

## Article

# The Chickpea Pod Borer, *Helicoverpa armigera* (Hübner): Yield Loss Estimation and Biorational Insecticide Assessment in Morocco

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**Abstract:** *Helicoverpa armigera* (Hübner) is considered one of the most destructive insect pests of chickpea crops in Morocco; however, the extent of the yield loss it causes in Morocco is unknown. This study assessed the yield losses and pod damage caused by the chickpea pod borer *H. armigera* on four improved Kabuli varieties with insecticide treatment at two different locations. The second part of this study investigated the contact and systemic toxicity of different biological and selective insecticides in the control of the larvae of *H. armigera* under controlled laboratory and field conditions. The results demonstrated that the yield losses due to *H. armigera* infestation were in the range of 14.3–31.2%. Chickpea pod borer infestation resulted in losses in the total seed weight for all the chickpea varieties, with the highest yield losses for Zahor (F84-145C) being 31.18% at Allal Tazi followed by Farihane (F84-79C) with 27.38% at the Marchouch station. Emamectin benzoate at 250 g/ha showed a high level of larvicidal and systemic activity, with 100% mortality 24 h after application. Indoxacarb at 25 mL/100 L water, recorded 100% and 92% larval mortality in larvicidal and systemic activity, 48 h after application, respectively. The bioinsecticide spinosad in 30 mL/100 L water resulted in 88% and 92% larval mortality in contact and systemic activity, 48 h after application, respectively. Under field conditions, the two insecticides emamectin benzoate and indoxacarb were found to be highly effective in reducing the *H. armigera* larval population and pod damage after two sprays. Both insecticide treatments significantly increased grain yields compared with the untreated plots, with 25.8% and 24.5%, respectively. These findings showed that two applications of the selective chemical insecticides emamectin benzoate or indoxacarb with a week interval starting from the pod setting could be incorporated into the management strategies for the control of *H. armigera*.



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**Keywords:** chickpea pod borer; yield losses; emamectin benzoate; indoxacarb; spinosad; Morocco

## 1. Introduction

Chickpea (*Cicer arietinum* L.) is one of the most globally important legumes. In Morocco, it is considered the second major food legume after the faba bean. It is cultivated in different zones and climatic conditions such as rainfed areas and dry zones of the country [1]. The field area covered by chickpeas was 53,599 ha in 2020 with a production of 49,714 tonnes [2]. Chickpea is characterized by a high content of protein and fat, and it is a good source of soluble fiber and micronutrients. Additionally, chickpea plays a key role in the crop rotation system with cereals to improve soil fertility [3,4].

Unfortunately, chickpea productivity remains lower in Morocco than the world average. This is mainly attributed to abiotic limiting factors, such as terminal drought stress, and various biotic stresses, such as insects, diseases, and weeds that negatively impact crop production in terms of quality and quantity [5]. Among several biotic constraints, the chickpea pod borer *H. armigera* (Hübner) (Lepidoptera: Noctuidae) is a major field insect pest affecting chickpea

production in several agro-ecological zones. *H. armigera* is a widely polyphagous species feeding on more than 180 hosts in 70 plant families, and it is widely distributed in Europe, Africa, Asia, and Oceania [6,7]. The first instar larvae of *H. armigera* cause damage by feeding on the tender portion of the green leaves and later on flower buds and pods. Thus, the loss of flower buds and flowers results in a reduction in the yield. Under high pest infestation, the whole crop may get defoliated. After the formation of pods, the third instar larvae make a hole in the pod and move inside to feed on the green grains [8]. A single larva of *H. armigera* can destroy up to 40 pods throughout its larval stage on chickpea crops [9]. The yield loss caused by the pest can reach 400 kg/ha [10]. Insecticides are commonly applied to manage this pest all over the world, and the annual costs of this application can exceed more than USD 1 billion [6]. Over USD 328 million in losses have been attributed to *H. armigera* in chickpea production in semi-arid tropical regions [11].

Integrated pest management strategies have been emphasized by several researchers in different parts of the world to minimize *H. armigera* damage, which includes the use of resistant cultivars. Furthermore, the adoption of recommended cultural practices, such as early sowing with optimum planting density and fertilizer levels, intercropping with trap crops (mustard, coriander, marigold, sunflower, sorghum, and linseed), and installing animated bird perches and T-perches at 2 m distance of predatory zones, in addition to the use of biological agents and the application of biological and chemical control measures [12,13]. In Morocco, the chickpea leaf miner (*Liriomyza cicerina* R.) (Diptera: Agromyzidae) and the chickpea pod borer are considered the main damaging pests of chickpeas [14–16]. Many winter chickpea varieties well-adapted to different agro-ecological areas in Morocco were developed and released, with high yield potential and resistance to several biotic stresses. The chickpea plants damaged during their vegetative, flowering, and early podding stages have a remarkable capacity to recover from pod borer damage. Several chickpea genotypes with less susceptibility to *H. armigera* or the genotypes that have the capacity to recover from pod borer damage are not well identified and studied in Morocco. In view of the limited success in developing crop cultivars with resistance to this *H. armigera*, there is a need to identify varieties with different mechanisms of resistance such as tolerance or less preference.

Currently, there is no insecticide registered against *H. armigera* on chickpeas in Morocco. In order to control the chickpea pod borer, farmers apply larger amounts of insecticides; however, the indiscriminate or irrational use of pesticides has resulted in residues, the development of insecticide resistance, in addition to undesirable adverse side effects on nontarget organisms, humans, and the environment [16,17]. Various studies mentioned the great effectiveness of several selective chemical insecticides and bacterial insecticides that can be used to minimize the damage caused by *H. armigera*, and their use can reduce exposure to toxic and broad-spectrum insecticides that may affect beneficial insects [18,19].

The aim of the present study was to determine the yield losses caused by *H. armigera* and their preference towards different winter-sown chickpea varieties in Morocco. In addition, to evaluate the insecticidal efficacy of several selective insecticides under laboratory and field conditions, with multiple modes of action for the effective management of this lepidopteran insect with lesser residues and lower environmental threat. The results of our studies will help in spreading knowledge about the approximate crop loss caused by *H. armigera* in the major chickpea-growing regions of Morocco and the best biological or chemical insecticides to be used to avoid yield losses in chickpea, thus maximizing its yield and net returns.

## 2. Materials and Methods

### 2.1. Yield Loss Assessment for Chickpea Pod Borer in Relation to Different Chickpea Varieties and Locations

Four local winter varieties of chickpea, namely Moubarak (F84-182C), Farihane (F84-79C), Rizki (FLIP 83-48C), and Zahor (F84-145C), were planted at the Marchouch Experimental Station of the International Center for Agricultural Research in the Dry Areas (ICARDA) (33°61' N and 6°71' W, with an altitude of 410 m) on 13 December 2016, in a

split-plot design with four repetitions in six lines of 4 m length, with a spacing of 60 cm. The same experiment was also conducted in the Allal Tazi Research Station (34°30' N, 6°19' W, with an altitude of 10 m) located 30 km from Kenitra city, on 28 December 2016. The second-year trials during 2017–2018 were not considered in the two research stations since the infestation by *H. armigera* was negligible. Normal agronomic practices were followed for raising the crop using a seeding rate of 100 kg/ha. Treatment fungicide against *Ascochyta* blight was applied with Curator (Azoxystrobin + Chlorothalonil) with a dose of 2 L/ha during the second week of February 2017. Proclaim 05 SG (Syngenta Morocco) with active ingredient emamectin benzoate was applied weekly with a dose of 250 g/ha, starting from the early vegetative stage, using a low-pressure backpack sprayer 16 L to avoid the infestation of pods in the untreated plots. The data were recorded on pod borer damage at the harvest, and the infestation was estimated from ten random plants on the border of each plot after counting the total number of pods and the number of pods damaged. After harvest, the data on biomass, the total weight of the seeds, and the total weight of 100 seeds were recorded separately for each plot. The percentage yield loss for each plot was determined according to the following Equation [20]:

$$\text{Percentage yield loss (\%)} = \frac{[\text{yield of treated variety} - \text{yield of untreated variety}]}{[\text{yield of treated variety}]} \times 100 \quad (1)$$

The percentage increase in yield was calculated using the following formula:

$$\text{Percentage increase in yield over check(\%)} = \frac{[P1 - P2]}{[P1]} \times 100 \quad (2)$$

where P1 is the mean yield of protected plots (kg/ha), and P2 is the mean yield of unprotected plots (kg/ha).

## 2.2. Evaluation of Insecticides and Biopesticides for the Management of *H. armigera*

### 2.2.1. Insect Rearing

*H. armigera* larvae were collected from infested chickpea fields between April and July 2017 in the Marchouch ICARDA Experimental Station (33°56'10" N 6°69'21" W). The *H. armigera* larvae were reared according to Boulamtaf et al. [16] using the artificial diet, according to Koul et al. [21]. The larvae were reared under laboratory conditions (27 ± 2 °C; 75 ± 5% R.H; and photoperiod of 14 L:10 D). The larvae were kept separately in Petri dishes to feed on the artificial diet until pupation. The emerged adults were fed with 10% honey solution within glass cages after their emergence. After oviposition, the eggs were collected daily from the chickpea plants that were planted in small pots within cages. The harvested eggs were then transferred to the Petri dishes with the artificial diet. The third instar larvae were used in various bioassays.

### 2.2.2. Pesticides

The recommended doses of seven insecticides (two bioinsecticides and five chemical insecticides) were used for different bioassays. The selected insecticides belong to different families of insecticides with various modes of action (Table 1).

**Table 1.** Characteristics of the contact insecticides used to control the chickpea pod borer and their respective doses.

Trade Name	Active Ingredients	Chemical Class	Dose	Company
TRACER 480 SC	Spinosad (480 g/L)	Bacterial bioinsecticide	30 mL/100 L water	PROMAGRI
BACTOSPEINE HPWP	<i>Bacillus thuringiensis</i> -Kurstaki (serotype 3a–3b) (32,000 UI/mg)	Bacterial bioinsecticide	0.5 kg/ha	CPCM
Coragen	Chlorantraniliprole (200 g/L)	Anthranilic diamides	15 mL/100 L water	AGRIMATCO
AVAUNT 150 EC	Indoxacarb (150 g/L)	Oxadiazine	25 mL/100 L water	AGRIMATCO
Proclaim® 05 SG	Emamectin benzoate (5%)	Avermectines	250 g/ha	SYNGENTA MAROC
DECIS FLUXX	Deltamethrin (25 g/L)	Pyrethroid insecticide	30 mL/100 L water	BAYER SA
TAKUMI 20 WG	Flubendiamide (200 g/kg)	Benzene dicarboxamides	50 g/100 L water	MARBAR-CHIMIE

### 2.2.3. Laboratory Bioassays

#### Contact Bioassay

A topical application was prepared under laboratory conditions, at a temperature of  $26 \pm 2$  °C and relative humidity of  $72 \pm 3\%$  using the recommended dose of seven insecticides (Table 1). For each dose, five larvae of the third instar were used in a complete randomized design (CRD), with five replications. The control larvae were treated with distilled water containing 0.1% Triton X-100 and used as a surfactant to obtain a uniform distribution. A droplet of the treatment was applied to the thorax of the treated larvae. The treated and the control were kept on the artificial diet, and mortality rates were recorded until 6 days after treatment. The bioassays were repeated five times, and each replication consisted of 15 larvae per concentration. The larvae were considered dead if there was no movement when gently touched with a fine paintbrush.

#### Systemic Bioassay

The effects of the insecticides against the larvae of *H. armigera* were studied by incorporating different treatments into an artificial diet following a previous methodology [21]. The third instar larvae were starved for 4 h and then placed in Petri dishes comprising the artificial diet with mixed treatments. The recommended dose of seven insecticides was evaluated, including two biological and five synthetic insecticides. The experiment was laid out using five larvae in a complete randomized design (CRD), with five repetitions. The larval mortality caused by different insecticides was recorded daily, until 6 days after treatment. The insects were provided with an untreated artificial diet as the control.

### 2.2.4. Field Bioassay

A field experiment was performed in the Marchouch ICARDA Experimental Station, Morocco ( $33^{\circ}61' N$  and  $6^{\circ}71' W$ , with an altitude of 410 m) during the cropping season of 2017. The susceptible Kabuli chickpea variety Farihane (F84-79C) was planted in early spring (mid-February 2017), in 6 lines of 4 m length, with 60 cm spacing. The experiment was laid out following a randomized complete block design with 3 repetitions. Standard agronomic practices were followed throughout the season using a seeding rate of 100 kg/ha. The treatments were applied at the economic threshold level of larvae (one larva/meter row), while the plots treated with water were used as the control. The economic threshold level of larvae was based on the assessments made on larval counts prior to treatment applications. The most effective chemical insecticides and bioinsecticides selected from laboratory trials were emamectin benzoate, indoxacarb, spinosad, chlorantraniliprole, and water as the control.

All the insecticides were applied with a low-pressure backpack sprayer of 20 L, lifting 3 m space between the plots to prevent spray drift to adjacent plots. Two sprays were applied, starting from the early pod formation stage. The incidence of chickpea bod borer larvae was counted as the total number of larvae from one-meter row length (mrl-1), at three different sites one day before spray, while post-treatment counts were taken at 3, 5 and 7 days after the spraying of the insecticides. The larvae were counted from the whole above-ground parts in each plot. The pod damage was recorded 7 days after sprays by counting the total number of healthy and damaged pods by *H. armigera* larvae from 6 plants per plot that were assigned randomly. The grain yield was recorded after harvesting at the maturity stage and then converted into kilogram per hectare. The grain yield involved the whole harvestable plot area (excluding border rows).

### 2.3. Statistical Analysis

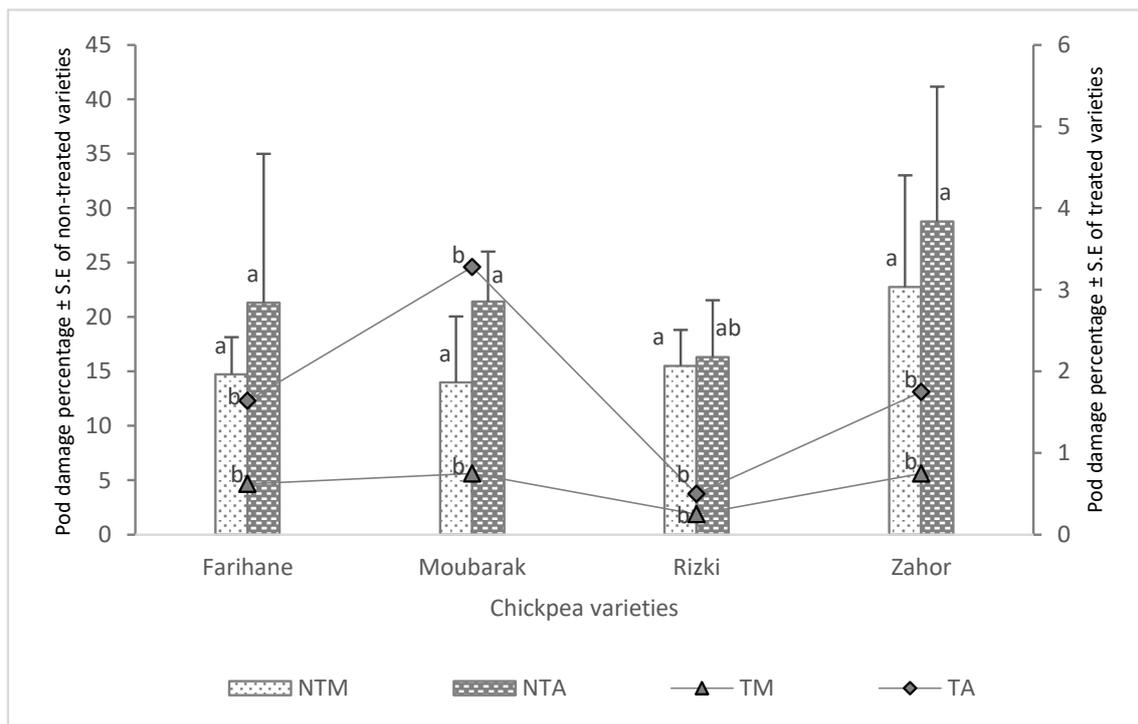
The mortality percentages of *H. armigera* larvae were transformed into angular values (arcsine  $\sqrt{P}$ ). The mean number of *H. armigera* live larvae was transformed into square-root values before the statistical analysis. A one-way analysis of variance (ANOVA) was used for both transformed values under laboratory conditions. The means were compared using Fisher's least significant difference (LSD) test at  $p < 0.05$ . Under field conditions,

a two-way repeated-measure analysis of variance (ANOVA) was used to determine the effects of the insecticides and exposure time. The computations were carried out using GenStat (19th Edition, VSN International, UK). The Pearson correlation coefficients (PCCs) were computed between the mean pods damaged by *H. armigera* per plot with grain yield/hectare using XLSTAT 22. The means of pod damage by *H. armigera* and the seed weight of each year were separated by Student–Newman–Keuls test at  $p < 0.05$  using XLSTAT 22.

### 3. Results

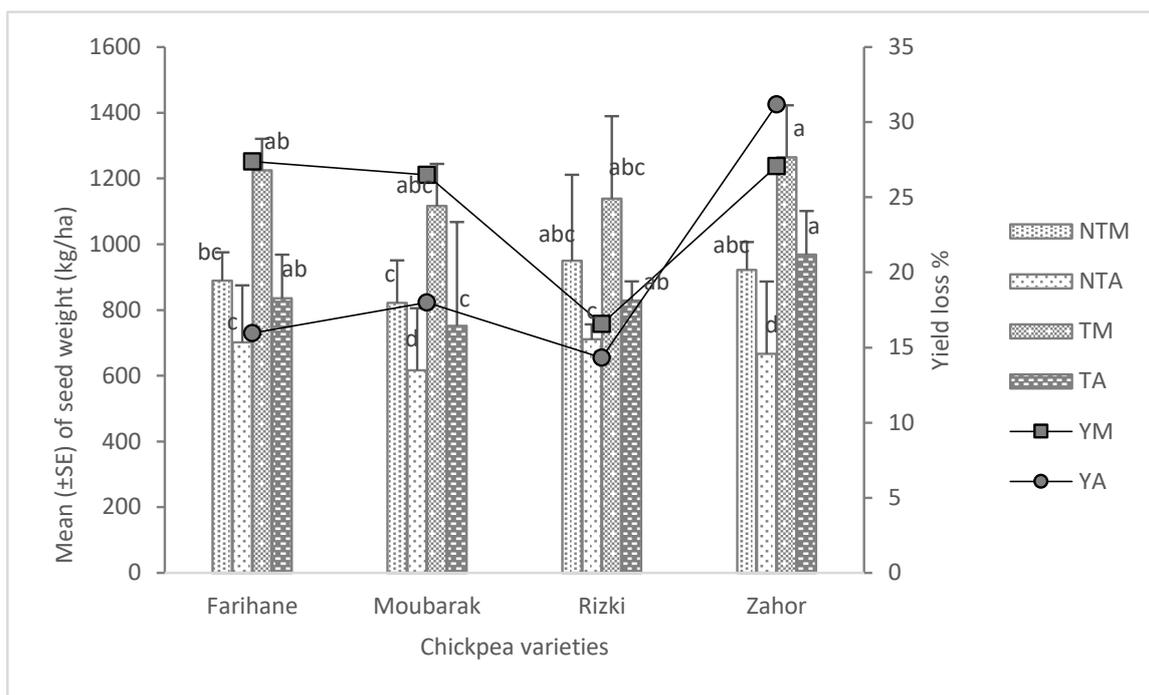
#### 3.1. Yield Loss Caused by Chickpea Pod Borer in Relation to Different Varieties and Locations

The main effects of variety ( $F = 352.1$ ;  $p < 0.001$ ) and location ( $F = 3643$ ;  $p < 0.001$ ) and the variety  $\times$  location interaction were highly significant ( $F = 33.03$ ;  $p < 0.001$ ). The interactions between variety  $\times$  treatment for plot protection were highly significant ( $F = 72.20$ ;  $p < 0.001$ ). The highest pod infestation was recorded for the Zahor variety in both stations, with 28.78% and 22.75% in Allal Tazi and Marchouch stations, respectively. The Farihane variety was recorded as having the second-highest pod borer damage, with 21.33% and 14.75% in Allal Tazi and Marchouch stations, respectively. The lowest pod borer damage was recorded for the Rizki variety with 16.32% and 15.50% in Allal Tazi and Marchouch stations, respectively (Figure 1). Higher seed yield was recorded in the protected plots than in the unprotected ones.



**Figure 1.** Percentage of pod infestation by chickpea pod borer for all varieties in different locations. NTM: non-treated variety at Marchouch station; NTA: non-treated variety at Allal Tazi station; TM: treated variety at Marchouch station; TA: treated variety at Allal Tazi station; means followed by the same letter(s) do not significantly differ at  $p < 0.05$ .

The yield losses due to the chickpea pod borer are presented in Figure 2. There was a higher mean seed yield of 1264.58 kg/ha for the protected plots of Zahor, followed by Farihane, with 1225.00 kg/ha. Both Zahor and Farihane showed losses due to *H. armigera*, with maximum yield losses of 29.13% followed by Moubarak and Farihane varieties, with 22.24% and 21.67%, respectively.



**Figure 2.** Effect of chickpea pod borer *H. armigera* infestation on seed weight and yield loss on different varieties at Marchouch and Allal Tazi stations during the cropping season of 2017. NTM: non-treated variety at Marchouch station; NTA: non-treated variety at Allal Tazi station; TM: treated variety at Marchouch station; TA: treated variety at Allal Tazi station; YM: yield loss at Marchouch station; YA: yield loss at Allal Tazi station; means followed by the same letter(s) do not significantly differ at  $p < 0.05$ .

The results revealed that the grain yield in most of the chickpea varieties tested was significantly and negatively associated with the chickpea pod borer damage (Table 2). The grain yield per hectare was significantly and negatively correlated with the *H. armigera* damage in Farihane, Zahor, and Rizki varieties ( $r = -1.25, -0.84$  and  $-0.32, p < 0.05$ , respectively) in the Allal Tazi station, and for the Moubarak variety in the Marchouch station ( $r = -0.99, p < 0.05$ ). By contrast, the correlation was non-significant and positive for Rizki in the Marchouch station.

**Table 2.** Simple correlations between chickpea pod borer damage and grain yield/hectare.

Variety/Location		Allal Tazi	Marchouch
		Grain Yield (kg/ha)	
Pod borer damage causing yield losses	Farihane	-1.25 *	-0.52
	Moubarak	-1.84 ns	-0.99 *
	Rizki	-0.32 *	0.90 ns
	Zahor	-0.84 **	-0.75

\* Significant at 5% probability level; \*\* significant at 1% probability level; ns: not significant.

### 3.2. Laboratory Bioassays

#### 3.2.1. Contact Toxicity

The contact toxicity of the insecticides was evaluated against the larvae of *H. armigera* (Table 3). All the tested insecticides significantly decreased the number of larvae ( $p < 0.001$ ) at different exposition intervals. The interaction between the two factors (contact application and exposure periods) with the mortality of *H. armigera* was highly significant ( $p < 0.001$ ) (Table S1). One day after application, the highest percentages (100% and 96%) of larval mortality were recorded for chlorantraniliprole in 15 mL/100 L water and indoxacarb in 25 mL/100 L water, respectively (Table 3). Two days after application, the three chemical

insecticides (chlorantraniliprole, indoxacarb, and emamectin benzoate at 250 g/ha) induced the maximum larvicidal activity (100%), while the bioinsecticide spinosad in 30 mL/100 L water resulted in 88% and 100% larval mortality, two and four days after application, respectively. However, the lowest larval mortality occurred with the use of deltamethrin in 30 mL/100 L water and flubendiamide in 50 g/100 L water, with 76%. The biological insecticide *Bacillus thuringiensis* kurstaki (serotype 3a–3b) at 0.5 kg/ha achieved very low toxicity, with 44% mortality six days after application.

**Table 3.** Larval mortality over time after exposure to different insecticides against third instar larvae of *H. armigera* using the contact application.

Treatments	Mean (%) Larval Mortality					
	1 DAS	2 DAS	3 DAS	4 DAS	5 DAS	6 DAS
<i>Emamectin benzoate</i>	100 ± 0.00 <sup>a</sup>	100 ± 0.00 <sup>a</sup>	100 ± 0.00 <sup>a</sup>	100 ± 0.00 <sup>a</sup>	100 ± 0.00 <sup>a</sup>	100 ± 0.00 <sup>a</sup>
<i>Indoxacarb</i>	96 ± 2.45 <sup>a</sup>	100 ± 0.00 <sup>a</sup>	100 ± 0.00 <sup>a</sup>	100 ± 0.00 <sup>a</sup>	100 ± 0.00 <sup>a</sup>	100 ± 0.00 <sup>a</sup>
<i>Chlorantraniliprole</i>	72 ± 2.00 <sup>b</sup>	100 ± 0.00 <sup>a</sup>				
<i>Deltamethrin</i>	60 ± 3.16 <sup>bc</sup>	64 ± 2.40 <sup>b</sup>	68 ± 2.57 <sup>b</sup>	68 ± 2.57 <sup>b</sup>	72 ± 3.50 <sup>b</sup>	76 ± 1.50 <sup>b</sup>
<i>Flubendiamide</i>	36 ± 7.48 <sup>cd</sup>	48 ± 2.00 <sup>bc</sup>	52 ± 2.45 <sup>bc</sup>	60 ± 3.16 <sup>b</sup>	64 ± 5.50 <sup>b</sup>	76 ± 1.00 <sup>b</sup>
<i>Bacillus thuringiensis</i>	16 ± 2.00 <sup>d</sup>	24 ± 3.18 <sup>c</sup>	28 ± 3.00 <sup>c</sup>	32 ± 3.00 <sup>c</sup>	32 ± 3.00 <sup>c</sup>	44 ± 2.00 <sup>b</sup>
<i>Spinosad</i>	20 ± 2.10 <sup>d</sup>	84 ± 3.78 <sup>a</sup>	92 ± 4.00 <sup>a</sup>	100 ± 0.00 <sup>a</sup>	100 ± 0.00 <sup>a</sup>	100 ± 0.00 <sup>a</sup>
S.E.M	5.81	8.52	5.62	4.75	4.27	5.74
L.S.D. (5%)	16.94	17.59	16.41	13.87	12.46	16.75

Means in the same column followed by different letter(s) are significantly different based on Fisher's protected LSD test ( $p < 0.05$ ). DAS: days after spraying; S.E.M: Standard error of the mean; L.S.D: Least significant difference.

### 3.2.2. Systemic Activity

The mean percentage mortality rates of the *H. armigera* larvae exposed by systemic activity to different insecticides are listed in Table 4. Our analysis revealed highly significant effects of the tested insecticides ( $p < 0.001$ ), exposure periods ( $p < 0.001$ ), and their interaction ( $p < 0.001$ ) on the mortality of *H. armigera* (Table S1). The mortality percentages were compared using LSD, and the results showed that emamectin benzoate (at 250 g/ha) resulted in the highest percentage (100%) of mortality one day after treatment, while spinosad and indoxacarb resulted in a 92% mortality rate two days after application (Table 4). The results showed that emamectin benzoate, indoxacarb, flubendiamide, and spinosad induced the highest toxic activity in adult females, ranging between 88% and 100% mortality, three days after treatment, while on the sixth day after treatment, the statistical analysis showed no difference ( $p = 0.11$ ) in terms of mortality between all the treatments.

**Table 4.** Systemic toxicity over time after exposure to different insecticides against third instar larvae of *H. armigera* using the systemic application.

Treatments	Mean (%) Larval Mortality					
	1 DAS	2 DAS	3 DAS	4 DAS	5 DAS	6 DAS
<i>Emamectin benzoate</i>	100 ± 0.00 <sup>a</sup>	100 ± 0.00 <sup>a</sup>	100 ± 0.00 <sup>c</sup>	100 ± 0.00 <sup>a</sup>	100 ± 0.00 <sup>a</sup>	100 ± 0.00 <sup>a</sup>
<i>Indoxacarb</i>	84 ± 3.5 <sup>b</sup>	92 ± 2.40 <sup>ab</sup>	92 ± 2.40 <sup>c</sup>	92 ± 2.40 <sup>ab</sup>	92 ± 2.40 <sup>ab</sup>	96 ± 2.45 <sup>a</sup>
<i>Chlorantraniliprole</i>	52 ± 6.78 <sup>c</sup>	52 ± 3.70 <sup>c</sup>	60 ± 2.00 <sup>b</sup>	72 ± 2.00 <sup>b</sup>	76 ± 2.45 <sup>b</sup>	92 ± 2.40 <sup>a</sup>
<i>Deltamethrin</i>	8 ± 1.50 <sup>d</sup>	12 ± 1.50 <sup>d</sup>	28 ± 3.00 <sup>a</sup>	32 ± 2.40 <sup>c</sup>	84 ± 1.95 <sup>b</sup>	88 ± 2.00 <sup>a</sup>
<i>Flubendiamide</i>	52 ± 2.00 <sup>bc</sup>	76 ± 2.00 <sup>bc</sup>	88 ± 3.74 <sup>c</sup>	88 ± 2.00 <sup>ab</sup>	92 ± 2.40 <sup>ab</sup>	92 ± 2.40 <sup>a</sup>
<i>Bacillus thuringiensis</i>	0 ± 0.00 <sup>d</sup>	16 ± 1.80 <sup>d</sup>	32 ± 2.45 <sup>a</sup>	68 ± 3.16 <sup>b</sup>	100 ± 0.00 <sup>a</sup>	100 ± 0.00 <sup>a</sup>
<i>Spinosad</i>	64 ± 2.00 <sup>bc</sup>	92 ± 2.40 <sup>ab</sup>	100 ± 0.00 <sup>c</sup>	100 ± 0.00 <sup>a</sup>	100 ± 0.00 <sup>a</sup>	100 ± 0.00 <sup>a</sup>
S.E.M	5.80	6.91	6.85	5.78	4.67	4.64
L.S.D. (5%)	16.94	20.18	20.00	16.86	13.63	13.54

Means in the same column followed by different letter(s) are significantly different based on Fisher's protected LSD test ( $p < 0.05$ ). DAS: days after spraying; S.E.M: Standard error of the mean; L.S.D: Least significant difference.

### 3.3. Field Bioassay

The statistical analysis showed highly significant effects of the tested insecticides ( $p < 0.001$ ), the exposure time ( $p < 0.001$ ), and their interaction ( $p < 0.001$ ), with the mean number of live larvae of *H. armigera* (Table S2). The data in Table 5 show the effect of the different insecticides tested, compared with the control (water), in terms of the mean number of live larvae of *H. armigera* after two applications.

**Table 5.** Larvicidal activity of the insecticides, 3, 5, and 7 days after two sprays against the larvae of *H. armigera* under field conditions in Marchouch Station, 2017.

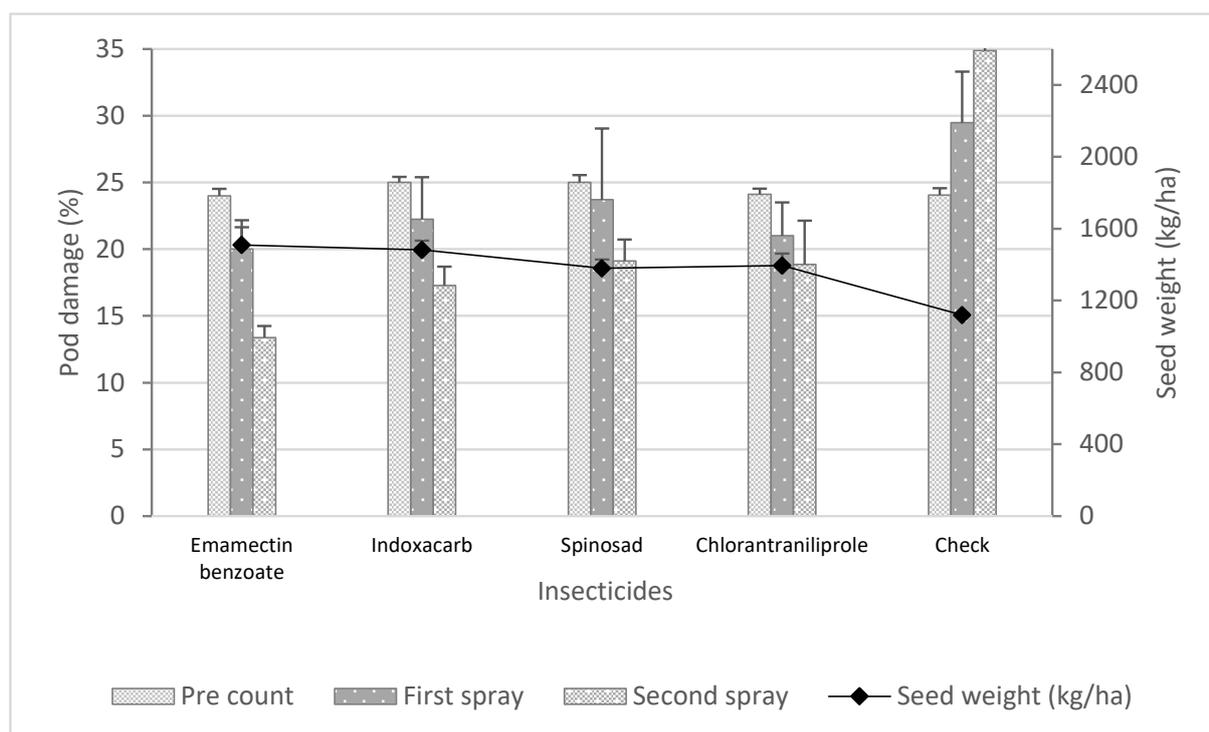
Treatments	Pre Count	Mean No. of Live Larvae per mrl					
		First Spray			Second Spray		
		3 DAS	5 DAS	7 DAS	3 DAS	5 DAS	7 DAS
<i>Emamectin benzoate</i>	3.25 ± 0.47 <sup>a</sup>	0.5 ± 0.28 <sup>a</sup>	0.25 ± 0.10 <sup>b</sup>	0.25 ± 0.10 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>b</sup>	0.00 ± 0.00 <sup>a</sup>
<i>Indoxacarb</i>	3.00 ± 0.40 <sup>a</sup>	1 ± 0.00 <sup>b</sup>	0.50 ± 0.28 <sup>b</sup>	0.50 ± 0.28 <sup>b</sup>	0.250 ± 0.10 <sup>a</sup>	0.00 ± 0.00 <sup>b</sup>	0.00 ± 0.00 <sup>a</sup>
<i>Spinosad</i>	2.5 ± 0.64 <sup>a</sup>	1.25 ± 0.25 <sup>ab</sup>	0.75 ± 0.25 <sup>b</sup>	0.50 ± 0.28 <sup>ab</sup>	0.250 ± 0.10 <sup>a</sup>	0.00 ± 0.00 <sup>b</sup>	0.00 ± 0.00 <sup>a</sup>
<i>Chlorantraniliprole</i>	3.00 ± 0.40 <sup>a</sup>	1.2 ± 0.25 <sup>b</sup>	0.75 ± 0.25 <sup>b</sup>	0.50 ± 0.28 <sup>b</sup>	0.50 ± 0.28 <sup>a</sup>	0.25 ± 0.10 <sup>b</sup>	0.25 ± 0.10 <sup>a</sup>
Check (water)	3.00 ± 0.40 <sup>a</sup>	3 ± 0.40 <sup>c</sup>	3.25 ± 0.47 <sup>a</sup>	3.25 ± 0.47 <sup>c</sup>	4 ± 0.40 <sup>b</sup>	4.00 ± 0.40 <sup>a</sup>	4.25 ± 0.47 <sup>b</sup>
S.E.M	0.0939	0.889	0.286	0.1544	0.1888	0.1206	0.1343
L.S.D. (5%)	0.2894	2.740	0.882	0.4759	0.5817	0.3717	0.4138

Means in the same column followed by different letter(s) are significantly different based on Fisher's protected LSD test ( $p < 0.05$ ). DAS: days after spraying; mrl: meter row length; control: plot treated with water.

The statistical analysis indicated differences in the total number of live larvae across all treatments and the control after 3, 5 and 7 days for each spray. The lowest number of larvae (0.5 larvae) was recorded with emamectin benzoate, one day after treatment (Table 5), while the highest number of larvae (3 larvae) was counted in the control, one day after treatment. After 5 days of the first spray, the average number of live larvae compared using LSD, showed that all the tested insecticides were separated as a different group from the control. After 6 days of the first spray, emamectin benzoate showed the highest reduction in the live larvae (0.25 larvae) compared with the other treatments, including the control (3.25 larvae).

In the second spray, our results showed apparent differences in the number of live larvae between all the tested biological and chemical insecticides and the untreated control (unsprayed plots), after 3, 5, and 7 days of the application. The mean number of live larvae, which was compared using LSD, showed that all the tested insecticides were similar in reducing the larval population of pod borer in different exposure intervals. Seven days after application, emamectin benzoate, indoxacarb, and spinosad remained effective in decreasing the number of larvae (0 larvae), compared with the untreated check (4.25 larvae).

The data in Figure 3 show the effect of the different tested insecticides compared with the control (water) in terms of the percentage of pod damage and yield. The statistical analysis showed no difference in the percentage of pod damage by the chickpea pod borer between all the treatments seven days after the first spray. However, there were highly significant differences between all the tested insecticides and the control ( $p < 0.001$ ) seven days after the second spray. Among the different insecticides, emamectin benzoate showed the lowest percentage of pod damage (13.39%), compared with the untreated plots (34.87%). The highest seed yield was recorded for the plots treated with emamectin benzoate (1509 kg/ha) and indoxacarb (1482 kg/ha), compared with the control (1119 kg/ha). The bioinsecticide spinosad (1380 kg/ha) was found effective in increasing the seed yield similar to chlorantraniliprole (1394 kg/ha).



**Figure 3.** Effectiveness of different insecticides on the percentage of pod damage and yield of chickpea in Marchouch Station, 2017.

#### 4. Discussion

The current study showed that chickpea pod borer infestation resulted in losses in the total seed weight for all the varieties, with the highest yield losses for Zahor at 31.18% in Allal Tazi followed by Farihane at 27.38% in the Marchouch station. Both Zahor and Farihane varieties showed the maximum pod damage by *H. armigera* and yield losses in different locations. Yield losses due to pod borer varying from 10% to 60% were reported in the chickpea in India under normal weather conditions [22]. While, under favorable weather conditions, 50–100% of the yield loss was estimated [23]. Patel [24] observed that the yield loss increased from 6.09% to 34.71% when the larval density was increased from one to ten larvae of *H. armigera* in caged gram plants. The present study's findings showed a significant and negative correlation between grain yield and pod borer damage in unprotected plots. Similarly, a significant association was reported earlier between the yield and pod damage by Lakshmi et al. [25] and Sreelatha [26].

Most of the tested varieties are well-adapted to different agro-ecological areas, with high yield potential and tolerance to *Ascochyta* blight [5]. This study revealed significant variation among the tested chickpea varieties in their response to *H. armigera* damage. These variations are in accordance with the findings of other researchers based on which they were associated with the genetic diversity in the major qualitative and quantitative traits present in chickpea plants [27]. The development of cultivars resistant or tolerant to *H. armigera* has considerable potential for use in integrated pest management. Several chickpea accessions such as ICC506EB, ICC10667, ICC10619, ICC4935, ICC10243, and ICCV95992 with resistance to *H. armigera* and lines such as ICCV7, ICCV10, and ICCL86103 with moderate resistance have been identified [28]. In line with the findings of our study the results obtained by Deshmukh et al. [29] revealed 30.6% to 31.5% pod damage in chickpea genotypes. Several studies indicated chickpea genotypes that presented the lowest and highest susceptibility to *H. armigera* [30–32]. The present study showed that the Rizki variety recorded the lowest pod damage and yield loss compared with the other winter chickpea varieties. An understanding of the mechanisms (antixenosis, Antibiosis, and

tolerance) and inheritance of resistance for this variety will help to develop strategies for improving the grain yield and developing pod-borer-resistant cultivars in chickpeas.

The second part of the current study revealed that all the tested insecticides resulted in higher larval mortality than the untreated control. The insecticide applications of emamectin benzoate at 250 g/ha and indoxacarb at 25 mL/100 L water were very effective in reducing pod damage and the total number of *H. armigera* larvae in chickpeas. In addition, the two insecticides recorded the highest seed yield over all the treatments.

The present findings are in agreement with the results of Sarnaik and Chiranjeevi [33], who reported the chemical insecticide emamectin benzoate 5% WG at 15.0 g a.i./ha as the most effective treatment to reduce *H. armigera* larvae (0.13 larvae per plant) and with the lowest percentage of pod damage (5.83%) after seven days of spraying, respectively. In a previous study, Chaukikar et al. [34] reported that emamectin benzoate 5% WG applied at different doses of 9.4 and 8.1 g a.i./ha recorded maximum reductions in *H. armigera* larvae and pod damage (1.28% and 1.29%, respectively). Kumar and Sarada [35] reported that emamectin benzoate 5 SG (2.85%) is highly effective in reducing pod damage with a 79.1% reduction over the control. Emamectin benzoate (EB) is an important derivative of the avermectin family, isolated from the fermentation broth of the soil actinomycete, *Streptomyces avermitilis* [36]. This active ingredient acts effectively through ingestion and contact, targeting the neuromuscular system by binding to the GABA and glutamate H receptors, causing the rapid paralysis and death of lepidopterous pests [37,38]. Emamectin benzoate 5% WG sprays showed no adverse effects on predators such as ladybird beetle and lacewings, and the chickpea plants were not affected by any phytotoxic symptoms [34,39]. The recommended field concentration of emamectin benzoate was classified as slightly harmful and moderately harmful for *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae). However, this study recommended that an emamectin benzoate application should be avoided when the adult population of this important biocontrol agent, i.e., *T. brassicae*, is at its highest level in agricultural ecosystems [40].

Economic analysis revealed that emamectin benzoate sprays were cost-effective and demonstrated a higher cost-benefit ratio than the other tested chemical insecticides in chickpea crops [35,41]. In Morocco, the field evaluation of the efficacy of different chemical and biological insecticides against defoliating caterpillars, including *H. armigera*, showed that chlorantraniliprole (15 mL/100 L water) and emamectin benzoate (250 g/ha) were most effective in the reduction in the larval population of different defoliating caterpillars, including *H. armigera* (89.4% and 82.5%, respectively) in spearmint crops [42].

The current results agree with the findings of Kambrekar et al. [39], who indicated that indoxacarb 14.5% SC @ 75 g a.i./ha registered the second-highest larval mortality after emamectin benzoate, leading to lower damage to pods and higher chickpea yield grain. The effectiveness of several insecticides has been reported by Mihretie et al. [43], where a three-time application of indoxacarb 0.3 L/ha with a week interval starting from the pod setting was highly effective and gave maximum protection to pods, which resulted in grain yield prevention (48.11%). In fact, indoxacarb is characterized by neurotoxic effects, which induce paralysis by blocking voltage-dependent sodium channels, eventually leading to the paralysis and death of lepidopteran pests and coleopteran species, as well as certain homopteran pests [44,45]. In addition, indoxacarb has low side effects on non-target insects including several predators and immature wasp parasites [46,47]. Previous works by Pashte and Patil [48,49] reported that indoxacarb was the most toxic compound for honeybee *Apis mellifera*, acting through direct contact. It is, therefore, suggested that the application of indoxacarb must be used only with the greatest care, as they destroy bees, including non-target insects that are essential for pollination.

The current study revealed that the bioinsecticide spinosad was very effective in reducing the number of *H. armigera* and pod damage 7 days after the second spray under field conditions, acting through contact and especially ingestion. Furthermore, the effectiveness of spinosad in the present study is also supported by previous studies under both laboratory and field conditions. Chlorpyrifos and spinosad have been demonstrated in laboratory

bioassays to be effective against the third instar *H. armigera* larvae through both contact and ingestion [19]. Ahmed et al. [50] indicated that spinosad (60 mL/acre) was the most toxic against the chickpea pod borer followed by indoxacarb (150 mL/acre). Spinosad is a naturally derived insecticide, produced via the fermentation of *Saccharopolyspora spinosa*, and it is a neurotoxin comprising a mixture of spinosyns A and D. Its mechanism of action is primarily by targeting nicotinic acetylcholine receptor, which causes excitement in the nervous system of insects, leading to muscle contraction, paralysis, and ultimately death [51,52]. However, Mihretie et al. [43] showed that three applications with a week interval of spinosad (Tracer 480 SC) 0.15 L ha<sup>-1</sup> under field conditions in Ethiopia can provide the maximum protection, with 43.37% of yield increase over the control. Furthermore, spinosad in 25 mL/100 L water showed a significant impact to reduce the number of chickpea leafminer *L. cicerina* larvae under field conditions, with little or no effect on *L. cicerina* parasitoids, including the species from Braconidae (Hymenoptera) and Eulophidae families [24,53].

The response of the field populations of *H. armigera* to several insecticides using a leaf-dip bioassay showed no or very low levels of resistance to spinosad, Abamectin, and emamectin benzoate. Resistance to indoxacarb ranged from moderate during 2003–2006 to no resistance during 2015 and 2016, corresponding to the reduced use of indoxacarb in Pakistani agriculture [54].

## 5. Conclusions

The results revealed a negative correlation between the grain yield per hectare and the pod damage by *H. armigera*, which revealed that a high infestation rate of pod borer might cause great yield losses in the chickpea. The current study showed that *H. armigera* caused an average yield loss in the total grain between 15.96% and 31.18%, and the Rizki variety showed the lowest pod damage and yield loss compared with the other tested winter chickpea varieties. These losses justify the development of integrated management options for the control of this pest in Morocco. The bioassays conducted in the laboratory showed that all the tested insecticides significantly reduced the mean number of the third instar *H. armigera* larvae both via contact and ingestion. Among the various tested insecticides, emamectin benzoate (Proclaim<sup>®</sup> 05 SG) with 250 g/ha and indoxacarb (Avaunt 150 EC) with 25 mL/100 L water, and the bioinsecticide spinosad (TRACER 480 SC) with 30 mL/100 L water showed the lowest percentage of pod damage in chickpeas and the maximum reduction in the larval population under field conditions, which provided the highest seed yield over all the treatments. Furthermore, the application of the bioinsecticide spinosad can be effective to manage chickpea leafminer larvae at the same time. A two-time application of these selective insecticides with a week interval starting from the pod setting should be an effective option for the management of *H. armigera* in chickpea crops in Morocco.

**Supplementary Materials:** The following are available online at <https://doi.org/10.5281/zenodo.7327834>. Table S1: Analysis of variance for the effect of insecticides as a contact and systemic applications and their combinations on *H. armigera* larvae. Table S2: Analysis of variance for the effect of insecticides tested under field conditions on mean number of *H. armigera* larvae and on pod damage and their combinations with exposure time.

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