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ORIGINAL RESEARCH ARTICLE

Individual and combined treatments with imidacloprid and spinosad disrupt survival, life-history traits, and nutritional physiology of *Spodoptera littoralis*

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Abstract
The Egyptian cotton leafworm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) is a pervasive agricultural polyphagous insect pest. Because of the negative side-effects of conventional pesticides used in agricultural fields, safer alternatives for insect pest management are required. We evaluated here susceptibility, biological features, and nutritional indices of *S. littoralis* after treatment of 4th-instar larvae with the neonicotinoid imidacloprid and the spinosyn spinosad separately or in combination. Larvae were fed for three successive days on treated leaves of castor-bean *Ricinus communis* using leaf-dip technique (treatment period). In addition, in case of nutritional indices study, treated leaves were replaced by fresh untreated leaves for two successive days (recovery period). Spinosad was more toxic than imidacloprid, and their combination revealed additive effects based on the co-toxicity factor. Individual and combined treatments significantly decreased pupation rate, adult emergence rate, pupal weight, number of eggs laid per female, egg-hatch, and female longevity, compared to those of controls. Pupal and adult malformations were recorded. During the treatment period, nutritional indices were insecticide- and time-dependent. On the 2nd day of recovery, all nutritional indices of treated larvae were not significantly different, compared to those of controls. The results presented herein may help in developing more effective crop protection methodologies within integrated pest management of this insect.

Keywords Biological features · Egyptian cotton leafworm · Neonicotinoids · Nutritional indices · Spinosyns · Toxicity

Introduction

The Egyptian cotton leafworm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctiudae) is one of the most injurious and destructive polyphagous insect pests. It infests about 90 host plant species belonging to 40 families, including economically valuable vegetables and crops (Shaurub et al. 2020a). In Egypt, this insect occurs throughout the year and is considered one of the most destructive pests to cotton, the key economic crop in the country (Shaurub et al. 2020a).

The extensive use of broad-spectrum synthetic insecticides has given rise to problems, such as residual toxic effects in the environment, development of pest resistance, and harmful effects on beneficial insects (Pathak et al. 2022). To overcome these problems, new insecticidal groups that mimic natural products or originate from biotic agents, with new modes of action, selective, and eco-friendly (i.e., biorational insecticides) have been developed and registered as alternatives for use in integrated management approaches (Haddi et al. 2020). Imidacloprid and spinosad are among the biorational insecticides (Haddi et al. 2020).

Imidacloprid is one of the best-selling neonicotinoids worldwide (Nugnes et al. 2023). It is a chloronicotynil systemic insecticide agonist of the nicotinic acetylcholine receptors (nAChRs) increasing Na^+ entrance and K^+ exit, causing irreversible blockage of postsynaptic receptors, resulting in convulsions and paralysis, leading to death of insects (Matsuda et al. 2001; Nugnes et al. 2023). Imidacloprid is among the most promising and effective insecticides against lepidopterous insect pests in different modes of application, including foliar, and seed or soil treatments (El-Sheikh et al. 2018; Sabry et al. 2013, 2021). Horowitz and

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Ishaaya (2004) concluded that imidacloprid has mild effects on beneficial insects, especially as a foliar agent, its efficacy for controlling insect pests and its versatile use render it an important component in integrated pest management (IPM) and integrated risk management (IRM) programs. Nevertheless, a recent study (Nugnes et al. 2023) revealed that chronic toxicity risk quotient values of imidacloprid against the pelagic organisms, the rotifer *Brachionus calyciflorus* Pallas (Ploima: Brachionidae) and the cladoceran crustacean *Ceriodaphnia dubia* Richard (Anomopoda: Daphniidae) as well as the benthic ostracod crustacean *Heterocypris incongruens* (Ramdohr) (Podocopida: Cyprididae) were generally below to a threshold value of 1, with no consequential environmental concern other than for the Canadian areas. On the contrary, the genotoxicological risk quotient values were found higher than the threshold value in all continents. Moreover, this pesticide is highly toxic to pollinators, particularly honey bees (*Apis mellifera* L.) (Hymenoptera: Apidae) due to activating oxidative stress (He et al. 2021).

Spinosad is a spinosyn bioinsecticide, it is a mixture of two macrocyclic lactones (spinosyn A and spinosyn D) isolated from the soil actinomycete *Saccharopolyspora spinosa* under natural fermentation conditions (Copping and Menn 2000). It has a relative low mammalian toxicity and a favorable environmental profile (Sparks et al. 1996). Although spinosad is toxic to pollinators, including *A. mellifera* (Mayes et al. 2003; Rabea et al. 2010), natural enemies (Horowitz and Ishaaya 2004), and aquatic invertebrates (Duchet et al. 2009; Monteiro et al. 2019, 2020) in direct applications, with some precautions it can be used in IPM programs (Horowitz and Ishaaya 2004). Its mode of action is similar to that of the neonicotinoids, as its primary target site appears to be a subtype of the nAChRs with a proposed secondary target site at the gamma-aminobyturic acid (GABA)-gated chloride channel, causing tremors and involuntary muscle contractions, leading to paralysis and death of insects (Salgado 1998). Spinosad is effective on various economically important lepidopteran pests (Abouelghar et al. 2013; Abd El-Samei et al. 2019; Ishadi et al. 2022).

We hypothesized that mixtures of pesticides with different modes of action would complement the action of each other for killing the target pests with minimal doses, leading to enhancing the spectrum of pest control and meanwhile limiting the development of resistance and environmental pollution. There are a number of papers on effects of imidacloprid and spinosad on *S. littoralis* separately (Abouelghar et al. 2013; Sabry et al. 2021) or in combination with other pesticides (El-Sheikh 2015; Ismail 2018; Abd El-Samei et al. 2019). On the contrary, to the best of our knowledge, no studies have been undertaken to elucidate the effects of combined imidacloprid and spinosad on *S. littoralis*.

In view of the above-mentioned background, in the present study, we evaluated the toxicity of imidacloprid and spinosad separately or in combination to *S. littoralis* larvae. We also evaluated the effects of these treatments on biological features and nutritional indices.

Materials and methods

Insect culture

A stock colony of *S. littoralis* was initiated with eggs obtained from the Research Division of the Cotton Leafworm, Plant Protection Research Institute, Assiut, Egypt. Before starting the experiments, larvae were reared in the insectaries of the Zoology Department, Faculty of Science, Assiut University for 30 generations on leaves of castor-bean *Ricinus communis* L. (Euphorbiaceae). Adults were fed on a 10% sucrose solution. Insects were maintained at 27 ± 2 °C, 65 ± 5% relative humidity, and 16-h light: 8-h dark photoperiod according to Shaurub et al. (2020a). A branch of oleander *Nerium oleander* L. (Apocynaceae) was placed in the cage as an oviposition site. Egg-masses were collected daily and kept in 90-ml plastic cup until hatching.

Insecticides

Imidacloprid (Imaxi 35% SC) and spinosad (Tracer 24% SC) were produced by Syngenta Agrochemical Co., Ltd and Dow AgroSciences Co., UK, respectively.

Bioassays of individual insecticides

The susceptibility of *S. littoralis* larvae to imidacloprid and spinosad separately was evaluated using leaf-dip technique. Newly molted 4th-instar larvae (33.75 mg each, < 1-day-old) were selected for bioassays according to Shaurub et al. (2020a). Six aqueous concentrations of each insecticide were prepared in distilled water (50, 150, 250, 550, 750, and 1000 ppm). Before treatment, larvae were starved for 4 h. They were then released into a 500-ml plastic cup, lined with 10-cm diameter filter paper (Whatman # 1, Sigma-Aldrich), and covered with a piece of muslin cloth, held in position by a rubber band. Larvae were offered 5-cm diameter leaves of castor-bean *R. communis*, separately dipped for 20 s in each concentration, and air-dried at room temperature. Mortality count was made after 24, 48, and 72 h of treatment. Each concentration was replicated three times with independently treated leaves and 50 larvae each. A parallel control experiment of larvae fed on leaves dipped in distilled water only was also conducted. Each control experiment was replicated three times with 50 larvae each. Percentage of mortality was corrected by Abbott's formula (Abbott 1925) as follows:

$$\% \text{ corrected mortality} = (\% \text{ mortality in treatment} - \% \text{ mortality in control}/100 - \% \text{ mortality in control}) \times 100.$$

The LC $_{25}$, LC_{50}, and LC_{90} of each insecticide after 24, 48, and 72 h of treatment was estimated by probit analysis (Finney 1971).

Bioassays of combined insecticides

The LC_{25} values of imidacloprid and spinosad separately after 72 h of treatment (271.02 and 99.26 ppm, respectively), as determined above, were chosen. The toxic action of binary combination of the LC_{25} of imidacloprid + LC_{25} of spinosad (vol: vol) was conducted using leaf-dip technique as described above. Larval mortality was recorded after 72 h of treatment. The interaction between imidacloprid and spinosad, in relation to larval mortality, was differentiated according to the co-toxicity factor (Mansour et al. 1966) as follows:

$$\%\,\text{co}-\text{toxicity factor} =$$

$$(\%\,\text{observed mortality} - \%\,\text{expected mortality}\,/\,\%\,\text{expected mortality}) \times 100,$$

where:

% expected mortality = sum of % mortality of each insecticide alone at the same level of concentration used in the combination while % observed mortality = % mortality of combination. Co-toxicity factor of a positive value of 20 or more is considered synergistic, co-toxicity factor of a negative value of 20 or more is considered antagonistic, and co-toxicity factor of intermediate value between – 20 and + 20 is considered additive.

Biological studies

For separate treatment with imidacloprid and spinosad, their LC_{50} values after 72 h of treatment (352.18 and 175.34 ppm, respectively), as determined above, were chosen. While for the combined treatment, the combined LC_{25} values of each insecticide after 72 h of treatment (271.02 and 99.26 ppm, respectively) (vol: vol) were chosen. The biological activities resulting from individual or combined treatment of newly molted 4th-instar larvae of *S. littoralis* were carried out. Three replicates with 100 larvae each were tested for each treatment. A parallel control of untreated larvae was also run. Each control experiment was replicated three times with 100 larvae each. Larvae were fed on treated leaves for 72 h. The survivors were then transferred into clean jars (10 cm in diameter, 21 cm in height), covered with a piece of muslin cloth, and provided daily with fresh untreated castor-bean leaves until larvae either died or pupated. Two-day old pupae were weighed individually and transferred into jars for adult emergence. Larval period, pupal period, pre-oviposition period, oviposition period, post-oviposition period, pupal weight, pupation, adult emergence, fecundity (number of eggs /♀), and fertility (% egg-hatch) were recorded for each treatment. Percentages of pupal and adult malformations were also recorded. For the fecundity assays, 10 pairs of moths (1 ♀ × 1 ♂ per each pair) that emerged on the same day from each treatment were collected and housed into a glass jar, covered with a muslin cloth, and provided with a branch of oleander *N. oleander* as an oviposition site, and a piece of cotton wool soaked in a 10% sucrose solution for moth nutrition. The egg-masses laid were counted daily, and oleander branches were replaced every two days until the death of the females. To evaluate the fertility, egg-masses obtained from each treatment were observed daily for hatching, then the hatch percent was counted. Number of eggs hatched was assessed after 4 days when the egg-hatch was completed in control. Sterility was calculated according to Toppozada et al. (1966) as follows:

$$\%\,\text{sterility} = (100 - \text{a} \times \text{b}/\text{A} \times \text{B}) \times 100,$$

where:

A = number of eggs laid/female in control, a = number of eggs laid/female in treatment,

B = % egg-hatch in control, and b = % egg-hatch in treatment.

Nutritional physiology studies

Three groups of newly molted 4th-instar larvae of *S. littoralis* were confined separately to a 500-ml plastic cup, lined with filter paper, and covered with a piece of muslin cloth, held in position by a rubber band. The 1st and 2nd groups consisted of larvae fed for three successive days on leaves of castor-bean *R. communis* of a known weight and treated respectively with the LC_{50} of imidacloprid and spinosad (352.18 and 175.34 ppm, respectively) using leaf-dip technique as described above. The 3rd group consisted of larvae fed for three successive days on leaves of a known weight and treated with a combination of the LC_{25} of imidacloprid (271.02 ppm) + LC_{25} of spinosad (99.26 ppm) (vol: vol) using leaf-dip technique. Each treatment was replicated three times with 100 larvae each. A parallel control experiment of untreated larvae was also conducted. Each control was replicated three times with 100 larvae each. After treatment, treated leaves were removed, and fresh leaves of a known weight were provided for two successive days (recovery period) until larvae either died or pupated. Thus, the whole experimental period lasted 5 days, 3-day-treatment period and 2-day-recovery period according to Shaurub et al. (2020b). To minimize experimental errors often associated in calculating the nutritional indices, only enough food was supplied so that at least 80% of the available food was consumed during the experiment (Schmidt and Reese 1986). Dead larvae were discarded, and the fresh mass of survivors, feces, and consumed leaves in each rearing cup were recorded daily throughout the whole experimental period. To estimate the actual loss of moisture, fresh leaves were kept in a similar rearing cup under the same experimental conditions, which was used for calculating the corrected weight of consumed leaves. Food consumption and utilization were calculated according to the equations of Waldbauer (1968) as follows:

$$\text{Consumption index (CI)} = \text{F/TA},$$

$$\text{Relative growth rate (RGR)} = \text{G/TA},$$

$$\text{Approximate digestibility (AD)} = (\text{F} - \text{E/F}) \times 100,$$

$$\text{Efficiency of conversion of ingested food to biomass (ECI)} = (\text{RGR/CI}) \times 100, \text{ and}$$

$$\text{Efficiency of conversion of digested food to biomass (ECD)} = (\text{G/F} - \text{E}) \times 100,$$

where:

A = fresh mean weight of larvae during the feeding period (mg),

E = fresh mass of feces (mg),

F = fresh weight of food ingested (mg),

G = fresh weight gain of larvae at the end of the feeding period (mg), and

T = duration of the feeding period (days).

Statistical analyses

The LC_{25}, LC_{50}, LC_{90}, slope, and the 95% confidence limits were estimated using probit analysis (Finney 1971) through Polo-PC Plus, version 3.1 statistical software (LeOra Software, Berkeley, CA, USA).

All datasets of biological characteristics and nutritional indices were first assessed for normality using the Shapiro–Wilk test and subsequently expressed as the mean ± standard error (SE) for analysis. Group means of adult stage characteristics (pre-oviposition period, oviposition period, post-oviposition period, number of eggs per female, percentage of egg-hatch, sterility, and female longevity) were compared by multivariate analysis of variance (MANOVA) followed by separate one-way analysis of variance (ANOVA). A separate ANOVA only was conducted for immature stage characteristics (larval duration, pupal duration, and pupal weight).

Group means of nutritional indices were compared by the Repeated Measures ANOVA. In all statistical analyses (adult stage characteristics, immature stage characteristics, and nutritional indices), multiple comparisons were carried out using post hoc least significant difference (LSD) test. Significance level was set at $P \leq 0.05$ for all the above-mentioned tests. All statistical calculations were conducted using IBM-SPSS Statistics, v. 25 (IBM, Armonk, New York, NY, USA).

Results

Toxicological studies

The susceptibility of *S. littoralis* larvae to imidacloprid and spinosad is shown in Table 1. There is direct correlation between insecticide toxicity and exposure period as the toxicity increases with increasing exposure period. Spinosad was more toxic than imidacloprid. After 24, 48, and 72 h of treatment, spinosad was 2.66, 2.54, 2.73; 2.10, 1.86, 2.01; 1.35, 1.03, 1.12 times more toxic than imidacloprid at the LC_{25}, LC_{50}, and LC_{90} level, respectively. The binary mixture of imidacloprid + spinosad revealed additive effect, with co-toxicity factor of -12.39% (Table 2).

Biological studies

Life history characteristics of *S. littoralis* individually treated as 4th-instar larvae with imidacloprid and spinosad or in combination is shown in Table 1. As to adult stage characteristics (pre-oviposition period, oviposition period, post-oviposition period, number of eggs per female, percentage of egg-hatch, sterility, and female longevity), there was a significant difference among all treatments and controls (Roy's Largest Root = 11.08, $F_{6,33} = 60.98$, $P = 0.0001$) (Table 3).

Treatments with imidacloprid, spinosad, and their combination significantly decreased the number of eggs laid per female ($F_{3,36} = 49.10$, $P = 0.00001$, LSD = 381.98), percentage of egg-hatch ($F_{3,36} = 5.87$, $P = 0.0023$, LSD = 30.910), oviposition period ($F_{3,36} = 19.20$, $P = 0.00001$, LSD = 1.34), female longevity ($F_{3,36} = 4.030$, $P = 0.0143$, LSD = 1.40), and pupal weight ($F_{3,28} = 23.90$, $P = 0.00001$, LSD = 0.013), compared to controls. However, these treatments didn't affect larval duration ($F_{3,12} = 0.270$, $P = 0.840$), pupal duration ($F_{3,16} = 1.010$, $P = 0.412$), pre-oviposition period ($F_{3,36} = 1.158$, $P = 0.3389$), and post-oviposition period ($F_{3,36} = 2.690$, $P = 0.0608$), compared to controls. Data revealed that imidacloprid and spinosad separately or in combination induced significant female sterility ($F_{3,36} = 47.2$, $P = 0.00001$, LSD = 16.08).

Although treatments with imidacloprid and spinosad separately or in combination drastically suppressed pupation rate (~50% decrease, compared to controls), adult emergence rate was not appreciatively suppressed (~7–13% decrease, compared to controls).

Table 1 Susceptibility of *S. littoralis* larvae treated as 4th-instar larvae with imidacloprid and spinosad for different times

Treatment	Time of treatment (h)	LC_{25} (ppm)	LC_{50} (ppm)	LC_{90} (ppm)	Slope ± SE
Imidacloprid	24	395.91 (356.03–432.92)	574.62 (532.69–616.38)	1166.00 (1069.57–1291.65)	3.73 ± 0.05
	48	287.19 (239.8–329.6)	390.14 (341.05–439.5)	698.27 (605.11– 841.58)	4.21 ± 0.05
	72	271.02 (247.6–293.1)	352.18 (327–378)	579.42 (532.25– 640.64)	5.68 ± 0.05
Spinosad	24	149.00 (101.6–196.9)	273.88 (210.4–340.8)	860.74 (671.11–1210.30)	2.38 ± 0.07
	48	113.19 (66.56–159.2)	209.95 (147.13–277.80)	679.03 (499.003–1064.430)	2.35 ± 0.08
	72	99.26 (63.0–134.0)	175.34 (129.0–224.6)	516.83 (393.005–763.140)	2.66 ± 0.08

Figures between brackets are the 95% confidence limits of the respective LC

Individual treatment with imidacloprid and spinosad, and their combination revealed pupal and adult malformations. Percentage of adult malformations were 3.04, 1.40, and 1.64 times more than pupal malformations, respectively (Table 1). Pupal malformations included degeneration of appendages (Fig. 1B), melanization of the body (Fig. 1C), larval-pupal intermediates (Fig. 1D), and detachment of appendages from the integument (Fig. 1E). However, adult malformations were represented only by folding of wings (Fig. 1G-J).

Nutritional physiology studies

Consumption index

Across the time course (5 days post-treatments), Repeated Measures ANOVA indicated significant differences among the means of the CI (Greenhouse–Geisser, $F_{2.15,17.25} = 109.83$, $P = 0.0001$). The effects of treatments (imidacloprid, spinosad, imidacloprid + spinosad, and controls) on the means of the CI across time was significant ($F_{3,8} = 20.89$, $P = 0.0001$). The effect of interaction of time * treatments was significant (Greenhouse–Geisser, $F_{6.46,17.25} = 10.39$, $P = 0.0001$).

The pairwise comparisons indicated that there were significant mean differences in the CI on the 1st day post-treatment versus other days separately (i.e., the 2nd and 3rd day post-treatment, and the 1st and 2nd day of recovery) ($P \leq 0.006$). However, there were no significant mean differences between the 2nd and 3rd day post-treatment ($P = 0.767$). On the 1st day post-treatment, imidacloprid, spinosad, and their combination significantly decreased the CI, compared to controls ($P = 0.00001$, LSD = 0.047). This decrease was ~78, 54, and 44%, respectively. On the 2nd day post-treatment, imidacloprid and combination of imidacloprid + spinosad significantly decreased the CI, with no change in case of treatment with spinosad, compared to controls ($P = 0.0035$, LSD = 0.117). On the 3rd day post-treatment, imidacloprid or spinosad significantly decreased the CI, with no change in case of their combination, compared to controls ($P = 0.0009$, LSD = 0.134). On the 1st day of recovery, all treatments significantly increased the CI, compared to controls ($P = 0.0062$, LSD = 0.265). But on the 2nd day of recovery, all treatments didn't affect the CI, compared to controls ($P = 0.2663$) (Fig. 2A).

Relative growth rate

Results indicated significant mean differences in the RGR across the five days post-treatments (Greenhouse–Geisser, $F_{1.58,12.70} = 165.27$, $P = 0.0001$). The effect of treatment groups on the average RGR across time was statistically significant ($F_{3,8} = 47.08$, $P = 0.0001$). This effect was qualified by a significant time * treatments interaction effect (Greenhouse–Geisser, $F_{4.76,12.70} = 10.68$, $P = 0.0001$).

Table 2 Interaction between imidacloprid and spinosad against *S. littoralis* larvae treated as 4th-instar larvae

Treatment	Binary combination	% expected mortality	% observed Mortality	% co-toxicity factor	Type of interaction[3]
Imidacloprid + Spinosad	$LC_{25} + LC_{25}$	51.89	45.46	- 12.39	Additive

Table 3 Biological characteristics of *S. littoralis* treated as 4th-instar larvae with imidacloprid and spinosad separately or in combination

Treatment	Larval duration* (days)	Pupal duration (days)	% pupation	Pupal weight (mg)	% adult emergence	% pupal malfor-mation	% adult malfor-rmation	No eggs /♀	% egg hatchability	% sterility	Pre-oviposition period (days)	Oviposition period (days)	Post-oviposition period (days)	Female longevity (days)
Control	7.75 ±0.25a	9.60 ±0.25a	95.00	0.25 ±0.003a	98.25	0	0	3201.00 ±73.22a	98.81 ±0.25a	0.00b	0.70 ±0.15a	9.60 ±0.22a	1.80 ±0.44a	12.10 ±0.48a
Imidacloprid	8.00 ±0.41a	10.40 ±0.40a	46.00	0.21 ±0.004b	91.54	5.80	17.65	1068.20 ±133.15c	57.82 ±0.14b	81.15 ±6.06a	1.30 ±0.37a	5.40 ±0.62b	3.20 ±0.59a	9.90 ±0.41b
Spinosad	8.25 ±0.48a	10.20 ±0.37a	46.33	0.20 ±0.004b	85.48	10.79	15.09	1422.70 ±184.02c	37.17 ±0.12b	79.78 ±6.93a	1.60 ±0.56a	5.40 ±0.60b	3.20 ±0.51a	10.20 ±0.47b
Imidacloprid + Spinosad	8.00 ±0.41a	10.00 ±0.32a	52.67	0.21 ±0.006b	88.11	9.49	15.56	1877.30 ±118.29b	54.68 ±0.16b	66.94 ±6.39a	1.00 ±0.21a	5.70 ±0.30b	3.80 ±0.51a	10.50 ±0.58b

*Calculated starting from 4th-instar larvae. Means within the same column followed by the same letters are not significantly different according to the LSD test ($P = 0.05$)

The pairwise comparisons indicated that there were significant mean differences among the 5 days post-treatments ($P \leq 0.03$). The RGR was significantly decreased on the 1st day post-treatment with imidacloprid, spinosad, and their combination, compared to controls ($P = 0.00001$, LSD = 0.011). On the 2nd day post-treatment, imidacloprid and combination of imidacloprid + spinosad significantly decreased the RGR, with no change in case of treatment with spinosad, compared to controls ($P = 0.00001$, LSD = 0.022). On the 3rd day post-treatment, all treatments significantly decreased the RGR, compared to controls ($P = 0.0021$, LSD = 0.079). On the 1st day of recovery, all treatments significantly increased the RGR ($P = 0.0015$, LSD = 0.108). On the 2nd day of recovery, all treatments didn't affect the RGR, compared to controls ($P = 0.7256$) (Fig. 2B).

Approximate digestibility

There were significant mean differences in the AD across the five days post-treatments (Sphericity Assumed, $F_{4,32} = 48.35$, $P = 0.0001$). The main effect of treatment groups on the average AD across time was statistically significant, ($F_{3,8} = 36.83$, $P = 0.0001$). This effect was qualified by a significant time * treatments interaction effect (Sphericity Assumed, $F_{12,32} = 9.22$, $P = 0.0001$).

The pairwise comparisons revealed that there were no significant mean differences between the 1st and the 2nd day post-treatment ($P = 0.149$), and between the 2nd and the 3rd day post-treatment ($P = 0.063$). However, there were significant mean differences between the 1st and the 3rd day post-treatment, between the 1st day post-treatment and the 1st day of recovery, and between the 1st day post-treatment and the 2nd day of recovery ($P \leq 0.004$). Individual treatment with imidacloprid significantly increased the AD on the 1st day post-treatment, whereas individual treatment with spinosad significantly decreased it, with no change in case of combined treatment with imidacloprid + spinosad, compared to controls ($P = 0.0001$, LSD = 4.782). On the 2nd day post-treatment ($P = 0.0005$, LSD = 4.391) and the 3rd day post-treatment ($P = 0.00001$, LSD = 3.763), imidacloprid significantly increased the AD, whereas spinosad significantly decreased it, with no change in case of combined treatment with imidacloprid + spinosad, compared to controls. On the 1st day of recovery, imidacloprid significantly increased the AD, with no change in case of treatment with spinosad alone or combined imidacloprid + spinosad, compared to controls ($P = 0.0429$, LSD = 4.240). On the 2nd day of recovery, all treatments didn't affect the AD, compared to controls ($P = 0.1434$) (Fig. 2C).

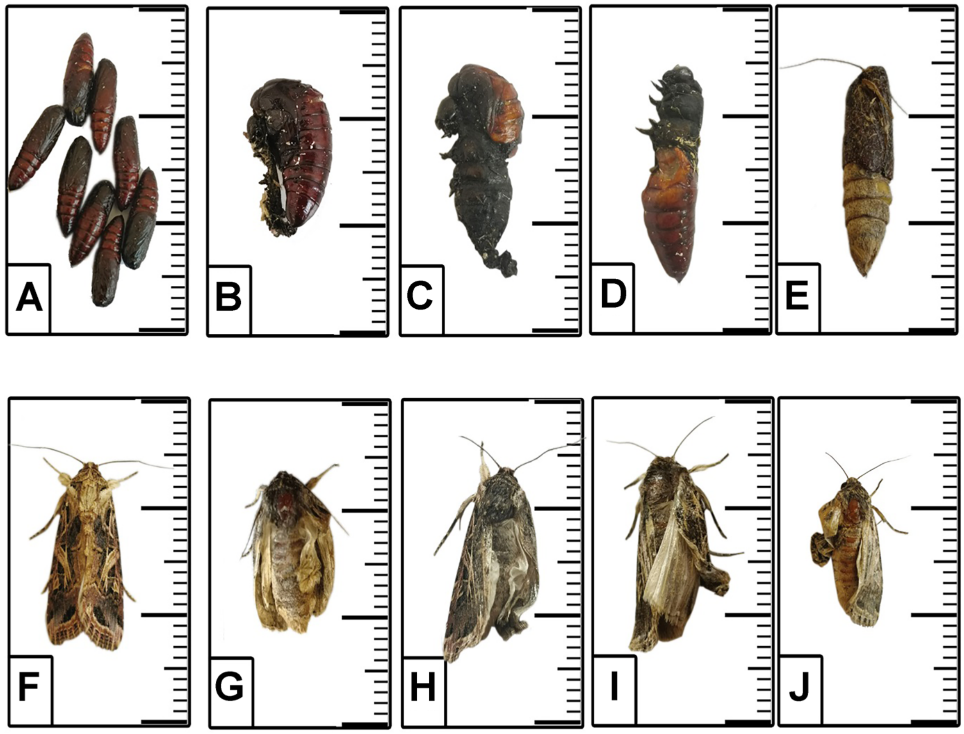


Fig. 1 Malformations of *S. littoralis* treated as 4th-instar larvae with imidacloprid and spinosad separately, or in combination. **A** Normal untreated pupae. **B**-**E** Malformed pupae: **B** Degeneration of appendages, **C** Melanization of the body, **D** Larval-pupal intermediates, **E** Detachment of appendages from the integument. **F** Normal untreated adults. **G**-**J** Malformed adults showing different forms of wing folding. Scale bar = 30 mm

Efficiency of conversion of ingested food to biomass

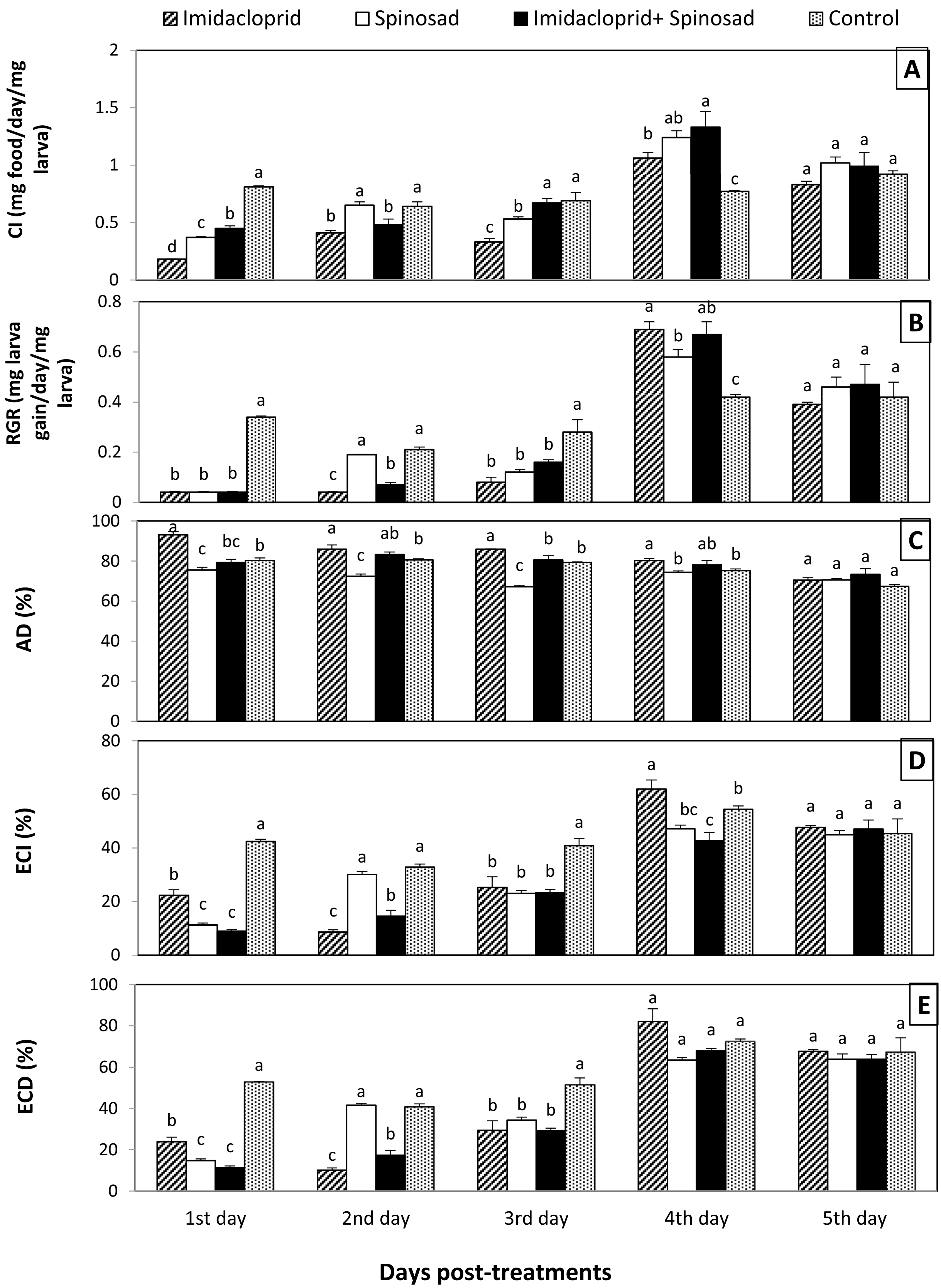
Results revealed significant mean differences in the ECI across the five days post-treatments (Greenhouse–Geisser, $F_{1.85,14.86}=80.05$, $P=0.0001$). The effect of treatment groups on the average ECI across time was statistically significant ($F_{3,8}=63.69$, $P=0.0001$). The effect of time * treatments interaction was significant (Greenhouse–Geisser, $F_{5.57,14.86}=6.82$, $P=0.001$).

The pairwise comparisons indicated that there were no significant mean differences between the 1st and the 2nd day post-treatment ($P=0.75$). However, there were significant mean differences between the 1st day post-treatment and the remaining days separately (the 3rd day post-treatment, and the 1st and 2nd day of recovery) ($P\leq 0.003$). Treatment with imidacloprid, spinosad, and their combination significantly decreased the ECI on the 1st day post-treatment ($P=0.00001$, LSD = 3.984) and the 3rd day post-treatment ($P=0.0031$, LSD = 8.280), compared to controls. On the 2nd day post-treatment, imidacloprid and combined imidacloprid + spinosad significantly decreased the ECI, with no change in case of treatment with spinosad, compared to controls ($P=0.00001$, LSD = 4.570). On the 1st day of recovery, imidacloprid significantly increased the ECI, whereas combined imidacloprid + spinosad significantly decreased it, with no change in case of treatment with spinosad, compared to controls ($P=0.002$, LSD = 8.146). On the 2nd day of recovery, all treatments didn't change the ECI, compared to controls ($P=0.9259$) (Fig. 2D).

Efficiency of conversion of digested food to biomass

There were significant mean differences in the ECD across the five days post-treatments (Greenhouse–Geisser, $F_{1.86,14.95}=103.94$, $P=0.0001$). The main effect of treatment groups on the ECD across time was statistically significant ($F_{3,8}=38.90$, $P=0.0001$). This effect was qualified by a significant time * treatments interaction effect (Greenhouse–Geisser, $F_{5.60,14.86}=6.82$, $P=0.002$).

The pairwise comparisons revealed that there were no significant mean differences between the 1st and the 2nd day post-treatment ($P=0.106$), and between the 1st and the 2nd day of recovery ($P=0.237$). However, there were significant mean differences between the 1st day post-treatment and the remaining days separately (the 3rd day post-treatment, and the 1st and 2nd day of recovery) ($P\leq 0.001$). The pattern of ECD was identical to that of ECI during the three days of treatment. ECD was significantly decreased on the 1st day post-treatment ($P=0.00001$, LSD = 4.044) and the 3rd day post-treatment with imidacloprid, spinosad, and their combination ($P=0.0024$, LSD = 9.861), compared to controls. On the 2nd day post-treatment, imidacloprid and combined imidacloprid + spinosad significantly decreased the ECD, with no change in case of treatment with spinosad, compared to controls ($P=0.00001$, LSD = 5.098). On the 1st day of recovery ($P=0.3573$) and the 2nd day of recovery ($P=0.8419$), all treatments didn't affect the ECD, compared to controls (Fig. 2E).


Imidacloprid
Spinosad
Imidacloprid+ Spinosad
Control
CI (mg food/day/mg larva)
A
RGR (mg larva gain/day/mg larva)
B
AD (%)
C
ECI (%)
D
ECD (%)
E
1st day
2nd day
3rd day
4th day
5th day
Days post-treatments

◂**Fig. 2** Nutritional indices of *S. littoralis* larvae treated as 4^{th}-instar larvae with imidacloprid and spinosad separately or in combination for 3 days, and then recovered for 2 days. **A** Consumption index, CI; **B** Relative growth rate, RGR; **C** Approximate digestibility, AD; **D** Efficiency of conversion of ingested food to biomass, ECI; **E** Efficiency of conversion of digested food to biomass, ECD. 1^{st}, 2^{nd}, and 3^{rd} day are the treatment period, i.e., the period during which larvae were fed on treated leaves. 4^{th} and 5^{th} day are the recovery period, i.e. the period during which treated leaves were replaced by untreated ones. Means followed by the same letters are not significantly different according to the LSD test ($P=0.05$)

Discussion

Using IPM protocols to provide effective protection against pest infestations is among the wise pest management programs. Acute toxicity of spinosad and imidacloprid to *S. littoralis* larvae has been considerably variable among different studies. Our results revealed that the LC_{50} of spinosad after 24 h of treatment of *S. littoralis* 4^{th}-instar larvae using leaf-dip technique was 273.88 ppm, compared to 70.7 ppm (Ragaei and Sabry 2011) and 28.86 ppm (El-Sheikh 2012) after 24 h of treatment the same instar using the same technique. Abouelghar et al. (2013) reported that the LC_{50} of spinosad after 72 h of treatment of *S. littoralis* 4^{th}-instar larvae using leaf-dip technique was 57.8 ppm, compared to 175.34 ppm which was obtained in the present study for the same instar using the same technique. Ismail (2018) showed that the LC_{50} of imidacloprid against *S. littoralis* 4^{th}-instar larvae was 6.42 ppm after 72 h of treatment, compared to 352.18 ppm which was obtained in our study. Sabry et al. (2021) attained LC_{50} of 66.5 ppm for *S. littoralis* larvae treated as 2^{nd}-instar larvae with imidacloprid for 7 days using leaf-dip technique. These variations may be due to variations in insecticide formulations.

Toxicity of spinosad and imidacloprid to *S. littoralis* larvae was time-dependent, as it increases with increasing the exposure period to insecticides. These results agree with those reported by El-Sheikh (2015) and Ismail (2018) for imidacloprid- and spinosad-treated *S. littoralis* larvae. In the present study, spinosad was more toxic than imidacloprid against *S. littoralis* larvae. This may be due to the unique mode of action of spinosad, it acts primarily on nAChRs and further on GABA resulting in paralysis of insects, leading to death (Salgado 1998).

The toxic effect of combined imidacloprid + spinosad on *S. littoralis* larvae was additive. Similarly, most studies revealed that combining imidacloprid or spinosad with other pesticides were additive effects. El-Sheikh (2015) found that the combined effect of emamectin benzoate or lufenuron + spinosad was either additive or antagonistic on 3^{rd}- and 5^{th}-instar larvae of *S. littoralis*. Korrat et al. (2012) showed that the effects of profenofos combined with emamectin benzoate or spinosad were additive against 2^{nd}-instar larvae of *S. littoralis* using leaf-dip technique. In the same way, Sherby et al. (2010) found that the combinations of spinosad with chlorpyrifos resulted in an additive or antagonistic effect on *S. littoralis*. Abd El-Samei et al. (2019) attained additive effect on *S. littoralis* 3^{rd}- and 5^{th}-instar larvae when spinosad was combined with *Bacillus thuringiensis* at the level of LC_{25}. Radwan et al. (2009) elucidated that combinations of profenofos with spinosad in different mixing ratios lead to an antagonistic effect, they attributed this result to the different modes of action between combined insecticides. Whereas, Ismail (2018) found that combination of imidacloprid + lufenuron at the LC_{25} level resulted in a synergistic effect on 2^{nd}-instar larvae of *S. littoralis*. When two compounds are applied to an insect, one might interfere with the other's activation, with its detoxication reaction or with both. Antagonism results where interference with the activation mechanisms occurs, while synergism results where interference with the detoxication takes place. If both reactions encounter interference, synergism or additive effect could result depending on the degree of interference with the different reactions (Hewlett 1960; DuBois 1969).

The current study revealed that treatments with the LC_{50} of imidacloprid and spinosad separately or in combination ($LC_{25}+LC_{25}$) caused significant negative effects on most the life history characteristics of *S. littoralis*. Similar results have been demonstrated on the diamondback moth *Plutella xylostella* L. (Lepidoptera: Plutellidae) (Yin et al. 2008), *S. littoralis* (Abouelghar et al. 2013) and cotton bollworm *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) (López et al. 2011), when larvae were treated with sublethal concentrations of spinosad. Sublethal effects, such as suppression of larval weight and reproductive potential of survivors could negatively affect population dynamics (Knight 2000; Pineda et al. 2004). Although insecticide hormoligosis is known to occur in some insects, leading to pest resurgence (Luckey 1968), hormoligosis was not observed in our study as sublethal treatments with imidacloprid and spinosad separately or in combination negatively affected the biotic characteristics of *S. littoralis*.

In control programs of lepidopteran pests, larvae are the target as they are the harmful stage. So, the decrease in larval duration is in the favor of controlling such pests. In our study, treatment with imidacloprid and spinosad separately or in combination did not affect duration of larvae of *S. littoralis*. Nevertheless, this result is considered in the favor of controlling this pest, compared to significantly increased larval duration of *S. littoralis* treated with the juvenile hormone analogue (JHA) pyriproxyfen (Shaurub et al. 2020a).

The precise nature of the interaction between imidacloprid or spinosad and the physiological processes that are significant in reducing fecundity remains obscure. El-Sheikh (2012) reported that ovarioles of *S. littoralis* females resulting from 4^{th}-instar larvae fed on castor-bean leaves treated with the LC_{50} of spinosad showed reduced size, degenerating yolk, and the follicular epithelium lost its organization. The decrease in fecundity and fertility of *S. littoralis* treated with imidacloprid and spinosad separately or in combination was concomitant with the decrease in oviposition period.

Lu et al. (1978) hypothesized that the accumulation of toxic xenobiotics in any organism may be expected to affect longevity, which is a complicated balance of such factors as absorption, excretion, intoxication, and detoxication. This hypothesis may be applied to decreased longevity of *S. littoralis* female moths treated with imidacloprid and spinosad separately or in combination.

Interesting results were obtained here that separate or combined treatment with imidacloprid and spinosad induced malformations to pupae and adults. Pupal malformations included detachment of appendages from the integument, degeneration of appendages, melanization of the body, and formation of larval-pupal intermediates. While adult malformations were only folding of wings. Staal (1972) reported that the changes in the exoskeleton are actually only part of the overall morphological abnormalities. Recently, it has been reported that the JHA pyriproxyfen may inhibit normal pupal development in *S. littoralis* by modulating the effects of 20-hydroxyecdysone (20E) (El-Sheikh et al. 2016). A reduction in ecdysone titer following JHA exposure may affect larval–pupal or pupal–adult metamorphosis in *S. littoralis* (El-Sheikh et al. 2016). The reduction in ecdysone titer could result from a block in the release of prothoracicotropic hormone (PTTH), which in turn would result in a block in the secretion of ecdysone (Dedos and Fugo 1999). Our results suggest the interference of imidacloprid and spinosad with the release of PTTH.

The present study demonstrated that food consumed was dramatically reduced in case of treatments with imidacloprid and spinosad separately or in combination on the 1st day of treatment. The decrease in food consumption rate has been reported in the beet armyworm *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) larvae fed on lettuce leaves treated with spinosad (Yee and Toscano 1998). The reason for the decrease in food consumption here is not immediately apparent, but it may be directly related to the mechanism of action of imidacloprid and spinosad. As neurotoxic pesticides, imidacloprid and spinosad cause paralysis of insects (Salgado 1998; Matsuda et al. 2001), consequently a cessation of feeding. Even though feeding cessation and malformations of the mouthparts of *S. littoralis* larvae fed on imidacloprid-and spinosad-treated leaves were not measured in the current study, both effects were visually observed. These effects are very important from a practical point of view because the larval-feeding-damage to crops would be lessened (Pineda et al. 2006). The significant increase in food consumed by treated *S. littoralis* larvae on the 1st day of recovery indicates a recovery of normal behavior and therefore it may have been an attempt by the treated larvae to compensate the reduction in food consumed during the treatment period. The non-change in food consumed on the 2nd day of recovery might be due to larvae that were approaching the pupal stage and thus stopped feeding. Spinosad degrades quickly, and generally shows little residual insecticidal activity 3–7 days after application (Williams et al. 2004). This finding points to the necessity of more than one spray of spinosad in the field to ensure that larvae will ingest this pesticide throughout their duration.

The decrease in larval weight here was concomitant with the decrease in food consumed. Reduction in larval weight has been reported for spinosad-treated *S. exigua* (Yee and Toscano 1998) and spinosad-treated *S. littoralis* (Abouelghar et al. 2013). In insects, poor nutrition during development typically leads to undersized adults (Scriber and Slansky 1981; Awmack and Leather 2002; Colasurdo et al. 2009; Dmitriew and Rowe 2011). Decreased larval weight of *S. littoralis* in the present study may explain reduced pupal weight.

Abouelghar et al. (2013) showed that treatment of *S. littoralis* larvae with spinosad resulted in degeneration in the epithelial cells of the midgut and the peritrophic matrix. Such histological alterations may be responsible for the overall reduction in growth, digestion, and gross food utilization (ECI and ECD) caused by spinosad in the current study. Timmins and Reynolds (1992) attributed reduction in the efficiency of food utilization to increased energetic costs arising from a reduced ability to utilize dietary nitrogen, which would not necessarily interfere with absorption from the gut. Furthermore, Senthil-Nathan and Kalaivani (2005) reported that the reduction in food utilization may be a result of a diversion of energy from biomass production into induction of enzymes involved in detoxification of the candidate pesticide.

In the present study, imidacloprid increased the digestibility during the three days of treatment and on the 1st day of recovery. Barnby and Klocke (1987) suggested that an elevated digestibility may be attributed to higher retention of a food bolus in the gut for a longer period and therefore longer exposure to digestive enzymes. This will allow for greater digestion and absorption of nutrients for normal biomass production.

Overall, spinosad more negatively affected most biotic parameters investigated than imidacloprid. This might be attributed to the fact that imidacloprid acts only on nAChRs (Matsuda et al. 2001; Nugnes et al. 2023), whereas spinosad acts on both nAChRs and GABA-gated chloride channel, leading to involuntary muscle contractions, tremors, paralysis, and death (Salgado 1998). Higher GABA levels in artificial dietary supplements of *S. littoralis* in turn affect the performance of feeding *S. littoralis* larvae (Scholz et al. 2015). GABA is also suggested to be involved in plant defense against herbivorous insects (Mithöfer and Boland 2012). This hypothesis is based on the fact that GABA is known as an inhibitory neuromuscular transmitter acting at

GABA-gated chloride channels in insects, where it could affect normal development when ingested by feeding (Bown et al. 2006). Thus, the presence of GABA might deter feeding of herbivorous insects (Ramputh and Bown 1996).

Conclusions

Treatment of *S. littoralis* larvae with imidacloprid and spinosad separately or in combination would affect population dynamics of survivors via reducing moth emergence and reproductive potential in particular. Consequently, a population would be maintained below a level of economic loss. These treatments also stopped larvae from feeding shortly after exposure (i.e., after 24 h), leading to reduction of crop damage. The results presented herein may help in developing more effective crop protection methodologies within IPM of *S. littoralis*. The laboratory findings obtained need to be confirmed with some field trials on *S. littoralis*.

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Author contributions EHS and AIT conceived and designed the research. EHS wrote the manuscript. AME carried out the experiments and statistical analyses and prepared the figures. All authors read and approved the manuscript.

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Data availability All data related to this study are present in the paper.

Declarations

Competing interests No conflicts of interest are reported by the authors.

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