BioChemMed

Technium BioChemMed Vol. 11, pp.8-15 (2024)
ISSN: 2734-7990
www.techniumscience.com

Effect of different storage periods on pathogenic bacteria isolated from wheat grains contaminated with serrated grain beetle Oryzaephilus surinamensis L. (Coleoptera: Silvanidae) stored in Al-Rifai city

Ashjan Agar Nasser AL-Shwelly*

University of Sumer collage of Basic Education

Abstract

The study was carried out in the College of Science, University of Sumr, an insect lab during the year 2022. The current study was conducted to isolate and diagnose some types of aerobic pathogenic bacteria during different time periods 3 months, 6 months and 9 months from wheat grains infected with the serrated grain beetle and to know the effect of changing the periods on the level of contamination with pathogenic bacteri Nine types of pathogenic bacteria have been isolated (*Bacillus cereus*, *Escherichia coli* , *Proteus mirabils* , *Enterococcus faecalis* , *Enterobactor aerogenes* , *Pseudomonas aerugenosa, Klebsiella pneumonia, Staphlococcus aureus, Staphlococcus epidermidis)* from mechanically damaged wheat samples infected with the insect under the influence The factor of the difference in the duration of storage, and it was found that the period of 6 months is relatively superior.

Introduction

Warehouse insects are among insects of great economic importance, For being it causes financial losses estimated at millions annually, and is one of the dangerous insect pests due to their many types and spread Widespread in all countries of the world Under various circumstances The feeding of these insects is not limited to one food only, but rather they infect and feed on everything that is stored, and this is the reason for their wide spread(Al-Qazzaz *et al*., 2012) Grain products are the main source of human food in various countries, especially poor ones, whose citizens find it difficult to obtain animal protein, as they constitute the largest part of the food basket for many people in the world because they contain a high percentage of carbohydrates, in addition to containing large quantities of proteins and fats and nutrients (Alnoso *etal.,2011).* Stored grain insects are among the most severe pests that share food with humans, and they have a major impact on the country's economic and commercial returns due to the large losses they cause to storage materials. The annual loss as a result of infection with this insect may reach 36 million tons annually in the world(Weston ,2000) .

Technium BioChemMed Vol. 11, pp.8-15 (2024)
ISSN: 2734-7990
www.techniumscience.com

It is a saw-chested beetle *Oryzaephilus surinamensis* is a pest the important insect that causes paralytic infestation Stocks, it also infects wheat, barley,y ellow corn, and oilseeds such as sesame, as well For dry fruits such as dates, dried figs, and even Tobacco (Al- Qazzaz *et al*., 2012).

The insect(*Oryzaephilus surinamensis)* is among the secondary insects that do not infect grains unless they have been previously infested by primary insects such as *Sitophilus spp. or Rhyzorpertha dominica* (Trematerra *et al.*, 2012).

Many circumstances played a fundamental role in the spread and distribution of the insect on a large scale, including globalization, internationalization, and commercial activities related to the transport and distribution of warehouse grains. This, in addition to hot climatic conditions in particular, led to the development and growth of this global pest(Rees, 2004).

Insects in general are important vectors of pathogens, whether bacterial or fungal, so their presence in stored foodstuffs is considered a serious problem (Chalfine *et al*., 2000).

The factor of pollution and the damage it causes to foodstuffs is one of the most widespread problems at the present time that can be caused during any of the stages of food production and marketing, such as harvesting, storage, transportation, etc. There are factors that help the growth of microorganisms in stored food, which is affected by two types of factors. The first type relates to the properties of the food itself in terms of acidity, pH, humidity, water percentage in the food...etc., which are called intrinsic or actual factors. The second type is related to storage conditions such as temperature, humidity, and storage pests, which are called non-essential (accidental) factors (Hocking, 2003; Willey.*et al*., 2008).

Materials and methods

Preparing a permanent farm for a beetle of *Oryzaephilus surinamensis L*. (Coleoptera: Silvanidae)

The insect was collected from infected stored grains and was raised and propagated in the laboratory by preparing glass bottles with dimensions of (15 * 9) cm and placing 100 grams in each. of crushed wheat, then infested with a sufficient number of adult insects, placed in containers, covered with pieces of gauze, and placed in a refrigerated incubator (temperature 30±2°C and humidity 70±5g/cm3).

It was left to multiply and this farm was re-perpetuated from time to time in the previous manner. The insect was identified on the basis of its appearance By Dr. Rasha Al-Taie, a lecturer at the University of Kufa

Technium BioChemMed Vol. 11, pp.8-15 (2024)
ISSN: 2734-7990
www.techniumscience.com

Qualitative diagnosis and quantitative enumeration of bacterial species isolated from the types used:

The total numbers of bacteria isolated from food were estimated according to the method of Harrigan and McCane, 1966v , as follows:

10 grams of each grain sample was weighed and placed in a Blender electric blender after sterilizing it, and 90 milliliters of sterile dilution solution containing 0.85% sodium chloride was added to it. The sample was mixed for 1-5 minutes, then left for 1-2 minutes. This represents the first dilution 1 -10 I attended a series of scares up to $10c^6$ prepared a set of sterile tubes to put 9 ml of physiological saline solution in them, then added to the first tube 1 ml of the mixed sample, and the final range was 10 ml. I repeated the process with the rest of the tubes.) Transfer 1 or 0.1 ml of the dilutions to sterile Petri dishes, then add Its culture medium is Nutrient agar

The dishes were moved clockwise and counterclockwise to homogenize the dilution with the culture medium. They were then placed in the incubator at a temperature of 37°C for 24-48 hours. After the growth of the bacterial colonies, they were counted using a Colony Counter device to count isolated colonies on Nutrient agar and Blood agar dishes, where the numbers of bacteria were recorded. present in the grain samples, and the colonies were counted according to concentrations.

Diagnosis

The isolated bacteria were diagnosed based on morphological characteristics, cultural characteristics, and biochemical tests.

The effect of different storage periods on the level of contamination with aerobic pathogenic bacteria

Tested three storage periods for wheat grains,. The periods were (3, 6, and9) months to determine their effect on the level of bacterial contamination. 100 grams of wheat were placed in glass bottles with dimensions of (15*9) cm, and 5 pairs of insects were inserted into each bottle. Newly emerged (male and female) were placed in an incubator at a temperature of 30±2°C and a humidity of 70±5 g/cm3 for the time periods mentioned above, with three replicates for each case, along with three replicates of each insect-free wheat (control treatment), and at the end of each period. Bacteria are isolated and diagnosed and their numbers estimated

BioChemMed

Technium BioChemMed Vol. 11, pp.8-15 (2024)
ISSN: 2734-7990
www.techniumscience.com

Results and discussion

Table 1: Shows the types and numbers of bacteria (colony/gm) *10^6 isolated from healthy grains of wheat infected with the serrated grain beetle *Oryzaephilus surinamensis L.* (Coleoptera: Silvanidae). In the numerical level (5 pairs) and for two generations during different time periods during the months of storage

Total summation	9Months		6 Months		3 Months		Isolated bacteria
	Infected	Non Infected	Infected	Non Infected	Infected	Non Infected	
58.6	11.0	12.7	16.0	6.6	9.0	3.3	*E. coli*
21.0	0.0	0.0	16.7	4.3	0.0	0.0	*B. cereus*
36.9	0.0	8.6	11.6	10.0	6.7	0.0	*E. aerogens*
36.6	0.0	0.0	18.0	10.0	8.6	0.0	*E. faecalis*
36.2	0.0	0.0	17.0	8.6	10.6	0.0	*K. pneumonia*
40.9	0.0	0.0	20.3	9.0	11.6	0.0	*P. mirabilis*
21.0	0.0	0.0	18.0	3.0	0.0	0.0	*P. aerugenosa*
27.0	0.0	0.0	21.0	6.0	0.0	0.0	*S. aureus*
19.0	0.0	0.0	12.0	7.0	0.0	0.0	*S. epidermidis*
297.2	11.0	21.67	150.6	64.5	46.5	3.3	total summation

$CalX^2$= 10.35	$TabX^2$= 13.27	P. value= 0.03	DF= 4	According to the status of wheat for the month 3
$CalX^2$= 8.07	$TabX^2$= 20.09	P. value= 0.42	DF= 8	According to the status of wheat for the month 6
$CalX^2$= 6.18	$TabX^2$= 6.63	P. value= 0.01	DF= 1	According to the status of wheat for the month 9

Table 2: Shows the total number of bacterial colonies isolated from healthy andinfested wheat grains during the storage months (3,6,9) months

Total summation	**Infected**	**Non Infected**	**Wheat**
			Examination dates / sample status
150	140	10	**3 Months**
637	452	185	**6 Months**
97	33	64	**9Months**
884	625	259	**total summation**

$CalX^2$= 100.11	**$TabX^2$= 9.21**	**DF= 2**	**P. value= 0.001**	**Total interference**

BioChemMed
Technium BioChemMed Vol. 11, pp.8-15 (2024)
ISSN: 2734-7990
www.techniumscience.com

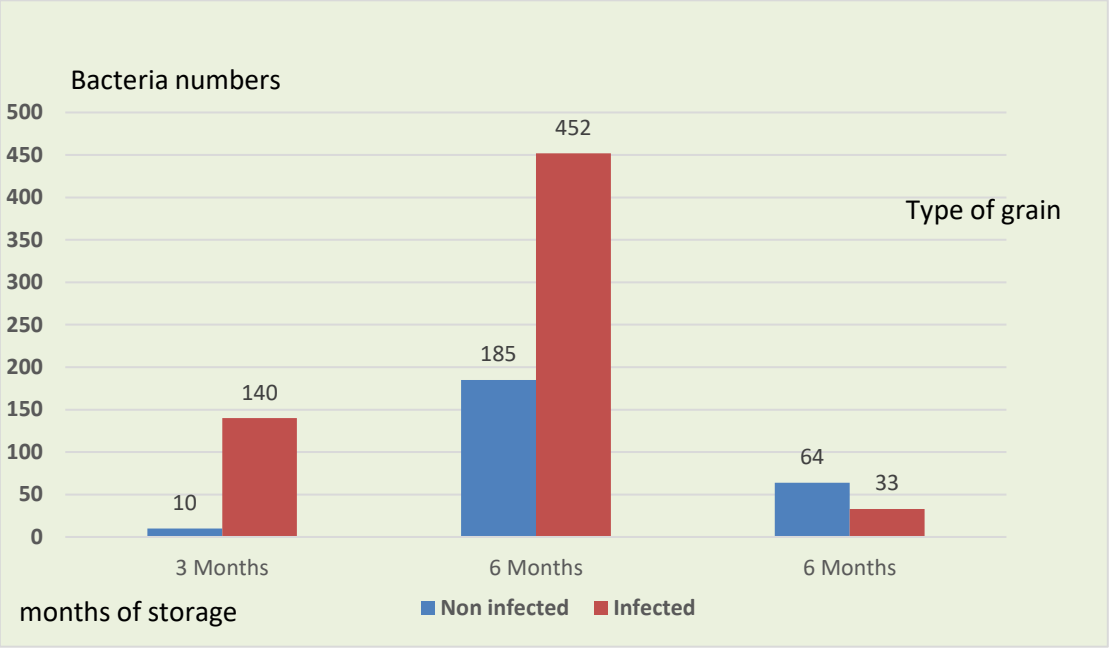

Figure 1. The total average number of bacteria isolated from healthy and infected wheat during the months of storage (9,6 ,3)months.

A-The effect of different storage periods on the level of qualitative and quantitative bacterial contamination in wheat grains

1-Specific pollution:

Table (1) shows the types and numbers of bacterial colonies that were isolated during storage periods of (3, 6,9) months from healthy wheat infected with serrated grain beetle *Oryzaephilus surinamensis L.* The most important isolated species were *Echerichia coli ,Enterobactera aerogens,Enterococcus faecalis , S.aureus, S.epidermidis,Klebsiella.pneumonia,Proteus mirabilis,Psedomonas aerugenos*

The species differed in their appearance points in terms of numbers, and the type was *E.coli* distinguished from two parts, as the total number of its colonies during the total storage period was(9) nine months (58.6)*10^6 colony/gm , while the lowest number of colonies was for *S.epidermidis* at the same period (19.0) *10^6 colony/gm . This difference is explained by the resistance of some bacterial species to storage conditions and the low It was also found that many types of bacteria had a delayed appearance starting from infection and did not appear until six months later, as is the case with the species *P.aerogenosa, S.aureus, and S.epidermidis*, this indicates their slow reproduction and growth compared to other species

It did not appear after (6) months and was few compared to other types. As for the total number of bacterial colonies recorded during the storage periods, it was(297.2)*10^6

Technium BioChemMed Vol. 11, pp.8-15 (2024)
ISSN: 2734-7990
www.techniumscience.com

colony/gm as shown in Table No. (1) during storage periods (9, 6,4) months , The results of the statistical analysis indicated that there were significant differences in the numbers of bacterial species between the infected and healthy grains during the three storage periods, while the remaining species appeared It appeared during the storage period (6) and disappeared during the two periods (3,9) Such as the following types *S. epidermidis, S. aureus, P. aerugenosa.* Where was the total number of colonies isolated in a respectively, (19.0,27.0,21.0))*10^6 colony/gm Note that some species appear during storage periods (3 and 9) only. The results of the statistical analysis indicated that there were significant differences in the numbers of bacterial species between the infected and healthy grains during the three storage periods. It is clear from the table that the total number of bacterial colonies of some isolated species decreased as the storage period increased, as the last storage period (9)months was the least in terms of appearance. Bacterial species excluding bacteria *E. coli* It was observed to appear during the last storage period, The lack of water content is what explains the death of bacterial species during the last periods of storage Which leads to a lack of water activity

When Saudi and Jadoua (2011) studied the effect of different storage periods of (12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1) months on the water content of the seeds included in the study, they found that the two storage periods (11 and 12) months gave the lowest average moisture content of 10.65% and 10.66%, respectively, while the content was 15.05% at the first month. This result was consistent with the findings of Al-Awadi (2008), where microorganisms were isolated from samples of some healthy legume species infected with the southern cowpea beetle, and the last storage period was the period in which the appearance of microorganisms decreased. his result also agreed with the findings of Al-Dhahabi (2009), where the two storage periods (3.6) months were superior in terms of the appearance of types of microbes on brown flour, and the last storage period (9) months was the least apparent in terms of the appearance of types of microorganisms. The main reason for the increased growth of microorganisms on seeds infected with insects during the first storage periods compared to the long storage periods may be due to the increase in the moisture content of the grains, as it is higher during the first months of storage than it is during the long months of storage.

Quantitative pollution:

Table 2shows the total number of colonies that appeared on healthy and infected grains during the different storage periods. The total number of colonies during the storage periods reached (884*10^6) colonies/gm. The results of the statistical analysis showed significant differences between the storage periods, with the highest total number of bacterial colonies reaching (637*10^6) colonies/gm at a storage period of (6) four months, and the least during a storage period of (3) two months, where it reached (97*10^6) colonies/gm

The high level of quantitative contamination clearly appears after the storage period (6) months, and is caused by the drying of the stored seeds as the storage period advances and

the lack of reproduction and activity of insects, which reflects negatively on the reproduction of bacterial species, as Wielly *at el* (2008) found that the growth of some bacterial species is affected by two types of Factors including those related to the type and degree of food pH, some of which are related to other factors, including storage conditions such as temperature and humidity. The results of the statistical analysis also indicated that there were statistically significant differences between infected and healthy grains

REFERENCES

[1] Al-Dhahabi, Zainab Hadi Farhud. (2009): Fungi associated with the rusty flour beetle Tribolium castaneum (Herbst) (Coleoptera; Tenebrionidae) contaminating some types of flour and the effect of some plant powders on them. Master's thesis, College of Education for Pure Sciences, Dhi Qar University, p. 91.

[2] Alonso-Amelot, M. E., & Avila-Núñez, J. L. (2011). Comparison of seven methods for stored cereal losses to insects for their application in rural conditions. *Journal of stored products research*, *47*(2), 82-87.

[3] Al-Shwelly, A. A. N., & Al-Gizy, S. T. A. (2021). Contamination with Aerobic Bacteria Accompanying to Seeds of Triticum Aestivum. L that are Stored and Infected with Different Numerical Levels of Rusty Flour Beetle Insect Tribolium. Castaneum (Herbest) Coleoptera: Tenebrionidae. *Annals of the Romanian Society for Cell Biology*, 959-971.

[4] Chalfine, A.; Timsit, J. F. and Acar, J. (2000). Antibiotic resistance in nosocomial pulmonary pathogens. In Seminars in respiratory and critical care medicine (Vol. 21, No. 01, pp. 0045-0052). Thieme Medical Publishers, Inc., 333 Seventh Avenue, New York, NY 10001, USA. Tel.:+ 1 (212) 584-4662.

[5] Hocking, A. D. (2003). Microbiological facts and fictions in grain Preston, Harley and Klein's, Microbiology (pp: 840-842).

[6] Maguire, K. M., Sabarez, H. T., & Tanner, D. J. (2003). Postharvest preservation and storage. In *Handbook of vegetable preservation and processing* (pp. 65-97). CRC Press.

[7] Mislit, H. M., Mohammed, A. A., & Kareem, A. A. (2020). Evaluation of the efficacy of local and commercial entomopathogenic fungal isolates against Oryzaephilus surinamensis (Coleoptera: Silvanidae). *Plant Archives*, *20*(2), 5672-5676.

[8] Rees, D. P. (2004). *Insects of stored products*. CSIRO Publishing.

[9] Saudi, Ahmed Hamid and Khudair Abbas Jadoua (2011): The effect of packing methods and storage duration on the qualitative characteristics of grains of rice varieties Oryza sativa L, Journal of the College of Education for Pure Sciences, 1 (3), pp. 54-64.

Technium BioChemMed Vol. 11, pp.8-15 (2024)
ISSN: 2734-7990
www.techniumscience.com

[10] Trematerra, P., Throne, J. E., Fernandez, M., & Knox, R. 2012. Insect and mite pests of durum wheat. In M. Sissons, B. A. Marchylo, & M. Carcea (Eds.), *Durum wheat chemistry and technology* (2nd ed., pp. 73-83). AACC International, Inc.

[11] Weston, P. A. and Rattlingourd, P. L. (2000). Progeny production by*Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) on maize previously infested by *Sitotroga cerealella* (Lepidoptera: Gelechiidae). Journal of Economic Entomology, 93(2): 533-536.

[12] Wiley, J. M.;Sherwood, L. M. and Woolverton, C. J. (2008). In