Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 959 - 971
Received 05 March 2021; Accepted 01 April 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

**Ashjan Agar Nasser AL-Shwelly[*1], Sadik Th. Ali AL-Gizy[*2]**

University of Sumer collage of Basic Education / University of Thi-Qar College of Science

## ABSTRACT

When studying stored grain *(Triticum aestivum*) healthy and injured with numerical levels 5,10,20 apair of whole bugs of an insect *Tribolium. castaneum*(Herbest) and for two generations it is found that there is aspecific contamination of the following an aerobic bacterial species*Escherichia.coli, Pro.mirabili , Klebsiella.pneumonia,Staphloccocus aureaus , S. epidermids , P. aerugenosa*this level of quantitative contamination of the above bacterial species differed according to the numerical level of the initial infecation with the insect, where the highest rate tocontamination at the infection level 20 pairs where it was($1225*10^3$) colony/ g and lower him at level 5 pair ,where it was ($392*10^3$) colony/ g.

Injured wheat seeds surpassed healthy ones in terms of numbers as it reached the total population of bacterial colonies isolated in the infected wheat $(1885)*10^3$colony/ g , while the total number of colonies on healthy grains $(615)*10^3$ colony/ g .

And the highest rate of bacterial contamination was at the exit of the second generation ,as the rates were $(392,883,1225)*10^3$ colony / g ,for level 5,10,20 pairs of insects respectively.

**Keywords:**seeds of *Triticum aestivum.L* ; rusty flour beetle insect *Tribolium. Castaneum* (Herbest) Coleoptera: Tenebrionidae.

## INTRODUCTION

Cereal products are the main source of human food in vaious countries , especially the poor ones ,which diff icult for their citizens to get animal protein as they from the most part of the food basket for many people of the world because they contain ahigh percentage of carbohydrates as wellas contain large quantities of proteins, fast and elements ( Alnoso *et al.*,2011).

Large quantities of wheat and other grains are exposed to damage periods, as the extent of damage is between 5-10 % and may reach more than 20 to 30 % in tropical area,

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 959 - 971
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because it is infected with various insects, including the rusty flour beetle , which causes damage to approximately 10-40% of the stored crop ( AL-Jaber, 2006).

Red flour beetles ( *Tribolium castaneum* ) ,in the order Coleoptera , is a cosmopolitan pest of stored products (Rees, 1996; Nenaah, 2014).it belongs to the family of darkling beetles Tenebrionidae. Tenebrionidae is a vast and varied group of small beetles containing more than 10,000 species, with nearly 100 of them being pests on diverse stored products (Haines, 1991; Rees, 1996; Sallam, 2008). The term Tenebrionid means those that are Tenebrio, a word which later literally denotes seekers of dark places (Haines, 1991).
Adults of the species in the Tenebrionidae family infesting stored products are of variable colours, ranging from black to reddish-brown and dark brown (Rees, 1996; Singh and Prakash, 2015). They possess parallel-sided and flattened shape with body length ranging from 3-10 mm long (Haines, 1991; Rees, 1996) and are mostly polyphagous (Singh and Prakash, 2015).
The most predominant pestivorous species of Tenebrionids that are perfectly adapted to live on dried commodity are *T. castaneum.*

The insect is distinguished by its ability to resist harsh environmental conditions because its larvae have the ability to survive for aperiod of 23 months without food , where the damage occurs due to feeding the larvae on the grians and destroying them , as well as contaminating the stroed grains with molten skins and excrement(FAO,1995).

The loss of dry weight and nutritional value is the most important damage caused by warehouse insects , especially the Red flour beetles *Tribolium castaneum* ( Sinha,R.N.andWatters,F.L1986).

The stored grain insects are among the most severe pests that humans share with their food, which have agreat impact on the economic and commercial return of the country because of the great losses they cause on storage materials , the annual loss as a result of infection with this insect may reach 36 million tons annually in the world ( Weston ,2000).

The moral loss is not limited to the presence of the insect with the grains in the storage places only, the storage conditions also play the main role in infecting those grains , such as humidity and heat , which leads to the growth of molds , especially those that secrete toxins such as *Cladosperium sp* , *A pergillus spp* , *penicillium sp* (Magan *et al*.,2003;

Allotey ,1996).

In addition to the Carsinognic compounds that the insect can secrete when the infection is severe (Hodgest et al., 1996).Insects in general are considered important vectors for pathogens , whether bacterial or fungal , so their presence is considered aserious problem (Chalfine *etal*., 2000).

The pollution factor and the food spoilage it causes is one of the most prevalent problems at the present time , which can cause during the harvesting and marketing stages of food

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such as harvesting , storage , transportation etc , there are factors that help the growth of microorganisms in the stored food and it is affected by two types of factors , the first type relates to the properties of the food itself in terms of pH, humidity, water percentage in the food item ect , which called Intrinsic Factors, as for second type , it is related to the storage and humidity , storage pests, which are called Extrinsic Factor (Hocking,2003;Willey.*et al*.,2008).

(Frazier and Westhoff,1978) pointed out that in addition to what is received by the grains from micro – organisms during the growth of plants in the field and from the soil and from other natural sources , the circulation of the grain from the harvesting process to the end of the manufacturing or storage processes leads to an increase in micro-organisms they contain .

## MATERIALS AND METHODS

### 1- Ceate a permanent farm(culture) for rusty flour beetle *( Tribolium castaneum)*

The insect was collected from the infected stored grains , and it was reared and multiplied in the laboratory by preparing glass bottles with dimensions of (9*15)cm and put in each of them( 100 g) of crushed wheat and then infected it with asufficient number of this insect and put it in containers and covered with pieces of muslin cloth with rubber bandge at laboratory temperature and left to multiply and re-perptuate this the culture , from time to time , in previous method, identified the insect on the basis of phenotypic characteristics.

### 2- Studies samples:

In this study, wheat samples collected from the local markets of AL-Rifai city during the year 2019 were used , and the sample weighed one kilogram, the wheat was transported in sterile polyethylene bags and placed adegree of zero c°, for 24 hours, to get rid of any role of insects that may be present on or inside the seeds (AL-Saady,2001), and then diagnosed .

### 3-The effect of three different numerical levels of *Tribolium castaneum* In the level of contamination with pathogenic bacteria .

numerical levels of the insect were tested which (5,10,20)pairs it isThree

a modern full- fledged insect (males and females) on the wheat grains , 100 gm of crushed wheat are placed in glass bottles with dimensions of (15*9) cm and released in each bottle fresh emerging insects full of the levels mentioned above and with three replicates for each level and closed with muslin cloth with rubber peroids bandge , then placed in the laboratory and with it three replicates of crushed wheat , but without infestation by insects, (control ) treatments are followed for two generations . where samples were taken from healthy and infected grains on the following peroids:

1-two weeks after the injury .

2- after four weeks of starting injury .

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3- two weeks after the exit of insects of the first generation .

4- after four weeks of the first generation exit .

Where bacteria is estimated at every date above .

*The numerbers of bacteria in the type tables are divided by (3) to show the true value of the observation.

The bacteria count is diagnosed as follows :

**4- Qualitative and quantitiative diagnosis of bacterial species isolated in the food used:**

The total numbers of bacteria in wheatgrass seeds were estimated by following a method (McCane, Harrigan 1966)as follows:

10g of each food sample was weighed and placed in an electric mixer(blander) after being disolved , 90 % ml of sterile dilution solution containing 0.85% of Nacl was added to it , The sample was mixed for 15 minutes , leave it for 1-2 minutes , and this represents the first dilution ,aseries of decimal easements were performed until $10^6$ transfer 1or 0.1 milliliter of dilution to sterile petri dishes , then add the culture medium to it Nutrient agar , the dishes are moved clockwise and reverse to smooth the dilution with the culture medium and later put in the incubator at a temperature 37c° for an hour , and after the growth of bacteria colonies were counted using adevice (Colony Counter) to count the isolated colonies on plates Nutrient agar , Blood agar, where the number of bacteria present in the food samples were recorded and the colonies were also counted according to the concentrations.

**5- Bacteria diagnosis**

The bacteria were diagnosed using biochemical tests, according to (collee at el.,1996).

**6-** ***Reservation of bacterial isolates*** .

After diagnosing the bacteria , it was purified on MaCconkey -agar and nutrient blood agar medium , samples were transfeered from the developing bacterial colonies to sterile , sealed glass vials ,container on (Slant Nutrien Agar ), and incubated at 37c° in for 24 hours and kept at 4c° in the refrigerator and renewed the isolates from time to time (Harley and Prescott,1996).

**7- Statistical analysis** .

Current study results were analyzed using Microsoft Excel version 2010 and SPPSS statistical program and kay square law for indepence at asignificant level 0.05>.

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## RESULTS AND DISCUSSION

### First: qualitiative pollution.

The tables (1,2,3) show thespieces and numbers of pathogenic bacteria in uninfected and infected grains of wheat *Tribolium castaneum*with numerical levels (5,10,20) rust beetle pests a pair of insects full and for two generations. Where (8) eight spieces of pathogenic bacteria were isolated.

We note that the number of registered bacterial species varies according to the numerical level of infection the primary, where it appeared at the numerical level (5) pairs of three species, namely, *E. coli*, *P. mirabilis*, *K. pneumonia*then, the number of registered species increased with the increase in the numerical level of the primary infection.

Where all the species appeared at the numerical level (10) pairs except for the speieces*P. aerugenosa* and at the numerical level (20) pairs, there was a clear appearance of all the species.

In terms of the number of species, *E. coli* was distinguished from the rest of the bacterial species, where its numbers reached to ($70.7 * 10^3$) colony / gm and $73.0 * 10^3$) colony / gm at the two levels, 20,5 pairs at straight.

While the spieces of *E. aerogens* was the most numerous at the level (10) pairs where the number was ($52.7*10^3$) colony/gm.

This can be explained by increasing the numbers of primary infestation, leading to an increase in the resulting insect population they reported that they multiplied throughout the storage period, and this in turn led to increased contamination with excrement, residues and skins the alienation resulting from the growth of insects, which creates a suitable environment for increasing the proliferation of bacteria and other microorganisms (Al-Dhahabi, 2009).

This is in agreement with what (Hussny ,2020) found when studying the effect of using numerical levels of House flies in the amount of food contamination with Salmonella and Shigella bacteria, where it was found Food contamination increases with the increase in the number of flies, as it reached the highest amount of pollution at the numerical level (20) pairs of flies was $188 * 10^6$ with Salmonella bacteria and $119 * 10^6$ with Shigella bacteria.

This conclusion was in agreement with his findings (Ali,2010) where it was found that the level of contamination of legumes with aerobic bacteria increases with increasing numberical level of *Callosobruchus maculatus*, where the hightest level of contamination legumes was at the numerical level (15) pair of insects in the fact that the species appears in adifferent way and with an increase in number according to the numerical level of contamination, in which it differed in the fact that *P. aerugenosa*

Bacteria appear at the levels (10,20) pairs of insects**.**

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It has been observed in studies that most types of bacteria isolated from stored grains are (Nonpathogenic)bacteria , as for contamination by pathological bacteria such as *Salmonella* ,as well as bacteria *E.coli,B.*cereus they are intestinal aerobic bacteria (Enteric bacteria) however , their presence on the stored grains may be the result of biological factors such as insects, birds and rodents , which it may occur as a result of storage , transportation and harvesting (Hoching,2003).

The growth of micro-organisms can also be attributed to the ability of some of their types to survive and compete , as most of their individuals are highly competitive because are they do not need special nutritional requirements and their ability to produce decomposing enzymes (Koch,1999).

**Second : quantitative pollution** .

Table (4) shows the total number of bacterial colonies isolated from healthy and infected wheat by *Tribolium castaneum* under the influence of numerical levels (20,10,5) pair of insects. the total number differed according to the studied factors, which are the numeric level of the insect, and the check up of times the samples, the state of wheat grains, and with regard to the numerical level of the insect, we note that the total the isolated bacterial colonies increase with the increase in the number of the insect, where the total is observed the total number of bacterial colonies on wheat grains is at the numerical level (5) and in its two healthy and infected states
During the two generations it reached (392 * $10^3$) colony / g, while we observe the positive increase of the colonies bacterial by increasing the numerical level of the insect, and this is evident in the numerical level (10), where the total was reached the total number of colonies is (883 * $10^3$) colony / g, as well as in the numerical plane (20) pairs of Insects The total number of bacterial colonies within the last level was (1225 * $10^3$). Colony / g. In terms of sampling dates, it was the highest total of bacterial colonies Isolated at the date four weeks after the emergence of members of the first generation where the total was
(1012 * $10^3$) colony / g, and at least two weeks after the start of the infection, when it reached (327 * $10^3$) Colony / g, where the significant differences were observed between the two above dates as for the state of grains, the highest total of bacterial colonies was in infected wheat, where it was the numbers for the three levels of the affected case respectively (978,644,293) * $10^3$ colony/g, as for the normal was the total number of bacterial colonies for the three levels in a row (247,239,129) * $10^3$ colonies / g.
Figure No. (1) shows the overlap between the numerical level and the times of sample examination, as it showed a difference
Significantly, we note that the highest rate of bacterial numbers is four weeks after the appearance of members of the first generation
At the numerical level (10) pairs, where (883 * $10^3$) colony / gm, either at least after two weeks
From the start of infection at the numerical level (5) pair, where (63 * $10^3$) colony / gm.
Figure (2) showed the overlap between the numerical level and the state ofseeds, which showed significant differences

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Where the highest rate of bacteria in infected wheat was at the numerical level (10) pair, as it reached(644 * $10^3$) colony / gm, either in comparison wheat, where the highest rate of bacterial numbers was also reached.

At the level (10) pairs, where it reached (239 * $10^3$) colony / gm.
Either figure No. (3) shows the triple overlap between the three factors, and significant differences were recorded
The highest rate of bacterial numbers was in the infected seeds four weeks after the emergence of the individuals of the generation The first is at level (10) pairs.
This means that the insect's numerical level is favorable to the emergence of the largest number of bacterial colonies that occur In the period before the emergence of the second generation in each of (20,10,5) pairs where the numbers are higher and from Then its offspring will be large, while at level (5) pair the number of bacteria continues to increase continuously the population of the insect reaches its maximum. It is evident from the above table and figure that increasing the numerical level of the initial injury leads to building a population greater than the roles of the insect, and this is a major reason for increased pollution in neighborhoods the microscopic ones, which in turn multiply as a result of the metabolic activities of the insects, as the latter sheds their excreta and skins from their offspring on the host they are on one side, and what this high population causes from a change from the internal environment factors (intrinsic factors) to the grain content, especially with regard to
By temperature, the level of relative humidity, and creating a suitable environment for different reproduction and spread Microorganisms, including bacteria.(Embaby and Abel Galil,2006 ; Agrawal and Sinclai,1997;Belko,1994).
This study agrees with what Al-Awadi (2008) found, when it was found that increasing the density of insects is one of the vital processes on the increase Infection with warehouse fungi, as it plays an important role in the transmission and spread of fungi from infected to healthy seeds.
Also (Abdel Razik et al., 1986) indicated that the average number of fungi in infected seeds
The southern cowpea beetle, *Callosobruchu macuatess*, was found in the second month of infection, higher than the average in the first month.
the current result is consistent with his findings (Al-Dhahabi, 2009). Where he found the rate of germs The flour increases with the increase in the numerical levels of the rusty flour beetle and the generations the numbers of microorganisms in the second generation after the insect individuals emerged were higher than their numbers in the first generation.

**Table 1:** Types and numbers of bacteria colony/$gm^3$ isolated from healthy and infected wheatby the rusty flour beetle *T. castaneum* in numerical level 5 pairsfor a period of two generations.

| Sample examination time | First generation | | | | Second generation | | | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | **Two weeks after infection** | | **Four weeks after infection** | | **Two weeks after exit first generation insect** | | **Four weeks after exit first generation insect** | | |
| Bacterial type | **Wheat grain state** | | | | | | | | |
| | **healthy** | **Infected** | **Healthy** | **Infected** | **healthy** | **infected** | **healthy** | **Infected** | |
| *E. coli* | 5.3 | 6.7 | 6.0 | 7.3 | 6.7 | 11.0 | 13.3 | 14.3 | 70.7 |
| *E. aerogens* | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *E. faecalis* | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

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| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *K. pneumonia* | 0.0 | 3.7 | 0.0 | 4.3 | 0.0 | 6.7 | 0.0 | 11.7 | 26.3 |
| *P. mirabilis* | 0.0 | 5.3 | 5.0 | 6.0 | 6.7 | 10.7 | 0.0 | 0.0 | 33.7 |
| *P. aerugenosa* | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *S. aureus* | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *S. epidermidis* | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Total | 5.3 | 15.7 | 11.0 | 17.7 | 13.3 | 28.3 | 13.3 | 26.0 | 130.7 |
| Overlap between time and grain state | | | | **DF= 6** | **Sig = 0.174** | | **TabX$^2$= 11.07** | | **CalX$^2$= 8.987** |
| Overlap between the generations and the state of the grain | | | | **DF= 6** | **Sig = < 0.01** | | **TabX$^2$= 11.07** | | **CalX$^2$= 35.554** |
| Total overlap of the state of the grains | | | | **DF= 14** | **Sig = < 0.01** | | **TabX$^2$= 23.68** | | **CalX$^2$= 45.387** |

**Table2:** Types and numbers of bacteria colony/gm$^3$ isolated from healthy and infected wheatby the rusty flour beetle *T. castaneum* in numerical level 10 pairsfor a period of two generations.

| Sample examination time / Bacterial type | First generation | | | | Second generation | | | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | **Two weeks after infection** | | **Four weeks after infection** | | **Two weeks after exit first generation insect** | | **Four weeks after exit first generation insect** | | |
| | | | | | | | **Wheat grain state** | | |
| | **healthy** | **infected** | **healthy** | **infected** | **healthy** | **infected** | **healthy** | **Infected** | |
| *E. coli* | 6.0 | 7.7 | 6.3 | 8.3 | 7.3 | 11.7 | 0.0 | 0.0 | 47.3 |
| *E. aerogens* | 0.0 | 6.3 | 0.0 | 12.7 | 0.0 | 15.0 | 3.7 | 17.3 | 55.0 |
| *E. faecalis* | 0.0 | 5.0 | 0.0 | 10.3 | 0.0 | 14.3 | 4.3 | 18.7 | 52.7 |
| *K. pneumonia* | 0.0 | 4.3 | 6.0 | 6.7 | 5.3 | 7.3 | 5.3 | 12.0 | 47.0 |
| *P. mirabilis* | 2.0 | 2.7 | 5.3 | 7.7 | 6.0 | 10.0 | 0.0 | 0.0 | 33.7 |
| *P. aerugenosa* | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *S. aureus* | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 14.3 | 20.0 | 34.3 |
| *S. epidermidis* | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 7.7 | 16.7 | 24.3 |
| Total | 8.0 | 26.0 | 17.7 | 45.7 | 18.7 | 58.3 | 35.3 | 84.7 | 294.3 |
| Overlap between time and grain state | | | **DF= 12** | **Sig = < 0.01** | | **TabX$^2$= 21.03** | | **CalX$^2$= 27.028** | |
| Overlap between the generations and the state of the grain | | | **DF= 18** | **Sig = < 0.01** | | **TabX$^2$= 28.87** | | **CalX$^2$= 120.03** | |
| Total overlap of the state of the grains | | | **DF= 42** | **Sig = < 0.01** | | **TabX$^2$= 58.10** | | **CalX$^2$= 203.99** | |

**Table 3:** Types and numbers of bacteria colony/gm$^3$ isolated from healthy and infected wheatby the rusty flour beetle *T. castaneum* in numerical level 20 pairsfor a period of two generations.

| Sample examination time / Bacterial type | First generation | | | | Second generation | | | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | **Two weeks after infection** | | **Four weeks after infection** | | **Two weeks after exit first generation insect** | | **Four weeks after exit first generation insect** | | |
| | | | | | | | **Wheat grain state** | | |
| | **healthy** | **infected** | **healthy** | **infected** | **healthy** | **infected** | **healthy** | **infected** | |
| *E. coli* | 7.0 | 11.0 | 6.7 | 11.7 | 6.0 | 14.0 | 0.0 | 16.7 | 73.0 |
| *E. aerogens* | 0.0 | 9.0 | 4.0 | 10.3 | 0.0 | 0.0 | 0.0 | 20.0 | 43.3 |
| *E. faecalis* | 0.0 | 7.0 | 4.3 | 10.7 | 0.0 | 11.7 | 4.3 | 12.7 | 50.7 |
| *K. pneumonia* | 0.0 | 9.3 | 0.0 | 11.0 | 0.0 | 14.3 | 5.3 | 25.0 | 65.0 |
| *P. mirabilis* | 3.7 | 7.0 | 0.0 | 7.7 | 0.0 | 10.7 | 0.0 | 12.0 | 41.0 |
| *P. aerugenosa* | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 8.3 | 0.0 | 10.7 | 19.0 |
| *S. aureus* | 0.0 | 0.0 | 0.0 | 0.0 | 13.3 | 0.0 | 13.7 | 18.7 | 45.7 |
| *S. epidermidis* | 0.0 | 0.0 | 0.0 | 11.7 | 0.0 | 20.0 | 14.0 | 25.0 | 70.7 |
| Total | 10.7 | 43.3 | 15.0 | 63.0 | 19.3 | 79.0 | 37.3 | 140.7 | 408.4 |
| Overlap between time and grain state | | **DF= 15** | **Sig = < 0.01** | | **TabX$^2$= 25.0** | | **CalX$^2$= 39.016** | | |
| Overlap between the generations and the state of the grain | | **DF= 21** | **Sig = < 0.01** | | **TabX$^2$= 32.67** | | **CalX$^2$= 115.46** | | |
| Total overlap of the state of the grains | | **DF= 49** | **Sig = < 0.01** | | **TabX$^2$= 66.32** | | **CalX$^2$= 242.67** | | |

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 959 - 971
Received 05 March 2021; Accepted 01 April 2021.

**Table 4**: total summation of bacteria colony / gm3 isolated from healthy wheat grains infected by the rusty flour beetle *T. castaneum* stored for two generations, with anumerical level (5,10,20) pairs

| Numerical level / Time of Examination | Five Pairs | | | Ten Pairs | | | Twenty Pairs | | | Overall total | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Health** | **Infect** | **Total** | **Health** | **Infect** | **Total** | **Health** | **Infect** | **Total** | **Health** | **Infect** |
| Two week after infection | 16 | 47 | 63 | 24 | 78 | 102 | 32 | 130 | 162 | 129 | 263 |
| Four week after infection | 33 | 53 | 86 | 53 | 137 | 190 | 45 | 189 | 234 | 239 | 644 |
| Two weeks after exit first generation insect | 40 | 85 | 125 | 56 | 175 | 231 | 58 | 237 | 295 | 883 | 422 |
| Four weeks after exit first generation insect | 40 | 78 | 118 | 106 | 254 | 360 | 112 | 422 | 543 | 1251 | 1329 |
| Total | 129 | 263 | 392 | 239 | 644 | 883 | 247 | 978 | 1225 | | 2580 |

| | | | | |
|---|---|---|---|---|
| Overlap between numeric level and time of examination | **P. value < 0.01** | Df= 6 | **TabX$^2$= 12.59** | **CalX$^2$= 27.977** |
| Overlap between numeric level and grain status | **P. value < 0.01** | Df= 15 | **TabX$^2$= 25.0** | **CalX$^2$= 32.974** |
| Total overlap | **P. value < 0.01** | Df= 24 | **TabX$^2$= 36.42** | **CalX$^2$= 60.973** |

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 959 - 971
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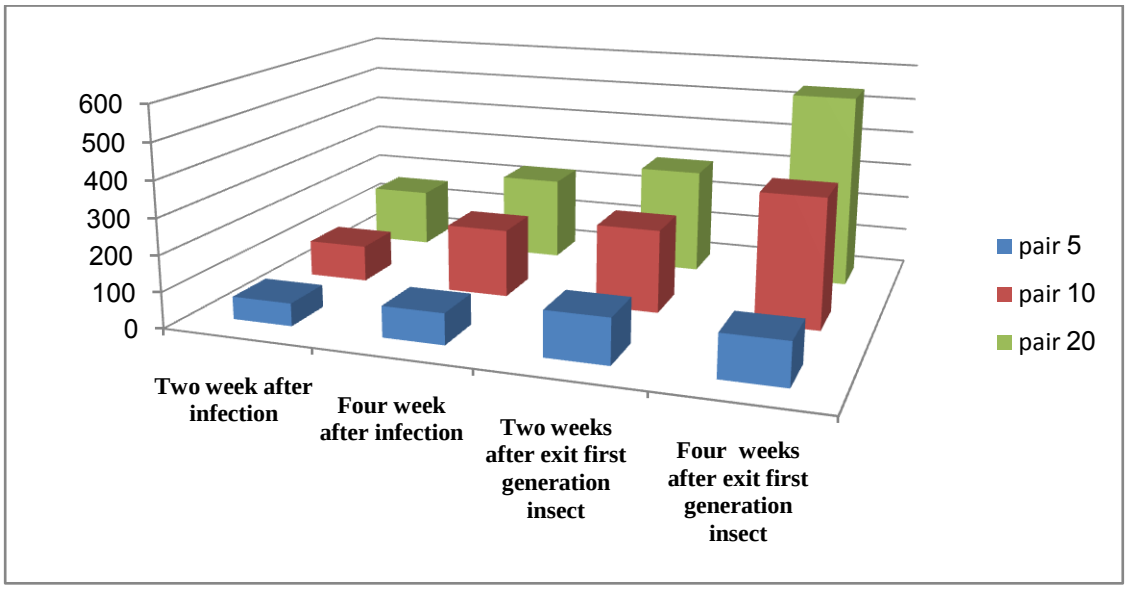


Figure No. (1) shows the overlap between the numerical levels (20,10,5) pair and the times of examining samples.

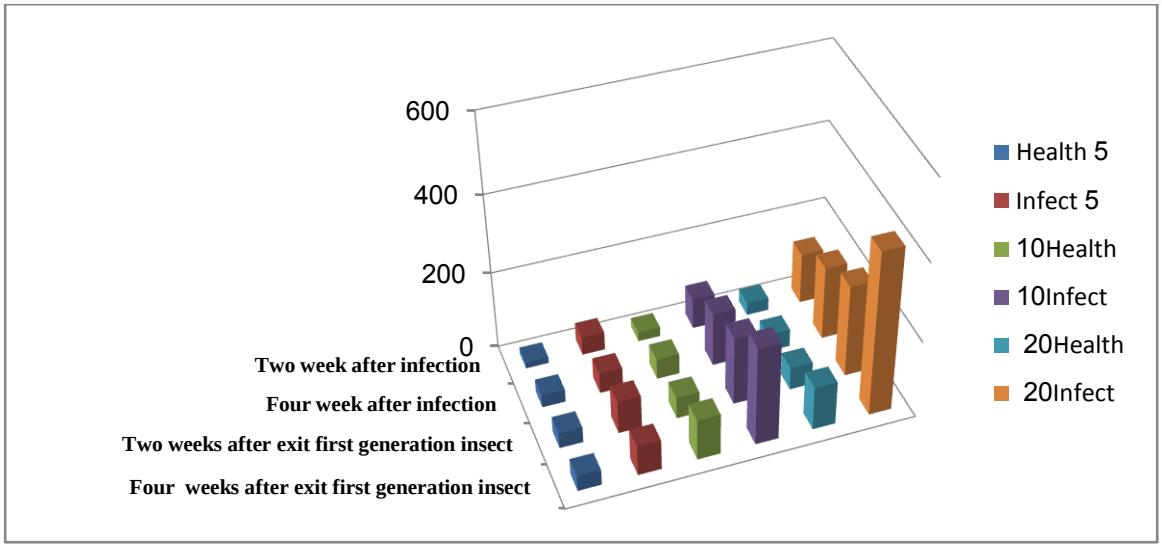


Figure No. (2) shows the overlap between the numerical level and the state of the seeds (healthy and infested).

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 959 - 971
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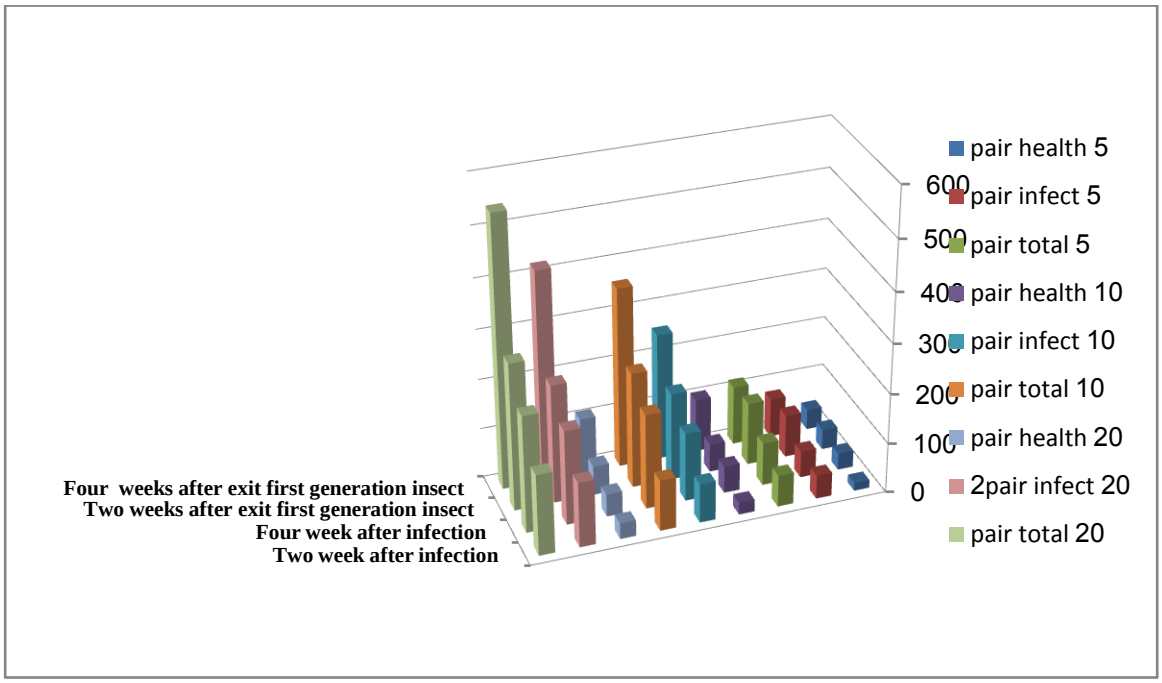


Figure No. (3) The triple overlap between the level, grain condition, and sampling times.

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