

**CHEMICAL COMPONENTS AND SUSCEPTIBILITY OF DATE PALM TREE VARIETIES TO INFESTATION WITH RED PALM WEEVIL, *Rhynchophorus ferrugineus* (OLIVIER)**

El-Deeb, M.A.<sup>1</sup> ; M.M. El-Zohairy<sup>1</sup> ; M.K.A. Abbas<sup>2</sup> ; T.R. Amin<sup>2</sup> and Olfat E. Arafa<sup>2</sup>

1. Plant Protection Dept., Fac. Agric., Zagazig Univ., Egypt

2. Plant Protection Res. Inst., (A R C), Dokki, Giza, Egypt



### ABSTRACT

The red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera, Curculionidae) is the most serious insect pest of cultivated palm trees in several countries. The purpose of the present work is to study Susceptibility of five date palm trees varieties to infestation with red palm weevil, *Rhynchophorus ferrugineus*(OLIVIER) during four successive years (2010-2013) and quantitative variations of some chemical components. The results appeared that Zaghloul& Hayani varieties were the more preference for infestation than other varieties, The percentages of infestation were about (27, 16.5, 16.8 and 13.8 %) for Zaghloul, (25, 15.3, 11.2 and 11.3 %) for Hayani , (19, 8, 5 and 2 %) for Bent Eshia, (12, 5, 1 and 0 %) for Samani and (4, 3, 0 and 0%) for Amry variety during the four successive seasons, respectively. Chemical analysis for trunk tissues of date palm tree varieties showed clear variations between the amount of chemical components Phenols, Alpha esterase's, Phenol oxides, and total proteins appeared positive significant variation infested palm trees as compared with to total nitrogen, Free amino acids ,total carbohydrates ,Tannins, , B-glycosidase, which showed positive and non-significant variation in all infested date palm varieties.

### INTRODUCTION

Red palm weevil, *Rhynchophorus ferrugineus*, (Olivier) (Family: Curculionidae, Order: Coleoptera) was recorded for first time in Egypt in date palm plantations at Sharkia and Ismailia Governorates (Saleh, 1992; and Saleh and Gouhar, 1993). This weevil is the most distractive to palm trees between the different pests Masellam (1999) found correlation between some chemical constituents of the tested varieties and biological aspects under laboratory conditions in order to define the most tolerant variety where on insect reproduction is the lowest and its developmental period is the longest. Angeles-Barcelonet *al.*, (1983). determined that analyses are presented for free amino acids in the foliage of 18 palms, including dates, and for free amino acids in the phloem exudates of .The aim of the present work is to study the following aspects:

- 1- Susceptibility of date palm trees varieties to infestation by RPW
- 2- Chemical analysis of different varieties of health and infested palm trees.

## MATERIALS AND METHODS

### 1-Susceptibility of date palm trees varieties to infestation.

Susceptibility of date palm trees varieties to infestation with RPW was estimated by the differences in the number and percentage of infested palm trees varieties (Hayani, Zaghloul, Bent-asha, Samani and Army) at Abo-Nagi, EL-Kassassien district, Ismailia Governorate during the period from 2010 to 2013.

Five hundred of uninfested palm trees (100 of each variety) of same state, age and vigor of trees under field conditions were chosen. The monthly numbers of infested palm trees of each variety with RPW were counted and avoided from the previous monthly number remainder of health trees of chosen trees.

Percentage of infestation was determined as follows:

$$\% \text{ Infestation} = \frac{\text{No. of Infested Trees / Variety}}{\text{Beginning no. of chosen trees}} \times 100$$

### 2- Chemical analysis of the tested date palm varieties:

Equal pieces of trunk from each tested palm variety were taken for determining some chemical constituents. The pieces were cut as fine slides and left for aerial drying under laboratory normal temperature, until weight constant in Yousry El-Sebay Laboratory of red palm weevil at Kasassien. The prepared sample, were determined in insects physiology department, Plant protection Research Institute, Dokki, Giza, Egypt. The plant sample (weighing 0.1-0.4g) stored at -20C, and then processed as described by Ni et al. (2001). Briefly, The enzymes from the frozen plant samples were extracted using cold potassium phosphate buffer (0.1M, pH 7.0) containing 1% (w/v) poly vinyl pyrrolidone and 1% (v/v) Triton X-100. The samples were macerated with 1 ml of the extracting buffer. Samples were further ground with another 1ml of the extracting buffer. In total, 2ml of the extracting buffer was used each sample. An aliquot (1.5ml) of the extract was centrifuged at 10000g for 10min at 4C. The supernatant was immediately frozen for future enzyme activity assays and the constituents were assessed as follows:

1. Peroxides activity was determined according to Vetter *et al.* (1958).
2. Beta-glycosidase activity was measured by assaying glucose liberated by enzymatic hydrolysis of salicin as described Lindorth (1988).
3. Phenoloxidase activity was determined according to a modification of Ishaaya (1971)
4. Non specific esterases Alpha esterase ( $\alpha$ -esterase) and beta esterase ( $\beta$ -esterases) were determined according to Van Asperen (1962).
5. Total proteins were determined by the method of Bradford (1976).
6. Total free amino acids determination, were calorimetrically assayed by ninhydrin reagent according to the method described by Lee and Takabashi (1966).
7. Total carbohydrates were estimated in acid extract of plant seedling by the phenol-sulphuric acid reaction of Dubois *et al.*, (1956). Total

carbohydrates were extracted from the plant and prepared for assay according to Crompton and Birt (1967).

8. Nitrogen was determined according to Sadasivam and Manickam (1991).
9. Determination of phenols: Extracion was performed as described by Kahkonenet *al.*, (1999).The amount of total phenolics in extracts was determined by Folin-Ciocateu method as modified by Singelton and Rossi (1965).
10. Determination of Tannins: Extraction was performed as described by Sadasivam and Manickam (1991).The amount of Tannins in extracts was determined by Folin-Ciocateu method as modified by Singelton and Rossi (1965).

#### **Statistical analyses**

The obtained results were statistically analyzed using a computer program, Costat programs were used to determine the Significance among treatments. The utilized analyses were: analysis of variance, T-test, Correlation analysis and regression analysis. (S.A.S., 1985). Differences in each parameter were evaluated by Analysis of Variance (ANOVA).when F values were significant ( $P < 0.05$ ), means were compared using the Least Significance Difference test (LSD) for all parameters.

## **RESULTS AND DISCUSSION**

### **I-Susceptibility of date palm trees varieties to infestation.**

Monthly infestation to RPW of different examined date palm varieties (Hayani,Zaghloul , Bent-Esha , Samani and Amry) during four successive seasons 2010, 2011,2012 and 2013 were recorded in Tables (1 and1a).The results cleared that the susceptibility of trees to infestation according to different varieties, were varied as total number of infested date palm trees in each verity, though the different seasons. Zaghloul & Hayani varieties were the more susceptibility for infestation than other varieties where, the percentages of infestation during four successive seasons were about (27,16.5,16.8 and 13.8 %) for Zaghloul , (25,15.3,11.2 and 11.3 %) for Hayani , (19,8,5 and2 %) for Bent Eshia ,(12,5, 1and 0 %) for Samani and (4, 3, 0 and0%) for Amry respectively. The total percentages of infestation for date palm varieties arrived that 62.8, 74.1,34,18 and 7 with Zaghloul, Hayani, Bent Eshia, Samani and Amry during the total period. These results are agreed with that obtained by Abdel-Salam *et al.*, (2008).and AbdEl-Fattah (2010) who found that The highest infestations were recorded with Zaghloul, variety, followed by Hayani, while the lowest infestations were observed Aglan, Amry, Bent –Esha and Samani varieties during the two seasons.

Table (1). Susceptibility of date palm varieties to infestation with red palm weevil, *Rhynchophorus ferrugineus* (Oliv.) during four successive seasons 2010, 2011, 2012 and 2013 (100 date palm each variety).

Months	2010						2011					
	No. of infested palms	The varieties of infested palms					No. of infested palms	The varieties of infested palms				
		H./100 Date palm	Z./100 Date palm	B./100 Date palm	S./100 Date palm	Amr./100 Date palm		H./98 Date palm	Z./97 Date palm	B./100 Date palm	S./100 Date palm	Amr./100 Date palm
Jan.	0	0	0	0	0	0	4	2	2	0	0	0
Feb.	8	2	2	2	2	0	2	1	1	0	0	0
Mar.	10	1	4	2	2	1	7	2	2	2	1	0
Apr.	7	3	3	1	0	0	0	0	0	0	0	0
May.	9	3	3	2	1	0	3	2	1	0	0	0
Jun.	7	2	2	2	1	0	0	0	0	0	0	0
July.	5	2	2	1	0	0	0	0	0	0	0	0
Aug.	10	3	2	2	2	1	2	0	2	0	0	0
Sep.	7	2	2	1	1	1	5	2	2	1	0	0
Oct.	7	2	2	2	1	0	6	2	2	1	1	0
Nov.	12	3	3	3	2	1	10	2	2	2	2	2
Dec.	5	2	2	1	0	0	8	2	2	2	1	1
Total	87	25	27	19	12	4	47	15	16	8	5	3
Total %	17.4	25	27	19	12	4	9.4	15.3	16.5	8	5	3
S.E. ±	0.89	0.26	0.28	0.23	0.25	0.14	0.96	0.28	0.26	0.26	0.19	0.18

Table (1a). Cont.

Months	2012						2013					
	No. of infested palms	The varieties of infested palms					No. of infested palms	The varieties of infested palms				
		H./98 Date palm	Z./95 Date palm	B./100 Date palm	S./100 Date palm	Amr./100 Date palm		H./97 Date palm	Z./94 Date palm	B./98 Date palm	S./100 Date palm	Amr./100 Date palm
Jan.	2	0	2	0	0	0	0	0	0	0	0	0
Feb.	2	1	1	0	0	0	2	1	1	0	0	0
Mar.	5	2	2	1	0	0	4	2	2	0	0	0
Apr.	0	0	0	0	0	0	0	0	0	0	0	0
May.	2	1	1	0	0	0	2	1	1	0	0	0
Jun.	0	0	0	0	0	0	0	0	0	0	0	0
July.	0	0	0	0	0	0	0	0	0	0	0	0
Aug.	2	0	2	0	0	0	2	0	2	0	0	0
Sep.	3	1	2	0	0	0	2	1	1	0	0	0
Oct.	5	2	2	1	0	0	4	2	2	0	0	0
Nov.	7	2	2	2	1	0	6	2	2	2	0	0
Dec.	5	2	2	1	0	0	4	2	2	0	0	0
Total	33	11	16	5	1	0	26	11	13	2	0	0
Total %	6.7	11.2	16.8	5	1	0	5.3	11.3	13.8	2	0	0
S.E. ±	0.66	0.26	0.26	0.19	0.08	0	0.58	0.26	0.26	1.67	0	0

H=Hayani S=Samani

Z=Zaghloul

B=Bent Eshia

## **II- The variations in amounts of chemical components between healthy and infested palm varieties by *Rhynchophorus ferrugineus* :**

The chemical components for trunk tissues of date palm varieties (healthy and infested) are illustrated in table (2). The obtained constituent of chemical components was: B-glycosidase, Peroxidase, phenol, Alpha esterases, Phenol oxidase estimated as follows:

### **1-Peroxidase:**

Highly significant variance of this material in different varieties ( $F = 43.37^{**}$ ) was observed the means of peroxides were 5.76 and 7.16 mg with healthy and infested palm trees respectively. Obtained values of peroxides were higher in infestation trees from healthy trees. Positive insignificant correlation in infested trees while negative insignificant correlation with healthy palms Table (2)

### **2 -Alpha esterases**

Highly significant variance of this material in different varieties ( $F = 59.94^{**}$ ) was obtained the means of alpha esterases were 64.8 and 96.666 mg with healthy and infested palm trees. Quantities of Alpha esterase's were high in infestation trees compared with health trees. Positive insignificant correlation in infested while negative insignificant correlation with recorded healthy palm Table (2)

### **3-Phenol oxidase**

Highly significant variance was recorded of this material in different varieties ( $F = 41.6^{**}$ ) the means of peroxidase were 2.012 and 2.742 mg with healthy and infested palm trees. Obtained values for Phenol oxidase were higher in infestation trees from healthy trees. Negative insignificant correlation in was obtained with infested trees while negative insignificant correlation observed with health palms trees Table (2)

### **4-B-glycosidase**

Highly significant variance was recorded of this material in different varieties ( $F = 30.43^{**}$ ), the means of B-glycosidase were 4.697 and 7.808 mg with healthy and infested palm trees. Quantity of B-glycosidase was high in infested trees compared with healthy trees. Positive highly significant correlation observed with infested trees while positive insignificant correlation was recorded with healthy palms trees Table (2)

### **5- Phenols**

Highly significant variance obtained of this material in different varieties ( $F = 24.13^{**}$ ), the means of B-glycosidase were 1215.53 and 2100 mg with healthy and infested palm trees. Quantity of Phenols was high infested trees compared with health trees. Positive significant correlation observed in infested trees while positive highly significant correlation noticed with healthy palms trees Table (2)

In this respects, data in Table (3) showed some other that the chemical components for trunk tissues of date palm trees and variations amount of these components in healthy and infested date palm varieties. The most obtained constituent were: nitrogen, Free amino acids, total carbohydrates, Tannins, Total proteins, total nitrogen, estimated as follows:

2-

### **1-Tannins**

No significant variance of this material in different varieties, the means of tannins were 110.2 and 110.13 mg with healthy and infested palm trees. Quantity of tannins no different between infested trees and healthy trees. Negative insignificant correlation in with infested while positive highly significant correlation with healthy palm trees Table (3)

### **2-Total carbohydrates**

Highly significant variance recorded of this material in different varieties ( $F = 56.872^{***}$ ), the means of total carbohydrates were 1215.53 and 2100 mg with healthy healthy and infested palm trees. Quantity of total carbohydrates was high in infested trees compared with healthy trees. Positive highly significant correlation was observed in infested while negative in significant correlation with healthy palm trees Table (3)

### **3-Total proteins**

Highly significant variance was noticed of this material in different varieties, ( $F = 94.99^{***}$ ) the means of total proteins were 110.17 and 165.87 mg with healthy and infested palm trees. Quantity of total proteins was high in infested trees compared health trees. Positive insignificant correlation in infested trees while was negative significant correlation with healthy palm trees Table (3)

### **4- Total nitrogen**

Highly significant variance was noticed of this material in different varieties ( $F = 66.48^{***}$ ), the means of total nitrogen were 466.13 and 567.80 mg with healthy and infestation palm trees. Quantity of total nitrogen was high in infestation trees compared with healthy trees. Positive insignificant correlation recorded in infested trees while negative highly significant correlation was obtained with healthy palm tree Table (3)

### **5- Free amino acids**

Insignificant variance recorded this material in different varieties; the means of free amino acids were 317.33 and 317.87 mg with healthy and infested palm trees. Quantity of free amino acids was different between infested and healthy trees. Negative highly significant correlation in infested while negative highly significant correlations with healthy palm trees Table (3) General there were increase of amount in peroxides, B-glycosidase, Total carbohydrates and free amino acid in infested palm compared with healthy palm, trees in the other direction the decrease amount of phenols, tannins and total proteins in infested palm.

Farazmand (2002) Found that determined that the reasons of food preference of RPW, the vascular tissues of different cultivars of date palms trees (Mazafati, Rabbi, Halileh, Zardan and Pimazoo) and a native wild palm (*Nannorrhops ritchiana*) were analyzed and dry weight, crude fiber, total sugar, fat and 12 chemical elements the interaction of various nutrient components affected the vital qualifications of *R. ferrugineus*. The most effective were sugar and calcium. Sugar was correlated with growth, daily oviposition and reduction in mortality, while increase in calcium clearly inhibited RPW growth record and Mesallam, *et al.* (2010). Revealed that all tested parameters in significantly influenced by changing the contents of total carbohydrates except with larval period which demonstrated highly

significant and sex ratio as well as pre-oviposition period that proved to be significantly correlated at 0.05 level of probability. While the total sugars content exhibited negative and insignificant correlations with the examined biological aspects with the exception of larval period, adult emergence, sex ratio and oviposition period that proved to have insignificant and positive correlation coefficients.

## REFERENCES

- Abd El-Fattah, S.M.(2010). Study of some recent trends on *Rhynchophorus ferrugineus* (Oliv.) infesting date palm trees in Sharkia Governorate.Ph.D.Thesis, Fac. of Agric., Zagazig University: 188 310-311.11ref.
- AbdEl -Salam ,A.H.;S.S.Awadalla and K.M.Abdel-Hamid (2008) . Evaluation of infestation degrees ,age,stem height and occurrence of the red palm weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) on certain date palm ultivars in Egypt , Journal of Agricultural Science,Mansoura University,33(10):7549-7567.
- Abdel-Megeed, M.E.; H.A. Zedan and G.B. El-Saadany (2004). The integrated management for controlling pests of date palm. Kenza Group Pub., Egypt, 483 pp (Arabic Language).
- Aly, A.G. and E.A. Elwan (1995). Survey of insect pests and mites infesting palm trees in Oman Sultanate. Egypt. J. Appl. Sci., 10 (4): 261-264.
- Azam,K.M.;S.A.Razvi and Issa Al-Mahmuli (2003). Survey of red palm weevil, (*Rhynchophorus ferrugineus* Olivier) infestation in date palm in Oman Int.Conf.on Date palm and Joint Events, Kingdom of Saudi Arabia, Ministry of Higher Education, King Saud Univi., Qaseem Branch, College of Agric. &Vet Med. Sept., 16-19, Pa.239-245.
- Barranco, P.; J. Pena; M.M. Martin and T. Cabello (2000). Host rank for *Rhynchophorus ferrugineus* (Olivier, 1790) and host diameter. (Coleoptera: Curculionidae). Boletin de Sanidad Vegetal Plagas, 26 (1): 73-78.
- Bradford, M.M. (1976).A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principle of protein –dye binding .Anal.Biochem. 72:248-254.
- Brand, E. (1917). Coconut red weevil, some facts and fallacies. Trop. Agric., Peradeniya, XIX (1): 22-24.
- Crompton, M.and Birt, L.M. (1967). changes in the amounts of carbohydrates, phosphagen, and related compounds during the metamorphosis of the blowfly, *Lucilia cuprina*.J.Insect physiol., 13:1575-1595.
- Dembilio, O; J.A. Jacas and E. Llacer (2009). Are the palms *Washingtonia filifera* and *Chamaerops humilis* suitable hosts for the red palm weevil, *Rhynchophorus ferrugineus* (Col., Curculionidae). J. Appl. Entomol., 133, 565–567.
- Dubios,M.;Gilles ,K.A.;Hamilton ,J.k.;Rebers,P.A.and Smith, F. (1956).Colorimetric method for determination of sugars and related substances.Analyt.Chem.,28:350-356.



- Eissa, L.S. (1986). Insect pests attacking date palm in the State of Qatar. The Second Symposium on Date Palm in Saudi Arabia, II: 304-314.
- EPPO (European and Mediterranean Plant Protection Organization) (2008). Data sheets on quarantine pests, *Rhynchophorus ferrugineus*. EPPO Bull., 38: 55 – 59.
- Farazmand, H. (2002). Investigation on the reasons of food preference of red palm weevil, *Rhynchophorus ferrugineus*( Oliv. ) . [ Persian] Applied Entomology and Phytopathology. 70: 1, Pe49-Pe61, 11-12. 6 refs.
- Ghosh, C.C. (1912). Life histories of Indian insects: III The rhinoceros beetle (*Oryctes rhinoceros*) and red palm weevil (*Rhynchophorus ferrugineus*). Memoirs of the Department of Agriculture, India, II (10): 205-217.
- Hammad, S.M.; A.A. Kadous and M.M. Ramadan (1986). Preliminary studies on the population density of some date-palm insects at Al-Hassa region (Saudi Arabia) using a mercury-vapour light trap. Proc. of the Second Symposium on Date Palm in Saudi Arabia II: 321-330.
- Ishaaya, I. (1971). Observations on the phenoloxidase system in the armored scales *Aonidiella aurantii* and *Chrysomphalus aonidum*. Comp. Biochem. Physiol., V.39B:935-943.
- Kähkönen, M.P.; Hopia, A.I.; Vuorela, H.J.; Rauha, J.P.; Pihlaja, K.; Kujala, T.S. and Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. J. Agric. food chem., 47:3954-3962.
- Lee, Y.P. and T. Takabashi (1966). An improved colorimetric determination of amino acids with the use of ninhydrin. Anal. Biochem., 14:71-77.
- Leefmans, S. (1920). De palmsnuitkever (*Rhynchophorus ferrugineus* Oliv.) [The palm weevil, *R. ferrugineus*]. Meded. Inst. Plantenziekten Buitenzorg, 43: 90 pp.
- Lindroth, R.L. (1988). Hydrolysis of phenolic glycosides by mid gut  $\beta$ -glucosidase in *Papilio glaucus* subspecies. Insect biochem. V. 18:789-792.
- Liver, R.J.A.W. (1969). Pests of the coconut palm. FAO Rept., Rome: 113-117.
- Mesallam, T.I.I. (2010). Effect of different date palm varieties on some biological aspects of the red palm weevil, *Rhynchophorus ferrugineus* (Olivier) and its control. Ph.D. Thesis, Faculty of Agriculture, Zagazig University: 293 pp.
- Ni, X.; Quisenberry S.S.; T. Heng-Moss; J. Markwell; G. Sarath; R. Klucas and F. Baxendale (2001). Oxidative responses of resistant and susceptible cereal leaves to symptomatic and nonsymptomatic cereal aphid (Hemiptera: Aphididae) feeding. Journal of Economic Entomology 94:743-751.
- Nirula, K.K. (1956). Investigations on the pests of coconut palm. IV- *Rhynchophorus ferrugineus* F.. Indian Coconut J., 9 (4): 229-247.
- S.A.S Institute (1985). SAS user's guide: statistics, 5th Ed SAS Institute, Cary, NC
- Sadasivam, S. and A. Manickam (1991). Amino acids and proteins. In biochemical methods for agricultural sciences (Wiley eastern limited and Tamil Nadu agricultural university, Coimbatore). P.33-95.





Table (2): Enzymes analysis of different varieties of date palm trees (healthy and infested) with *R.ferrugineus*.

Chemicals analysis	Varieties										Mean	F Inter action	r Infestation palm	r Health palm	
	Kinds		Hayani	Bent Ashia	Amri	Samani	Zaglou								
	Healthy	Infest.													
Peroxidase (Ug GA/gdw)	Healthy		7.03	7.92	6.29	5.98	5.76	6.596 <sup>b</sup>			43.37				
	Infest.		9.06	8.56	7.96	5.66	7.16	7.68 <sup>a</sup>			***				-0.0152ns
Alpha esterase's (Ug.a-naphthol /min/gdw)	Healthy		67	58.67	70	65	63.33	64.8 <sup>b</sup>			59.94				
	Infest		66.33	94.33	74	124	124.33	96.666 <sup>a</sup>			***				-0.123ns
Phenol oxidase (O.D.units /min/gdw)	Healthy		2.44	2.47	0.59	3.39	1.17	2.012 <sup>b</sup>			41.63				
	Infest.		2.48	2.86	1.31	4.77	2.29	2.742 <sup>a</sup>			***				-0.02934 ns
B-glycosidase	Healthy		12.90	9.22	5.32	5.81	5.79	4.697 <sup>b</sup>			30.43				
	Infest.		5.82	4.55	3.82	4.14	5.17	7.808 <sup>a</sup>			***				+0.9137**
Phenols (Ug GA/gdw)	Healthy		1380	1082.67	1143.33	1191	1280.67	1215.53 <sup>b</sup>			30.43				
	Infest.		1536.67	2447.67	1181	2788.33	2546.33	2100 <sup>a</sup>			***				+0.7207**

Table (3): Chemical analysis of resistant materials in different varieties (healthy and infested) of date palm with *R.ferrugineus*.

Chemicals analysis	Varieties mg/ gm										Mean	F Inter action	r Infestation palm	R Health palm	
	Kinds		Hayani	Bent Ashia	Amri	Samani	Zaglou								
	Healthy	Infest.													
Tannins (Ug tannic acid/gdw)	Healthy		112	97.67	112	115.67	113.33	110.13							
	Infest.		120	96.33	108.67 <sup>33 bc</sup>	126.33	99.67	110.13 <sup>a</sup>							-2403
Total carbohydrates (mg/gdw)	Healthy		162.33	149.67	255	256	242.33	110.17			56.872				
	Infest.		65.33	126.33	246.67	255	136	165.87 <sup>a</sup>			***				+0.82524**
Total Proteins (mg/gdw)	Healthy		1.91	2.32	2.97	4.10	3.14	2.888 <sup>b</sup>			94.99*				
	Infest.		2.20	3.56	2.86	4.52	5.03	3.636 <sup>a</sup>			**				+0.17012ns
Total nitrogen (Ug/gdw)	Healthy		313	371.33	477.33	665.33	503.67	466.13 <sup>b</sup>			66.48				
	Infest.		340.67	544.67	458.67	713.33	781.67	567			***				+0.011824 ns
Free amino acids (Ug alanine/min/gdw)	Healthy		253	266.67	340.67	403	323.33	317.33 <sup>a</sup>			3.5*				
	Infest.		247	272.33	345.67	412	312.33	317			***				-0.166*