

## Response of Three Soybean Genotypes to Lima Bean Pod Borer (*Etiella zinckenella*) Infestation Using Some Bio and Chemical Insecticides

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### Abstract

The present investigation was carried out at Giza Agricultural Experiments and Research Station, Agricultural Research Center (ARC), Giza, Egypt during the two successive seasons 2018 and 2019 to evaluate three soybean genotypes (Giza 35, Crawford and DR10I) to infestation with lima bean pod borer using four bio and chemical insecticides (Diple-2x 6.4% DF, Biover10 % WP, Suncide Agri-pest and Lannate 25% WP) for increasing seed yield and net return. The treatments were four insecticides (Diple-2x 6.4% DF, Biover10 % WP, Suncide Agri-pest and Lannate 25% WP) beside water as control and three soybean genotypes (Giza 35, Crawford and DR10I). Split-plot distributions in a randomized complete block design with three replications were used. Insecticide sources were randomly assigned to main plots and soybean genotypes were allocated in subplots. The results showed that the bacterial insecticide Diple-2x 6.4% DF recorded lower pod infestation and seed damage than the other insecticides. Soybean genotype DR10I had lower pod infestation and seed damage, meanwhile the reverse was true for soybean genotype Crawford. Soybean variety Giza 35 recorded higher most yield traits than the soybean genotypes Crawford and DR10I. Soybean genotypes x insecticide sources interaction was significant for pod infestation, seed damage, seed yield per plant, 100-seed weight, seed yield per ha, and HI in both seasons. Spraying of the bacterial insecticide Diple-2x 6.4% DF in the flowering stage of soybean variety Giza 35 enhanced its tolerance to lima bean pod borer infestation with higher seed yield and net return than the other treatments.

**Keywords:** Soybean Genotypes; Insecticides Treatments; Lima Bean Pod Borer; Net Return

### Introduction

Among the different control measures such as cultural, mechanical, biological and chemical methods, the farmers prefer the use of chemicals to control pests because it gives quick results. Over 300 trade name pesticides, manufactured by over 50 chemical companies, were used [1] where soybean [*Glycine max (L.) Merr.*] crop was attacked by many insects such as spider mites, aphids, cotton leaf worm and many other pests [2]. Soybean is considered one of

the important food and industrial crops on the international level, owing to containing about 30% of cholesterol free oil, and about 40% of protein which is similar in its nourishing value to the animal protein [3]. However, excessive use of chemicals and synthetic insecticides is not only expensive but also results in series problems like the development of insect resistance to insecticides, harm to other natural enemies of insects and toxic effects on plants, soil and human being [4].

Lima bean pod borer, *Etiella zinckenella* Treitschke, is one of the major insect pests of soybean in many parts of the world [5]. Applications of systemic insecticides must be precisely timed to ensure that larvae receive a lethal dose in the 25- 90 minutes prior to entering the pods [6]. In this concern, Aganon [7] found that the most critical stages when crop was damaged by insect was during the pod filling (53 days after planting) and beginning seed formation (60 days after planting), where this insect caused 70% yield loss [8] through larvae that can destroy the seeds during development inside a pod [9]. Consequently, chemical pesticides are not effective against this insect [10] because larvae are well protected in floral parts and pods [11]. Thus, alternative control methods should be considered to control insect damage.

Exposure to light leads to opening of stomata for CO<sub>2</sub> uptake, although the conductivities are affected by the light spectrum [12]. This leads to a diurnal pattern of opening where stomata of C<sub>3</sub> plants typically have high stomatal conductance during the day and low during the night. The stomatal conductance is further regulated through opening and closing, influenced by various environmental conditions [13].

Various pathways for penetration of an insecticide within a plant have been summarized by Mitchell, *et al.* [14] through the walls of root hairs or the epidermal cells of roots; through the cuticle of hairs on aerial parts or on epidermal cells; after penetration of stomata, through the cuticle of cells of the spongy mesophyll; through the cuticle and walls into epidermal cells associated with bundle sheath extensions; through lenticels, or cracks in the cuticle and periderm into cells of the phellogen; or through the cuticle into the middle lamella between adjacent epidermal cells. They added that aqueous solutions or suspensions enter these openings and penetrate the plasma membrane by way of the thin-walled cells of the mesophyll. However, we will just study the penetration of insecticides to leaves of soybean plants through their stomata in our study. Biological control is the use of living beings (control auxiliaries) or their products (inert bio pesticides) to fight pests or disease vectors. While inert biopesticides are generally derived from bacteria or fungi and they are the quickest biodegradable [15]. *Bacillus thuringiensis* var *íétékrustaki* is one of these inert biopesticides. It is a bacterium recognized by its protein crystal included in the cytoplasm (O-endotoxin) which is toxic to mosquito

larvae [16]. On the other hand, photosensitizers and nanocomposites represent a possible alternative to traditional chemical compounds [17], where Indig, *et al.* [18] indicated that the process of photosensitization in photodynamic therapy leads to the generation of singlet oxygen and of several radicals (R·) and reactive oxygen species (ROS). These species damage membranes, proteins and deoxyribonucleic acid (DNA), resulting in several mechanisms of cell death [19]. Thus, photoactive compounds usually used for photosensitization might be effective as pesticide agents, with low impact on the environment, being non-toxic and not mutagenic. Consequently, photosensitizer accumulates within the insect body and, following exposure to visible light, induces lethal photochemical reactions and death [20]. Moreover, chlorophyll is a raw material in the synthesis of various photosensitizers used in photodynamic therapy such as synthetic chlorins [21,22].

Accordingly, Amro, *et al.* [23] indicated that soybean varieties Clark, Giza 22 and Toano equipped higher infestation by *E. zinckenella* than soybean varieties Hagen 32 and S5. They added that the highest damage percentage appeared on soybean variety Toano while the lowest one appeared on soybean variety Hagon 32. There were three varieties and two germplasm accessions that can be used as gene sources for improving the resistance of the varieties. The three varieties are able to be cultivated directly in field to decrease the *E. zinckenella* occurrence [24]. Moreover, Abdel-Wahab, *et al.* [25] reported that there were three susceptible soybean varieties (Giza 21, Giza 22 and Crawford), two moderately resistant soybean varieties (Giza 35 and Giza 111) and one resistant soybean variety (Dr-101) in the first season. Meanwhile, soybean varieties Giza 111 and Crawford were susceptible, soybean varieties Giza 21 and Giza 35 were moderately resistant, and soybean varieties Giza 22 and Dr-101 were highly susceptible and resistant, respectively, in the second season. So, the usage of a tolerant soybean variety can be played an important role to decrease pesticide residue in the environment and economically benefit.

### Objective of the Study

The objective of the present study was to evaluate three soybean genotypes (Giza 35, Crawford and DR101) to infestation with lima bean pod borer using four bio and chemical insecticides (Diple-2x 6.4% DF, Biover10 % WP, Suncide Agri-pest and Lannate 25% WP) for increasing seed yield and net return.

## Materials and Methods

The experiment was conducted at Giza Agricultural Experiments and Research Station (Lat. 30° 00' 30" N, Long. 31° 12' 43" E, 26 m a. s. l), Agricultural Research Center (ARC), Egypt during the two successive seasons 2018 and 2019. The objective of the present study was to evaluate three soybean genotypes (Giza 35, Crawford and DR101) to infestation with lima bean pod borer using four bio and chemical insecticides (Diple-2x 6.4% DF, Biover10 % WP, Suncide Agri-pest and Lannate 25% WP) for increasing seed yield and net return. The treatments were combinations between four insecticide treatments (Diple-2x 6.4% DF, Biover10 % WP, Suncide Agri-pest, Lannate 25% WP) beside water as control and three soybean genotypes (Giza 35, Crawford and DR101). Table 1 shows the common names, origin, pedigree, maturity group and growth habit of the studied soybean genotypes. The studied soybean genotypes were sown on June 12<sup>th</sup> and 3<sup>rd</sup> in 2018 and 2019 summer seasons, respectively. Soybean plants were grown in one row on ridges 60 cm width with leaving two plants per hill distanced at 20 cm. Furrow irrigation is the irrigation system in the region. All cultural practices of growing soybean were conducted according to the crop recommendation.

Soybean genotype	Origin	Pedigree	Maturity group	Growth habit
Crawford	USA	Williams x Columbus	IV	Indeterminate
DR101	Egypt	Selected from Elgin	V	Determinate
Giza 35	Egypt	Crawford x Celest	III	Indeterminate

**Table 1:** The common names, origin, pedigree, maturity group and growth habit of the studied soybean genotypes.

The tested insecticides are two commercial products of bio-insecticides (Diple-2x, *Bacillus thuringiensis* var. Kurstaki and Biover, *Beauveria bassiana*), one Suncide Agri-pest (Photosensitizer, Magnesium chlorophyllin (Mg-Chl)), one recommended chemical insecticide (Lannate) as a standard check material and the control 'water' (Table 2).

Spraying was carried out at 50% flowering stage and directed to the plants using a small hand pressure sprayer at the morning hours in both seasons. Split-plot distributions in a randomized

Trade name	Active ingredient	Used rate
Diple-2x 6.4% DF	<i>B. thuringiensis</i> var. Kurstaki	2.0 g/L
Biover10 % WP	<i>B. bassiana</i>	2.0 g/L
Suncide Agri-pest (photosensitizer Magnesium chlorophylline (Mg-Chl))	Mg-Chl	0.35 g/L
Lannate 25% WP	Methomyl	1.0 g/L
The control	Water	Water

**Table 2:** Trade name, active ingredient and used rate of the studied insecticides.

complete block design with three replications were used. Insecticide treatments were randomly assigned to main plots and soybean genotypes were allocated in subplots. Each plot was 9m<sup>2</sup> (5 ridges, 3m in length and 0.6m in width).

### The studied traits

#### Stomata dimensions in soybean leaves

Soybean leaves were taken at flowering stage for three soybean genotypes to estimate stomata dimensions. Stomata dimensions were expressed as length (µm) and width (µm) per 30 µm. Stomata size was calculated as follows: stomata length x stomata width. This analysis was done by using SEM Model Quanta 250 FEG (Field Emission Gun) in the Egyptian Mineral Resources Authority Central Laboratories Sector.

#### Lima bean pod borer assemblages in soybean pods and seeds

Samples were collected from the diagonal of every plot, consisting of 90 random green pods (30 pods/each plot). The green pods were kept in a paper bag then transferred to the laboratory. These experiments were carried out to determine resistance status of the tested soybeans to lima bean pod borer. The mean number of the larval escaping holes on the green and dry soybean pods was considered as an indicator of the infestation percentage caused by lima bean pod borer. Attacking percentage was calculated as follows:

### Soybean seed yield and its attributes

At harvest, the following traits were measured on ten plants from each plot: Plant height (cm), number of branches per plant, number of pods per plant, seed yield per plant (g) and 100-seed weight (g). Biological yield per ha (ton): it was recorded on the basis of experimental sub plot area by harvesting all plants of each plot. Straw yield per ha (ton): it was recorded on the basis of experimental sub plot area by harvesting all plants of each plot. Seed yield per ha (ton): it was recorded on the basis of experimental sub plot area by harvesting all plants of each plot. Harvest index 'HI' (%): Harvest index was calculated by Clipson, *et al.* [26] as follows:

Note: Damaged seeds were excluded from yield traits.

### Economic return

Economic return was used to compare costs and returns amongst different target insects control treatments. Average of production costs of soybean per ha were recorded from Bulletin of Statistical Cost Production and Net Return [27]. The production costs were 607 USD/ha and sale price of soybean was 534 USD/ton (market price). It was estimated that irrigation, fertilization and crop field service treatment required 237 USD/ha. It was found that pest control required 36 USD/ha. The cost of harvesting, transporting the crop and expenses were 99 USD/ha. The costs of renting the land was 209 USD/ha. Application of Diple-2x 6.4%DF treatments required 28 USD/ha. While application of Biover10 % WP treatments required 24 USD/ha. Lannate 25%WP was 47 USD/ha. The Photosensitizer Magnesium chlorophyllin (MgChl) was not registered commercially and it was difficult to calculate the net return so it was calculated as general pest control that mentioned in the Bulletin (36 USD/ha). Net returns (USD/ha) were calculated by subtracting the cost of plant protection along with other costs (USD/ha) from the gross returns. Benefit Cost (B:C) ratio was obtained by taking the ratio of gross returns to the financial costs including the plant protection measures.

### Statistical analysis

Analysis of variance of alive larvae, seed yield, and its attributes of each season was performed. The measured variables were analyzed by ANOVA using MSTATC statistical package [28]. Mean comparisons were performed using the least significant differences (L.S.D) test with a significance level of 5% [29].

## Results and Discussion

### Stomata dimensions in soybean leaves

Soybean genotypes differed significantly for stomata length and size (Figure 1 and table 3), meanwhile stomata width was not differed among them. Stomata length in leaves of soybean genotypes Crawford, Giza 35 and DR101 recorded 12.02, 9.69 and 9.08  $\mu\text{m}$ , respectively. These results show that leaves of soybean genotype Crawford had higher stomata length than Giza 35 variety or DR101 genotype. It is important to mention that there were no significant differences between soybean genotypes Giza 35 and DR101 for stomata length. Also, stomata size in leaves of soybean genotypes Crawford, Giza 35 and DR101 recorded 31.37, 29.74 and 26.69  $\mu\text{m}^2$ , respectively. These results reveal that leaves of soybean genotype DR101 had smaller stomata than Giza 35 or Crawford genotype. It is important to mention that there were no significant differences between soybean genotypes Crawford and Giza 35 for stomata size. These results indicate that the insecticide efficacy appears to be negatively affected by stomata size.

**Figure 1:** Stomata dimensions in leaves of the studied soybean genotypes.

### Lima bean pod borer assemblages in soybean pods and seeds Soybean genotypes

Data in table 4 presents percentages of pod infestation and seed damage caused by lima bean pod borer in three soybean genotypes. With respect to pod infestation, it ranged from 12.73 to 20.94% in the first season and from 15.97 to 24.28% in the second one.

Stomata dimension	Crawford	Giza 35	DR101	L.S.D. 0.05
Stomata length	12.02 $\mu\text{m}$	9.69 $\mu\text{m}$	9.08 $\mu\text{m}$	1.56
Stomata width	2.61 $\mu\text{m}$	3.07 $\mu\text{m}$	2.94 $\mu\text{m}$	N.S.
Stomata size	31.37 $\mu\text{m}^2$	29.74 $\mu\text{m}^2$	26.69 $\mu\text{m}^2$	2.88

**Table 3:** Stomata dimensions in leaves of the studied soybean genotypes.

Soybean genotype DR101 recorded lower pod infestation (12.73% in the first season and 15.97% in the second season), meanwhile, soybean variety Giza 35 came in the second rank (16.53% in the first seasons and 19.82% in the second season) than soybean genotype Crawford (20.94 and 24.28% in the first and second seasons, respectively). With respect to seed damage, it ranged from 10.00 to 18.43% in the first season and from 11.64 to

Soybean genotype	Insecticide	Pod infestation (%)		Seed damage (%)	
		First season	Second season	First season	Second season
Crawford	Biover10 % WP	19.16	23.73	23.46	25.13
	Diple-2x 6.4% DF	10.23	13.88	9.98	11.40
	Suncide Agri-pest	21.56	24.14	17.16	18.72
	Lannate 25% WP	11.83	14.21	10.02	11.98
	Control	41.92	45.46	31.53	32.68
	Mean	20.94	24.28	18.43	19.98
Dr-101	Biover10 % WP	8.83	12.14	7.61	9.26
	Diple-2x 6.4% DF	8.66	11.83	8.25	10.03
	Suncide Agri-pest	4.83	8.16	4.79	6.15
	Lannate 25% WP	8.66	11.58	7.25	8.92
	Control	32.67	36.18	22.14	23.84
	Mean	12.73	15.97	10.00	11.64
Giza 35	Biover10 % WP	10.33	12.02	9.18	11.88
	Diple-2x 6.4% DF	9.00	13.99	9.34	10.22
	Suncide Agri-pest	17.35	20.21	19.64	21.70
	Lannate 25% WP	11.33	13.62	10.66	11.09
	Control	34.66	39.29	23.17	24.69
	Mean	16.53	19.82	14.39	15.91
Average of Biover10 % WP		12.77	15.96	13.41	15.42
Average of Diple-2x 6.4% DF		8.02	12.00	8.03	9.25
Average of Suncide Agri-pest		14.58	17.50	13.86	15.52
Average of Lannate 25% WP		10.60	13.13	9.31	10.66
Average of Control		36.41	40.31	25.61	27.07
L.S.D. 0.05 Soybean genotypes		3.07	2.88	2.22	2.38
L.S.D. 0.05 Insecticide treatments		2.63	2.15	1.84	1.97
L.S.D. 0.05 Interaction		3.46	3.29	2.63	2.76

**Table 4:** Average numbers of lima bean pod borer larvae in pods of three soybean genotypes after treated with the tested insecticides in both seasons.

19.98% in the second one. Soybean genotype DR101 gave lower seed damage (10.00% in the first season and 11.64% in the second season), meanwhile, soybean variety Giza 35 came in the second rank (14.39 and 15.91% in the first and second seasons, respectively) than soybean genotype Crawford (18.43 and 19.98% in the first and second seasons, respectively). The results showed that there were two tolerant soybean genotypes (DR101 and Giza 35) and one susceptible soybean genotype (Crawford) for lima bean pod borer infestation.

Tolerance or susceptibility among the studied soybean genotypes to lima bean pod borer infestation could be due to differences in their stomata size (Figure 1 and table 3) and pod pubescences. These results are in parallel with those observed by Abdel-Wahab, *et al.* [25] who found that pods of soybean genotypes DR101 and Giza 35 were denser than those of soybean genotype Crawford. These results are in harmony with those obtained by Talekar and Lin [30] who showed that the soybean accession PI 227687 is consistently resistant to lima bean pod borer. Also, Amro, *et al.* [23] indicated that the tested soybean varieties Clark, Giza 22 and Toano equipped higher infestation by lima bean pod borer with an average of 4.30, 3.54 and 9.13% respectively, than the tested varieties Hagen 32 and S5 by 2.38 and 3.21%, respectively. Moreover, Kuswanto, *et al.* [24] found that five soybean genotypes were resistant and seventeen soybean genotypes were moderately resistant.

### Insecticide treatments

Data in table 4 show percentages of pod infestation and seed damage caused by the lima bean pod borer with the application of the tested insecticides. It was observed that all the tested insecticides reduced significantly pod infestation and seed damage compared with the control treatment. With respect to pod infestation, the bacterial insecticide Diple-2x 6.4% DF gave lower pod infestation (8.02 and 12.00% in the first and second seasons, respectively), meanwhile, the chemical insecticide Lannate came in the second rank (10.60 and 13.13% in the first and second seasons, respectively) than the others. The fungal insecticide Biover10 % WP recorded 12.77% in the first season and 15.96% in the second season, meanwhile the biological insecticide Suncide Agri-pest recorded 14.58% in the first season and 17.50% in the second season for pod infestation. Conversely, the control treatment had the highest pod infestation (36.41% in the first season and 40.31% in

the second season) compared with the others. It is worthy to note that there were no significant differences between the fungal insecticide Biover10 % WP and the chemical insecticide Lannate for pod infestation in the first season. Meanwhile, there were no significant differences between the bacterial insecticide Diple-2x 6.4% DF and the chemical insecticide Lannate for pod infestation in both seasons.

With respect to seed damage, the bacterial insecticide Diple-2x 6.4% DF gave lower seed damage (8.03% in the first season and 9.25% in the second season), meanwhile, the chemical insecticide Lannate came in the second rank (9.31 and 10.66% in the first and second seasons, respectively) than the others. The fungal insecticide Biover10 % WP recorded 13.41% in the first season and 15.42% in the second season, meanwhile the biological insecticide Suncide Agri-pest recorded 13.86% in the first season and 15.52% in the second season for seed damage. Conversely, the control treatment had the highest seed damage (25.61 and 27.07% in the first and second seasons, respectively) compared with the others. It is important to mention that there were no significant differences between the bacterial insecticide Diple-2x 6.4% DF and the chemical insecticide Lannate for seed damage in both seasons.

With respect to the chemical insecticide Lannate, the active ingredient (methomyl) is toxic (Table 2) to insects such as beetles, aphids, thrips, leafhoppers, and caterpillars and particularly loopers, beet armyworm, and corn earworm as mentioned by Harvey, *et al.* [31]. Consequently, the chemical insecticide Lannate had a positive effect on pod infestation and seed damage compared with the biological insecticides. With respect to the bacterial insecticide Diple-2x 6.4% DF, the active ingredient of Diple-2x 6.4% DF that is *B. thuringiensis* var. *Kurstaki* (Table 2) was toxic to young and mature larvae probably due to gut paralysis caused by bacterium spores and crystal protein [11]. Consequently, it is likely that the chemical insecticide Lannate did not kill all the larvae over time, as a result of escaping some larvae from their natural enemies that the chemical insecticide Lannate has killed.

With respect to the fungal insecticide Biover10 % WP, the active ingredient of Biover10 % WP is *B. bassiana* (Table 2). It is known that fungicides are reported to contribute to the disappearance of epizootics of entomopathogenic fungi and have exerted control on key pests in an orchard [32]. So, the fungal insecticide works ef-

fectively in plants that have extended crop canopy (leafy and totally covering soil) to provide a humid microclimate conducive to the development of the fungus [11]. This greatly demonstrated the relatively positive effect of the fungal insecticide Biover10 % WP on pod infestation and seed damage. On the other hand, the active ingredient of the biological insecticide Suncide Agri-pest is Mg-Chl (Table 2) that accumulates within the lima bean pod borer body and, following exposure to visible light, induces lethal photochemical reactions and death [20]. The data confirmed that the biological insecticide Suncide Agri-pest was the least impact on lima bean pod borer larvae because it is an insecticide that works when light and oxygen are available, so some larvae may resort randomly to hide from light and oxygen, which increases the infestation, as well as some larvae, may escape from the natural enemies that the biological insecticide Suncide Agri-pest has killed. These data reveal that the bacterial insecticide Diple-2x 6.4% DF was more effective in reducing pod infestation and seed damage larvae in soybean pods than the chemical insecticide Lannate.

#### The interaction between soybean genotypes and insecticide treatments

Pod infestation and seed damage were affected significantly by the interaction between soybean genotypes and insecticide treatments in both seasons (Table 4). With respect to pod infestation and seed damage, applying the biological insecticide Suncide Agri-pest on the canopy of soybean genotype DR101 recorded the lowest pod infestation and seed damage compared with the other treatments in both seasons. Although the maturity group of soybean genotype DR101 is V (Table 1), the bacterial insecticide Diple-2x 6.4% DF efficacy remained high during that period. These results could be due to bacteria of Diple-2x 6.4% DF reduced the fertility of lima bean pod borer and the rate of hatching through protein crystal of *B. thuringiensis* var. Kurstaki where these microscopic crystals are ingested and transformed into toxic protein molecules (of-endotoxin) which destroy stomach walls; insects usually stop feeding within hours [33]. It is likely that leaves of soybean genotype DR101 that had smaller stomata size (Figure 1 and table 3) opened faster than large stomata of soybean genotypes Crawford and Giza 35 which contributed effectively to receive of the biological insecticide Suncide Agri-pest. Thus, it is expected that soybean genotype DR101 which is a determinate type (Table 1) will furnish more time to damage living tissues of lima bean pod borer by inducing lethal photochemical reactions and death [20].

However, it is important to mention that there were no significant differences among the fungal insecticide Biover10 % WP, the biological insecticide Suncide Agri-pest and the chemical insecticide Lannate for pod infestation and seed damage of soybean genotype DR101 in both seasons (Table 4). With respect to the fungal insecticide Biover10 % WP, soybean genotype DR101 is a determinate type (Table 1) meaning no extended crop canopy to furnish a humid environment for fungus development which reflected negatively on the efficacy of this insecticide over time. With respect to the chemical insecticide Lannate, it is likely that the efficacy of this insecticide destroyed the natural enemies of lima bean pod borer larvae that reflected positively on its vitality over time.

Applying of the bacterial insecticide Diple-2x 6.4% DF, the fungal insecticide Biover10 % WP or the chemical insecticide Lannate on the canopy of soybean variety Giza 35 recorded the lowest pod infestation and seed damage compared with the other treatments in both seasons. These results may be due to bacteria of Diple-2x 6.4% DF that parasitized larvae of lima bean pod borer without negative effects on the natural enemies due to its short growth period (Table 1) that also contributed to killing the harmful insect. With respect to the fungal insecticide Biover10 % WP, it is known that soybean variety Giza 35 has a bigger canopy compared with the other varieties [25] which formed a humid umbrella to furnish suitable environment for the fungus effectively and thereby raising the efficacy of the fungal insecticide Biover10 % WP against larvae of lima bean pod borer. With respect to the chemical insecticide Lannate, although this insecticide destroyed all harmful and beneficial insects, the short growth period for soybean variety Giza 35 has mainly contributed to raising the efficacy of this insecticide. Moreover, the biological insecticide Suncide Agri-pest came in the second rank for reducing pod infestation and seed damage in soybean variety Giza 35, probably due to this insecticide depended on the light penetration within the canopy of Giza 35.

#### Seed yield and its attributes Soybean genotypes

From table 5, the data reveal that varietal differences were statistically significant with respect to seed yield and its attributes. Soybean variety Giza 35 gave higher biological yield per ha (16.91 and 16.90 ton in the first and seconds, respectively), meanwhile soybean genotype Crawford came in the second rank (11.78 ton in the first season and 11.29 ton in the second one) than DR101 genotype.

Soybean genotype	Insecticide treatment	Biological yield/ha (ton)		Straw yield/ha (ton)		Plant height (cm)		Number of branches/plant		Number of pods/plant	
		First season	Second season	First season	Second season	First season	Second season	First season	Second season	First season	Second season
Crawford	Biover10 % WP	12.20	11.86	9.83	9.58	107.62	105.92	3.62	3.43	123.74	121.17
	Diple-2x 6.4% DF	11.97	11.62	9.49	9.26	107.46	106.16	3.48	3.50	123.29	121.32
	Suncide Agri-pest	11.36	10.92	9.21	8.86	107.69	106.01	3.55	3.36	123.38	121.09
	Lannate 25% WP	11.56	11.34	9.14	8.99	107.38	105.90	3.46	3.49	123.65	120.92
	Control	11.83	10.71	9.80	8.73	107.74	105.99	3.66	3.40	123.45	121.21
	Mean	11.78	11.29	9.49	9.08	107.57	105.99	3.55	3.43	123.50	121.14
DR101	Biover10 % WP	7.94	8.01	5.95	6.09	75.93	74.41	2.96	2.91	68.19	67.64
	Diple-2x 6.4% DF	8.81	8.66	6.55	6.46	76.02	74.29	2.89	2.78	68.33	67.48
	Suncide Agri-pest	8.13	7.88	5.99	5.87	75.88	73.98	2.82	2.89	67.92	67.82
	Lannate 25% WP	8.32	8.07	6.40	6.22	75.76	74.14	3.01	2.99	68.27	67.58
	Control	7.70	7.45	5.92	5.78	76.10	74.22	2.88	2.80	67.97	67.90
	Mean	8.18	8.01	6.16	6.08	75.93	74.20	2.91	2.87	68.13	67.68
Giza 35	Biover10 % WP	16.74	16.63	13.16	13.12	110.78	110.02	5.02	4.96	115.55	112.77
	Diple-2x 6.4% DF	17.14	17.24	13.47	13.69	110.59	109.84	5.13	4.83	115.76	113.14
	Suncide Agri-pest	17.06	16.87	13.64	13.55	110.66	109.92	4.97	4.77	114.41	113.02
	Lannate 25% WP	16.51	16.55	13.02	13.13	110.81	110.10	4.99	4.81	115.72	112.87
	Control	17.11	17.22	13.90	14.09	110.70	109.89	4.89	4.91	115.28	112.99
	Mean	16.91	16.90	13.43	13.51	110.70	109.95	5.00	4.85	115.34	112.95
Average of Biover10 % WP		12.29	12.16	9.64	9.59	98.11	96.78	3.86	3.76	102.49	100.52
Average of Diple-2x 6.4% DF		12.64	12.50	9.83	9.80	98.02	96.76	3.83	3.70	102.46	100.64
Average of Suncide Agri-pest		12.18	11.89	9.61	9.42	98.07	96.63	3.78	3.67	101.90	100.64
Average of Lannate 25% WP		12.13	11.98	9.52	9.44	97.98	96.71	3.82	3.76	102.54	100.45
Average of Control		12.21	11.79	9.87	9.53	98.18	96.70	3.81	3.70	102.23	100.70
L.S.D. 0.05 Soybean genotypes		5.17	4.84	4.93	4.68	7.83	7.59	1.67	1.44	18.84	18.62
L.S.D. 0.05 Insecticide treatments		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
L.S.D. 0.05 Interaction		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Soybean genotype	Insecticide treatment	Seed yield/plant (g)		100-seed weight (g)		Seed yield/ha (ton)		HI (%)	
		First season	Second season	First season	Second season	First season	Second season	First season	Second season
Crawford	Biover10 % WP	32.06	29.94	15.69	15.46	2.37	2.28	19.42	19.22
	Diple-2x 6.4% DF	33.19	31.03	16.09	15.89	2.48	2.36	20.71	20.31
	Suncide Agri-pest	31.78	29.61	15.54	15.31	2.15	2.06	18.92	18.86
	Lannate 25% WP	32.77	30.50	15.86	15.62	2.42	2.35	20.93	20.72
	Control	29.63	27.34	15.22	15.04	2.03	1.98	17.16	18.48
	Mean	31.88	29.68	15.68	15.46	2.29	2.20	19.43	19.52

DR101	Biover10 % WP	25.42	23.04	18.70	18.43	1.99	1.92	25.06	23.97
	Diple-2x 6.4% DF	26.71	24.40	19.21	18.96	2.26	2.20	25.65	25.40
	Suncide Agri-pest	26.18	23.96	19.02	18.74	2.14	2.01	26.32	25.50
	Lannate 25% WP	25.90	23.70	18.87	18.59	1.92	1.85	23.07	22.92
	Control	22.93	20.72	18.32	18.12	1.78	1.67	23.11	22.41
	Mean	25.42	23.16	18.82	18.56	2.01	1.93	24.64	24.04
Giza 35	Biover10 % WP	36.37	34.16	18.19	17.86	3.58	3.51	21.38	21.10
	Diple-2x 6.4% DF	36.76	34.49	18.30	18.14	3.67	3.55	21.41	20.59
	Suncide Agri-pest	35.49	33.31	17.90	17.56	3.42	3.32	20.04	19.68
	Lannate 25% WP	36.22	33.97	18.04	17.71	3.49	3.42	21.13	20.66
	Control	33.44	30.99	17.68	17.36	3.21	3.13	18.76	18.17
	Mean	35.65	33.38	18.02	17.72	3.47	3.38	20.54	20.04
Biover10 % WP		31.28	29.04	17.52	17.25	2.64	2.57	21.95	21.43
Diple-2x 6.4% DF		32.22	29.97	17.86	17.66	2.80	2.70	22.59	22.10
Suncide Agri-pest		31.15	28.96	17.48	17.20	2.57	2.46	21.76	21.35
Lannate 25% WP		31.63	29.39	17.59	17.30	2.61	2.54	21.71	21.43
Control		28.66	26.35	17.07	16.84	2.34	2.26	19.67	19.69
L.S.D. 0.05 Soybean genotypes		1.17	1.06	0.32	0.25	0.16	0.14	1.54	1.47
L.S.D. 0.05 Insecticide treatments		0.89	0.78	0.19	0.12	0.12	0.11	1.23	1.12
L.S.D. 0.05 Interaction		1.28	1.19	0.37	0.32	0.21	0.19	1.67	1.61

**Table 5:** Average seed yield and its attributes of three soybean genotypes after treated with insecticides in both seasons.

This variation in biological yield could be attributed to differences in yielding ability of the studied soybean genotypes that reflected on biological yield per unit area. Also, soybean variety Giza 35 recorded higher straw yield per ha (13.43 ton in the first season and 13.51 ton in the second one), meanwhile soybean genotype Crawford came in the second rank (9.49 ton in the first season and 9.08 ton in the second one) than DR101 genotype. These results are in accordance with those of Noureldin, *et al.* [34] and Abdel-Wahab, *et al.* [25] who found significant variation among soybean genotypes for biological and straw yields per unit area.

There was a remarkable significant difference in respect of plant height among all the soybean genotypes. Soybean variety Giza 35 gave taller plants (110.70 cm in the first season and 109.95 cm in the second one), meanwhile soybean genotype Crawford came in the second rank (107.57 cm in the first season and 105.99 cm in the second one) than DR101 genotype. In general, the plant height of soybean variety Giza 35 could be increased as a result

of increasing internodes number and elongation through plant hormones. Similar results were observed by Morsy, *et al.* [35] who reported that soybean genotypes L 105, L 153, and L 155 gave the tallest plants, meanwhile, the determinate exotic variety Holladay produced the shortest plants. This variation in plant height could be attributed to the difference in the genetic make-up of soybean genotypes completed with a growth environment [25].

Also, table 5 shows the recorded data on the number of branches per plant of the soybean genotypes. Soybean variety Giza 35 recorded a higher number of branches per plant (5.00 and 4.85 in the first and seconds, respectively), meanwhile, soybean genotype Crawford came in the second rank (3.55 in the first season and 3.43 in the second one) than DR101 genotype. These results may be attributed to the fact that soybean variety Giza 35 benefited greatly from environmental climatic and edaphic resources which reflected positively on more photosynthetic activities and accumu-

lation of dry matter during growth and development stages. These results are in parallel with those observed by Abd El-Mohsen, *et al.* [36] who found that the highest number of branches per plant was achieved by soybean variety Giza 111 compared with others.

With regard to the number of pods per plant, the soybean genotypes Crawford and Giza 35 recorded higher number of pods per plant (123.50 and 115.34 in the first season and 121.14 and 112.95 in the second one, respectively) than DR101 genotype. Hence, it may be possible that different parts of the plant organs of soybean variety Giza 35 were more adapted to environmental conditions than the others, indicating the high efficiency of the photosynthetic process which provided a good opportunity for yield improvement. These results are in parallel with those observed by Morsy, *et al.* [35] who found that soybean variety Giza 111 and lines L 105 and L 153 produced the highest number of pods per plant, while soybean varieties Giza 83 and Clark produced the lowest one.

Also, soybean variety Giza 35 had higher seed yield per plant (35.66 and 33.38g in the first and second seasons, respectively), meanwhile, soybean genotype Crawford came in the second rank (31.88g in the first season and 28.68g in the second one) than DR101 genotype (Table 5). These results may be due to plants of soybean variety Giza 35 were more efficient in utilizing solar energy through higher numbers of branches and pods per plant than those of other varieties. It is important to note that seed yield is a function of yield attributes and thereby increase in seed yield per plant was the cumulative effect of increase in numbers of branches and pods per plant.

On the other hand, soybean genotype DR101 recorded higher 100-seed weight (18.82g in the first season and 18.56g in the second one), meanwhile soybean variety Giza 35 came in the second rank (18.02g in the first season and 17.72g in the second one) than Crawford genotype. These results probably attributed to the maturity group and growth habit of soybean genotype DR101 (Table 1) had the longest period of soybean growth during available normal climatic conditions from stem elongation to pollination and until late seed filling compared with other varieties. Seed weight is a genetic characteristic and is also affected by the environmental situation [37]. These results are in accordance with Abdel- Wahab, *et*

*al.* [25] who reported that soybean genotype DR101 had higher 100-seed weight, meanwhile soybean varieties Giza 22 and Giza 111 gave higher seed yield per plant than the others.

Furthermore, seed yield per ha varied significantly among the soybean genotypes (Table 5). soybean variety Giza 35 gave higher seed yield per ha (3.47 and 3.38 ton in the first and second seasons, respectively) meanwhile soybean genotype Crawford came in the second rank (2.29 ton in the first season and 2.20 ton in the second one) than DR101 genotype. These results reveal that genetic make-up of soybean variety Giza 35 interacted positively with environmental resources and translated to performance of its growth habits during vegetative and reproductive stages. Physiological maturity is a point where there is the stabilization of dry matter translocation to the seed [38]. However, soybean genotype DR101 recorded higher HI (24.64% in the first season and 24.04% in the second one), meanwhile, soybean variety Giza 35 came in the second rank (20.54% in the first season and 20.04% in the second one) than Crawford genotype. These results were due to soybean genotype DR101 had lower pod infestation and seed damage than the others which reflected positively on HI. HI of soybean should not be less than 20%, because the data of HI < 20% is defined as the abnormal values and those values were mostly affected by biological or non-biological factors interference [39]. Accordingly, these results reveal that soybean genotypes DR101 and Giza 35 are more tolerant to lima bean pod borer infestation than soybean genotype Crawford.

#### Insecticide treatments

Data in table 5 show seed yield and its attributes treated by the studied insecticide treatments. Seed yield per plant, 100-seed weight, seed yield per ha and HI were affected significantly by insecticide treatments, meanwhile biological and straw yields per ha, plant height and numbers of branches and pods per plant were not affected in both seasons. It was observed that the tested insecticides increased significantly seed yield per plant, 100-seed weight, seed yield per ha and HI compared with the control treatment. The bacterial insecticide Diple-2x 6.4% DF gave higher seed yield per plant (32.23 and 29.97g), 100-seed weight (17.86 and 17.66g), seed yield per ha (2.80 and 2.70 ton) and HI (22.59 and 22.10%) in the first and second seasons, respectively than the other insecticide treatments.

Also, the fungal insecticide Biover10 % WP recorded 31.28g in the first season and 29.04g in the second season for seed yield per plant, 17.52g in the first season and 17.25g in the second season for 100-seed weight, 2.64 ton in the first season and 2.57 ton in the second season for seed yield per ha, as well as 21.95% in the first season and 21.43% in the second season for HI. It is important to mention that there were no significant differences among the fungal insecticide Biover10 % WP, the bacterial insecticide Diple-2x 6.4% DF and the chemical insecticide Lannate for seed yield per ha and HI in both seasons.

The action of biological insecticides maintained natural enemies than the chemical insecticide Lannate. It seems that the studied biological insecticides reduced yield losses due to the control of the lima bean pod borer attack on soybean. However, the results show that all the studied insecticides suppressed larvae population of lima bean pod borer that reflected positively on seed yield per plant, 100-seed weight, and HI compared with the control treatment. The bacterial insecticide Diple-2x 6.4% DF was superior for seed yield per plant, 100 - seed weight, seed yield per ha, and HI in both seasons. These findings are in line with Dhaka, *et al.* [40] who showed that *B. thuringiensis* was recorded the highest seed yield comparable control. Also, Vinod [41] studied the impact of insecticides and biorationals on lima bean pod borer larval population and seed yield of soybean. He found that the seed yield was 1511 and 1449 kg/ha when treated with *B. thuringiensis* and *B. bassiana*. However, all the treatments recorded significantly higher seed yield than untreated control (1356 kg/ha).

#### The interaction between soybean genotypes and insecticide treatments

Seed yield per plant, 100-seed weight, seed yield per ha, and HI were affected significantly by soybean genotypes x insecticide treatments interaction, meanwhile biological and straw yields per ha, plant height, numbers of branches and pods per plant were not affected in both seasons (Table 5). Applying all the studied insecticides on the canopy of soybean variety Giza 35 recorded higher seed yields per plant and per ha than the other treatments in both seasons. However, applying the bacterial insecticide Diple-2x 6.4% DF on the canopy of soybean genotype DR101 recorded higher 100-seed weight and HI followed by soybean variety Giza 35 then soybean genotype Crawford ranked the third than the other treat-

ments in both seasons, indicating the bacterial insecticide Diple-2x 6.4% DF contributed positively with all the studied genotypes to tolerate the lima bean pod borer infestation.

It is worthy to note that there were no statistically significant differences between the fungal insecticide Biover10 % WP and the bacterial insecticide Diple-2x 6.4% DF in seed yield per plant, 100-seed weight, seed yield per ha and HI for soybean variety Giza 35. Consequently, the fungal insecticide Biover10 % WP was more suitable for soybean variety Giza 35 to tolerate the lima bean pod borer infestation than the soybean genotypes DR101 and Crawford. Also, there were no statistically significant differences between the bacterial insecticide Diple-2x 6.4% DF and the biological insecticide Suncide Agri-pest in seed yield per plant, 100-seed weight, seed yield per ha and HI for soybean genotype DR101.

Thus, the biological insecticide Suncide Agri-pest was more suitable for soybean genotype DR101 to tolerate the lima bean pod borer infestation than the soybean genotypes Giza 35 and Crawford probably due to stomata of leaves of soybean genotype DR101 opened faster than those of the other genotypes. Moreover, there were no statistically significant differences between the bacterial insecticide Diple-2x 6.4% DF and the chemical insecticide Lannate in seed yield per plant, 100-seed weight, seed yield per ha and HI for soybean genotype Crawford. Hence, the chemical insecticide Lannate was more suitable for soybean genotype Crawford to tolerate the lima bean pod borer infestation than the soybean genotypes Giza 35 and DR101. These results imply that soybean genotypes responded differently to insecticide treatments for seed yield per plant, 100-seed weight, seed yield per ha, and HI.

#### Economic return

Data in table 6 and figures 2-4 show economic returns of three soybean genotypes after treated with insecticides in both seasons. In general, the biological insecticides had higher net returns and B:C ratio than the chemical insecticide Lannate in both seasons. Applying the bacterial insecticide Diple-2x 6.4% DF or the fungal insecticide Biover10 % WP on the canopy of soybean variety Giza 35 recorded higher gross and net returns, as well as B:C ratio than the other varieties in both seasons.

With respect to soybean genotype DR101, applying the bacterial insecticide Diple-2x 6.4% DF or the biological insecticide Suncide

Soybean genotype	Insecticide treatment	Gross returns (USD/ha)		Financial costs (USD/ha)		Net returns (USD/ha)		B:C ratio	
		First season	Second season	First season	Second season	First season	Second season	First season	Second season
Crawford	Biover10 % WP	1265.58	1217.52	573	573	692.58	644.52	2.20	2.12
	Diple-2x 6.4% DF	1324.32	1260.24	569	569	755.32	691.24	2.32	2.21
	Suncide Agri-pest	1148.10	1100.04	581	581	567.10	519.04	1.97	1.89
	Lannate 25% WP	1292.28	1254.90	592	592	700.28	662.90	2.18	2.11
	Control	1084.02	1057.32	545	545	539.02	512.32	1.98	1.94
DR101	Biover10 % WP	1062.66	1025.28	573	573	489.66	452.28	1.85	1.78
	Diple-2x 6.4% DF	1206.84	1174.8	569	569	637.84	605.80	2.12	2.06
	Suncide Agri-pest	1142.76	1073.34	581	581	561.76	492.34	1.96	1.84
	Lannate 25% WP	1025.28	987.90	592	592	433.28	395.90	1.73	1.66
	Control	950.52	891.78	545	545	405.52	346.78	1.74	1.63
Giza 35	Biover10 % WP	1911.72	1874.34	573	573	1338.72	1301.34	3.33	3.27
	Diple-2x 6.4% DF	1959.78	1895.70	569	569	1390.78	1326.70	3.44	3.33
	Suncide Agri-pest	1826.28	1772.88	581	581	1245.28	1191.88	3.14	3.05
	Lannate 25% WP	1863.66	1826.28	592	592	1271.66	1234.28	3.14	3.08
	Control	1714.14	1671.42	545	545	1169.14	1126.42	3.14	3.06

**Table 6:** Economic returns of three soybean genotypes after treated with insecticides in both seasons.

**Figure 2:** Gross returns of three soybean genotypes after treated with insecticides in both seasons.

**Figure 3:** Net returns of three soybean genotypes after treated with insecticides in both seasons.

Agri-pest on canopy of this genotype gave higher gross and net returns, as well as B:C ratio than the other insecticide treatments in both seasons. However, applying the bacterial insecticide Diple-2x 6.4% DF or the chemical insecticide Lannate on canopy of soybean genotype Crawford recorded higher gross and net returns, as well as B:C ratio than the other insecticide treatments in both seasons. These results show that applying the bacterial insecticide Diple-2x

6.4% DF on canopy of Giza 35 was more profitable and should be recommended.

The present findings are in line with findings of Mahalakshmi, *et al.* [42] observed the highest seeds yield (0.74 ton/ha) was obtained from treatment with flubendiamide (0.2 ml/l). They added

**Figure 4:** B:C ratio of three soybean genotypes after treated with insecticides in both seasons.

that rynaxypyr (100 ml/l) was excellent in its bio-efficacy in managing the insect pests of soybean, which recorded the highest B: C ratio of 1.83. Also, Vinod (2015) found that the treatments exposed to biorationals recorded lower net returns in comparison with insecticides.

### Conclusion

It can be concluded that the choice of an insecticide that targets lima bean pod borer (*Etiella zinckenella*) depends on the maturity group of a soybean genotype. Applying the bacterial insecticide Diple-2x 6.4% DF on the canopy of soybean variety Giza 35 that tolerated lima bean pod borer infestation and recorded higher seed yield and net return than the other varieties should be recommended. The soybean genotype DR101 is a tolerant genotype for lima bean pod borer infestation and it should be included in breeding programs to improve tolerance in the sensitive soybean genotypes.

### Conflict of Interest

No declare for financial interest or any conflict of interest exists.

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