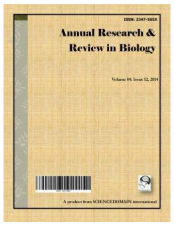

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Comparative Study on Some Biological Indices of *Agrotis ipsilon* (Lepidoptera: Noctuidae) Larvae Treated with Three Control Agents under Laboratory Conditions

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MAG and ARE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author ARE managed the analyses of the study. Authors NYS and WLA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Sensitivity of *Agrotis ipsilon* towards three control agents: Runner, Tracer and local raw diatomaceous earth (silica nano-particles) were investigated.
Study Design: Evaluation of the effects of some insecticides on some nutritional indices of the black cutworm *A. ipsilon.*
Place and Duration of Study: Pests and Plant Protection Department, National Research Centre, Dokki, Giza, Egypt, within 2016-2017.
Methodology: Newly moulted 4th instar larvae were transferred individually into plastic cups (5 cm in diameter and 5 cm height) to avoid the cannibalistic behaviour then covered with muslin. These cups were divided into three groups each one was marked for one tested material, while the 4th group was marked as check (untreated control). Each one of the three groups was subdivided into

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three subgroups, each subgroup comprised 30 individuals to be represented for three concentrations (High, Medium and Low concentration). Each cup was provided with known weighed of fresh castor bean leaves treated with one of the abovementioned concentration of each tested material.
Nutritional indices were calculated using the following items: food consumption, weight gain, developmental time and percentage of mortality as well. Larvae were weighted before the experiment and repeated daily until the pupation or death. Weights were recorded daily; at the end of the experiment, all above recoded weights were expressed as percentage of dry masses and reweighted to reach a constant weight. The calculation of the nutritional indices as the following: Relative growth rate (RGR), the conversion of ingested food (Approximate digestibility (AD)), Efficiency of conversion of ingested food to body substance (ECI), consumption index and Efficiency of conversion of digested food into growth (ECD) were carried out according to the summarized equations.
Results: All the tested agents were found to be toxic to *A. ipsilon*, inducing significant mortality percentage at almost all tested concentrations. The physiological parameters (nutritional indices) affected by the tested agents were evaluated. Results showed that treated larvae reflected significant reduction in consumed food and growth. The approximate digestibility of treated larvae was insignificantly high for all treatments. Silica nano-particles insignificantly enhance the rapid mortality as compared with Tracer (insecticide) and/or Runner (IGR) at all tested concentration.
Conclusion: The growth rate was decreased when larvae consumed castor bean leaves dipped in IGR by increasing the concentration. This decrease in consumption rate is due to antifeedant, deterrent nature and toxic mode of action of IGR. Rearing of *A. ipsilon* larvae on host plant treated with IGR induced reduction in growth rate, efficiency in convert food to body mass and also, reduction in pupal weight. Depending on the obtained results, the IGR has adverse effect on *A. ipsilon* larvae. Thus, it can be used in the control program for the present insect pest, since the highest AD and the low ECD values lead to the retardation of growth.

Keywords: Black cutworm; nutritional parameters; silica nano-particles; tracer; runner.

1. INTRODUCTION

Agrotis ipsilon (Hüfn.) (Lepidoptera: Noctuidae) was marked as a dangerous destructive lepidopterous pest, that because it attacks almost all plant families inducing great damages to the planted seedlings which leads to re-plantation of the destroyed plots or complete re-plantation of the whole field, where larvae cut-off the seedlings of any plant of either vegetable and/or crops 3-5cm of soil surface, that leading finally to duplicate the costs [1]. Therefore, great attention was paid to find safer methods used for controlling such pest as an alternate to chemical insecticides.

The permanent uses of chemical pesticides to protect field crops, a significant negative aspects were raised up, such as developing of insecticide resistant strains [2,3]. The detection of these risks has increased interest to find out alternative protectants to replace the synthetic chemical insecticide [4]. Among such alternatives is the utilization of Insect Growth Regulators which have substantial effects on several pests and also, because it have a little mammalian toxicity and little environmental pollutions [5,6]. Among IGR effects, the hinder of chitin production which was reported [7] to be more professional in killing immature stages of different insect species.

This study aims to estimate the effects of three control agents (Runner, Tracer and silica nano-particles) to control *Agrotis ipsilon* under laboratory conditions.

2. MATERIALS AND METHODS

Natural poisonous materials, insect growth regulator (IGR) in addition to other compounds were examined.

2.1 Tested Control Agents

- **Local raw diatomaceous earth (Silica nano-particles):** was manufactured by Refractories, Ceramics and Building material Department, NRC, Giza, Egypt.
- **Spinosad: Tracer (24% SC)**. The recommended concentration is 250 ml/100L water.
- **IGR: Methoxyfenazide, 240 SC** (**Runner**). The recommended concentration is 150 ml/200L water.

2.2 Treated Insect

Fourth larval instar of *A. ipsilon* was obtained from NRC Permanent Rearing Lab. maintained for several generations on castor bean leaves at constant conditions (25±2°C, 75±5% RH).

2.3 Bioassay and Technique of the Treatment

Newly moulted *A. ipsilon* 4th instar larvae were transferred individually into plastic cups (5cm in diameter and 5cm height) to avoid the cannibalistic behaviour [8] then covered with muslin. These cups were divided into 4 groups'. The first three groups of them each one was marked for one tested material, while the 4th group was marked as check (untreated control). Moreover, each one of these three groups was subdivided into three subgroups, each subgroup comprised 30 individuals to be represented for three concentrations (High, Medium and Low concentration) as explained in Table 1.

Each cup was provided with known weighed of fresh castor bean leave disks treated with one of the abovementioned concentration of each tested material.

2.4 Nutritional Indices Calculation

Nutritional indices were calculated using the following items: food consumption, weight gain, developmental time and percentage of mortality as well. Larvae were weighted before the experiment and repeated daily until the pupation or death. Weights were recorded daily; at the end of the experiment, all above recoded weights were expressed as percentage of dry masses and reweighted to reach a constant weight. The nutritional indices were calculated and expressed as follow: Relative growth rate (RGR), the conversion of ingested food (Approximate digestibility (AD)), Efficiency of conversion of ingested food to body substance (ECI), consumption index and Efficiency of conversion of digested food into growth (ECD) were carried out according to equations summarized by [9,10,11].

2.5 Statistical Analysis

Percentage of mortality was corrected by applying Abbott's formula [12]. Analysis of variances (ANOVA) F-test was carried out through the SPSS Computer program to discriminate between treatments and/or concentrations used. Differences between mean were compared using Duncan's Multiple Range test [13].

3. RESULTS AND DISCUSSION

Impacts of the tested control agents on various nutritional indices for *A.ipsilon* 4th instar larvae were summarized in Table (2). Our study showed that, these control agents have significant diverse effects on tested nutritional parameters of *A.ipsilon* larvae, which were matched with that mentioned on *Helicoverpa armigera* (Hübner) [14].

Results clarify that, although the treated 4th instar larvae were capable to sustain the approximate digestibility (AD), *i.e.,* increased throughout the experiment, they could not preserved the relative growth rate (RGR) during larval development. The maximization of AD might be referred to the longer retention of food in gut because of the larvae of *Spodoptera littoralis* demand more nutrients [15,16]. The highest mean of AD value may be referred to the increase of larval instar duration, where more food must be allotted to maintain its metabolism, as reported about their work on *Taragama siva* [17]; on *Lymantria dispar* [18] and on *Chillo partellus* [19].

In general, all tested control agents caused high AD values, this result was in contrary with that reported for *Antheraea mylitta* [20] and with [21] who said that low AD values could be the cause of the disruption in the metabolic rate of insects.

Abdel-Aal & Abdel-Khalek [22] mentioned that IGRs reduced approximate digestibility (AD) when the 2nd larval instar of *S. littoralis* fed on treated plants compared to the check individuals.

Table 1. The concentrations used for each tested material

Concentrations	IGR	Tracer	Silica
High	75 µl/100 ml water	250 µl/100 ml water	4 g/L water
Middle	37.5 µl/100 ml water	125 µl/100 ml water	2 g/L water
Low	18.75 µl/100 ml water	62.5 µl/100 ml water	1 g/L water

High approximate digestibility percentage decline of RGR, where it reached its lowest values in larvae that treated with IGR (all tested concentrations) then followed by all concentrations of the tested agents except the Silica nano-particles treated leaves at the low concentration (Table 2). It may be inferred from the previous studies that, the fourth larval instar feed on IGR had high AD value and lowest RGR value coupled with decrease of larval growth. The lowest RGR values perhaps resulted from either decrease the food consumption or its metabolism or both.

Among the three tested control agents, the highest insignificant value of the consumption index (CI) for the 4th larval instar that fed on castor bean leaves were dipped in high and low Tracer concentrations, followed by Silica nano-particles treatment (Middle concentration) and IGR (High concentration). Where the highest values of CI were achieved when *Agrotis* larvae were fed on treated castor bean leaves with Tracer (low concentration), while the lowest significant value of CI resulted from feeding larvae on Silica nano-paticles (low concentrations), this result was matched with that mentioned on *S. littoralis* [15, 23].

The highest percentage of mortality (80%) was recorded for larvae reared on IGR, while Silica nano-particles treated leaves gave the lowest value (10%) (Table 2).

Feeding of *Agrotis* larvae on castor bean leaves dipped in IGR significantly reduced either larval or pupal growth rate if compared to the check (control). This reduction was irrespective of any significance in consumption index at high concentration, but showed significant decline at both Middle and Low concentrations compared with the check larvae (Table 2).

The consumption index (CI) reduction, lowering the ECD and ECI of *Agrotis* larvae when treated with IGR as the food ingested was decrease, the insects become smaller and suffer from reduction in growth. The obtained data were matched with that reported on *Agrotis ipsilon* [24]; on *S. littoralis* [25,26]; on *H. armigera* [27] and on *L. dispar* [18], while it was mismatched with that reported when *H. armigera* were reared on different bean cultivars [28].

Also, the poorer rate of growth could be due to the damage occurred to the midgut lumen cellular surfaces as mentioned by Jansen and Groot [29]. While the lower values of either ECI or ECD probably lead to a retard in larval development, percent of pupation and formation of diminished pupae as reported on *S. littiralis* [25,30]. In addition, the reduction percentage of ECD and ECI values resulted from the deficiency of conversion reduces growth through the conversion of energy from biomass production into detoxification [31].

The highest ECI & ECD values for 4th larval instar treated with the highest concentration of Silica nano-particle and the lower one of Tracer, which reflect the enormous efficiency in converting the ingested and digested food into body mass and resulted in increasing larval weight as matched with that reported on *S. littoralis* [11,15] and on forest insect pests *Hyblaea puera* and *Eutectona machaeralis* [32].

The lowest significant value of Feeding Deterrence Index (FDI) was recorded in all concentration of Silica nano-particle treatment, and the highest concentration in case of IGR treatment. The higher FDI in *A.ipsilon* larvae was perhaps due to deterrence releases by chemical sensila on the mouth parts of larvae or retracted pulses coming from the stomodael nervous system after ingestion, as mentioned on *Spodoptera litura* [33]. Moreover, because of the existence of some chemical compounds, *i.e.,* flavonoids, terpenestannins and sterols as reported for their work on *S. littoalis* [34], or behave toxic effect after ingestion. Khosravi *et al.* [35] in their field trials on *Glyphodes pyloalis* with IGR deterrence effect mentioned that retards in the treated insect growth rate, and extends the time of insect's search for food, which leads to increase the percent mortality of the insect.

The prolongation of the 4th larval instar in addition to the reduction in both pupal formation and weight in all concentrations of the tested IGR may be the result of the reduction in ECI & ECD obtained in this study. Delay in larval growth and formation of smaller pupa which have a direct relation to fecundity and longevity of the adult insect and make them susceptible to diseases and natural enemies [35]. Similar results were observed on *S. littoralis* [36].

Table 2. Effects of three control agents applied at three concentrations on nutritional indices of *A. ipsilon* larvae

Treatment	Concentration	AD	ECD	CI	ECI	FDI	RGR	Mortality (%)
		Mean ± SE						
IGR	High	99.30±0.30 a	-3.32±2.57 c	1.03±0.24 a	-3.37±2.57 c	42.74±6.16 a	-0.05±0.03 c	70
Tracer		99.07±0.24 a	7.21±4.21 b	1.14±0.36 a	4.53±5.20 b	33.56±12.50 a	0.02±0.03 b	80
Silica		98.62±0.12 a	19.64±2.36 a	0.85±0.09 a	20.35±1.70 a	3.67±3.88 b	0.15±0.01 a	70
Control		99.19±0.13 a	17.83±1.38 a	0.95±0.07 a	17.59±0.88 a	0.00±0.00 b	0.16±0.01 a	0
F-value		**1.404 NS**	**20.448****	**0.261 NS**	**22.339****	**15.293****	**33.015****	
IGR	Middle	86.95±9.69 a	-5.39±2.47 c	0.39±0.10 b	-5.24±2.40 c	57.13±8.07 a	-0.03±0.03 c	60
Tracer		98.06±0.59 a	8.69±2.15 b	0.86±0.20 a	8.56±2.09 b	37.80±12.68 ab	0.10±0.10 b	60
Silica		98.11±0.33 a	12.56±4.18 ab	1.05±0.21 a	12.99±3.52 ab	14.99±9.60 bc	0.11±0.02 ab	40
Control		99.19±0.13 a	17.83±1.38 a	0.95±0.07 a	17.59±0.88 a	0.00±0.00 c	0.16±0.01 a	0
F-value		**1.147 NS**	**13.054****	**3.770***	**16.308****	**8.829****	**21.399****	
IGR	Low	88.09±9.79 a	4.53±2.91 b	0.53±0.09 b	5.05±2.84 b	40.28±5.14 a	0.04±0.02 b	50
Tracer		98.98±0.19 a	19.17±3.10 a	1.13±0.19 a	18.94±3.04 a	14.78±3.47 b	0.18±0.02 a	20
Silica		96.67±0.42 a	12.58±2.21 a	0.23±0.04 b	12.92±2.24 a	38.70±4.95 a	0.02±0.00 b	10
Control		99.19±0.13 a	17.83±1.38 a	0.95±0.07 a	17.59±0.88 a	0.00±0.00 c	0.16±0.01 a	0
F-value		**0.900 NS**	**6.331****	**14.425****	**5.958****	**25.630****	**26.999****	

***= Highly significant   *= Significant   NS= Not Significant*

Means in a column followed with the same letter(s) are insignificantly different at 5% level of probability.

Concentrations	IGR	Tracer	Silica
High	75 µl/100 ml water	250 µl/100 ml water	4g/L water
Middle	37.5 µl/100 ml water	125 µl/100 ml water	2g/L water
Low	18.75 µl/100 ml water	62.5 µl/100 ml water	1g/L water

Finally, the highest percentage of mortality (80%) was recorded for larvae reared on IGR, while silica nanoparticles treated leaves gave the lowest value (10%) (Table 2).

4. CONCLUSION

The growth rate was decreased when larvae consumed castor bean leaves dipped in IGR by increasing the concentration. This decrease in consumption rate is due to antifeedant, deterrent nature and toxic mode of action for IGR. Rearing of *A. ipsilon* larvae on host plant treated with IGR induced reduction in growth rate, efficiency in convert food to body mass and also, reduction in pupal weight.

Depending on the obtained results, the IGR has adverse effect on *A. ipsilon* larvae. Thus, it can be used in the control program for the present insect pest, since the highest AD and the low ECD values lead to the retardation of growth.

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COMPETING INTERESTS

All authors have declared that, there is no competing interests exist in this work.

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