

Field trial of new Bt-base bioinsecticide formula, Bashield[®], for controlling *Spodoptera frugiperda* J.E. Smith on maize

Achmad Djunaedy¹, Syaiful Khoiri^{1*}, Nuriya Firdaus¹, Dita Megasari², Giyanto³

¹Program study of Agroecotechnology, Faculty of Agriculture, Universitas Trunojoyo Madura, Bangkalan, East Java, Indonesia

²Program study of Agrotechnology, Faculty of Agriculture, Universitas Pembangunan Nasional Veteran Jawa Timur, Surabaya, East Java, Indonesia

³Department of Plant Protection, Faculty of Agriculture, IPB University, Bogor, West Java, Indonesia

Abstract. Maize is one of the important staple crops. The process of cultivating corn cannot be separated from pests and plant diseases, one of the attacking pests is *Spodoptera frugiperda*, which can attack the growing points of plants causing a decrease in corn production. Based on previous research conducted in vitro, bioinsecticide of *Bacillus thuringiensis*-base (Bt-base) liquid formula (namely Bashield[®]) was able to reduce pupal weight, the proportion of larvae-pupae, and pupal becomes abnormal. Action that can be taken to control this attack is by utilizing biological agent, *B. thuringiensis* strain BT2, as an alternative control. This research aims to evaluate or to determine the effectiveness of a new bioinsecticide of Bt-base liquid formula (Bashield[®]), as a control agent for *S. frugiperda* on maize in the field. This research was prepared at the Laboratory of Plant Protection and Environment, Universitas Trunojoyo Madura, and was conducted at the Horticultural Seed Garden, Socah District, Bangkalan Regency, East Java, Indonesia (7°05'17.2"S 112°42'32.0"E). The design used was a complete randomized block design (CRBD) with 4 treatments and 6 replications. The data were analyzed using 5% ANOVA and if there was a significant effect, then a DMRT follow-up test was carried out with a 5% level. The results showed that the 15% treatment was able to kill test larvae up to 78% with a high virulence level, and reduced damage up to 100%. In conclusion, the new Bt-base liquid formula, Bashield[®], has good potential for effective biocontrol and contributed as a new biopesticide for *S. frugiperda*.

Keywords: Bioinsecticide, *Bacillus thuringiensis*, liquid formula, *Spodoptera frugiperda*.

1 Introduction

The fall armyworm, *Spodoptera frugiperda* J.E. Smith is a new invasive pest all over the world [1]. In African and European countries, the economic losses caused by this pest amount to 20.6 million tonnes per year with a total loss of US\$ 2.5-6.2 billion [2]. In Indonesia, which currently attacks various regions. The first reports of *S. frugiperda* attacks in Indonesia were recorded in 2019, precisely in Lampung [3]. *S. frugiperda* infestation threatens successful maize production and causes extensive damage due to its existence as a new invasive pest. This pest can explore up to 100 km with wind thrust in one week, this pest is also considered a more active and more voracious pest compared to other types of armyworms [4-5]. Around 60% of sweet hybrid corn in Petir Village, Dramaga District, Bogor Regency, West Java, Indonesia was damaged by *S. frugiperda* attacks [6]. Another report, the damage caused by the *S. frugiperda* attack also occurred in Tuban Regency, East Java with a damage range of 58% to 100% and can result

in a decrease in corn yields of up to 18 million tons per year [7-8].

Generally, the controlling of *S. frugiperda* was carried out using synthetic insecticides [9] but several studies have reported that pest resistance builds up quickly and makes synthetic insecticides less effective [10]. Pest resistance is caused by mutations in genes that may be resistant to pyrethroids, organic phosphates, and carbamates [11]. Therefore, an alternative control is needed such as using entomopathogenic bacteria [12]. An example is by utilizing entomopathogenic bacteria, *Bacillus thuringiensis*. The use of *B. thuringiensis* as a biological agent is more environmentally friendly because the crystal protein is selective [13].

B. thuringiensis strains were reported effective as biological control [14-16]. In our previous study, we successfully isolated potential strain, namely *B. thuringiensis* strain BT2, and its effective to kill *S. frugiperda*. This strain was formulated in liquid form, namely Bashield[®] [in press]. Formulations can maintain population density and can increase the effectiveness of active ingredients [17-18]. In addition,

* Corresponding author: syaiful.khoiri@trunojoyo.ac.id

the right formula can assist in availability, mass propagation, storage, transportation, packaging, application, and marketing [18-19].

The Bashield® was effective in controlling *S. frugiperda* at laboratory and greenhouse tests. But, in the field the data is not yet available. So, this research aims to check the ability of Bashield® formula in field for controlling *S. frugiperda*. The contributions of this research are to evaluate new Bt-base liquid formula, Bashield®, on controlling *S. frugiperda* as an environmental friendly technology.

2 Material and methods

2.1 Research location

This research was conducted in the Laboratory of Plant Protection and Environment, Faculty of Agriculture, Universitas Trunojoyo Madura for the preparation of the formula and at Horticulture Seed Garden, Socah, Bangkalan, East Java, Indonesia (7°05'17.2"S 112°42'32.0"E).

2.2 Materials

The materials used are Bashield® formulation ingredients (*B. thuringiensis* strain BT2, *S. frugiperda* larvae was provided by Balai Penelitian Tanaman Pemanis dan Serat (The Indonesian Sweetener and Fiber Crops Research Institute), Malang, Indonesia, nutrient broth, nitric acid, sodium nitrate, 20% glycerol, distilled water, TiO₂, 70% alcohol, distilled water), corn seeds var. Madura-3, NPK and urea fertilizer.

2.3 Methods

2.3.1 Bashield® preparation

B. thuringiensis strain BT2 was cultured on 250 mL nutrient broth and shaker at 250 rpm at 37°C for 48 hours. Bt cultures were harvested by centrifugation at a speed of 15000 rpm for 15 minutes. Bacterial pellets were resuspended with 20% (v/v) glycerol 20%. The Bashield® formula is prepared by mixing 50 mL prepared Bt, 3% TiO₂, 1% NaNO₃, 0.1% citric acid, 20% glycerol, and aquadest up to 500mL.

2.3.2 Field preparation and seed planting

Field preparation is carried out by loosening the soil to a depth of 30 cm using a tractor. This land preparation is carried out 1 week before planting to restore the fertility level of the soil. Next step, planting is carried out by planting corn seeds at a spacing of 80 cm x 20 cm in a plot measuring 1 m x 1 m. The planting hole is made 3 cm deep with the number of seeds per hole being 2 corn seeds.

Plant maintenance is carried out in several stages starting from replanting, watering, weeding, and fertilizing. Embroidery is done by replacing plants that

die or cannot grow well, this is done so that the plants can grow uniformly and is done twice in the morning until the plants are 7 days after planting (DAP). Watering is done twice a day in the morning and evening, but when it rains and the soil is damp, no watering is done. Fertilization is carried out when the plants are 7-10 days old by giving 10g NPK per plant and when the plants are 35-40 days old, 20g NPK per plant and 10 g urea per plant. Weeding is carried out on weeds that disturb the plants when the corn plants are 3 to 4 weeks old, weeding is carried out 2 times with weeding intervals once a week.

2.3.3 Larvae infestation

Artificial investment of *S. frugiperda* is carried out by inoculating 2nd instar larvae. Artificial investment is carried out so that the number of larvae and corn plants planted can produce uniform attacks, this is because natural attacks by *S. frugiperda* larvae are different. The number of larvae invested is 2 larvae per plant. The larvae are invested when the plants are 3 WAP.

2.3.4 Experimental design

This research used a complete randomized block design (CRBD) with 6 replications and 4 treatments including control (P0) (aquadest only), 5% (P1), 10% (P2), and 15% (P3). This research has 24 experimental units.

Bashield® application is carried out by spraying as much as 15 mL of liquid formula bioinsecticide per plant by spraying on the leaves in the afternoon according to the concentration treatment. Application is carried out once every 1 week within 2 weeks. Observations are carried out every 2 days in the morning. Observation parameters include *S. frugiperda* population, percentage of *S. frugiperda*-infested plants, and *S. frugiperda* infestation intensity.

Population observation of *S. frugiperda* is done by calculating how many dead *S. frugiperda* larvae are on plant shoots. The results of the larval count are then recorded according to the observation time, sample plant number, and sample plot. The insect mortality rate is carried out using the equation 1 [20], [21]:

$$\text{Mortality (\%)} = \frac{\text{The number of dead larvae}}{\text{Total larvae}} \times 100\% \quad (1)$$

The percentage of incidence is done by observing how many plants are attacked by *S. frugiperda*. Observation is carried out 3 times a week, with a period of 2 days after the spraying period. Observations were made at 2 days after application (DAA), 4 DAA, 6 DAA, 9 DAA, 11 DAA, 13 DAA. The formula used in determining the percentage of plants affected by *S. frugiperda* is presented in equation 2:

$$\text{Incidence (\%)} = \frac{\text{The number of maize attacked}}{\text{Total maize observed}} 100\% \quad (2)$$

The intensity of pest attacks is carried out to find out how much the level of pest attacks that occur or leaf feeding rates. Observation is carried out 3 times a week,

with a period of 2 days after the spraying period. Observations were made at 2 DAA, 4 DAA, 6 DAA, 9 DAA, 11 DAA, 13 DAA. The intensity scale uses Davis scale measurements [22]. The determination of the intensity of *S. frugiperda* attacks is calculated using the equation 3 [7], [23].

$$Intensity (\%) = \frac{\sum(n_i \cdot v_i)}{N \times Z} \times 100\% \quad (3)$$

Where *n* is the number of attacked plants in *i*, *v* is the scale in *i*; *N* is the number of all observed plants; and *Z* is the maximum score scale.

2.3.5 Data analysis

Data analysis was carried out using ANOVA on SPSS software to determine the effect of treatment. If there is a noticeable difference, a further DMRT test is carried out at $\alpha=5\%$.

3 Result and discussion

Mortality is the percentage mortality value of dead larvae from the total number of observed larvae. The highest mortality value occurred in treatment with a concentration of 15% with a mortality value of 78%, while the mortality value at concentrations of 10%, 5%, and 0% with mortality values of 65%, 54%, and 19% respectively (Table 1).

Table 1. Mortality *S. frugiperda* due to the application of Bashield® with different concentrations.

Treatment	Mortalitas (%)	Virulence
Bashield® 0% (P0)	19	Low
Bashield® 5% (P1)	54	High
Bashield® 10% (P2)	65	High
Bashield® 15% (P3)	78	High

Salaki & Watung [24] classify the virulence level based on the mortality value of the test insects, namely high virulence if the mortality value is above 50%, moderate virulence if the mortality value is 30% - <50%, low virulence if the mortality value is <30%, not virulence if the mortality value is 0%. High virulence levels were found in treatments with concentrations of 15%, 10%, and 5% with mortality values of 78%, 65%, and 54%, while low virulence levels were found in control treatments with mortality values of 19%. The mortality of *S. frugiperda* larvae that occurred in this study shows that *B. thuringiensis* contained in Bashield® formulas can work effectively. The dead larvae show symptoms of black color, dry out, shrivel, and gradually become destroyed (Fig. 1). The symptoms same with previous study that *S. frugiperda* which died after two days had a blackish body color, then a few days later the body of *S. frugiperda* became smaller, dried out, and became destroyed [25], [26]. The death of *S. frugiperda* is thought to be due to the presence of protein crystals produced by *B. thuringiensis* which are toxins for lepidopteran. Parasporal crystals of *B. thuringiensis* will

enter through the insect's digestive tract, these protein crystals will become δ -endotoxin protein or protoxin when activated by the alkaline environment in the digestive tract, then the insect protease enzyme will activate the protoxin to become a toxin and bind specifically to the receptor in the tract. Digestion, crytoxin which attaches to the peritropic membrane is what can cause death in insects because it is injurious and causes cytoplasmic leakage [23].

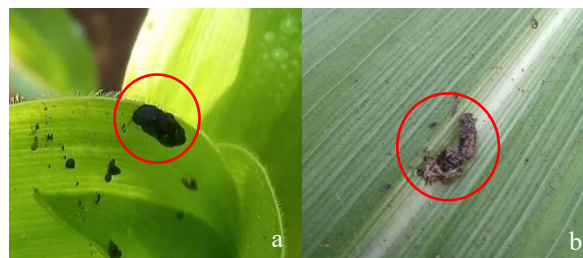


Fig 1. Symptoms of larval death (larvae die at 2 DAA (a), and larvae die at 4 DAA (b)).

Based on the recorded larval mortality data, it is known that the concentration estimation value is based on regression analysis with the equation $Y = a + bX$ with *a* and *b* values of -0.846 and 1.319 respectively with an R^2 value of 0.951. Thus, the higher the log concentration (*X*), the higher the mortality percentage (*Y*). The more the value of *R* is close to number 1, the stronger the influence between the independent variable (*X*) and the dependent variable (*Y*). The results of the probit analysis, LC_{50} from the Bashield® formula are 4.38%.

Table 2. Effects of treatment on the incidence of *S. frugiperda* attacks.

Treatment	Days after application (DAA)					
	2	4	6	9	11	13
Bashield® 0% (P0)	25.00a	27.78a	27.78a	25.00a	22.22b	22.22b
Bashield® 5% (P1)	55.56a	19.44a	13.89a	16.67a	8.33ab	5.56a
Bashield® 10% (P2)	36.11a	19.44a	22.22a	5.56a	2.78a	0.00a
Bashield® 15% (P3)	47.22a	25.00a	11.11a	2.78a	0.00a	0.00a

Note: Numbers accompanied by the same letter in the same column showed no significant difference based on the DMRT follow-up test of $\alpha=5\%$ ($P = 0.05$).

The results of observations of the occurrence of *S. frugiperda* attacks in four treatments showed that the control was significantly different from other treatments in the observations of 11 DAA and 13 DAA. The highest percentage of attacks occurred in 2 DAA of 52.78% in 5% Bashield® treatment and the lowest percentage of attacks occurred in 11 DAA of 0% in 15% Bashield® treatment (Table 2). Based on Table 2, each treatment experienced different decreases and increases at different times. The 15% Bashield® treatment decreased the occurrence of attacks by 0% faster than others in 11 DAA and 13 DAA. There is a decrease in attacks and an increase in re-attacks is thought to be due to the attack of *S. frugiperda* larvae moving from one plant to another

with the condition of the previous plant still in an infested state. Observations of 11 DAA showed a significant difference in P0 treatment with P2 and P3 treatment, while 13 DAA observations showed a real difference in 0% treatment (without Bashield®) with other treatments.

The percentage of *S. frugiperda* pest attacks is influenced by the age of the plant, pest attacks will continue to decrease when plants enter the generative phase because plants are stronger, although attacked during the vegetative phase plants can recover with good maintenance treatments such as fertilization and tillage [27].

The high attack of *S. frugiperda* is related to the high population level of larvae which also affects the intensity of attacks that occur [28]. The results of observations of *S. frugiperda* attack intensity in four treatments are presented in Table 3. The highest attack intensity occurred at 2 DAA of 27.16% in the 15% Bashield® treatment and the lowest attack intensity occurred at 11 DAA of 0% in the 15% Bashield® treatment. The results of data analysis showed that inter-concentration treatment on the attack intensity parameter of *S. frugiperda* had a significant effect with observation times of 9 DAA, 11 DAA, and 13 DAA, while at observation time, 2 DAA, 4 DAA, and 6 DAA had no significant effect.

Table 3. Effect of treatment on *S. frugiperda* intensity attack rates.

Treatment	Days after application (DAA)					
	2	4	6	9	11	13
Bashield® 0% (P0)	12.35a	20.06a	20.37a	19.14b	12.04b	7.10b
Bashield® 5% (P1)	22.53a	9.88a	8.64a	6.48a	3.09a	1.54a
Bashield® 10% (P2)	19.44a	13.89a	8.64a	2.78a	0.62a	0.00a
Bashield® 15% (P3)	27.16a	12.65a	3.70a	0.93a	0a	0.00a

Note: Numbers accompanied by the same letter in the same column showed no significant difference based on the DMRT follow-up test of $\alpha=5\%$ ($P = 0.05$).

Based on Table 3, at the beginning of the observation time, namely 2 DAA, 4 DAA, and 6 DAA did not have a significant effect, allegedly because *S. frugiperda* larvae still attack corn plants and dead larvae on affected plants need time to lower the attack intensity scale to restore plants on a low scale. However, the level of attack intensity at the time of this observation decreased. The observation time of 9 DAA, 11 DAA, and 13 DAA showed a significant difference in P0 treatment with other treatments, this is thought to be due to a decrease in *S. frugiperda* attacks due to dead larvae and affected plants that have given rise to new leaves. The effectiveness of *B. thuringiensis* in killing *S. frugiperda* is strongly influenced by the type of cry toxin, susceptibility to UV, and to reach pest for inducing toxin consumption[29-30]

This study was supported in part by The Institute of Research and Community Services (LPPM), Universitas Trunojoyo Madura with contract no. 203/UN46.4.1/PT.01.03/2022.

4 Conclusion

Based on the results, the new Bashield® formula can kill *S. frugiperda* larvae with the highest mortality of 78% in 15% Bashield® treatment. The LC₅₀ value in this study was 4.38% which shows that this bioinsecticide can effectively control the attack of *S. frugiperda*. The 15% Bashield® treatment is the best concentration that can reduce the occurrence and intensity of *S. frugiperda* attacks faster than other treatments.

5 References

1. CABI, *Spodoptera frugiperda* (Fall Armyworm). 2019. [Online]. Available: <https://www.cabi.org/ISC/fallarmyworm>.
2. FAO and CABI, "Community-based fall armyworm (*Spodoptera frugiperda*) monitoring, early warning and management: Training of trainers manual." FAO, and CABI USA, 2019.
3. Y. A. Trisyono, Suputa, V. E. B. Aryuwandari, M. Hartaman, and Jumari, "Occurrence of Heavy Infestation by the Fall Armyworm *Spodoptera frugiperda*, a New Alien Invasive Pest, in Corn in Lampung Indonesia," *Jurnal Perlindungan Tanaman Indonesia*, vol. 23, no. 1, pp. 156–160, 2019.
4. J. Capinera, "Fall Armyworm, *Spodoptera frugiperda* (JE Smith) (Insecta: Lepidoptera:Noctuidae)," *IFAS Extension, University of Florida*, 2017.
5. A. S. R. Bajracharya, B. Bhat, and P. Sharma, "Spatial and seasonal distribution of Fall Armyworm, *Spodoptera frugiperda* (JE Smith) in Nepal," *Journal of the Plant Protection Society*, vol. 6, pp. 192–201, 2020.
6. A. A. N. Lubis, R. Anwar, B. P. W. Soekarno, B. Istiaji, S. Dewi, and D. Herawati, "Serangan ulat grayak jagung (*Spodoptera frugiperda*) Pada tanaman jagung di Desa Petir, Kecamatan Daramaga, Kabupaten Bogor dan potensi pengendaliannya menggunakan *Metarizhium Rileyi*," *Jurnal Pusat Inovasi Masyarakat (PIM)*, vol. 2, no. 6, pp. 931–939, 2020.
7. D. Megasari and S. Khoiri, "Tingkat serangan ulat grayak tentara *Spodoptera frugiperda* J. E. Smith (Lepidoptera: Noctuidae) pada pertanaman jagung di Kabupaten Tuban, Jawa Timur, Indonesia," *Agrovigor: Jurnal Agroekoteknologi*, vol. 14, no. 1, 2021, doi: 10.21107/agrovigor.v14i1.9492.
8. S. Herlinda, O. Noni, S. Suwandi, and H. Hasbi, "Exploring entomopathogenic fungi from South Sumatra (Indonesia) soil and their pathogenicity against a new invasive maize pest, *Spodoptera frugiperda*," *Biodiversitas*, vol. 21, no. 7, 2020.

9. F. A. Paredes-Sánchez *et al.*, “Advances in control strategies against *Spodoptera frugiperda*. A review,” *Molecules*, vol. 26, no. 18, p. 5587, 2021.
10. T. Kumela *et al.*, “Farmers’ knowledge, perceptions, and management practices of the new invasive pest, fall armyworm (*Spodoptera frugiperda*) in Ethiopia and Kenya,” *Int J Pest Manag*, vol. 65, no. 1, pp. 1–9, 2019.
11. D. Boaventura, M. Martin, A. Pozzebon, D. Mota-Sanchez, and R. Nauen, “Monitoring of target-site mutations conferring insecticide resistance in *Spodoptera frugiperda*,” *Insects*, vol. 11, no. 8, p. 545, 2020.
12. A. Abbas *et al.*, “Biological Control of Fall Armyworm, *Spodoptera frugiperda*,” *Agronomy*, vol. 12, no. 11, 2022, doi: 10.3390/agronomy12112704.
13. A. Arsi, Y. Pujiastuti, S. Herlinda, S. H. K. Suparman, and B. Gunawan, “Efikasi Bakteri Entomopatogen *Bacillus thuringiensis* Barliner sebagai Agens Hayati *Spodoptera litura* Fabricus pada Lahan Pasang Surut dan Rawa Lebak,” in *Seminar Nasional Lahan Suboptimal*, 2019, pp. 254–263.
14. J. Luiz De Oliveira, I. Gómez, R. A. Polanczyk, and A. Bravo, “Performance of microencapsulated *Bacillus thuringiensis* Cry pesticidal proteins,” 2022, doi: 10.21203/rs.3.rs-1949207/v1.
15. G. Akhmad, I. Ilhamiyah, and J. Achmad, *Bacillus thuringiensis* *biologi, isolasi, perbanyakan dan cara aplikasinya*. Pustaka Banua, 2017.
16. L. Pinto *et al.*, “*Bacillus thuringiensis* monogenic strains: screening and interactions with insecticides used against rice pests,” *Brazilian Journal of Microbiology*, vol. 43, no. 2, pp. 618–626, 2012.
17. Y. Yulensri, “Efektifitas Formulasi Cair Konsorsium Bakteri sebagai Pengendali Hama dan Penyakit pada Padi Sawah Organik,” *Jurnal Ilmiah Inovasi*, vol. 20, no. 3, 2020.
18. D. R. Fravel, W. J. Connick Jr, and J. A. Lewis, “Formulation of microorganisms to control plant diseases,” in *Formulation of microbial biopesticides: beneficial microorganisms, nematodes and seed treatments*, Springer, 1998, pp. 187–202.
19. I. Wardati and D. N. Erawati, “Uji Formulasi *Beauveria Bassiana* Isolat Lokal sebagai Pengendali Hayati Hama Utama Kapas,” *Jurnal Ilmiah Inovasi*, vol. 15, no. 1, 2015.
20. N. N. Hidayati and N. K. Yuliani, “Pengaruh ekstrak daun suren dan daun mahoni terhadap mortalitas dan aktivitas makan ulat daun (*Plutella xylostella*) pada tanaman kubis,” *LenteraBio: Berkala Ilmiah Biologi*, vol. 1, pp. 95–99, 2013.
21. W. S. Abbott, “A method of computing the effectiveness of an insecticide,” *J. econ. Entomol*, vol. 18, no. 2, pp. 265–267, 1925.
22. F. M. Davis, S. S. Ng, and W. P. Williams, “Visual rating scales for screening whorl-stage corn for resistance to fall armyworm,” 1993.
23. L. I. Simionca Mărcășan *et al.*, “Comparative Analysis of Phenotypic and Molecular Data on Response to Main Pear Diseases and Pest Attack in a Germplasm Collection,” *Int J Mol Sci*, vol. 24, no. 7, 2023, doi: 10.3390/ijms24076239.
24. C. L. Salaki and J. Watung, “Biopesticide Application Of *Bacillus thuringiensis* Local Isolate To Control *Atherigona exigua* Pest On Corn Plants,” *Jurnal Agroekoteknologi Terapan*, vol. 3, no. 2, pp. 250–256, 2022.
25. R. Jani, S. Soedijo, and E. Liestiany, “Kemampuan *Bacillus thuringiensis* untuk Mengendalikan *Spodoptera frugiperda* JE Smith,” *JURNAL PROTEKSI TANAMAN TROPIKA*, vol. 6, no. 2, pp. 630–637, 2023.
26. D. Zulfiana, N. P. R. A. Krishanti, B. Wikantyoso, and A. Zulfitri, “Bakteri entomopatogen sebagai agen biokontrol terhadap larva *Spodoptera litura* (F.),” *Ber Biol*, vol. 16, no. 1, pp. 13–21, 2017.
27. J. M. E. Mamahit, J. Manueke, and S. E. Pakasi, “Hama infasif ulat grayak *spodoptera frugiperda* (JE Smith) pada tanaman jagung di kabupaten Minahasa,” in *Seminar Nasional Lahan Suboptimal*, 2020, pp. 616–624.
28. W. Wilyus, H. M. Siregar, and R. Aulia, “Perkembangan *Spodoptera frugiperda* JE Smith Pada Tanaman Jagung Manis (*Zea mays* L. Saccharata),” *Jurnal Media Pertanian*, vol. 6, no. 2, pp. 104–108, 2021.
29. R. Monnerat *et al.*, “Genetic variability of *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) populations from Latin America is associated with variations in susceptibility to *Bacillus thuringiensis* Cry toxins,” *Appl Environ Microbiol*, vol. 72, no. 11, pp. 7029–7035, 2006.
30. S. M. B. Da Silva *et al.*, “Characterization of novel Brazilian *Bacillus thuringiensis* strains active against *Spodoptera frugiperda* and other insect pests,” *Journal of Applied Entomology*, vol. 128, no. 2, pp. 102–107, 2004.