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Growth Inhibitor Effects of Bermuda Grass, *Cynodon dactylon* (L.) on *Schistocerca gregaria* (Forsk.) (Orthoptra: Acrididae)

El-Gammal, A. M.[*]; Gihan A. Mohamed[*]; M. T. Mohamed[*]; H. A . Selim[**] and N. Arif[**]
[*]Locust and Grasshoppers Research Department, PPRI, Agric. Res. Center, Dokki, Giza, Egypt
[**]Department of Plant Protection, National Res. Center, Dokki, Giza, Egypt

ABSTRACT

During the last plague of the desert locust, *Schistocerca gregaria* in the winter season of 2004 – 2005 to Egypt, it was observed that Bermuda grass, *Cynodon dactylon (L.)* argued to resist the infestations of this insect. To explore this observation, the present study was carried out in the laboratory in Locust and Grasshoppers Research Department, Plant Protection Res. Institute, Agriculture Res. Center, Ministry of Agriculture, Dokki, Giza, Egypt by feeding the 4th and 5th instar nymphs, *Schistocerca gregaria* on treated maize leaves with different extracts from leaves and roots of Bermuda grass in the solvents, methanol, acetone and etheoxyethanol. Results revealed that growth rate and metamorphosis of the treated nymphs were inhibited. Feeding of the 4th instar nymphs on the treated leaves of maize by methanol leaves extracted from grass leaves resulted in 8.3% mortality and 91.7% malformation of 5th instar nymphs which died after ecdysis. Also, treatment with methanol grass leaves extracts induced 40% mortality in the treated 4th instar nymphs, while the other 60.0% were molted to weak and small 5th instar nymphs. Feeding of 5th instar nymphs on the treated leaves of maize with methanol, acetone and ethoxyethanol leaves and roots of Bermuda grass resulted in different percentages of mortality ranged from 11.1% to 90.0%. the high mortality percent was obtained by ethoxyethanol leaves extract (90.0%). The lower one resulted after feeding on methanol leaves extract which induced the higher percent of adultoid insect (86.7%) followed by methanol and acetone root extracts (60.0%). The adultoid insects have had the intermediate features of the 5th instar and the adult stage.

Key Words: Growth inhibitor, Bermuda grass, *Cynodon dactylon*, *Schistocerca gregaria.*

INTRODUCTION

Swarms of the desert locust, *Schistocerca gregaria* (Forsk.) can cause complete crop loses after few hours of roosting especially during night. Prevention of this severe damage still relies on chemical insecticides. Plant-derived extracts have long been a subject of research since Pruthi (1937) to develop alternatives to conventional insecticides but with reduced health and environmental impacts, he discovered the repellent action of the neem tree *Azadirachta indica* aginst pests. Rao (1982) recorded this action in *Calotropis giganta,* against *S. gregaria.* The same was reported by El-Gammal *et al.* (1988), El-Gammal *et al.* and El-Gammal (1994) (1990) in *Argemon mixicana, Zygophyllum simplex, Calotropis procera, Withania somnifera, Solanum dopium and A. indica* against *S. gregaria.* In a further study, Rembold *et al.* (1989) found that injection of Azadirachtin -A, an insect growth inhibitor derived from *A. indica,* into female adults of *Locusta migratoria* inhibited ovarian development. He concluded that azadirachtin may influence the release of tropic hormones from the corpus cardiacum, leading to alternations in the timing and titre of morphogenetic hormone pools. Mordue (2004) recorded that azadirachtine is a strong antifeedant, repellent and growth regulator for a wide variety of phytophagous insects. Jogar *et al.* (2006) studied the effects of the natural insecticidal substances on the development and physiological state of insects. They found that, the timing of the normal and failed ecdysis as well as length of iterecdysial periods in *Tenebrio molitor* were prolonged after treatment with *Ledum palustre* plant extract. The treated pharate pupae transformed into extra-pupal instars, which is a symptom of juvenilizing effect. Also, Akhtar *et. al.* (2008) stated that bioactivities of some plant extracts belonging to the family Maliaceae proved to be strong growth inhibitors and contact toxins.

Thus, the present study is an attempt to find out these properties in Bermuda grass, *Cynodon dactylon L.* against *S. gregaria.*

MATERIALS AND METHODS

1. Experimental insects:

Newly molted 4th and 5th instar nymphs of *S. gregaria* were segregated from the stock colony of Locust and Grasshoppers, Department (LGD), Plant Protection Research institute, which was maintained under the crowded conditions of Hunter-Jones (1961) for several years. These nymphs were kept in wooden cages of 30x30x30 cm and fed on the Egyptian clover, *Triofolium alexandrinum* L.

2. Preparation of plant extracts:

Bermuda grass, *Cynodon dactylon* was planted in clay soil in the experimental plots at LGD. After one month, the grass was picked up by hands and the leaves were separated from the roots. They were allowed to dry in shadow for 10 days at room temperature. Air dried leaves and roots were grounded separately by a house hold grinder.

One hundred grams of every part were soaked in 200 ml of each solvent of methanol, acetone and ethoxyethanol for 48 hours, with continuous stirring.

Filtration was carried out after soaking through fine gauze to get the supernatant of each extract for testing against the 4th and 5th instar nymphs of *S. gregaria.*

3. Treatments:

Newly molted 4th and 5th instar nymphs were fed for 6 days on treated leaves of maize using dipping technique for 30 seconds for all tested extracts m.a. Three replicates of each instar were fed on definite quantities of treated maize leaves. The nymphs were allowed to feed on the treated leaves of maize for 6 days, the untreated control nymphs were fed on clean maize leaves disks, the numbers of treated and untreated 4th and 5th instars nymphs are illustrated in tables 1 and 2.

4. Determination of *Schistocerca gregaria* response to Bermuda grass:

After feeding of the 4th and 5th instars nymphs for six days on the treated leaves of maize, the nymphs were normally fed on clover along the remained period to the next molt. Mortality percentages were calculated; also the abnormality percentages were measured during ecdysis to 5th instar and adult stage in the treated and untreated 4th and 5th instars, respectively. These nymphs were weighted daily to evaluate their growth rate in comparison to the untreated control nymphs. The duration of 4th and 5th instars were calculated by Dembester equation (1957).

RESULTS AND DISCUSSION

1. Effects of Bermuda grass on 4th instar nymphs:

The newly molted 4th instar nymphs of *S. gregaria* were fed on treated leaves of maize with extracts from leaves and roots of Bermuda in methanol and acetone, only.

Table (1) shows that feeding 4th instar nymphs on treated maize leaves dipped in methanol extracts of bermuda grass leaves resulted in 8.3% mortality and 91.7% abnormal 5th instar nymphs, which died after ecdysis, to induce 100.0% mortality. Meanwhile, the feeding of 4th instar nymphs on treated maize leaves with methanol leaves extract of Bermuda grass induced 40% malformed 5th instar nymphs which died during ecdysis. Also, table (1) shows that feeding 4th instar nymphs on treated leaves of maize dipped in methanol extract of bermuda grass leaves resulted in 8.3% mortality and 91.7% abnormal 5th instar nymphs, which died after ecdysis, to induce 100% total mortality after this treatment. Meanwhile, feeding of 4th instar nymphs on treated maize leaves with methanol roots extract of Bermuda grass induced 40% malformed 5th instar nymphs which were died during ecdysis. Also, table (1) reveals that, feeding 4th instar nymphs on treated maize leaves dipped in acetone roots extract resulted in 100% weak 5th instar nymphs that survived to the adult stage. The treated 4th instar nymphs were inactive, small in size and weight compared to the untreated control (Fig. 1-A). The reduction in mean body weights of these nymphs was more pronounced than in the untreated control, 4th instar nymphs were not affected by treatment with methanol and acetone extract except slight prolongation after treatment with methanol roots extracts. The other extracts of Bermuda grass in ethoxyethanol, didn't exhibit any kind of biological activity in the treated 4th instar and the resulted 5th instar nymphs which survived normally to the adult stage.

2. Response of 5th instar nymphs to Bermuda grass:

Table (2) indicates the toxicity and the biological activities of different extracts from leaves and roots of the bermuda grass, *C. dactylon* on the 5th instar nymphs, *S. gregaria* . Feeding of these nymphs for 6 days on leaves of maize treated with methanol leaves extract induced 13.3% mortality and 86.7% adultoid insects which eventually died to give 100% mortality after this treatment. Also, feeding of 5th instar nymphs on leaves of maize treated with methanol roots extracts resulted in 40.0% mortality, 60.0% adultoid insects and 100% mortality of the adultoid insects. No perfect adults were produced after treatment with the previous extracts, which produced 100% mortality in the treated 5th instar nymphs.

Table (1): Effects of Bermuda grass (*Cynodon dactylon* L.) on some biological activities of the 4th instar nymph of *Schistocerca gregaria* (Forsk).

Biological parameters	Methanol extracts of:		Acetone roots extracts*	Untreated Control
	Leaves	Roots		
No. treated nymphs:	24	20	24	20
Mortality percent:	8.3 (2)	0.00	0.00	0.00
Abnormality percent:	91.7 (22)	40.00 (8)	0.00	0.00
Total mortality % :	100.00 (24)	40.00 (8)	0.00	0.00
Duration (days)**:	6.9	7.8	6.8	6.1
Resulted 5th instar:	0.00	60.00 (12)	100.00 (24)	100.00 (20)

* The resulted 5th instar nymphs after treatment with acetone roots extracts were weak because their mean body weights were affected by this treatment. The other extracts in ethoxyethanol didn't exhibit any biological activity in the 4th instar nymphs.

** Duration was calculated by Dembester equation (1957).

Table (2): Toxicity and some biological activities of Bermuda grass, *Cynodon dactylon L.* extracts on the 5th instar nymph of *Schistocerca gregaria* (Forsk).

Biological parameters	Methanol extracts of		Acetone extracts of		Ethoxyethonol extracts of		Untreated Control
	Leaves	Roots	Leaves	Roots	Leaves	Roots	
No. Insects:	30	30	36	20	30	30	40
Mortality %:	13.3(4)	40.0(12)	11.1(4)	40.0(8)	90.00(27)	80.00(24)	0.00 (-)
Adultoids %:	86.7(26)	60.0(18)	00.0(-)	60.0(12)	00.0(-)	00.0 (-)	0.00 (-)
Total mortality % :	100.0(30)	100.0(30)	11.1(4)	100.0(20)	90.0(27)	80.0 (24)	0.00 (-)
Duration (days)*:	9.3	8.8	10.5	8.9	15.1	10.2	8.8
Resulted adults **%:	00.0(-)	00.0	88.9(32)	00.0(-)	10.0 (3)	20.0 (6)	100.00 (40)

* Duration of the treated and untreated 5th instar nymphs were calculated by Dembester equation (1957).

** The resulting adults from the treated 5th instar nymphs have had slight malformed wings, but they survived normally along their life span

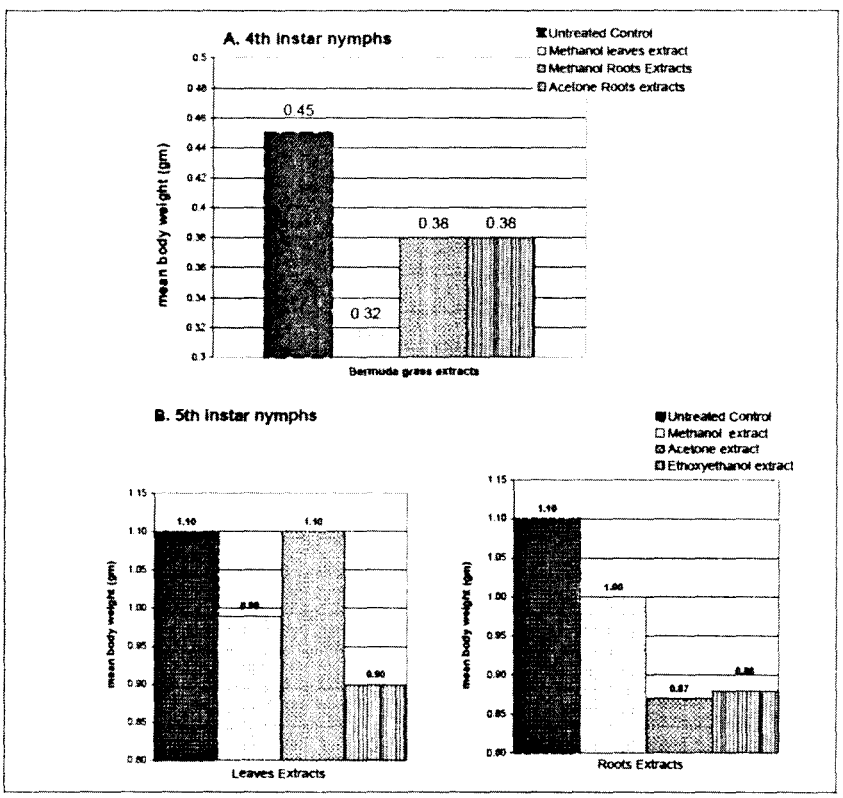
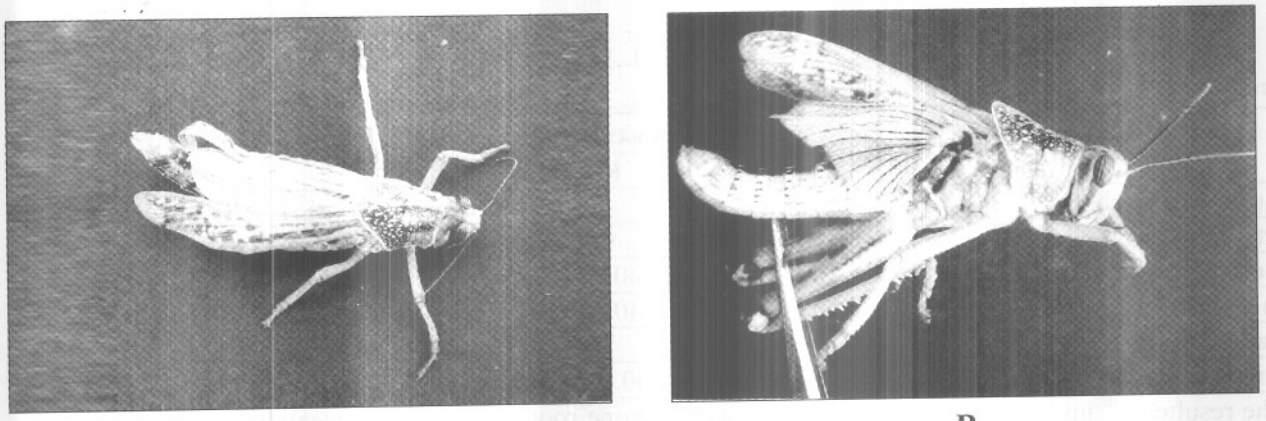


Fig. (1): Effect of different solvents extracts from Bermuda grass, *Cynodon dactylon* (L.) on mean body weights of the late nymphal instars of *Schistocerca gregaraia*, A: 4th instar and B: 5th instar nymphs.



A B

Fig. (2): The adultoids of *Schistocerca gregaria* as an indicator for the juvenilizing effect of the Bermuda grass, *Cynodon dactylon* L. on the 5th instar nymphs.

Also, table (2) shows that, feeding the 5th instar nymphs on the maize leaves treated with acetone leaves extract induced 11.1% mortality, 00.0% adultoid insects, and 88.9% perfect adults. On the other hand, feeding these nymphs on leaves of maize treated with acetone roots extracts resulted in 40.0% mortality, 60.0% adultoid insects and 100% total mortality. No adultoid insects were produced by feeding of the 5th instar nymphs on the maize leave treated with ethoxyethanol extracts from roots or leaves, but high percentages of mortality were obtained, they were 90.0 and 80.0% after treatment with ethoxyethanol extracts from leaves and roots, respectively, whereas the remaining percentages from each treatment were perfect adults. Moreover, treatment with ethoxyethanol extracts extended the interecdysial periods to the adult stage; it was also happened only with treatment by acetone leaves extract. The means body weight of the treated 5th instar nymphs with methanol and ethoxyethanol leaves extracts and methanol, acetone and ethoxyethanol root extracts were reduced during the 6 days of treatment, (Fig. 1-B). The producing intermediate insects (adultoids) (Fig. 2) had the abdomen, pronotum, head capsule and the legs of the 5th instar nymphs, beside the elytrones of the adult stage, all these body parts were green in color like the solitary phase of *S. gregaria* compared to the normal gregary phase and adult stage.

In conclusion, the solitary green color in the produced extra-nymphal instar (adultoid insects – Fig.2) and the prolongation in interecdysial periods of the treated 4th and 5th instar nymphs, *S. gregaria* fed on maize leaves treated with Bermuda grass extracts in the present study could be considered as symptoms of the high level of Juvenile hormone (JH) in their haemolymph. This conclusion coincides with Rembold *et. al.* (1989) who stated that , injection of azadirachtin-A, an insect growth inhibitor derived from neem tree seeds, into female adults of *Locusta migratoria* influenced the release of trophic hormones from their corpus cardiacum leading to alterations in the timing and titer of morphogenetic hormone pools. Also, Jogar *et. al.* (2006) have tested, JH-like effect extract of *Ledum polustre* plant against *Tenebio molitor*, they found an obvious alteration in timing of the normal and failed ecdysis as well as length of interecdysial periods to pupal stage. The treated pharate pupae transformed into extra-pupal instars, which is a symptom of juvenilizing effect. Thus, the extracts of bermuda grass may have some phyto-chemicals caused some alterations in the hormonal conditions of the treated 4th and 5th instar nymphs of *S. gregaria* leading to solitary features in the adultoid insects. In early study, Cassier and Delmore-Joulie (1976) stated that, the presence of ecdysone hormone alone during the last two days of the 4th instar nymphs and the early days of 5th instar, *S. gregaria* favour gregarious features, while the presence of ecdysone and JH together resulted in solitarious features development. Moreover, Simonds *et al.* (1994) found that, ecdysteroid was affected in favor of JH in azadiracthin treated nymphs of *L. migratoria*, for the same purpose.

The prolongations in the interecdysial periods were also obtained by Mohamed and El-Gammal (2002) after injection of azadirachtin into one day old 4th instar nymphs of *S. gregaria* and the resulting 5th instar.

Generally, such Juvenilizing effects against the nymphal instars of *S. gregaria* in the present study may explain why Bermuda grass argued to resist the infestation of swarms of this insect during its plague in Egypt, 2004 .May be it is due to the existence of some phytochemicals in this grass acted as growth inhibitors in this insect, as Akhtar *et al.* (2008) concluded that, most of plant extracts proved to be strong growth inhibitors and contact toxins to insects.

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الملخص العربى

التأثيرات المثبطة للنمو للنجيل من النوع "برمودا *Cynodon dactylon* على الجراد الصحراوي *Schistocerca gregaria* (Orthoptera: Acrididae)

عبد العظيم الجمـــال* & جيهان علي محمد* & محمد توفيق محمد* & حمدي سالم & نعمان عارف****

*قسم بحوث الجراد، معهد بحوث وقاية النباتات، مركز البحوث الزراعية، الدقي، الجيزة، مصر

**قسم وقاية النباتات، المركز القومي للبحوث، الدقي، الجيزة، مصر

أثناء وباء الجراد الصحراوي في شتاء ٢٠٠٤ – ٢٠٠٥ ، لوحظ أن النجيل من النوع "برمودا" يتحمل الاصابة بهذه الحشرة. و لتوضـيح أسـباب ذلـك أجريت هذه الدراسة بالمعمل بتغذية العمرين الرابع و الخامس لحوريات الجراد الصحراوي على أوراق أذرة معاملة بعدد من المستخلصات من أوراق و جذور هذا النجيل في المذيبات، الميثانول، و الأسيتون و الإيثوكسي إيثانول كل على حدة. أوضحت النتائج أن النمو و التطور لهذه الحوريـات قـد تـم تثبيطه . فتغذية حوريات العمر الرابع على أوراق الذرة المعاملة بمستخلص الميثانول من أوراق النجيل أدى إلى موت ٨,٣% و تـشوه النـسبة الباقيـة وهي ٩١,٧% ، و التي ماتت بدورها بعد الانسلاخ إلى العمر الخامس متأثرة بهذه المعاملة . و بمعاملة حوريات نفس العمر بمستخلص جـذور النجيـل في الميثانول نتج عنها ٤٠% موت، و لكن ٦٠% انسلخت إلى العمر الخامس حيث كانت ضعيفة و صغيرة الحجم. و بتغذية حوريات العمـر الخـامس على أوراق الذرة المعاملة مستخلص أوراق النجيل في المذيبات ، الميثانول و الأسيتون و الإيثوكسي إيثانول ، أدى ذلك إلى نسب مختلفة مـن المـوت تراوحت من ١١,١إلى ٩٠,٠%، كان أعلاها بالمعاملة بمستخلص الإيثوكسي لإيثانول (٩٠,٠%) و أقلها نتج عن المعاملة بمستخلص أوراق النجيل فـي الإيثانول، و الذي أدى إلى أعلى نسبة من الحشرات الوسطية التطور و التي تجمع بين صفات حوريات العمر الخامس و الحشرة الكاملة (٨٦,٧%) جاء بعدها مستخلص جذور النجيل في الميثانول و الأسيتون (٦٠,٠%). أوقفت هذه المعاملات عمليات التطور و النمو في الحوريات المعاملة نتيجة لارتفـاع هرمون الشباب في دم هذه الحوريات. و قد يرجع ذلك إلى وجود بعض المركبات النباتية في النجيل و التي تؤدي إلى ظهور مؤشـرات ارتفـاع هـذه الهرمونات مثل إنتاج الحشرات الوسطية و اللون الأخضر المميز للحوريات الانفرادية في جميع أجزاء هذه الحشرات و التي تظهر نتيجة لهذا الهرمـون بها و الذي قام بتثبيط نمو حوريات الجراد الصحراوي في هذه الدراسة.