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BIOLOGICAL AND CONTROL STUDIES ON *RHOPALOSIPHUM PADI* INFESTING WHEAT PLANTS*, TRITICUM AESTIVUM* AT MENOUFIA GOVERNORATE, EGYPT

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ABSTRACT: **Seasonal fluctuations of wheat aphid, *Rhopalosiphum padi* L., infesting five wheat varieties, were investigated under field conditions along 2020/2021 season at Menoufia governorate, as well as, biological and biocontrol studies on this pest were conducted, moreover, residual of pesticides were determined in seeds, straw and soil of treated wheat plants.**

As for the population density of *R. padi*, the highest numbers were recorded in March 2021 on Gemiza 11 variety. The life cycle of *R. padi* lasts 10.6 days ± 0.5 , the adult viviparous female lived 12.2 ± 0.4 days spends two days without putting larvae, put 28.4 ± 1.3 individuals during the reproductive period (10.2± 0.4) days, the life span lasts 20.8 ± 0.8 days.

Abamectin 3.6 % EC (double dose) and Congest 15% SC were effective in reducing the number of aphids per leaf, recording 46.67- 100% reduction and recording residues in wheat straw and wheat seeds less than the maximum residues limits. The tested treatments did not affect the germination process. The tested bio agents as nonconventional insecticide could be considered suitable for aphid control and safe for human and environment. It could be recommended the use of Abamectin and Imidacloprid in integrated aphid management programs.

Key words: **Biology, Ecology, Biocontrol, Bio-assessment, residual analysis, Wheat aphid.**

INTRODUCTION

Wheat crop, *Triticum aestivum* is considered the main aspect of food safety in Egypt; it is the largest importer of wheat cosmopolitan. Egyptians depend on bread as the main component of the meal (FAO, 2015). Wheat plants attacked with many pests causing great loss in the yield. The aphid, *Rhopalosiphum padi*, is one of the most harmful pests that affect wheat production all over the world; it has two ways of damage, the direct damage caused by the insects themselves when they suck the plant sap, and the indirect damage caused by the viruses transported by the aphid mouthparts (Tantawi *et al*., 1986, Blackman and Eastop 1986). The late sown wheat faces aphid outbreaks causing great damage (Bhambhro 2002). EL-Mitwally *et al*., 2013 studied the density of *R. padi* on the five wheat varieties: Giza 168, Sids 1, Gemiza 7, Gemiza 9, and Sakha 93 for two years in Egypt, and found that there were significant differences among the five tested varieties for *R. padi* population dynamic and density, reporting that Sids 1 was the most susceptible to *R. padi*, while Sakha 93 variety was the least. Araya *et al.,* 1987 elucidated after a study of *R. Padi* population along two seasons

that, the most convenient weather is in mid-October and early March, while Ullah *et al.,* 2020 indicated that the highest population of *R. Padi* recorded after the mid of March and the least numbers recorded during the first week of February. The increase in wheat seeds size, without any changes on grain number, results great increases in a yield boost under field conditions (Calderini *et al.,* 2020).

From the previous review, the present study was conducted to throw the light on the ecology, biology and control of wheat aphid, *Rhopalosiphum padi* L., infesting five wheat varieties under field conditions, as one of the important pests of wheat in Egypt.

MATERIALS AND METHODS

1- Ecological studies

Air temperature and relative humidity were monthly counted (Table 1) noted daily during the study period.

Wheat varieties under study were Misr1, Sids 14, Giza 171, Gemiza 11, and Gemiza 12, were obtained from shopping center of the Ministry of Agriculture, planted as broadcasting method on 30 October 2020.

Fifteen days after planting, from each wheat variety, five wheat plants as a sample were collected from the study fields in cloth bags, samples were replicated five times, transferred to the laboratory and directly examined with a hand lens 10 x and the numbers of aphid stages were counted. Weekly samples were continued till the end of the experiment.

2- Biological Studies

Rearing cages (Fig 1) were modified to be lighter and smaller to not harm the plant leaf, (A plastic cylinder with a diameter of 2 cm and a height of 1 cm covered with a layer of muslin gauze and mounted on a 6 cm metal hair clip with a circular base of 3 cm made of foam to act as a support for the plant sheet to strength the weight of the hollow part of the cages).

Ten adult females (viviparous) were selected as the source of the obtained larvae. The individuals (50 larvae) were put separately under the modified cage and daily examined with a hand lens 10 x with the aid of a fine brush, and the results were tabulated as Darwish (1983a), Gautam and Verma (1983), and Swelam (2012) elucidated.

3- Control Studies:

The experiment was applied under field conditions to obtain a clear image about the benefits or the disadvantages of the pesticide application that will have been happen.

Table (1): Temperature and relative humidity during the season of 2020/2021.

Month	Temperature °C		R.H%	
	min.	max.	min.	max.
October, 2020	24.1	28.9	33.2	84.6
November, 2020	17.1	23.2	43.7	78.4
December, 2020	14.5	21.6	38.1	78.3
January, 2021	13.3	21.7	37.9	83.0
February, 2021	9.9	22.5	34.4	87.0
March, 2021	13.8	31.4	30.5	73.4
April, 2021	15.0	34.5	19.8	71.6

Figure (1): The modified rearing cage where the larva put under it to be examined daily.

Two pesticides (Table 2) were applied as a spray with the aid of dorsal ordinary sprayer (20 L) on wheat plants (Gemiza 11 variety) 120 days old in three isolated areas of the field, and a control unit was accompanying the three treatments which were sprayed with water only, every treatment was replicated five times.

Abamectin 3.6 % EC:

Abamectin is a widely used insecticide and anthelmintic, a member of the Avermectin family and is a natural fermentation product of soil dwelling actinomycete Streptomyces avermitilis, was obtained from KZ Company for Agricultural pesticides and fertilizers, Egypt.

Congest 15% SC:

Congest is systematic, a mixture of Abamectin 3% EC, Imidacloprid 12% SC, used to control agriculture pests targeting nervous system of insects, obtained from Starchem Company for chemicals, Egypt.

4- Residual Analysis:

At the end of control experiment, samples were collected from soil, seeds, and straw after harvest. Samples were put in poly-ethylene bags and transferred to the laboratory where it was immediately subjected to analysis in order to determine the Abamectin and Imidacloprid residues.

Abamectin (3.6% EC) and Congest 15%SC (Abamectin 3% + Imidacloprid 12% SC), was obtained from local pesticide stores.

The analytical standard of Imidacloprid (99.9% purity) was purchased from Sigma-Aldrich and abamectin analytical applied for the residue standards was purchased from Dr Ehrenstorfer (LGC Standards; Augsburg, Germany). All solvents were purchased from Merck (Darmstadt, Germany).

Extraction cleanup:

Two grams of homogenized samples (wheat seeds, wheat straw and soil) were placed into 50 mL centrifuge tubes and 10 ml of deionized water mixed with the weighed portion. Vortex was used for mixing the mixture (5 s) then left for 10 min for hydration. Acetonitrile (10ml) was added and mixed with sample by using Sample Prep 2010–230 Geno/Grinder (SPEX Sample Pre, UK) at 700 rpm for 4 min. After addition of buffer–salt mixture the sample was centrifuged for 5 min at 4000 rpm, and the aliquot was filtrated by syringe filter (0.45 µm) before LC-MS/MS injection (Lie *et al.*, 2020).

Table (2): Common names and used concentrations of tested treatments

Common name	Applied concentration	Rate/ feddan
Abamectin 3.6% EC	5 ml / 20-liter water	120 liters / feddan
Abamectin 3.6% EC	10 ml / 20-liter water	
Congest 15% SC	5 ml / 20-liter water	

Measurement and residues via HPLC:

Agilent 1260 Series instrument (HPLC) was used for separation coupled to an API 6500 Qtrap tandem mass spectrometer from AB Sciex with electrospray ionization (ESI) interface. C18 column was used for separation (ZORBAX Eclipse XDB-C18 4.6 × 150 mm, 5μm particle size) (Agilent, USA). The mobile phase was as follows: Solvent A: 10 mM ammonium format solution at pH 4 ± 0.1 in methanol–water (1:9); Solvent B: methanol. The linear gradient program was: start at 100% A; 0–13 min from 100% to 5% A; 13–21 min 5% A; 21–28 min from 5%to 100% A; 28–32 min 100% A at a flow rate of 0.3 ml/min. The source was adjusted in the positive mode while nitrogen nebuliser, curtain and other gas parameters were optimized according to the manufacturer recommendations. A source temperature (400°C) and ion spray potential (5500 V) was common for all compounds. Decluster potential and collision energy was in tune by injecting direct infusion from individual pesticide solutions into MS detector. Multiple reactions monitoring mode was used for quantitation and confirmation (Li *et al.*, 2020).

Standard solution preparation:

1000 mg/L of stock solutions for each pesticide were prepared in acetonitrile or methanol. According to the analysis, a stock of multi-standard solution containing 10 mg/L of each pesticide was prepared in acetonitrile then, stored at -20 °C. To avoid the degradation of the analytes, various concentrations of standard working solutions was needed by preparing daily appropriately diluting the stock multi-standard solutions in blank matrix extracts or acetonitrile. All solutions were filtered through membrane (0.22 μm) prior to analysis.

Matrix effect (ME):

To assess the matrix effect, serial concentrations (5 ng/ml, 20 ng/ml, 50 ng/ml, 100 ng/ml, and 200 ng/ml) of standards. Where ME was calculated as follow: ME (%) = kmatrix/ ksolvent × 100, kmatrix is the slope of the matrix-matched calibration curve and ksolvent is the solvent-only calibration curve.

5- Biological assay:

After harvesting the control treatments, one hundred seeds from the treated wheat variety, were weighted to compare with that obtained from the Ministry of Agriculture, as a check sample. Treatments were replicated five times.

The treated wheat seeds variety Gemiza 11 were estimated for germination, where five replicates consist of twenty seeds were put on a wet cotton pod in a 15 cm petri dish, covered with a dark cloth and irrigated as required. Germination percentages were calculated GP = A˚/A X100 (Al- Saady 2015) where (A˚) no. germinated seeds (A) Total number of seeds.

6- Statistical analysis:

Data were subjected to the analysis of variance test (ANOVA) as randomized complete blocks design. The least significant differences (LSD) at the 5% level were determined using a computer program (CoStat, 2008) and Duncan's Multiple Range testes and LSD 5% values were used to compare the average mean numbers.

Reduction percentages were counted according to Abbott's formula.

Increase or decrease % =
Control – treatment / Control x 100

Reduction percentages were counted according to the formula of Henderson and Tilton (1955) and (Fleming and Retnakaran, 1985).

Reduction %=

$$\left[1 - \frac{treament\ after}{treatment\ before} \times \frac{control\ before}{control\ after}\right] \times 100$$

RESULTS AND DISCUSSION

Population dynamics of wheat aphid, *R. padi,* on five cultivated wheat varieties:

The obtained results illustrated in Figure (2) revealed that Gemiza 11 variety was the most susceptible one to wheat aphid, and Giza 171 was less susceptible, while Gemiza 12, Misr 1, and Sids 14 have no significant differences in their susceptibility to *R. Padi*, meanwhile it could be reported that they have good tolerance to the infestation with *R. padi*. Furthermore, the highest numbers of *R. padi* were recorded in March month with 13.8- 31.4 °C and 30.5- 73.4 RH%.

These results are different from that elucidated by (El-Mitwally *et al*., 2013) who studied the density of *R. padi* on five wheat varieties: Giza 168, Sids 1, Gemiza 7, Gemiza 9, and Sakha 93 for two years in Egypt, and found that there were significant differences among the five tested varieties for *R. padi* population dynamic and density, reporting that Sids 1 was the most susceptible to *R. padi*, while Sakha 93 variety was the least.

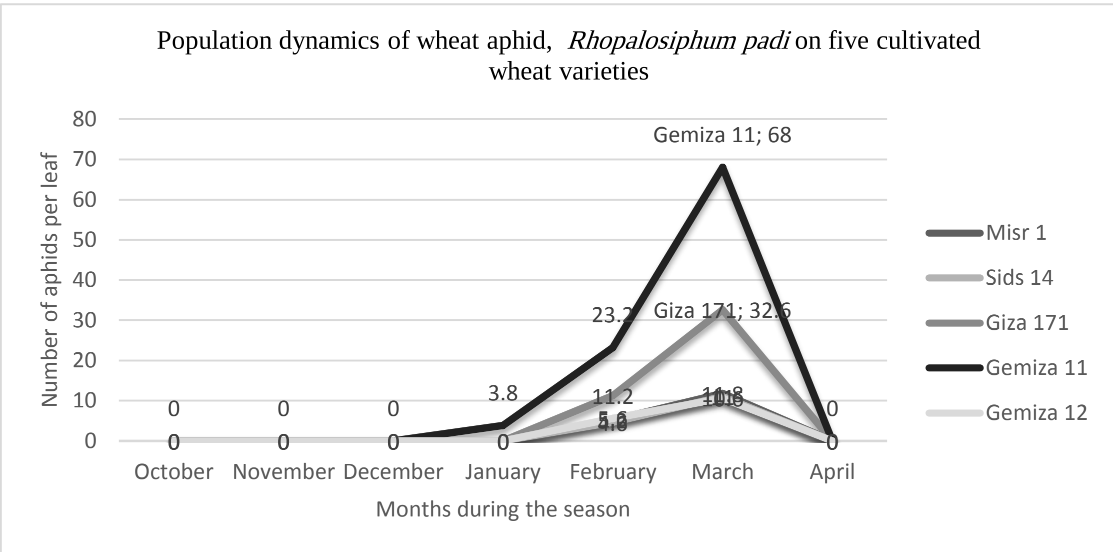

Figure (2): Population dynamics of wheat aphid, *R. padi* , on five wheat varieties

Biology of Rhopalosiphum padi on Triticum aestivum Gemiza 11 variety:

The obtained data in Table (3) elucidated that the life cycle of *R. padi* lasts for 10.6 days with ± 0.5 standard error and this means that the larva needs 10.6 ± 0.5 days to put another larva. The adult viviparous female lived 12.2 ± 0.4 days spends two days without putting larvae, one day was the pre-viviposition period and this period is from the last molt to the first larva put, the other day was the post-reproductive (post-viviposition) period is from the last larva put to the death, the adult viviparous female put 28.4 ± 1.3 individuals during the reproductive period 10.2 ± 0.4 days. The sum of the life cycle and the reproductive period and the post reproductive period called the life span which lasted 20.8 ± 0.8 days, this indicated to the high reproductive efficiency of this pest. So, *R. padi* causes huge losses when the suitable conditions are available.

Control studies:

Three treatments were tested against *R. padi* infesting wheat plants Gemiza 11 under field conditions. The data in Table (4) show that the highest reduction percentages of *R. padi*, after 24 hours were recorded at the treatment of Congest 15% with the concentration of 5 ml / 20 liters of water, followed by the treatment of Abamectin 3.6% (Double dose) while the treatment of Abamectin 3.6% occupied the least. The same trend was observed at 72 hrs, 7 days and 10 days of treatment.

Table (3): Biology of *R. padi* reared on wheat, in Meet mousa, Menoufia, March 2021

Biological aspects		Period in days
		Mean ± S.E.
Pre-reproductive	Immature stages	8.6 ± 0.5
	Pre-viviposition	1.0 ± 0.0
Reproductive	Viviposition	10.2 ± 0.4
Post-reproductive	Post-viviposition	1.0 ± 0.0
Life cycle		10.6 ± 0.5
Adult longevity		12.2 ± 0.4
Life span		20.8 ± 0.8
Mean no. of produced young's / female		28.4 ± 1.3

Values are means of 10 replicates of apterous viviparous adult's ± Standard Error.

Table (4): Reduction % in *R. padi* numbers per leaf after pesticide applications

Treatments	Reduction% in aphids/ leaf			
	1 day	3 days	7 days	10 days
Abamectin 3.6%	63.83	54.72	59.79	46.67
Abamectin 3.6% (double dose)	86.64	80.87	90.93	92.05
Congest15% (Abamectin 3 + Imidacloprid 12)	92.05	89.17	93.73	100

Biological assay:

Data in Table (5) show the weights of wheat seeds from each replicate of different treatments. Results indicated that the treatment of Abamectin 3.6% (double dose) gave the highest weights of seeds, followed by the treatment of Abamectin 3.6%, while the least weight was recorded with the treatment of Congest 15% (Abamectin 3% + Imidacloprid 12%) in comparison with untreated control.

Data in Table (6) show the germination percentage of wheat seeds from each treatment. Results indicated that the all the treatments have non-significant impact on the germination of the wheat seeds compared with the non-treated seeds.

Residual analysis:

The obtained results in Table (7) showed that the postharvest residues of the treatments of Abamectin 3.6% EC, Abamectin 3.6% EC (double dose) and Abamectin 3% EC were (0.001, 0.006 and 0.0038), (0.0014, 0.0072 and 0.0055) and (0.0035, 0.0046 and 0.0031) mg/ kg in soil, wheat straw and wheat seeds, respectively, compared with control which revealed no peak in the treatments.

As for the postharvest residues of the Imidacloprid 12% SC treatment, data in Table (8) showed that the residues were 0.0251, 0.0211 and 0.118 mg/ kg in soil, wheat straw and wheat seeds, respectively, compared with control which revealed no peak in the treatments.

Table (5): Mean values of the weights of wheat seeds after pesticides applications

Treatments	Weight g/ 100 seeds of wheat					Mean	± S.E.
	R1	R2	R3	R4	R5		
Abamectin 3.6%	7.5	7.6	7.5	7.5	7.6	7.54 b	0.02
Congest15% (Abamectin 3%+ Imidacloprid 12%)	6.8	7.1	6.4	7.1	6.7	6.82 d	0.13
Abamectin 3.6% (double dose)	7.9	7.7	7.7	7.9	8.4	7.92 a	0.12
Control	7.3	7.0	7.0	7.2	7.1	7.12 c	0.06
LSD 5%	0.29						

mean values followed by the same letter are not significantly different by (P=0.05) according to Duncan's multiple range test.

Table (6): Germination % of wheat variety Gemiza 11 after pesticide applications.

Treatments	Mean of Germination %	± S.E.
Abamectin 3.6%	96 a	4
Abamectin 3% + Imidacloprid 12%	98 a	2
Abamectin 3.6% *2	98 a	2
Control	98 a	2

Table (7): The residuals of the Abamectin 3.6% analyzed in soil, wheat straw, and seeds.

Treatments	Soil	Straw	Seeds
ST. Matrix 0.05 mg/L- Wheat	0.047	0.0303	0.0345
Spik sample Wheat	0.043	0.0289	0.0377
Abamectin 3.6% EC	0.0010	0.0060	0.0038
Abamectin 3.6% (double dose)	0.0014	0.0072	0.0055
Abamectin 3%	0.0035	0.0046	0.0031
Control	no peak	no peak	no peak

Table (8): The residuals of the Imidacloprid 12% analyzed in soil, wheat straw, and seeds

Treatments	Soil	Straw	Seeds
ST. Matrix 0.05 mg/L- Wheat	0.0425	0.0476	0.0733
Spik sample Wheat	0.0436	0.0489	0.0689
Imidacloprid 12% SC	0.0251	0.0211	0.0118
Control	no peak	no peak	no peak

Although, the maximum residues limits (MRLs) of Abamectin have not been established in wheat straw and wheat seeds, we believe that this level is sufficient for estimation of residue with expected MRLs between 0.02-0.05 mg / kg for wheat seeds and from 0.1-0.5 mg/ kg for wheat straw. So, from the obtained data it is obvious that the residues of Abamectin 3.6 % EC, double field recommended dose of Abamectin 3.6 %

EC and Abamectin 3 % EC in wheat straw and wheat seeds were less than expected MRL.

The residues of Imidacloprid 12% SC in wheat seeds were less than MRL 0.05 mg/ g, where the level of residues in straw was less in all treatments. Straw levels can be considered safe in animal feeding because of higher MRL of mg/ g as defined by Codex alimentraius.

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دراسات بيولوجية ومكافحة على حشرة منْ الشوفان التى تصيب نباتات القمح في محافظة المنوفية – مصر

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الملخص العربي

أجريت بعض الدراسات على حشرة منْ الشوفان *Rhopalosiphum padi* في محافظة المنوفية وذلك لتقليل الفقد الناتج في محصول القمح نتيجة اصابة نباتات القمح به. تمت دراسة التقلبات الموسمية لحشرة منْ القمح *Rhopalosiphum padi* L على خمسة أصناف من القمح منزرعة في الميعاد الموصى به من وزارة الزراعة بطريقة العفير تحت الظروف الحقلية بموسم 2021/2020 بمحافظة المنوفية وسجل من الشوفان أعلى أعداد له في شهر مارس وخاصة على النوع جميزة 11. وتم أخذ بعض القياسات البيولوجية على أكثر من 50 أنثى ولودة من حشرة منْ القمح *R. padi* والتى تمت تربية كل منها بشكل فردي داخل قفص معين خلال شهر مارس واستغرقت دورة الحياة للحشرة 10.6 يوم ، وكانت مدة وضع البيض 10.2 يوم وضعت خلالها 28.4 فرد جديد لتكون دورة الحياة الكلية للحشرة الواحدة 20.8 يوم، وكذلك تم اختبار فعالية اثنين من المبيدات الحشرية الحيوية ضد *R. padi* ، وفى نهاية التجارب تم تحليل عينات من التبن والبذور والتربة لتقدير كمية المتبقيات المتخلفة من المبيدات المستخدمة في التجربة، وكذلك تقدير نسبة إنبات البذور في المعمل وكذلك وزن البذور وكانت النتائج المتحصل عليها جيدة بالنسبة لكلا المبيدين حيث قللا من أعداد حشرات المن على أوراق النباتات بنسبة 46.67- 100٪ وكذلك فإن نسبة المتبقى من المبيد في كل من حبوب القمح والتبن والتربة تحت الحد المسموح به دوليا ولم تتأثر نسبة إنبات البذور بأي من المعاملات.

أسماء السادة المحكمين

أ.د/ رضا عليوه سند إبراهيـــــم معهد بحوث وقاية النباتات – الدقى جيزة

أ.د/ منال عبدالرؤوف عبد المجيد كلية الزراعة – جامعة المنوفية