



## Antifeedant activity and detrimental effect of Nimbecidine (0.03% Azadirachtin) on the nutritional performance of Egyptian cotton leafworm *Spodoptera littoralis* Boisd. (Noctuidae: Lepidoptera)

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(Published by Research Trend, Website: [www.biobulletin.com](http://www.biobulletin.com))

(Received 07 December 2016; Accepted 04 February 2017)

**ABSTRACT:** The present study was conducted to assess the antifeedant activity of Nimbecidine (0.03% Azadirachtin) against 4<sup>th</sup> instar larvae of the destructive phytophagous pest *Spodoptera littoralis* and investigate its disruptive effects on different nutritional parameters in both 4<sup>th</sup> and 6<sup>th</sup> (last) instar larvae. Fresh clean castor bean leaf discs were treated with sublethal concentrations (500, 100 & 10 ppm) of Nimbecidine and offered to the early 4<sup>th</sup> instar larvae for 24 hrs. Nimbecidine exhibited a serious antifeedant activity against 4<sup>th</sup> instar larvae in a dose-dependent course. A significant reduction of food consumed by the 4<sup>th</sup> and 6<sup>th</sup> instar larvae was recorded in an inverse relation to the concentrations. Enhanced approximate digestibility (AD) was recorded for 4<sup>th</sup> instar larvae, but remarkably prohibited for last instar larvae. A general inhibitory effect was exhibited by Nimbecidine on ECI and ECD of both 4<sup>th</sup> and 6<sup>th</sup> instar larvae with an exceptional case. Assimilation rate of 4<sup>th</sup> instar larvae was significantly induced at the higher two concentrations but considerably or slightly suppressed in last instar larvae. Significantly or slightly increasing relative metabolic rate was recorded. The relative weight gain was reduced, regardless the instar. The growth rate of 4<sup>th</sup> instar larvae was reduced parallel to the increasing concentration while a generally enhanced rate was recorded for last instar larvae. Nimbecidine exerted a prohibiting action on the excretory function in the 4<sup>th</sup> and 6<sup>th</sup> instar larvae which discharged drastically reduced amounts of fecal pellets.

**Keywords:** Assimilation, biomass, consumption, conversion, digestibility, frass, growth, larvae.

### INTRODUCTION

The Egyptian cotton leafworm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) is a polyphagous insect. It has long been a major polyphagous pest, widely distributed throughout Africa, Mediterranean Europe, and several parts of Asia (Azab *et al.*, 2001). Approximately 112 plant species belonging to 44 families are reported as hosts of this pest in tropical and temperate zones of the old world (Magd El-din and El-Gengaihi, 2000) or 73 species recorded from Egypt (Moufied *et al.*, 1960). In Egypt, this destructive phytophagous lepidopterous pest attacks cotton,

various vegetables and field crops all over the year (El-Khawas and Abd El-Gawad, 2002; Adham *et al.*, 2009). To control the attacks of *S. littoralis*, several types of insecticides have been used, including synthetic pyrethroids, organophosphates, and non-steroidal compounds (Casida and Quistad, 1998). In general, the extensive and indiscriminate use of these insecticides has caused resistant insect strains to emerge making their control even more difficult (Aydin and Gurkan, 2006; Mosallanejad and Smagghe, 2009) in addition to serious toxicological problems of the synthetic pesticides,

such as increased costs, handling hazards, several adverse effects on food, soil, ground water and air as well as carcinogenic, teratogenic and great threats to both human and environmental health (Costa *et al.*, 2008; Relyea, 2009; Garriga and Caballero, 2011). Over the past 25 years in Egypt, the intensive use of broad-spectrum insecticides against *S. littoralis* has led the development of insect resistance to many registered pesticides (Aydin and Gurkan, 2006). Owing to the socioeconomic importance of *S. littoralis*, the insect is subject to extensive research, much of which is focused on finding new ways to control it as a pest and to improve the effects of known pest control methods (Hussain, 2012). In this scenario, using new types of insecticides, originated from natural agents or products that disrupt the physiological processes of the target pest, could be useful alternatives in the integrated management approach (Smaghe *et al.*, 2003). To overcome those problems of synthetic pesticides, it is necessary to seek safe, convenient, environmental and low-cost alternative pest control methods among which are the botanicals. Plant extracts and plant based natural products in insect pest management programs are received much attention in recent years due to environmental pollution, pest resistance and resurgence, and undesirable effects to the non-target organisms caused by unsystematic use of synthetic pesticides. Several plant extracts or isolated active compounds have been shown to possess antifeedant activity (Ramya and Jayakumararaj, 2009). Some of plant derived products affect the feeding behavior of the insects and inhibit feeding (Chennaiyan *et al.*, 2016 a,b) while few others disrupt hormonal balance by inhibiting the growth, metamorphosis and reproduction. Several hundred plants have been reported as insect repellents, antifeedants, attractants, insecticides, ovicides and oviposition deterrents (Ekesi, 2000; Ulrichs *et al.*, 2008; Dubey *et al.*, 2010). The neem tree, *Azadirachta indica* A. Juss, is the most promising plant species being utilized for synthesis of biopesticides. Many compounds with biological activity have been extracted from its various parts, but seeds are the main source of bioactive compounds for neem-based insecticide formulations (Copping and Duke, 2007). Among the most important benefits of neem application are the insecticidal and feeding deterrent characteristics of its products (Morgan, 2009). The primary active ingredient of most neem-based pesticides is azadirachtin, a steroid-like tetra-nortriterpenoid, that exhibits a wide range of

bioactivity to hundreds of phytophagous insect species belonging to different orders. Along with direct toxicity, azadirachtin affects many different physiological events in insects, including regulation of growth, protein synthesis, reproduction, diapause, and behavior. Azadirachtin has hormonal effects, affecting both ecdysteroid and juvenile hormone titers (Abdullah and Subramanian, 2008; Morgan, 2009). Furthermore, azadirachtin interferes with chemoreception and exerts direct detrimental effects on many insect tissues such as muscles, fat body, and gut epithelial cells (Capinera and Froeba, 2007).

**Nimbecidine**<sup>®</sup> is a totally natural neem-oil based product containing 0.03% Azadirachtin as the major active ingredient in addition to other active compounds like Meliantriol, Salanin and Nimbin. Nimbecidine has a direct anti-feeding role due to its specific odour which directly affects gonadotropin production that eventually reduces the production of distinct ovarian protein (Wegener *et al.*, 2013; Amsalem *et al.*, 2014). After treatment of *Sphaerodema rusticum* with Nimbecidine, different metabolites were significantly affected in haemolymph and fat body (Shoba *et al.*, 2011, 2014). Nimbecidine inhibited the vitellogenesis of *Odontopus varicornis* via its effect on the neurosecretory cells, resulting in the malfunctioning of corpus allatum and the absence of its hormone (Ramya *et al.*, 2014). It influenced, also, the growth and development of *Helicoverpa armigera* (Wondafrash *et al.*, 2012) and caused significant reduction in fecundity, hatchability and adult emergence of *Earias vittella* (Bhardwaj and Ansari, 2015). Recently, Yasmin *et al.* (2016) reported that Nimbecidine acts as an insect repellent, antifeedant, growth regulator and mating disruptor. As an effective supplement for synthetic pesticides, it has been proved and recognized as ideal phytoproduct in the IPM program.

Feeding and reproduction in insects are very closely related to nutritional factors, the qualitative and quantitative aspects of which have impact on the rate of growth, development and fecundity. Since the amount, rate and quality of food consumed by a larva influences its performance, growth rate, development time, final body weight and survival (Slansky and Scriber, 1985). Therefore, an understanding of the nutritional indices in relation to the rate of ingestion, digestion assimilation and conversion by the growing larvae would be useful (Scriber and Slansky, 1981). Also, reduction in feeding activity of an insect may reduce normal development, weight gain, fecundity and increase mortality (Van Duyn, 1971).

It is important to point out that some of the natural products or synthetic chemicals disrupt the hormonal balance in insects by inhibiting the growth, metamorphosis and reproduction while other chemicals affect the feeding behavior of the insects and inhibit feeding. As defined by some authors (Yasui *et al.*, 1998; Lakshmanan *et al.*, 2012; Pavunraj *et al.*, 2012), antifeedant is a chemical that inhibits the feeding without killing the insect pest directly, while it remains near the treated foliage and dies through starvation. Some botanicals have been found as appetite inhibitors for insects. Because deterrence is the act of preventing a particular act or behavior from happening, these compounds and products can be described as food deterrents, phagodeterrents or antifeedants against insects. Antifeedant chemicals play a major role in the unsuitability of non host plants as food for insects. Isolation and structure elucidation of these active chemicals is important not only for understanding the ecological aspects of insect pests relationship, but also for their potential in insect pests control (Yasui *et al.*, 1998).

In insects, the physiological events that are linked to food consumption and utilization appear to be controlled by neural, endocrine and secretogogue mechanisms (Chapman, 1985). Hormones produced by the brain neurosecretory cells, the corpora cardiaca and corpora allata also control the digestive enzyme production (Prabhu and Sreekumar, 1994). With regard to the botanical influences on food metabolism of insects, many authors (Senthil-Nathan *et al.*, 2005, 2007) reported that the reduction of food consumption caused by botanicals has been reliant upon the insect species, type of botanical, and the concentration. However, the interferences of these materials with consumption, digestibility and conversion efficiency of food in several insect species after ingestion orally or by injection into their haemocoel are not consistent. Therefore, the current work was conducted aiming to assess the antifeedant activity of Nimbecidine and investigate its disruptive effects on the food consumption and utilization in 4<sup>th</sup> and 6<sup>th</sup> (last) larval instars of *S. littoralis*.

## MATERIALS AND METHODS

### A. Experimental insect

A sample of the Egyptian cotton leafworm *Spodoptera littoralis* pupae was kindly obtained from the culture of susceptible strain maintained for several generations in Plant Protection Research Institute, Agricultural Research Center, Doqqi, Giza, Egypt. In laboratory of Entomology,

Faculty of Science, Al-Azhar University, Cairo, a culture was reared under laboratory controlled conditions (27±2°C, 60-70% R.H., photoperiod 14 h L and 10 h D). Rearing procedure was carried out according to Ghoneim (1985) and improved by Bakr *et al.* (2010). Larvae were provided daily with fresh castor bean leaves *Ricinus communis*. The emerged adults were provided with 10% honey solution on a cotton wick as a food source. Moths were allowed to lay eggs on branches of *Nerium oleander*, then the egg patches were collected daily, and transferred into Petri dishes for another generation.

### B. Larval treatment with Nimbecidine

Nimbecidine® (Neem preparation with 0.03% EC Azadirachtin) was purchased from T. Stanes & company Ltd (Coimbatore, India). Most of the total food consumption and growth usually occur during the later larval instars and the performance values calculated for these instars tend to be representative of those calculated for the entire larval stage (Scriber and Slansky, 1981). Therefore, the 4<sup>th</sup> and 6<sup>th</sup> (last) larval instars of *S. littoralis* were chosen in the present study. In a preliminary experiment, 500, 100 and 10 ppm had been found as sublethal concentration levels of Nimbecidine against the 4<sup>th</sup> instar larvae.

Circular discs were cut from fresh clean leaves of the castor bean. After treatment of the leaf discs with each of these three concentrations, by dipping of leaf discs for 20 seconds and air drying for 5 minutes and then weighed, newly moulted 4<sup>th</sup> instar larvae were kept inside the Petri dishes (15 mm × 90 mm diameter) individually containing wet filter paper to avoid drying of the leaf disc. These larvae were starved for 3 hrs and enforced (no-choice method) to feed on the treated leaf discs for 24 hrs, then replaced with fresh untreated leaves along the larval stage (4<sup>th</sup>-6<sup>th</sup> instars). Control 4<sup>th</sup> instar larvae were provided with untreated leaves along the larval stage. Ten larvae were used as replicates for each treatment and control. The replicates were kept individually in 250 ml glass jars for observing and determining the nutritional parameters as described herein.

### C. Antifeedant activity

Antifeedant activity of Nimbecidine was recorded against the 4<sup>th</sup> instar larvae only because they were enforced to feed on the treated castor bean leaf discs. Antifeedant activity was assessed based on antifeedant index (AFI %). AFI was calculated according to the equation of Ladhari *et al.* (2013) as follows:  $AFI \% = [(C-T)/(C+T)] \times 100$  Where C: amount of food eaten by the control

insect. T: amount of food eaten by the treated insect.

#### D. Efficiencies of Food Metabolism

In the present work, food consumption, digestion, absorption, conversion efficiencies, assimilation, body weight gain, growth rate and frass output were determined through 4<sup>th</sup> and 6<sup>th</sup> larval instars of *S. littoralis*. Body weight of both treated and control was recorded before and after feeding, fresh food leaves were weighed before introduction to the larva, and then the fresh weight of remains was recorded after feeding every day. For calculating the corrected weight of consumed food, known weights of fresh food leaves were left without larva for 24 h, under the same laboratory conditions, and re-weighed at the end of this interval. Weight of faeces is the amount of frass produced by the larva during the last instar.

Relative weight gain (RWG) = mg weight gain during the instar/ days (Johnson and Mundel, 1987) with correction for a single instar.

Feeding rate is the amount of food consumed per instar along its feeding period; generally expressed on a "per day per unit body mass" basis (Slansky, 1993). Relative consumption rate (RCR) was calculated according to Slansky (1985) as follows: RCR = mg consumed food/ g mean fresh body weight/ day.

According to Waldbauer (1968), the following parameters can be calculated. Approximate digestibility (AD) = [Weight of ingested food - Weight of faeces / Weight of ingested food] X 100. Efficiency of conversion of ingested food to body substance (ECI) = [Weight gain / Weight of ingested food] X 100. Efficiency of conversion of digested food to body substance (ECD): [Weight gain / Weight of ingested food - Weight of faeces] x 100.

Assimilation rate (AR) = RCR x AD (Scriber and Slansky, 1981). Relative metabolic rate (RMR) was calculated according to Slansky (1980) but corrected for fresh weights and for a single nymphal instar as follows: RMR = (mg weight ingested food - weight of faeces) / g mean fresh body weight / day.

These parameters may help to clear the metabolic efficiencies which can affect growth (Hinks *et al.*, 1991). Growth rate (GR) can be calculated as follows: GR = fresh weight gain during feeding period / feeding period x mean fresh body weight of larvae during the feeding period (Waldbauer, 1968).

#### E. Statistical analysis of data

Data obtained were analyzed by the Student's *t*-distribution, and refined by Bessel correction

(Moroney, 1957) for the test significance of difference between means.

## RESULTS

### A. Antifeedant activity of Nimbecidine against *S. littoralis* larvae

In the present study, the newly moulted 4<sup>th</sup> instar larvae of *S. littoralis* were enforced to feed, in no-choice test, on Nimbecidine-treated castor bean leaf discs. Thus, the antifeedant activity of this plant product was assessed against this instar only. According to the antifeedant index (AFI) values arranged in Table 1, Nimbecidine exhibited a serious antifeedant activity against the 4<sup>th</sup> instar larvae. This antifeedant activity was found in a dose-dependent course (AFI: 30.10, 16.05 & 6.12%, at 500, 100 & 10 ppm, respectively).

**Table 1: Antifeedant activity of Nimbecidine against 4<sup>th</sup> instar larvae of *S. littoralis*.**

Conc. (ppm)	Antifeedant Index (%)
500	30.1
100	16.05
10	6.12
Control	---

### B. Effect of Nimbecidine on food consumption of *S. littoralis* larvae

Data distributed in Table 2 clearly revealed a significant reduction of food ingested and consumed by the 4<sup>th</sup> instar larvae in an inverse relation to the sublethal concentrations of Nimbecidine (206.3±7.4, 168.7±8.4 & 125.3±5.9 mg, at 10, 100 & 500 ppm, respectively, compared to 233.2±16.4 mg consumed by control larvae). Some of the treated 4<sup>th</sup> instar larvae successfully moulted to 5<sup>th</sup> instar and then to 6<sup>th</sup> instar. As obviously shown in Table 3, a similar reduction of food consumption was recorded for 6<sup>th</sup> instar larvae in an inverse relation to the Nimbecidine concentration (1340.0±79.4, 1140.7±114.3 & 843.0±50.9 mg, at 10, 100 & 500 ppm, respectively, vs. 1406.7±36.3 mg eaten by control congeners). Thus, no difference could be detected between these larval instars in response to the drastically reducing effect of Nimbecidine.

These data of food consumption could be expressed in relative consumption rate (RCR) and listed in Table 2. Pronouncedly decreasing RCR of 4<sup>th</sup> instar larvae was estimated (reduction %: 41.5, 31.9 & 5.9 at 500, 100 & 10 ppm of Nimbecidine, respectively). In addition, RCR of last instar larvae was unexceptionally suppressed by Nimbecidine (reduction %: 39.6, 12.7 & 6.7, at 500, 100 & 10 ppm, respectively). As easily seen,

the reducing effect of Nimbecidine on RCR (Table 3). intensified as the concentration was increased

**Table 2: Food ingestion and consumption of 4<sup>th</sup> instar larvae of *S. littoralis* as influenced by Nimbecidine.**

Conc. (ppm)	Food consumption (mg±SD)	RCR	Change (%)
500	125.3±5.9 d	1.91±0.23 d	-41.5
100	168.70±8.4 d	1.78±0.08 d	-31.9
10	206.30±7.4 c	1.27±0.11 d	-5.9
Control	233.20±16.4	1.35±0.06	---

Conc.: Concentration. Mean ± SD followed with (c): highly significantly different (P<0.01), (d): very highly significantly different (P<0.001). RCR: Relative consumption rate of food.

**Table 3: Food ingestion and consumption of last instar larvae of *S. littoralis* as influenced by Nimbecidine treatment of 4<sup>th</sup> instar larvae.**

Conc. (ppm)	Food consumption (mg±SD)	RCR	Change (%)
500	843.0±50.9 d	0.29±0.1 a	-39.6
100	1140.7±114.3 d	0.42±0.2 a	-12.7
10	1340.0±79.4 b	0.45±0.1 a	- 6.3
Control	1406.7±36.3	0.48±0.1	---

Conc., c, d, RCR: see footnote of Table (2). (a): not significantly different (P>0.05), (b): significantly different (P<0.05).

**C. Effect of Nimbecidine on food digestion, absorption and conversion efficiencies of *S. littoralis* larvae**

Response of the larval approximate digestibility (AD) to Nimbecidine depended on the larval instar because data of Table 4 clearly showed significantly or slightly enhanced AD of 4<sup>th</sup> instar larvae in no certain trend. The induced AD was determined in 4.6, 2.9 & 4.0, at 500, 100 & 10 ppm, respectively. In contrast, AD of last instar larvae was remarkably prohibited by Nimbecidine in an inverse correlation with concentration

(Reduction %s: 9.1, 6.1 & 2.6, at 10, 100 & 500 ppm, respectively, Table 5.

With regard to the efficiency of conversion of ingested food into biomass (ECI), Nimbecidine exhibited a general inhibitory effect on this efficiency of both 4<sup>th</sup> and 6<sup>th</sup> instar larvae. In the light of data assorted in Table 4, Nimbecidine exerted an inhibiting action on the ECI of 4<sup>th</sup> instar larvae, regardless the concentration. Reduction of ECI was considerable at the higher two concentrations (Change %s: -42.3 & -33.9) but low at the lowest concentration (Change %: -0.8).

**Table 4: Food digestion and absorption of 4<sup>th</sup> instar larvae of *S. littoralis* as influenced by Nimbecidine.**

Conc. (ppm)	AD	Change (%)	ECI	Change (%)	ECD	Change (%)
500	73.1±1.9 b	+4.6	14.3±1.7 d	- 42.3	19.2±1.9 d	- 45.9
100	71.9±2.5 a	+2.9	16.7±0.8 d	-33.9	24.2±2.05 d	-31.8
10	72.7±3.7 b	+4.0	24.6±2.3 a	-0.8	33.9±3.35 a	-4.5
Control	69.9±2.7	---	24.8±1.3	---	35.9±0.27	---

Conc, d: see footnote of Table (2). b: see footnote of Table (3). AD: Approximate digestibility. ECI: Efficiency of conversion of ingested food into biomass. ECD: Efficiency of conversion of digested food into biomass.

On the other hand, data arranged in Table 5 obviously revealed a contradictory effect of Nimbecidine on ECI of last instar larvae depending on the concentration since a drastic or slight inhibition of ECI was observed at the higher two concentrations (8.4±1.6 & 15.2±1.3 vs. 16.3±5.4 of control larvae) but an exceptional case of slightly

enhanced ECI was recorded at the lowest concentration (21.3±5.0 vs. 16.3±5.4 of control larvae).

In respect of the efficiency of conversion of digested food into biomass (ECD), data assorted in Table (4) unambiguously displayed an inhibitory effect of Nimbecidine on ECD of 4<sup>th</sup> instar larvae

(reduction %s: 45.9, 31.8 & 4.5 at 500, 100 & 10 ppm, respectively). Similar to its effect on ECI of last instar larvae, Nimbecidine exhibited inconsistent effects on ECD of last instar larvae, depending on the concentration since ECD was

slightly reduced at the higher two concentration levels (reduction %s: 19.5 & 1.3, at 500 & 100 ppm, respectively) but insignificantly increased at the lowest one (induction %: 44.3, Table 5).

**Table 5: Food digestion and absorption of last instar larvae of *S. littoralis* as influenced by Nimbecidine treatment of 4<sup>th</sup> instar larvae.**

Conc. (ppm)	AD	Change (%)	ECI	Change (%)	ECD	Change (%)
500	68.4±2.5 c	-2.6	8.4±1.6 c	-24.5	18.2±5.5 a	-19.5
100	68.3±4.5 b	-6.1	15.2±1.3 a	- 6.7	22.3±1.5 a	-1.3
10	66.1±5.1 b	-9.1	21.3±5.0 a	+30.7	32.6±1.9 a	+44.3
Control	72.7±2.1	---	16.3±5.4	---	22.6±7.1	---

Conc, c: see footnote of Table (2). a, b: see footnote of Table (3). AD, ECI, ECD: see footnote of Table (4) footnote of Table (3).

**D. Effect of Nimbecidine on the food assimilation by *S. littoralis* larvae**

For extensive investigation of the food metabolism, two additional metabolic parameters (assimilation rate, AR, and relative metabolic rate, RMR) may shed some light on the effect of Nimbecidine. As easily seen in Table 6, AR of 4<sup>th</sup> instar larvae was significantly induced by Nimbecidine at the higher two concentrations (13.8±1.41 & 12.1±1.02 at 500 & 100 ppm, respectively) but slightly regressed at the lowest concentration (8.4±0.99 vs. 9.4±0.77 of control larvae).

Just a look at data of Table 7, AR of last instar larvae was considerably or insignificantly suppressed by Nimbecidine (19.7±6.8, 25.8±6.7 & 27.5±8.1, at 500, 100 & 10 ppm, respectively, vs. 34.9±1.5 of control congeners). Thus, the last instar larvae were evidently more responsive to Nimbecidine than 4<sup>th</sup> instar larvae. Considering RMR, Nimbecidine generally promoted larvae of both 4<sup>th</sup> and 6<sup>th</sup> instars to attain significantly or slightly increasing RMR. The increasing RMR of 4<sup>th</sup> instar larvae had been found in a reverse trend of concentration while the increasing RMR of 6<sup>th</sup> instar larvae was recorded in no certain trend.

**Table 6: The correlation of AR and RMR to RWG and GR of 4<sup>th</sup> instar larvae of *S. littoralis* as influenced by Nimbecidine.**

Conc. (ppm)	AR	RMR	RWG	GR
500	13.8±1.41 d	2.8±0.13 a	4.9±0.71 d	4.0±0.71 d
100	12.1±1.02 d	2.9±0.06 a	8.1±0.73 d	6.0±0.44 d
10	8.4±0.99 a	3.0±0.18 b	15.7±0.95 c	9.8±0.46 c
Control	9.4±0.77	2.7±0.19	19.0±1.66	11.0±0.60

Conc, c, d: see footnote of Table (2). a, b: see footnote of Table (3). AR: Assimilation rate. RMR: Relative metabolic rate. RWG: Relative weight gain. GR: Growth rate.

**E. Effect of Nimbecidine on somatic growth and frass production by *S. littoralis* larvae**

Data of the relative weight gain (RWG) and growth rate (GR) of treated 4<sup>th</sup> instar larvae and their control congeners were distributed in Table 6 and data of RWG and GR of last instar larvae were arranged in Table 7. According to these data, Nimbecidine exhibited a conspicuous inhibitory effect on RWG of 4<sup>th</sup> instar larvae in a dose-dependent course (15.7±0.95, 8.1±0.73 & 4.9±0.71, at 10, 100 & 500 ppm, respectively, vs. 19.0±1.66 of control larvae). To a great extent, a

similar inhibitory effect of Nimbecidine was exhibited on RWG of last instar larvae.

Concerning the GR of 4<sup>th</sup> instar larvae, it was reduced parallel to the increasing concentration (9.8±0.46, 6.0±0.44 & 4.0±0.71, at 10, 100 & 500 ppm, respectively, vs. 11.0±0.6 of control larvae). On the contrary, a diverse effect of Nimbecidine on GR of last instar larvae was exhibited since it decreased at the highest concentration but increased at the other concentrations (2.5±0.44, 1.7±0.11 & 1.0±0.2, at 10, 100 & 500 ppm, respectively, vs. 1.4±0.5 of control congeners).

**Table 7: The correlation of AR and RMR to RWG and GR of last instar larvae of *S. littoralis* as influenced by Nimbecidine treatment of 4<sup>th</sup> instar larvae.**

Conc. (ppm)	AR	RMR	RWG	GR
500	19.7±6.8 d	3.7±0.10 d	11.4±4.01 d	1.0±0.20 a
100	25.8±6.7 c	2.5±0.30 c	29.6±0.33 a	1.7±0.11 a
10	27.5±8.1 a	2.6±0.19 d	41.9±8.84 a	2.5±0.44 c
Control	34.9±1.5	1.9±0.20	42.5±15.70	1.4±0.50

Conc., c, d: see footnote of Table (2). a, b: see footnote of Table (3). AR, RMR, RWG, GR: see footnote of Table (6).

**Table 8: Frass production (mg±SD) by *S. littoralis* larvae as influenced by Nimbecidine.**

Conc. (ppm)	4 <sup>th</sup> instar larvae	6 <sup>th</sup> instar larvae
500	69.9 ± 2.7 d	266.6 ± 36.9 d
100	108.4 ± 6.9 d	342.6 ± 57.5 b
10	123.9 ± 8.5 d	446.2 ± 57.1 a
Control	147.3 ± 9.3	446.9 ± 50.2

Conc., d: see footnote of Table (2). a, b: see footnote of Table (3).

Data of frass output by larvae of both 4<sup>th</sup> and 6<sup>th</sup> larval instars were summarized in Table 8. As shown in this table, Nimbecidine exerted a prohibiting action on the excretory function of larvae of both instars which discharged drastically reduced amounts of fecal pellets. The prohibiting action of Nimbecidine increased by increasing concentration, regardless the instar (Table 8). Larger amounts of fecal pellets discharged by 6<sup>th</sup> instar larvae than those discharged by 4<sup>th</sup> instar larvae had been observed in a positive correlation with the food consumption (Tables 2 & 3) and body weight gain of both instars (Tables 6 & 7).

## DISCUSSION

Food utilization efficiencies are useful for measuring the growth rate and development of the consumer (Scriber and Slansky, 1981). Several metabolic parameters were suggested and usually used to determine the food utilization. However, the common three parameters are: approximate digestibility (AD), efficiency of conversion of ingested food to biomass (ECI) and efficiency of conversion of digested food to biomass (ECD) (Waldbauer, 1968; Slansky, 1993). As described by Senthil-Nathan *et al.* (2005), ECI is an overall measure of an insect's ability to utilize the ingested food for growth and development and ECD is a measure of the efficiency of conversion of digested food into growth. ECD is sometimes called "Net growth efficiency" or "Metabolic efficiency" (Slansky and Scriber, 1985).

*A. Antifeedant efficacy of Nimbecidine against S. littoralis larvae*

In fact, extracts or products of several hundred plants have been reported as insect toxins, repellents, antifeedants, attractants, ovicides, oviposition deterrents (Ekesi, 2000; Ulrichs *et al.*, 2008; Dubey *et al.*, 2010) and growth inhibitors (Ekesi, 2000) as well as reproductive inhibitors against many pest species (Rai and Carpinella, 2006; Ben Hamouda *et al.*, 2015a, b, c). Therefore, plant extracts and plant based natural products are received much attention in recent years for the pest management programs in order to avoid the environmental pollution, pest resistance and resurgence, and undesirable effects to the non-target organisms caused by synthetic pesticides. Discovery of novel antifeedants from plant extracts has been recently emphasized as a potential method for the development of "ecologically safe pesticides" (Wheeler *et al.*, 2001). In other words, the quantification of antifeedant effect of botanicals is of great importance in the field of insect pest management (Pavunraj *et al.*, 2012). Antifeedant activity of botanicals against insects has been studied in many countries. Azadirachtin is the predominant biologically active chemical in most plant-based bioassays and is known as the 'most potent insect antifeedant discovered to date' (Miller *et al.*, 2006).

There is a rich literature on the antifeedant activity of extracts of several plants plant products against different insect pests. Significant feeding deterrence in larvae and adults of *Epilachna dodecastigma* had been recorded after treatment of 2<sup>nd</sup> instar larvae with the Neem oil nonidet (Anam *et al.*, 2006). Against *Lymantria dispar* larvae, ethanol extract of *Aesculus hippocastanum*

had strong antifeeding activity (Gvozdenac *et al.*, 2012). As reported by Wondafrash *et al.* (2012), the antifeedant activity of neem seed extract was greater than the neem leaf extract and the latter was stronger than Nimbecidine (0.03% Azadirachtin) against *Helicoverpa armigera* larvae. Against the 4<sup>th</sup> instar larvae of *Glyphodes pyloalis*, Achook (0.03% Azadirachtin) (Khosravi and Sendi, 2013) and essential oils of *Thymus vulgaris* and *Origanum vulgare* (Yazdani *et al.*, 2014) exhibited considerable antifeeding efficacies. Supercritical carbon dioxide extract of *Melia azedarach* fruits exerted a pronounced antifeedant action on *Spodoptera frugiperda* larvae at the higher concentrations (Scapinello *et al.*, 2014). A significant deterrence was observed for acetone extract of olive leaves against *Phthorimaea operculella* (Ben Hamouda *et al.*, 2015a). The seed and leaf extracts of *Solanum elaeagnifolium* had strong antifeedant activities against larvae of *Tribolium castaneum* (Ben Hamouda *et al.*, 2015b). The stem methanol extract of *Thevetia nerifolia* exhibited remarkably or slightly antifeedant efficacy against the early 4<sup>th</sup> instar larvae of *H. armigera*, depending on the concentration (Mishra *et al.*, 2015). Flavonoids (isolated from *Marchantia linearis*) exhibited feeding deterrent activity against 5<sup>th</sup> instar larvae of *Spodoptera litura* (Krishnan and Murugan, 2015). Recently, the petroleum ether, chloroform and ethyl acetate extracts of *Duranta erecta* leaves (Chennaiyan *et al.*, 2016a) and *Barleria longiflora* leaves (Chennaiyan *et al.*, 2016b) were assessed against *S. litura* larvae. The maximum antifeedant activity was recorded in ethyl acetate extract, followed by chloroform extract and petroleum ether extract.

In agreement with those reported results, the present study revealed a serious antifeedant efficacy of Nimbecidine against the 4<sup>th</sup> instar larvae of *S. littoralis* which had been enforced to feed, in no-choice test, on treated castor bean leaf discs. On the contrary, these results disagreed with those results reported the absence on antifeedant activity of Azadirachtin against *Peridroma saucia* (Koul and Isman, 1991) and *Manduca sexta* (Timmins and Reynolds, 1992). Also, the ethanol extracts of *Ambrosia artemisiifolia*, *Elodea canadensis* and *Daucus carota* exhibited no antifeedant activity against *L. dispar* larvae (Gvozdenac *et al.*, 2012).

For understanding the antifeedant efficacy of Nimbecidine against the 4<sup>th</sup> instar larvae of *S. littoralis*, in the current work, it is important to mention that the feeding behaviour depends upon both neural input from the insect's chemical

senses (taste receptors on tarsi, mouth parts, and oral cavity) and central nervous integration of this 'sensory code'. On the basis of Frazier and Chyb (1995)'s suggestion, insect feeding can be inhibited at three levels: preingestional (immediate effect associated with host finding and host selection processes involving gustatory receptors), ingestional (related to food transport and production, release, and digestion by salivary enzymes), and postingestional (long-term effects involving various aspects of digestion and absorption of food). Ben Hamouda *et al.* (2015a, b) reported that the active gradients present in certain plant species inhibit feeding behavior of the insect or make the food unpalatable or directly act on the chemosensilla of the insect resulting in feeding deterrence. However, several secondary metabolites contained in various plant species are known as antifeedants and possess food deterrence properties. These include sesquiterpene lactones, diterpenoids, triterpenoids, quinoline and indole alkaloids (Nawort *et al.*, 1986), cucurbitacines, quines and phenols (Norris, 1986) as well as glycoalkaloids and steroidal saponins (Wink, 2006). Koul (2005) reported that the considerably effective feeding inhibitors against insects come from terpenoids, alkaloids, saponins and polyphenols. In the current investigation, Nimbecidine is totally natural neem-oil based product containing 0.03% Azadirachtin as the major active ingredient in addition to other active compounds like Meliantriol, Salanin and Nimbin. One or more of these active compounds might prohibit the 4<sup>th</sup> instar larvae of *S. littoralis* to feed on treated food leaves.

#### *B. Food consumption by S. littoralis larvae as influenced by Nimbecidine*

With regard to the botanical influences on food metabolism of insects, many authors reported that the reduction of food consumption has been reliant upon the insect species, type of botanical, and the concentration. However, the interferences of these materials with consumption, digestibility and conversion efficiency of food in several insect species after ingestion orally or by injection into their haemocoel are not consistent (Senthil-Nathan *et al.*, 2005, 2007).

There is a large body of literature on the reported reduction of food consumption in many insects by several botanicals, such as reduction of consumption rate in larvae and adults of *E. dodecastigma* after feeding of 2<sup>nd</sup> instar larvae on Neem oil (nonidet)-treated leaves (Anam *et al.*, 2006), decreasing food consumption index after feeding of the 3<sup>rd</sup> instar larvae of *Spodoptera*

*exigua* on an artificial diet treated with Goniothalamine (isolated from *Goniothalamus wightii*) (Senthil-Nathan *et al.*, 2008). Also, food consumption of 4<sup>th</sup> instar caterpillars of *Spilarctia oblique* was gradually reduced with increase of the concentration of Nimbecidine (Ali *et al.*, 2008), food consumption of *Locusta migratoria* nymphs was significantly reduced in a dose-dependent manner by feeding on a diet treated with gibberellic acid (Abdellaoui *et al.*, 2009), food consumption index of *Schistocerca gregaria* adult females was reduced after treatment of last (5<sup>th</sup>) instar nymphs with Farnesol (plant product) (Awad *et al.*, 2013) and food consumption index of the 3<sup>rd</sup> instar caterpillars of *Hyposidra talaca* showed a decreasing trend after treatment with water extracts of *Polygonum hydropiper* and *Annona squamosa* (Roy *et al.*, 2015). In addition, treatment of the 2<sup>nd</sup> instar larvae of *Spodoptera eridania* with pure neem oil and Azatrol (1.2% Azadirachtin) through a synthetic diet resulted in reduction of consumption index, especially by the higher concentrations (Shannag *et al.*, 2015). Food consumption of *H. armigera* 4<sup>th</sup> instar larvae was significantly reduced when diet was treated with concentration level 5% of methanol extract of *Th. neriifolia* stems (Mishra *et al.*, 2015). The food consumption rate of *Plutella xylostella* 3<sup>rd</sup> instar larvae was significantly reduced by *O. vulgare* essential oil (Nasr *et al.*, 2015). Results of the present study are, to a great extent, in congruence with those reported results because a significant reduction of food consumed by 4<sup>th</sup> and 6<sup>th</sup> instar larvae of *S. littoralis* was observed in an inverse relation to the sublethal concentrations (500, 100 & 10 ppm) of Nimbecidine. No difference could be detected between the two larval instars in response to the reducing action of this neem product. On the other hand, results of the current work were in contrast with some reported results of significantly or slightly enhanced food consumption of some insects by different botanicals, such as *S. littoralis* 4<sup>th</sup> instar larvae after treatment with hexane extract of *Conyza dioscoridis* (Ebeid *et al.*, 2015), 3<sup>rd</sup> instar larvae of the same lepidopteran after treatment with methanol, ethanol and aqueous extracts of *Punica granatum* peel (Ben Hamouda *et al.*, 2015 c), *S. frugiperda* larvae after treatment with the a trypsin inhibitor (isolated from *Ricinus communis* leaves) (Carvalho *et al.*, 2015). In the present study, Nimbecidine prohibited *S. littoralis* larvae to consume normal amounts of food due to one or more of its components which directly or indirectly affected the 'centers' that control feeding and metabolism (Barnby and

Klocke, 1978). In addition, this remarkable reduction of food consumption of *S. littoralis* larvae can be attributed to a direct or indirect interference of Nimbecidine with the hormonal regulation of food intake (Calvez, 1981). It can be interpreted, also, by the partial avoidance of larvae to introduce Nimbecidine-treated food by the adversely affected mandibles and labrum or due to the blocked gut function such as prohibited proteases and -amylase (Khosravi and Sendi, 2013; Masih and Vaishya, 2014). Another suggestion can be accepted since Shekari *et al.* (2008) attributed the reduced food consumption to a stress of the botanical or some of its chemical constituents on the enzyme expression system to synthesize new and higher amounts of detoxification enzymes.

#### *C. Food digestive and absorptive capacities of S. littoralis larvae as influenced by Nimbecidine*

Special attention should be paid to another important nutritional parameter, AD, which expresses the digestion and absorption capacity of the insect. AD in insects is based on differences between the weight of ingested food and the weight of faeces, actually represents the food which is stored or metabolized. Therefore, the AD estimates the percentage of ingested food that is digested (Slansky and Scriber, 1985).

In the present study, enhanced AD was recorded for 4<sup>th</sup> instar larvae of *S. littoralis* as a response to the action of Nimbecidine, but remarkably prohibited AD in the last instar larvae, in an inverse relation to concentration. However, the enhancement of AD of 4<sup>th</sup> instar larvae came in agreement with several reported results of increasing AD of some insects by different botanicals, such as *Cnaphalocrocis medinalis* larvae after feeding on leaves treated with methanol extract of *M. azedarach* leaves (Senthil-Nathan, 2006), *G. pyloalis* 4<sup>th</sup> instar larvae after feeding on leaves treated with LC<sub>30</sub> of essential oils of *Th. vulgaris* or *O. vulgare* (Yazdani *et al.*, 2014), *H. talaca* 3<sup>rd</sup> instar caterpillars after feeding on food treated with water extracts of *P. hydropiper* or *A. squamosa* (Roy *et al.*, 2015), *S. litura* 5<sup>th</sup> instar larvae after treatment with flavonoids (isolated from *M. linearis*) (Krishnan and Murugan, 2015), *S. littoralis* 3<sup>rd</sup> instar larvae after treatment with methanol, ethanol and aqueous extracts of *P. granatum* peel (Ben Hamouda *et al.*, 2015c), *etc.*

On the contrary, the remarkably inhibited AD of 6<sup>th</sup> (last) instar larvae of *S. littoralis* as response to an inhibitory effect of Nimbecidine, in the present study, is in accordance with some reported results

of reduced AD in some insects by extracts of various plants or botanical products, such as *Earias insulana* larvae after treatment with garlic acid (Amr, 1986), *Agrotis ipsilon* 3<sup>rd</sup> instar larvae after treatment with LC<sub>50</sub> of oils of *Brassica napus* or *Sesamum indicum* (Ali, 2008), *Pieris rapae* larvae after treatment with methanol extract of *Silybium marianum* (Hasheminia *et al.*, 2013), *S. littoralis* 4<sup>th</sup> instar larvae after treatment with alcohol extract of *C. dioscoridis* (Ebeid *et al.*, 2015) and *S. eridania* 2<sup>nd</sup> instar larvae after treatment with pure neem oil or Azatrol, especially at the higher concentrations (Shannag *et al.*, 2015).

However, the enhancement of AD in 4<sup>th</sup> instar larvae of *S. littoralis* after treatment with Nimbecidine, in the current study, can be understood in the light of some suggestions reported herein. The increasing AD could be connected with a decrease rate of the passage of food in the gut owing to lack of tone in the muscles caused by Nimbecidine (Azadirachtin or some other active constituents) (Mordue (Luntz) *et al.*, 1985). Furthermore, AD may not, *after ali*, be closely connected with the retention time of food in the gut (Slansky and Wheeler, 1991; Slansky, 1993). It has been reported that adverse effect of a botanical, such as azadirachtin, on the midgut epithelial cells might disrupt the enzyme secretion and nutrient absorption (Nasiruddin and Mordue (Luntz), 1993).

On the opposite side, the decreased AD of last instar larvae of *S. littoralis* by Nimbecidine, in the current investigation, can be attributed to its toxic activity causing some cellular changes in the midgut epithelium. Therefore, it is reasonable to suggest that the reduced digestive and absorptive capacity is a result of the hypertrophy and displacement of the midgut epithelial cells from the basal lamina (Correia *et al.*, 2009). Also, Nimbecidine might exhibit a secondary effect on the normal gut function resulting in a reduction in the efficiency of protein digestion (Nasiruddin and Mordue (Luntz), 1994).

#### *D. Food conversion efficiencies of S. littoralis larvae as influenced by Nimbecidine*

From the metabolic view of point, the most important efficiencies of food metabolism are ECI and ECD. These efficiencies vary widely with the insect species. As for example, ECI and ECD of lepidopterous larvae are about double those of orthopterous larvae, while AD being about the same. The efficiencies of food utilization also vary with age (both within and between instars) and sex as well as with different environmental factors.

In the present study, Nimbecidine exhibited a general inhibitory effect on both ECI and ECD of both 4<sup>th</sup> and 6<sup>th</sup> instar larvae of *S. littoralis* with an exceptional case of slightly enhanced ECI and ECD of last instar larvae at the lowest concentration. The general reduction of ECI and ECD are concomitant to those reported results of drastically or slightly reduced food conversion efficiencies of several insect species after treatment with various botanicals, such as *Cn. medinalis* larvae after ingestion of the neem limonoids (Senthil Nathan *et al.*, 2005) or methanol extract of *M. azedarach* leaves (Senthil-Nathan, 2006), *Leptinotarsa decemlineata* larvae after feeding on diet treated with phenols and phenolic acid (Pavela, 2007); *S. exigua* 3<sup>rd</sup> instar larvae after feeding on an artificial diet treated with Goniotalamin (isolated from *G. wightii*) proportionally to the increasing concentration (Senthil-Nathan *et al.*, 2008), *G. pyloalis* 4<sup>th</sup> instar larvae after feeding on mulberry leaves treated with Achook (0.03% Aza) (Khosravi and Sendi, 2013) or after treatment with essential oils of *Th. vulgaris* and *O. vulgare* (Yazdani *et al.*, 2014), *H. talaca* 3<sup>rd</sup> instar caterpillars after treatment with water extracts of *P. hydropiper*, *A. squamosa*, *Clerodendrum viscosum*, *Argyreia speciosa* and *Leucas aspera* (Roy *et al.*, 2015), *P. xylostella* 3<sup>rd</sup> instar larvae after treatment with *O. vulgare* essential oil (Nasr *et al.*, 2015); *etc.*

The significantly reduced ECI and ECD, in the current work, may be due to the increased energetic costs arising from a reduced ability to utilize diet nitrogen which would not necessarily interfere with absorption from the gut (Timmins and Reynolds, 1992). In addition, the reduction in ECI and ECD results from a foodstuff conversion deficiency, which promotes growth, perhaps through a diversion of energy from the biomass production into detoxification (Senthil-Nathan, 2006; Senthil-Nathan *et al.*, 2007).

Concerning the exceptional case of increased ECI and ECD of last instar larvae of *S. littoralis* at the lowest concentration of Nimbecidine, in the present study, it was not the first record of enhanced food conversion efficiencies because some authors reported similar results for other insects after treatment with some botanicals (El-Malla and Radwan, 2008; Hasheminia *et al.*, 2013; Ebeid *et al.*, 2015). This exceptionally increasing ECI and ECD, in the present study, may be attributed to the fact that Nimbecidine-treated last instar larvae, at the lowest concentration, required large amounts of energy to deal with Nimbecidine toxicity. Unfortunately, we have no another

conceivable interpretation to this case of increased ECI and ECD right now!!

#### *E. Food assimilation and metabolism in S. littoralis larvae as influenced by Nimbecidine*

Some other nutritional parameters had been interestingly used in this area of study, viz. Assimilation rate (AR) and Relative metabolic rate (RMR). These parameters may help to clear the metabolic capacity which can affect the growth (Hinks *et al.*, 1991). As reported in the literature, AR attained by 2<sup>nd</sup> instar larvae of *S. eridania* was subjected to a reducing effect of the pure neem oil and Azatrol, especially at the higher concentrations (Shannag *et al.*, 2015). On the other hand, feeding of *H. virescens* larvae on tobacco plants, expressing potato proteinase inhibitors (PIN-2), resulted in no significantly affected AR (Brito *et al.*, 2001). In the present study, AR of 4<sup>th</sup> instar larvae of *S. littoralis* was significantly induced at the higher two concentrations of Nimbecidine but inhibited at the lowest one. AR of last instar larvae was considerably or slightly suppressed, regardless the concentration.

In respect of RMR, no reduction was reported in the available literature, irrespective of the insect species or botanical. On the contrary, RMR of the *S. frugiperda* 4<sup>th</sup> instar larvae increased after ingestion of a diet containing fractions from ethanol extract of *Toona ciliata* fruits and *Trichilia pallida* stems (Giongo *et al.*, 2015). In accordance with those reported results, the present results clearly revealed a significantly or slightly enhancing effect of Nimbecidine on RMR of *S. littoralis* larvae of both instars, regardless the concentration. On the other hand, no significantly affected RMR was recorded for larvae of *H. virescens* after feeding on tobacco plants treated with potato protease inhibitors (PIN-2) (Brito *et al.*, 2001) and for larvae of *S. frugiperda* after treatment with a trypsin inhibitor (isolated from *R. communis* leaves) (Carvalho *et al.*, 2015).

#### *F. Interrelationship between growth and nutritional performance of S. littoralis larvae under stress of Nimbecidine*

Taking into consideration that one of the principal goals of feeding is growth and development, it should be of great importance to determine the relative body weight gain (RWG). The available literature contains many reported results of drastically or insignificantly reduced RWG of various insects by different botanicals, such as *S. obliqua* 6<sup>th</sup> instar larvae after treatment of 4<sup>th</sup> instar larvae with Nimbecidine (Ali *et al.*, 2008), first two instars of *S. litura* after treatment with *S. indicum*

extracts (Sintim *et al.*, 2009), both 2<sup>nd</sup> and 4<sup>th</sup> instar larvae of *S. littoralis* after treatment with acetone extract of *M. azedarach* (Farag *et al.*, 2011) or fatty linoleic acid (Yousef *et al.*, 2013), *H. armigera* larvae after treatment with methanol and n-hexane extracts of *Artemisia annua* leaf (Anshul *et al.*, 2013), both 4<sup>th</sup> and 5<sup>th</sup> instar nymphs of *S. gregaria* after treatment with Neemazal or *Nigella sativa* seed extracts (Hamadah *et al.*, 2013), 5<sup>th</sup> instar nymphs of the same locust after treatment with *Ammi visnaga* fruit extracts (Ghoneim *et al.*, 2014), 4<sup>th</sup> instar larvae of *S. frugiperda* after feeding on a diet treated with ethanol extract of *T. pallida* leaves, *Trichilia pallens* stems and *T. ciliata* leaves and fruits (Giongo *et al.*, 2015), etc. In agreement with those reported results, RWG of *S. littoralis* larvae both 4<sup>th</sup> and 6<sup>th</sup> instars, in the present study, had been generally reduced after feeding of 4<sup>th</sup> instar larvae on castor bean leaves treated with sublethal concentrations of Nimbecidine.

Another point of interest is the affected growth rate (GR) or relative growth rate (RGR) which indicates the gain of insect biomass in relation to body weight per day. As obviously shown in the literature, GR or RGR of many insects had been suppressed by several botanicals, such as *Cn. medinalis* larvae after ingestion of the neem limonoids (Senthil Nathan *et al.*, 2005) or methanol extract of *M. azedarach* leaves (Senthil-Nathan, 2006), *L. decemlineata* larvae after treatment with phenols and phenolic acid (Pavela, 2007), *A. ipsilon* 3<sup>rd</sup> instar larvae after feeding on castor bean leaves treated with oils of *B. napus*, *Helianthus annuus* or *S. indicum* (Ali, 2008), *Helicoverpa zea* larvae after treatment with the powder of *Peumus boldus* (Silva-Aguayo *et al.*, 2010), *P. rapae* larvae after treatment with the methanol extract of *S. marianum* (Hasheminia *et al.*, 2013), larvae of *T. castaneum* and *Corcyra cephalonica* after treatment with some isoflavones derived from *Derris scandens* (Rani *et al.*, 2013), 1<sup>st</sup> instar larvae of *Plodia interpunctella* after treatment with extracts of *Mentha piperita* and *Mentha pulegium* (Saeidi and Hassanpour, 2014) or essential oils of *Satureja hortensis* and *Fumaria parviflora* (Shahab-Ghayoor and Saeidi, 2015), *Sitophilus zeamais* larvae after treatment with Polygodial, ugandensolide and warbuganal (isolated from oil extract of *Warburgia ugandensis*) (Opiyo *et al.*, 2015), *Trogoderma granarium* larvae after treatment with *Piper nigrum* extracts (Javed *et al.*, 2016), etc.

In conformity with those reported results, GR of 4<sup>th</sup> instar larvae of *S. littoralis* was significantly inhibited proportionally to the increasing

concentration of Nimbecidine, in the current study. In contrast, Nimbecidine generally enhanced GR of 6<sup>th</sup> (last) instar larvae of *S. littoralis*. This result, however, agrees with scarcely reported results of increased GR or RGR of some insects, such as *S. littoralis* larvae after feeding on Spinosad-treated food (El-Malla and Radwan, 2008).

However, the reduction of RWG in both 4<sup>th</sup> and last instar larvae as well as the inhibited GR of 4<sup>th</sup> instar larvae *S. littoralis*, in the present study, can be interpreted by the inhibitory effect of Nimbecidine on food intake, digestion and metabolism. Thus, the inadequate food and impaired metabolism might adversely affect certain endocrinal events resulting in an inhibited growth (Abouelghar *et al.*, 2013). It is important to point out that one or more of Nimbecidine constituents (*viz.*, Azadirachtin, Meliantriol, Salanin, Nimbin, etc.) might exhibit such inhibitory effect on the larval growth of *S. littoralis* through a reduction of the enzymatic activities of proteases and  $\alpha$ -amylase in the midgut (Martinez and Endem, 2001; Khosravi and Sendi, 2013). Also, the growth inhibition may be attributed to the use of food for purposes other than growth, such as detoxification enzymes synthesis (Giongo *et al.*, 2015). On the other side, the enhancing action of Nimbecidine on the growth of last instar larvae of *S. littoralis*, in the present study, cannot be acceptably interpreted right now!!

In connection with the frass production of *S. littoralis*, results of the current work clearly revealed a prohibiting action of Nimbecidine on the excretory function of larvae of both 4<sup>th</sup> and 6<sup>th</sup> instars which discharged little amounts of fecal pellets. In addition, the amount of fecal pellets discharged by 6<sup>th</sup> instar larvae was larger than that discharged by 4<sup>th</sup> instar larvae. This had been observed in a positive correlation with the food consumption and body weight gain of each instar. These results are, to a some extent, agreement with those reported results of decreasing fecal output of some insects under stress of different botanicals, such as *S. frugiperda* 4<sup>th</sup> instar larvae after feeding on an artificial diet treated with Arturmerone (extracted from rhizomes of *Curcuma longa*) (Tavares *et al.*, 2013) or on food treated with ethanol extract of *T. pallida* leaves, *T. pallens* stems and *T. ciliata* leaves and fruits (Giongo *et al.*, 2015). Also, prohibited fecal output was reported for *S. litura* 5<sup>th</sup> instar larvae after treatment with flavonoids (Krishnan and Murugan, 2015). However, the reduction of frass production of *S. littoralis*, in the present study, can be explained by an adverse action of Nimbecidine on the peristaltic movement of the gut (Broadway,

and Duffey, 1988) which was supported by Senthil-Nathan and Saehoon (2006).

## CONCLUSION

In the present study, Nimbecidine (0.03% Azadirachtin) appeared to possess anti-nutritional properties exhibiting considerable deterrence efficacy and predominant detrimental effect on the food consumption, digestion, absorption, conversion and assimilation reflecting on inhibited growth of *S. littoralis* larvae. Therefore, we would conclude that this natural neem product can be used as a new effective alternative to the conventional synthetic insecticides and may play a more prominent role in the integrated pest management programs against this dangerous agricultural pest in the future.

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