BIOASSAY AND PATHOGENICITY OF WHEAT SEED GALL NEMATODE *ANGUINA TRITICI*

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Abstract :

Highest infection percentage recorded in Abo-graib and wahe CVs. (83.3%) followed by Smeto (80.3%) while less infection percentage was in Rezgare and Al-Aez CVs. (33.3%). more galls (14 galls/ spike) formed in Sham-2 C.V. followed by Abo-graib, Smeto, Tammoz-2, Maxipak and Al-Aez respectively. highest number of nematode (12773) occurred in large galls, while the lowest (8145) in small galls. *A. tritici* population density increased dramatically with increase in galls size reached a peak (15112 second stage juveniles/gall) in big size gall. Results revealed that the vitality of second stage juveniles reached its maximum level (8.87%) at 12 Cº with no significant differences with the validity percentage at 6 Cº while it reached its minimum level (1%) at 20 Cº.

KEYWORDS: *Bioassay, pathogenicity, Wheat, Anguina tritici.*

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most economical important crops in the world. 65% of produced wheat is consumed by human, whereas 21% - 14% for animal feeding and industry (Gooding and Davies, 1977). According to USAID (2006), Iraq currently consumes nearly 4 million tons of wheat annually, yet only produces 500,000 tons of qualified wheat. Thus more than 85% of consumed wheat in Iraq imported, the current average is 800 Kg/h. The Iraqi Kurdistan Region produces about 50% of the wheat produced in Iraq.

According to Bhatti,*et al.*,(1978) Ear- cockle was the oldest reported disease of wheat which was caused by wheat seed gall nematode *Anguina tritici,* It is one of the major aerial diseases and causes sustainable losses in wheat crop of tropical and sub-tropical countries (Kort,1972). It is present wherever wheat is grown and this pest still common in Eastern Europe and in part of Asia and Africa (Agrios, 2005). From the first record (1921) of this nematode in Iraq (Rao, 1921), *A. tritici* remain the major pest in Iraq occurred in the most wheat growing areas by 22.9 to 45% on mexipac c.v. of Wheat.(Al-Beldaw; *et.al*.1974) increased to 75% on the same cultivar in Duhok Province in 1989 (Stephan and Antoon,1990), Ami, *et.al*.,(2004) reported that the percentage of infestation by galls reached its maximum value (50%) in bread wheat in Bashika – Kurdistan region of Iraq.

Ear- cockle disease reduces market price and human consumption of wheat (Paruthi and Bhatti, 1988), with significant reduction in the protein and gluten contents of the flour product of infested wheat with seed galls (Mustafa, 2009). This study aimed to test vitality of 2nd stage juveniles of *A. tritici* under different temperature and it's pathogenicity toward some common wheat cultivars in addition to its effect on quality of wheat flour.

Materials & Methods:

Seed galls (ear-cockle) collection: Galls used in this experiment were collected handmade from infested bread wheat samples brought from Faydia Silo.

Soil sterilization:

Soil was sterilized by Formalin 1% (1liter/m-³) after sieving to discard stones and soil lumps, and then placed on thick layer of polyethylene. Formalin was mixed thoroughly with soil which was covered by other pieces of polyethylene and closed tightly to prevent any escape of formalin, after 48 hours soil aerated for a week to remove residual of Formalin (Mustafee and Chattopadhyay, 1981).

Physical and chemical analysis of soil:

a- Soil texture: it was determined by hydrometer method to evaluate percentage contents of clay, silt and sand (Jaiswal, 2003).

b- Electrical conductivity (E.C) was estimated by using Conductivity Bridge of soil extract of 1:1.(Dipak and Haldar, 2005).

C- Percentage of organic matter: was measured in the soil by wet digestion method with

concentrated sulphuric acid according to (Black, 1965).
d-Soil pH by pH meter in the soil extract of 1:1 (Dipak and Haldar, 2005).

Susceptibility of wheat cultivar: nine wheat cultivars **(**Abo-qraib, Aba 95, Tammoz-2, Maxipak, Rezgare, Al-Aez, Wahe, Smeto, and Sham-2**)** were sown at the rate of 5 seeds/ pots (20 cm) in diameter containing sterilized soil which was infested directly with five galls, in 1cm deep holes between seeds at the rate of one gall/hole, Pots were plunged randomly in the trenches. This experiment consisted of 18 treatments (9 wheat C.V. X 2 levels of infestation) performed as factorial experiment in complete Randomized design (CRD) with 3 replication for each treatment, carried out in 2014 growing season, in one fields of the College of Agriculture, University of Duhok , Pots were irrigated when needed and the following infection criteria were calculated :

1- Infection percentage according to the following equation:-

$$\text{Infection percentage} = \frac{\text{Number of Infected plants}}{\text{Total number of plants in each pot}} \times 100$$

2-Number of galls/spike.
3-Number of seeds/spike.
4- Reduction percentage of seed number /infected spike according to the following:-

$$\frac{\textbf{Seed number /spike in control treatment – seed number/spike in infested soil}}{\textbf{Seed number in control treatment}} \times \textbf{100}$$

Effect of size and weight of galls on nematode population: Galls were divided according to their size to (Small, Moderate and large) and weight [18.3 milligram (19.6-16.5), 11.5mg (12.7-11.3) and 3.6mg (4, 1-3.1)]. Each galls was opened in 6.5 Cm diameter Petri-dishes filled with 2ml of distilled water (D.W) with the aid of two needles then transferred to a 250 ml beaker, volume was adjusted by adding D.W water and number of juveniles were calculated by the aid of counting dish and stereomicroscope.

Effect of different temperature on vitality of second stage juveniles: Nine Petri dishes were divided into 3 groups and 2ml of nematode suspension (200 second stage juveniles) was transferred into each Petri dish and after covering 1st and 2nd group placed in refrigerator at 6, 12 C° respectively while the third group left at laboratory conditions (20 c◦). Percentage of nematode vitality was calculated according to the following equation:-

Percentage of nematode vitality = (number of moving nematodes in each replicate ÷ Total number of nematode in the same replication) X 100. This experiment consists of 5 treatment and run out as CRD.

Effect of different temperature on the emergence of 2nd stage juveniles from galls: Four different temperatures including (5, 10, 15 and 20 C°) were selected to test their effect on juveniles emergence from their galls which were placed in Petri dish contained a small amount of moist soil at the rate of one gall / petri dish. Examining of galls was done after each 48 hrs. for the period of 20 days to determine the emergence of juveniles.

Results and Discussion:

1- Physical and chemical analysis of soil:

Results of soil analysis(Table,1) revealed that the texture was sandy loam which is suitable for nematode movement as well as chemical characters involving soil salinity, organic matter and soil pH were suitable for most plant parasitic nematode (Ismail, 1998).

Table (1): Physical and chemical analysis of soil.

Clay %	Silt %	Sand %	Texture	pH	Organic matter	Electric conductivity (salinity)
17.13	23.23	59.65	Sandy loam	8.13	1,38	0.52

2- Susceptibility of wheat cultivars:

All wheat cultivars used in this trial were infected by *Anguina tritici* with some significant differences in their susceptibility. Highest infection percentage recorded in each of Abo-ghraib and wahe CVs. (83.3%) followed by Smeto (80.3%) while less infection percentage was in Rezgare and Al-Aez CVs. (33.3%) (Fig.1) which means there were differences in susceptibility of those cultivars to ear-Cockle disease and also in ability of *Anguina tritici* to infect those cultivars.

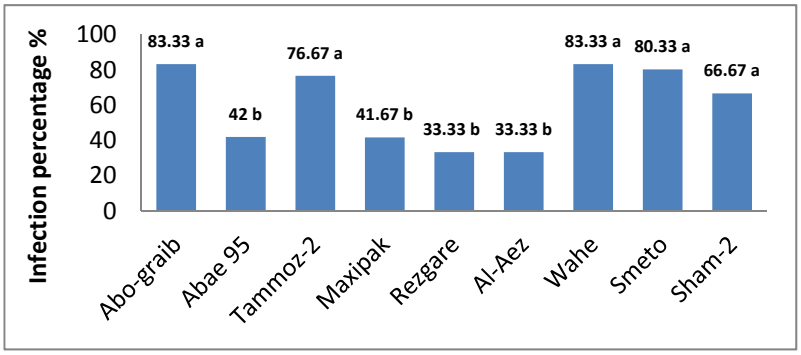

Figure (1): Infection percentage *Anguina tritici* with on different wheat cultivars.

Figure,2 illustrates that more galls (14 galls/ spike) formed in Sham-2 C.V. followed by Abo-graib, Smeto, Tammoz-2, Maxipak and Al-Aez respectively with significant differences between same cvs. No galls appeared on Abae 95 and Rezgare while both showed symptom of infection, this phenomenon indicated that *A. tritici* can't completed its life cycle after climbing of seedlings, which might be attributed that cultivar failed to supply adequate essential nutrients for nematode juveniles (Taher, 2012).

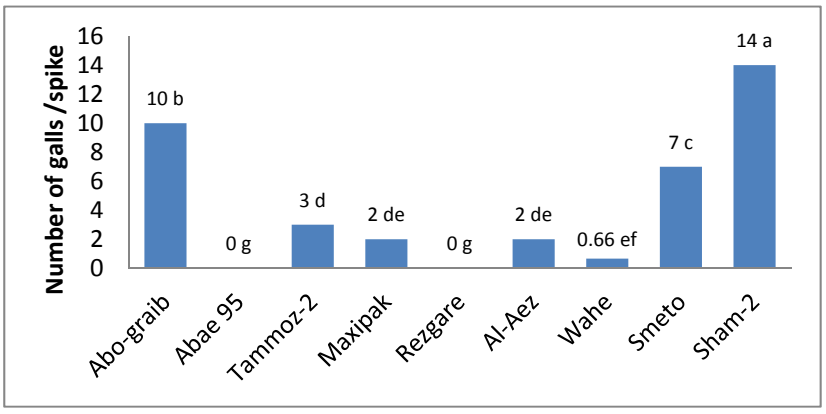

Figure (2): Number of galls / spike of different wheat cultivars

Differences in gall number/plant are also due to host genotypes and their susceptibility to *A. tritici* (Hamood and Fattah, 1989). Ear-cockle disease caused reduction in seed production in all tested cultivars. More reduction percentage which were 93.23, 86.93 and 86.93% was recorded from Rezgare, Abae 95 and Tammoz-2 respectively while the lowest were 36.67 and 38.87% recorded from Smeto and Al-Aez, the same results were recorded in different wheat cultivars according to the extent of their susceptibility (Al-Beldawi *et al.,* 1977**;** Fattah, 1988; Saleh and Fattah, 1990; Stephan *et al.,* 2000; Mustafa 2009; Taher, 2012).

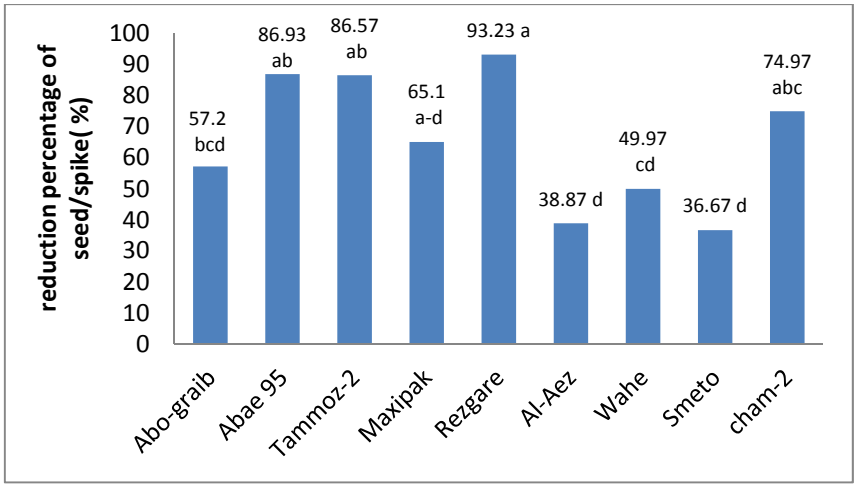

Figure (3): Reduction percentage of seeds/spike

3- **Effect of gall size and weight on nematode population density:**

Results indicate significant effect of gall size on the nematode population density, thus highest number of nematode(12773) occurred in large galls, while the lowest (8145) in small galls (Table2). This result consistent with finding of Ami, et al, (2004); Esser *et al*., 1991 and Taher, (2012).

Table (2): Effect of galls size on the population density of *Anguine tritici*.

Galls size	Nematode population density (Nematode/ gall)
Large	12773 a (10114 –13881)
Moderate	11919 a (9766 – 12088)
Small	8145 b (7255 – 9617)

Number of juveniles/gall depend on many factors such as wheat cultivar, gall size, environment conditions and soil biosphere characters in addition to their behavior in different geographical regions (Taher, 2012). Weight of galls also has noticeable effect on *A. tritici* population density which increasing dramatically with increase in galls size and reached a peak (15112 nematodes/gall) in big size gall, while number decreased to lowest level (3978 nematode/gall) in small size (table, 3) which means reduction in nematode population by 73.7%.

Table (3): Effect of galls weight on the population density of *Anguine tritici*.

Galls weight	Nematode population density (Nematode/ gall)
18.3(19.6-16.5)	15112 a
11.5 (12.7-11.3)	9440 b
3.6 (4.1-3.1)	3978 c

Regression analysis and correlation coefficient (Fig. 4) demonstrate positive relation between galls weight and nematode population density. From regression equation it was found that an increase of one unit (one mg of galls) in used galls followed by increasing in nematode population density by 803.19 with high determining factor, meaning that it can rely on this equation by 90.7%. Increase in nematode population density in big size galls and heavier ones might be due to the presence of more space in large galls to contain more juveniles during penetration of ovary wall (Ami, et al, 2004) on the other hand more weight and size of newly formed embryo supply more nutrient for feeding of juvenile and increase the ability of female to laying more eggs, otherwise big and heavey galls may belong to durum wheat Cultivars which are always bigger and have more protein contain that have effect in female fertility as we mentioned before.

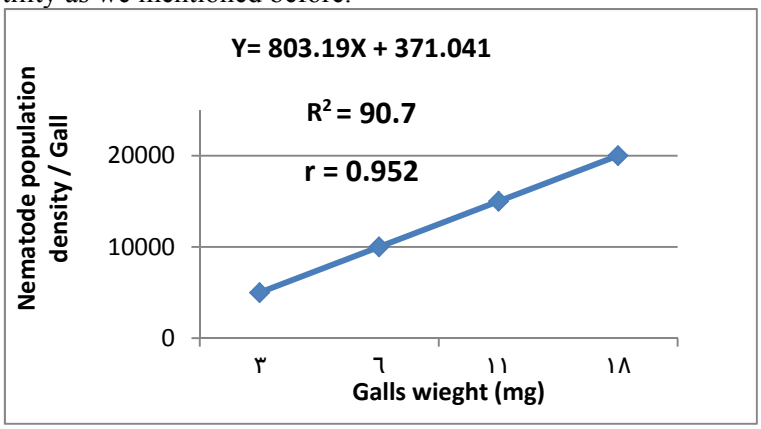

Figure (4): Linear relation, regression equation and correction coefficient between gall weight and nematode population density.

4- Effect of different temperature on vitality of second stage juveniles:

The results of revealed that the vitality of second stage juveniles reached its maximum level (8.87%) at 12 Cº with no significant differences with the validity percentage at 6 Cº while it reached its minimum level (1%) at 20 Cº as well as the vitality percentage increased slowly by the time and stretch 8.33% after 12 days from the time of gall opening with no significant differences with vitality percentage after 9 days (Table 4). On the other hand the interaction between temperature degrees and examining time showed its noticeable effect on vitality. In general maximum level of vitality was (13%) recorded after 12 days at 12 Cº with no significant differences with vitality after 9 days at the same temperature and with that after 9 and 12 at 6Cº while none of the juveniles moved after 3 days at 20 Cº. There are many studies on effect of planting date on infection and all are found that there are differences in infection behaviors of juveniles (Mustafa,2009; Radjender *et al.*, 2009; Taher, 2012) and most important factor in delay or early planting are temperature that influence activity of juveniles. The sudden high temperature may not be a catalyst to re-vitality of juveniles as they were in dormancy stage or cryptobiosis, in fact this study needs further confirmation and granting juveniles more time to check their vitality,

Table 4: Effect of different temperature degrees on the percentage of vitality of second stage juveniles of *A. tritici.*

Temperature	Percentage of vitality (%)				Effect of temperature degree
	1st Reading	2nd Reading	3rd Reading	4th Reading	
6 Cº	1.5 c	3 c	10 a	10 a	8.63 a
12 Cº	4c	7.5 bc	11 a	13 a	8.87 a
20 Cº	0.0 c	0.5 c	1.5 c	2 c	1 b
Effect of reading	1.83 b	3.67 b	7.5 a	8.33 a	

5- Effect of different temperature on the emergence of 2nd stage juveniles from galls: Results illustrated that the juveniles emerged from galls after 20, 16, and 14 days at 5, 10, 15 and 20 Cº respectively with the presence of moisture (Table 5)

Table5 : Effect of different temperature on the emergence of 2nd stage juveniles of *A. tritici* from the galls.

Temperature	Period of time in days after which juveniles emergence ware examined									
	2	4	6	8	10	12	14	16	18	20
5	-	-	-	-	-	-	-	-	-	+
10								+		
15	-	-	-	-	-	-	-	+		
20	-	-	-	-	-	-	+			

+ = period after which juvenile exited from galls. - = juveniles were not exited

-which indicates that the juveniles need a longer period of time for emergence at low temperature that means their vitality increased with increasing of temperature that stimulated juveniles for getting out from galls. That means that the temperature plays an important role in emergence of second stage juveniles.

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کورتی:

بەرزترین ریژا توشبوون هاتە تومارکرن ل هەردوو جورێن گەنمی ابو غریب و الواحە (٨٣.٣٪) لدیفدا سمیتو (٨٠.٣٪)، و کێمترین ریژا توشبوون ل هەر دوو جورێن رزگاری والعز ٣٣٪. زورترین ریژا دروستبونا گەنما رەشبوو (١٤ گەنما رەشبوو/گولا گەنمی) لجورێ شام– ٢ و لدیفدا أبو غریب، سمیتو، ٢ تموز، ماکسیباك. زوّرترین هژمارا نیماتودا (١٢٧٧٣) ل گەنمێن رەشبووییّن مەزن و کیمترین هژمار د گەنمێن رەشبووییّن بچوك (٨١٤٥) هاتیە تومارکرن. چرییا هژمارێن *Anguina tritici* زیّدە بوون دگەل زیّدەبوونا قەبارییّن گەنمێن رەشبوو و گەهشتیە بلندترین ئاست ١٥١١٢ (گەنما رەشبوو/گولا گەنمی) و هەر وەسا د ئەنجامێن ڤەکولینێ بو مە دیاردکەت چالاکی و لڤینا نیماتودا گەهشتیە بلندترین ئاست (٨.٨٧٪) ل پلا گرمیا ١٢{C و هەروەسا جیوازیەکا بەرچاف نەبو د چالاکی و لڤینا نیماتودا لژێر پلا گەرمیا ٦ C(١٪) ف ٢٠ C°. و گەهشتە کیمترین ریژە (١٪) لژێر پلا گەرمیا ٢٠ C°.

اختبار الحيوية والقدرة الإمراضية لنيماتودا تثألل حبوب اللحنطة *Anguina tritici*

الخلاصة:

سجلت أعلى نسبة الإصابة في صنفي أبو غريب و الواحة (٨٣.٣٪) تليها سميتو (٨٠.٣٪)، بينما كانت أقل نسبة الإصابة في صنفي ريزكاري والعز ٣٣٪. اعلى نسبة تكوين الثنااليل (١٤ ثألولة /سنبلة) في الشام– ٢ تليها أبو غريب، سميتو، ٢ تموز، ماكسيباك على التوالي. وصل عدد من يافعات الطور الثاني الى (١٢٧٧٣) في الثنااليل الكبيرة و في ثنااليل الصغيرة(٨١٤٥). الكثافة العددية *Anguina tritici* ازادة مع زيادة في حجم الثنااليل و بلغت ذروتها ١٥١١٢ (يافعات الطور الثاني/ ثألولة) في الثنااليل كبيرة الحجم. وكشفت النتائج أن حيوية يافعة الطور الثانية وصلت مستواه الأقصى (٨.٨٧٪) في درجة الحرارة ١٢C° مع اختلافات غير معنوية في حيوية اليافعات عند درجة الحرارة ٦ C° بينما وصلت إلى أدنى مستوى لها (١٪) في ٢٠ C°.