photorhabdus temperata and Xenorhabdus nematophila being used to manage sawtoothed grain beetle.

Methods

During present study, the pathogenicity of P. temperata and X. nematophila was evaluated against this crucial pest of stored grains. The bacterial concentrations were applied directly to the dates infested with sawtoothed grain beetles and data were recorded.

[0]Results

Significant results were observed for both bacterial treatments as the highest concentration of 10^8 cells/ml showed to be very effective against the beetle in terms of fecundity, adult emergence, and mortality. In terms of fecundity, 8.66 to 4.[24]33 mean number of eggs were

produced at bacterial P. temperata concentrations of 1×10^4 and 1×10^8 cells/ml, respectively. Similarly, the F_1 adult emergence for P. temperata was recorded 15.33 and 9 adults at concentrations of 1×10^4 and 1×10^8 cells/ml, respectively. Results showed a similar trend for the X. nematophila species.

Conclusions

These results indicate that utilizing nematodes as biological control agents can be advantageous for reducing insect populations in storage facilities and preventing the loss of grains and dates that have been stored in storage.

Keywords: Stored product insects, microbial control, entomopathogens, date fruits, Saudi Arabia

Abbreviation

First generation (F_1), integrated pest management (IPM), entomopathogenic nematodes (EPNs), toxins complexes (Tc's), gram (g), One-way analysis of variance (ANOVA)

1. Introduction

Dates contain a high rich protein and good source of rapid energy because of high amount of carbohydrates which is 70-80% (Ghnimi et al., 2017).[8] The sawtoothed grain beetle is a cosmopolitan pest found globally, it is responsible for sever losses, polyphagous feeding in nature and have broad range of stored goods, as well as cereal products, dried fruits, oil seeds and stored grains (Hashem et al., 2012). The sawtoothed grain beetle, infest dates which has low moisture content, with the calyx removed or those broken or with mechanical damage. Tunnels are made on the outer fruit skin and flesh by feeding of adults and larvae. During the high infestation, sawtoothed grain beetle consume all fruit contents leaving the skin or exo-carp integral. The beetle causes severe damage such as weight decrease, reducing the quality and quantity of the dates. Stored dry and semi dry dates face a basic issue of insect damage (Al-Dosary 2009). In stored products the date varieties are most infected by sawtoothed grain beetle and 40 to 75% losses have been reported (Mallah et al., 2016).

[0]Pesticides have been used as protectants all over the world because of their efficient pest control ability but on the other hand, the detrimental effects may include toxicity of stored food commodities causing sanitary and phytosanitary issues as well as life threatening effects on non-targeted organisms (Phillips and Throne 2010).[0] For the sake of human health and safety, environment friendly the implementation of less expensive control measures has become the need due to the lack of awareness of pesticides cost, their detrimental effects on human health and development of genetic resistance in insect species against these chemicals (Aggarwal et al., 2016).[9] Many countries are scrutinizing the traditional

3

fumigation procedures as they are posing threats which include ozone depletion, potential risk of carcinogenic methyl Bromide and phosphine. Alternative strategies must focus on the efficacy against limited target specific species which are biodegradable into non-toxic products and recommended to be use in integrated pest management programs as well as eco-friendly measures development are in discussion. Plant-based chemicals, insect growth regulators and the insect pathogens like entomopathogenic bacteria, fungi, viruses and protozoan are being assessed as better alternatives to chemical based prepared insect control programs. Biological control has great attention over past few decades, as alternative to chemical insecticides or as a constituent of integrated pest management (IPM) (Subramanyam and Hagstrum 2012). These constitute effective alternatives to chemicals without producing adverse effects on the environment.

Entomopathogenic bacteria, Photorhabdus temperata and Xenorhabdus nematophila are gram negative, motile rod-shaped bacteria which belongs to family Enterobacteriaceae and form symbiosis with entomopathogenic nematodes (EPNs) Steinernema and Heterorhabdus (Akhurst and Boemare 1988; Akhurst et al., 1996). Due to this symbiosis, nematodes provide shelter to bacteria in their guts where they seek protection from soil stressors as well as antagonists such as telluric bacterial consortia and bacteria inside insect guts that is why the isolation from soil samples is not possible without their nematode host. This pathogenic symbiosis is able to parasitize as well as to kill the larval stages of host from orders Diptera, Hymenoptera, Lepidoptera, Orthoptera, Coleoptera and Isoptera (Boemare 2002; Belien 2018). The toxins complexes (Tc's) and Photorhabdus insect-related toxins show oral activity against insect species despite of the fact that oral infection is not important to the biology of Photorhabdus or Xenorhabdus. Gram-positive and gram-negative bacteria produced toxins which have high molecular weight and multi subunit with insecticidal properties (Waterfield et al., 2001). Bacteria Photorhabdus or

4

Xenorhabdus encodes the toxins producing genes which co-exist with entomopathogenic nematodes. Strains of bacteria have been discovered with loci that encodes for toxins, some of which are associated to insect while others are not. The objective of this research is assessment of entomopathogenic bacterial toxicity towards sawtoothed grain beetle management and determining mortality of sawtoothed grain beetle in dates due to bacteria.

5

2. Materials and methods

2.1 Insects rearing

Oryzaephilus surinamensis adults were reared on dates and its healthy cultures were maintained in plastic jars, covered with the muslin cloth; tightened with the elastic rubber band. These jars were placed in a SANYO incubator, which was set at 27±2°C and 70 ± 5 percent relative humidity in the laboratory. Adult of sawtoothed grain beetle was identified as per the males have spine like structure on femur of meta-leg, which is absent in females.

2.2 Bacterial culture maintenance

Entomopathogenic bacteria namely Photorhabdus temperata and Xenorhabdus nematophila were obtained from Korean Agriculture Collection. At first the culture on supplement agar plates was streaked over at 25ºC for 2 to 4 days. Obtained culture was filtered by streaking individual colony on nutrient agar. Refined bacterial culture was replicated in nutrient broth for 24 hours. The serial dilution plate count method was used to draw a dilution curvature between the optical thickness and cells/ml in order to calculate and adjust the colony shaping units per unit volume. Distinctive concentrations of bacteria were prepared to apply against sawtoothed grain beetle.

2.3 Bacterial pathogenicity Bioassay

In each jar, 50g of dates was placed. The jars were secured with muslin cloth, fixed with elastic band and were placed in an incubator at temperature and humidity mentioned earlier. Following different concentrations, 1×10^4, 1×10^5, 1×10^6, 1×10^7 and 1×10^8 cells/ml of both bacteria were prepared and used for the experiments. In the control treatment distilled water was used. There were three replicates for each treatment, each jar representing one replicate, and there were five pairs of the young beetles in each jar. The effectiveness of entomopathogenic bacteria against sawtoothed grain beetle were studied according to the following parameters.

6

2.4 Eggs number

Eggs were counted on the third day following the bacterial post treatment. Three randomly selected dates were used to open each jar and count the number of eggs within. Average number of eggs were determined influence of different treatment on egg laying capacity (fecundity). Because the sawtoothed grain beetle's eggs are so tiny and delicate, therefore, only three dates from each jar were opened, and the eggs were counted in order to prevent any damage that would have an impact on the outcomes, such adult emergence.

2.5 Sawtoothed grain beetle adult emergence (F_1) and mortality%

After four weeks of the bioassay, all the jars were opened, emergence of adults (F_1) and the mortality of former adults employed for the bioassay and the new adults were counted. The number of adults' (F_1) were calculated in individual jar to check the repression of O. surinamensis appearance by using diverse concentrations of entomopathogenic bacteria.[0] In a similar manner, the mortality was assessed at 7, 14, and 21 days following treatment.[0] At each observation, the dead adults were removed from the jars, the number of dead and live adults were counted, and the mortality percentage was computed.

2.6 Statistical analysis

The data were collected, tabulated and analyzed. One-way analysis of variance (ANOVA) was done using bacterial concentrations as the main factor and eggs laid, adult mortality and new adult emergence as the response factor. The SAS 9.2 was used to analyze the data.

7

3. Results

3.1 Number of eggs laid by sawtoothed grain beetle in stored dates treated with various concentrations of P. Temperata

According to Table 1, all used concentrations of P. temperata Entomopathogenic Bacteria viz., 1×10^4, 1×10^5, 1×10^6, 1×10^7 and 1×10^8 cells/ml significantly reduced sawtoothed grain beetle fecundity as compared to the control. The least number of eggs were counted in the dates treated with the bacterial concentrations of 1×10^7 and 1×10^8 cells/ml in which the values were 5.00 and 4.00 respectively. However, the concentration 1×10^5, 1×10^6, 1×10^7 and 1×10^8 cells/ml were statistically similar to each other while 1×10^4 cells/ml concentration was dissimilar statistically from the all other used concentration. All bacterial concentration proved pathogenic against sawtoothed grain beetle fecundity biological trait and showed good results than the control.[0] Moreover, the results showed that the number of eggs laid by sawtoothed grain beetles got decreased with the increase in the entomopathogenic bacterial concentrations.

3.2 Sawtoothed grain beetle adult (F1) emerged in stored dates treated with different concentrations of P. temperata

All the bacterial concentrations of P. temperata 1×10^4, 1×10^5, 1×10^6, 1×10^7 and 1×10^8 cells/ml were better statistically in the F_1 adult emergence (table 2). The minimum number of 9.00 F_1 adults were emerged in the concentration of 1×10^8 cells/ml while 1×10^4 bacterial concentration showed maximum number of the 15.00 emerged adults. Moreover, the concentration 1×10^5, 1×10^6 and 1×10^7 cells/ml were showed statistically different results with one another. All bacterial concentrations indicated better F_1 adults inhibiting rate in stored dates as compared to the control.

8

3.3 Sawtoothed grain beetle adult (F1) emerged in stored dates treated with different concentrations of X. nematophila

Table 3, shows that highest numbers of sawtoothed grain beetle eggs 7.00 were observed when the date were treated with the bacterial concentration of 1×10^4 cells/ml. Similarly, the lowest number of the eggs was shown by the bacterial concentration 1×10^7 and 1×10^8 cells/ ml. All concentration gives a better result as compared to the control. The concentration 1×10^4, 1×10^5 and 1×10^6 were not statistically dissimilar while the 1×10^7 and 1×10^8 were similar among themselves and significantly different from the all other concentrations. Along with increase in the bacterial concentrations the number of eggs laid was reduced.

3.4 Number of adult F1 emerged sawtoothed grain beetle in stored dates treated with various concentrations of X. nematophila

The bacteria X. nematophila, showed significant inhibition of sawtoothed adult emergence. According to the Table 4, most effective concentrations which results in minimum number of new adults produced was observed to the 1×10^8 cells/ml while the least effective concentration was 1×10^4 cells/ml producing the greatest number of new emerged adults of sawtoothed grain beetle. However, all concentrations showed a smaller number of the adults as compared to the control. It was observed that when the higher concentration was used then a smaller number of sawtoothed grain beetle adults emerged.

3.5 Sawtoothed grain beetle adult mortality in stored dates treated with various concentrations of X. nematophila

Adult mortality was calculated in percentage by counting the dead and live adults in each jar at various time interval of the post treatment. The bacterial content and exposure time had a clear correlation with the mortality. The maximum adult mortality with 1×10^8 X. nematophila cells/ml concentrations after the seven days of post treatment was 30%. On the

9

other hand, the same treatment resulted in a maximum mortality rate of up to 70% at day 21 of post treatment (table 5).

10

4. Discussion

Dates are an extremely valuable fruit because they are only harvested once in a year with proper storage and insect prevention, may be eaten for several years. The usage of entomopathogenic organisms is very safe for people and other animals, including cattle that eat dates incorporated into their diets as an essential nutrient. The ability of entomopathogenic bacteria to inhibit the sawtoothed grain beetle from laying eggs has been proven by current data. Xenorhabdus spp., a symbiotic bacterium, is one of the well-known biocontrol agents used in pest management (Zhou et al., 2002; Gulcu et al., 2012). According to (Richards and Goodrich-Blair 2010), X. nematophila is an entomopathogenic bacterium with a wide host range and strong toxicity against insect pests; nonetheless, similar results were seen in our investigation. Similar findings were reported that P. temperata and X. nematophila were reported as deadly pathogens that produced proteins and secondary metabolites which prove toxic against a variety of insects (Dowling and Waterfield 2007; Sheets et al., 2011; Ng'ang'a 2015; Namsena et al., 2016). In particular, discussing X. nematophila's pathogenicity against adults of various insects has demonstrated that Drosophila melanogaster and Manduca sexta adults quickly died after being infected with X. nematophila (Kim et al., 2017).[29] These bacteria produce a number of compounds that are insecticidal and can be utilized to combat a variety of species, including insects (Abd-Elgawad 2022; Tomar et al., 2022).

In present study the emergence of F1 adults was very low in the treated dates as compared with the saw-toothed grain beetle adults where distilled water was applies as control treatment. It was noticed during observation that adults were not active in the treated dates as compared to the dates where water was applied. When adults were not active it reflects the deterrent properties of the metabolites produces by the bacteria. This deterrent property of bacteria has affected the normal activities like feeding, copulation, and fecundity. The

11

present data has shown the evidence of low fecundity and F1 adult emergence in the treated dates which have also been reported in other studies (Bode 2009; Kusakabe et al., 2022). Keeping continue, there are several natural products synthesized from the toxic produced by several species of Photorhabdus and Xenorhabdus, secretions and being used commercially for the management of several issues regarding human health and food storage and protection (Cimen et al., 2022). All these findings are in favor of the present results outcomes.

Conclusions

Present study results showed that bacterial effectiveness was strongly correlated with exposure period; larger concentrations of X. nematophila were more effective than lower concentrations against O. surinamensis. An investigation revealed that the entomopathogenic bacteria X. nematophila was more aggressive against O. surinamensis when applied to the dates infested with it. The study's findings led to a successful and secure biological control procedure and will in the future direct an effective IPM program for this crucial pest.

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Declaration of Competing Interest

The author declare that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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12

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14

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15