

INSECTICIDAL EFFECT OF BACILLUS THURINGIENSIS STRAINS AGAINST THE COTTON BOLLWORM (*HELICOVERPA (HELIOTHIS) ARMIGERA* HBN), WHICH CAUSES A SERIOUS THREAT TO COTTON CROPS

¹Alamuratov Rayimjon, ²Abdullayev Anvar, ³Aliyev Zafar, ⁴Safarov Husniddin

¹ Plant Protection and Quarantine Scientific Research Institute

^{2,3,4} Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan

<https://doi.org/10.5281/zenodo.8185641>

Abstract. Members of the Noctuidae family live in a variety of environments, and many species of this group are important agricultural pests, with one being *Helicoverpa armigera* Hbn. *Bacillus thuringiensis* strains 18fo, 31, 84, and 93, a safe alternative to broad-spectrum chemical insecticides for the control of major pests such as *H.armigera* due to environmental and regulatory concerns, were evaluated for their insecticidal activity against the second (L_2) and fourth (L_4) larval stages of the pest. According to the results of the research, Bt-18fo, Bt-93 and Bt-31 strains showed higher entomopathogenic activity on L_2 -year-old larvae than L_4 -year-old larvae of the pest, while Bt-84 strain was found to have low insecticidal activity.

Keywords: *Bacillus thuringiensis*, bacterium, insecticidal activity, *Helicoverpa armigera*, polyphagous, larva, spore, crystal, toxin.

Introduction. Cotton (*Gossypium hirsutum*) is a major source of fiber for textile industry, biofuel seed and oilseed production. USA, Pakistan, Brazil and Australia are leaders in cotton production. Pests and diseases cause 30% - 50% economic losses to cotton [2,3,15].

Helicoverpa armigera Hbn (*Lepidoptera: Noctuidae*) is a polyphagous phytophagous plant that infects about 200 plant species, including major crops such as cotton, corn, tomato, sunflower, soybean, pea, bean, and many others. Often 50-60% of crops are destroyed by this pest, resulting in a significant reduction in yield. [5,16,26].

Entomopathogenic drugs based on *Bacillus thuringiensis* (Bt) bacteria play a key role in biological protection of plants. The share of Bt in the global biopesticides market is about 85-90%. Over 50 years of toxicological studies worldwide have demonstrated the safety of Bt and its metabolites, including insecticidal proteins and other substances, allowing them to be widely used in plant protection practices [24].

Bacillus thuringiensis is a gram-positive, rod-shaped, spore-forming soil bacterium that is found in a variety of terrestrial ecosystems, including soil, water, dead insects, leaves of deciduous trees, endophytes of some plants, and milk. meeting is recorded [13].

B. thuringiensis bacteria produce a wide range of insecticidal proteins and are active against the larvae of various groups of insects, and sometimes it has been observed that they affect other types of insects. Strains belonging to the *B.thuringiensis* group produce protein-containing crystal (Cry) toxins during growth and development, and some strains produce cytolytic (Cyt) toxins, α - β - γ exotoxins, and Vip toxins. This, in turn, explains why products made on the basis of these bacteria become the most sold biological insecticides in the world today [14], for example, due to

the presence and use of genes encoding insecticidal proteins, it is possible to create new types of transgenic plants. [15].

In laboratory conditions, *B. thuringiensis* (Bt) strains showed 40.0 to 83.3% insecticidal activity against the cotton bollworm *Helicoverpa armigera*. Cotton yield increased by 2.4% compared to the control [19], when Bt and NPV were tested together against second and fourth instar larvae, larval susceptibility was reduced in adults, as second instar compared to fourth instar larvae (95,45%) recorded more deaths [12,23].

Several studies have shown that transgenic cotton plants containing Bt toxin genes are resistant to cotton blight, as observed in experiments [7,18], which were first conducted in the mid-1990s. The US company Monsanto has transgenic the *Bacillus thuringiensis* (Bt) gene into the cotton plant and commercialized it in cotton producing countries. Transgenic cotton killed some voracious insect pests and increased yield without harming the environment or human health [6,15], resulting in a reduction of more than 331 million tons of insecticide active ingredient. Transgenic cotton is highly selective, effective against many major *Lepidoptera* pests, environmentally friendly, and has become an important part of pest management (IPM) [10].

Research materials and methods.

Our research was carried out in the molecular biology laboratory of the 2023 Institute of Microbiology. An experiment was conducted to determine the biological effectiveness of *Bacillus thuringiensis* 18fo, 31, 84, 93 strains available in the institute's collection against the II- and IV-instar larvae (L₂-L₄) of the bollworm (*Helicoverpa armigera* Hbn) in laboratory conditions.

0.5 in a liquid solution to determine the effect of *B. thuringiensis* strains in 3 repetitions against the larvae of the 5 variants of the bollworm grown in laboratory conditions; 1.0%, that is, 10 ml of the strain was added to 1 liter of water, and 5 ml of the strain was added to 1 liter of water, and 0.5% was treated. The concentration of the solution of the bacterial strains used in the experiment was 1.0 µg ml⁻¹ 2x10⁸ in 1 ml and 0.001 ml of T-34 insecticide preparation was taken as a sample. Small-leaved sorrel (*Rumex crispus* L) and sedge (*Rumex acetosa* L) for larval feeding. We used the leaves of plants.

The plant leaves were kept at 4°C until the pest treatment in the laboratory. For isolation, the leaves are first washed with running water and dried for 10 minutes. A small piece (3 cm) is cut from the center of the leaf with sterilized scissors, then surface sterilized with 70% ethanol alcohol for 2 minutes and rinsed 3 times in sterilized water and dried for 10-15 minutes. Placed in cups and suspended in Bt strains. Bt toxins attach to specific binding sites in the midgut of insects, causing cell lysis. This lysis causes the insect to stop feeding and eventually die [9].

Larvae were fed on infected leaves for 48 h. Mortality was recorded every 48 h until pupation for both larval instars. Larvae were considered dead when probed with a blunt needle and unable to move [8].

The control variant was treated with water. The pests in the cups were placed in thermostats and kept at the same temperature and humidity. The experiments were kept at a temperature of 22-25 °C and a humidity of 45-50%.

Laboratory testing of new protective agents against pests of industrial crops was carried out using Khojayev's (2004) methodological manual [23], larval mortality was recorded before the experiment and 3, 5, 7, 10 and 14 days after the experiment. Pest Monitoring Bey-Biyenko (1965-69); Bondarenko (1978) and Volkov (1955) [17,18,19,20].

In laboratory experiments, biological efficiency was determined according to Abbot's formula (1925) [1]. $B_s = (A_b - B_a) / A_b \times 100\%$

B_s - biological effectiveness,

A is the number of pests before treatment in the experimental option;

a - the number of pests observed in the days after treatment;

B - the number (density) of the pest before treatment in the control (unsprayed) option;

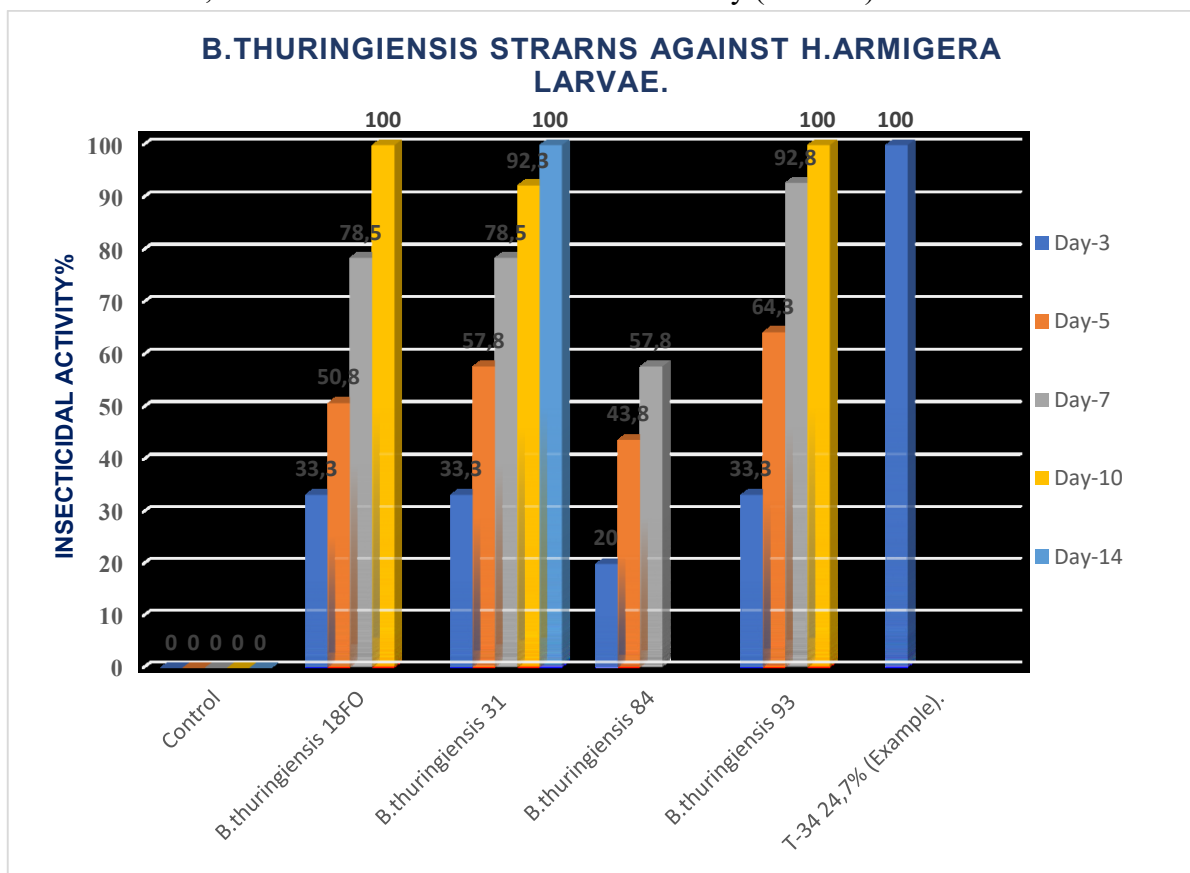
b - in the control option, the number of pests observed in the following days.

Research results and their discussion.

0.5 local strains of *Bacillus thuringiensis* 18fo, 31, 84, 93 against L₂ and L₄ larvae of bollworm or cotton bollworm (*Helicoverpa armigera* Hbn); It was treated at a concentration of 1.0%. After treatment with strains, live pest larvae were counted on days 3, 5, 7, 10 and 14, and biological efficiency was determined.

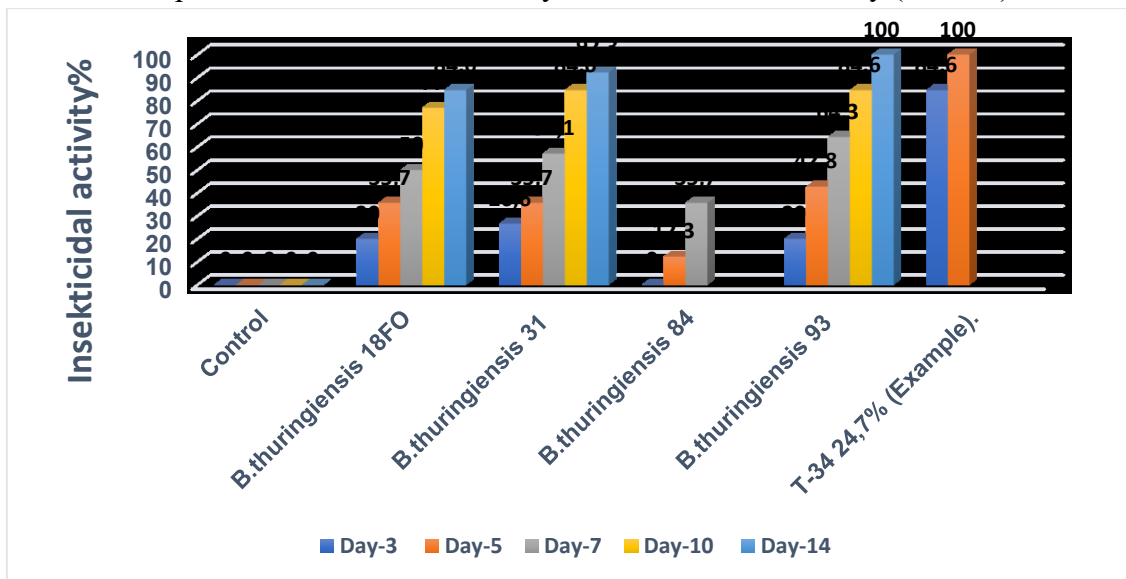
Bacillus thuringiensis 18fo strain against L₂ larvae of *H.armigera* was treated with food at a concentration of 1.0%, the effectiveness of the strain was 33.3% on 3 days, 50.8% on 5th day, 78.5% on 7th day, 10th day It was 100% fatal.

In strain Bt-31, 33.3% mortality, 57.8% mortality on 5th day, 78.5% mortality on 7th day, 92.3% mortality on 10th day, and 100% mortality on 14th day. In strain Bt-84, 3 days showed 20.0%, 5th day 43.8% and 7th day 57.8%. In strain Bt-93, 3 days was 33.3%, 5th day was 64.3%, 7th day was 92.8%, and 10th day was 100% death. When 0.001 ml of the T-34 insecticide used as a model was used, 100% death was observed on the 3rd day (Table-1).

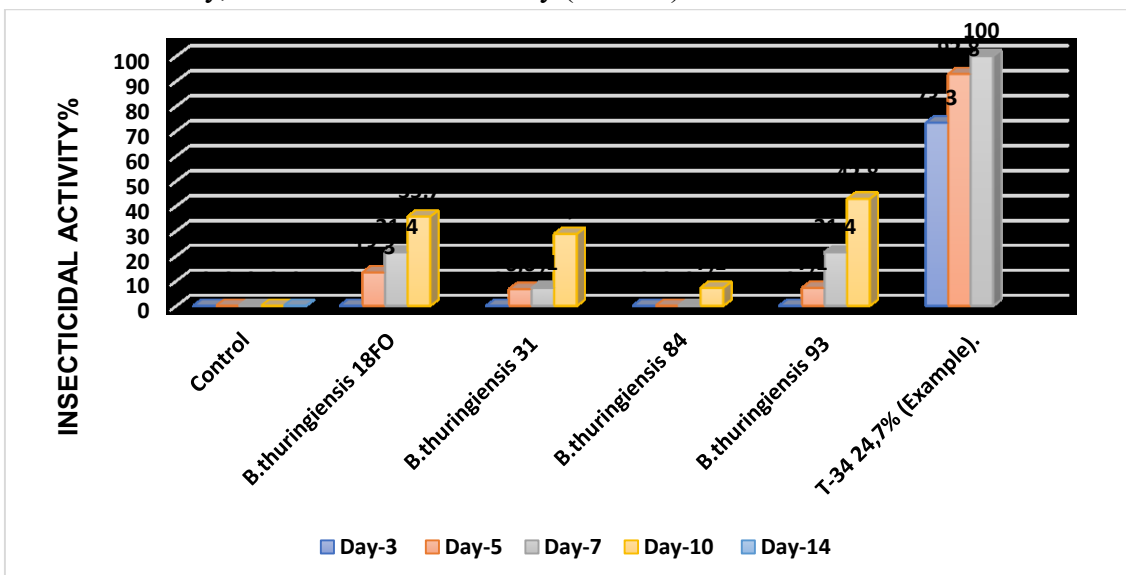


When treated against L₂ larvae of *H.armigera* at a concentration of 0.5%, strain 18fo was 20.0% on 3 days, 35.7% on 5th day, 50.0% on 7th day, 77.0% on 10th day, 14 and on -day it was 84.6%. In strain Bt-31, it was 26.6% on day 3, 35.7% on day 5, 57.1% on day 7, 84.6% on day 10, and 92.3% on day 14. In strain Bt-84, it was 14.3% on the 5th day and 35.7% on the 7th day. In

strain Bt-93, 20.0% on 3 days, 42.8% on 5th day, 64.3% on 7th day, 84.6% on 10th day and 100% on 14th day. When 0.0005 ml of chemical preparation T-34 was used as a template, it was observed that 84.6% of the pest was killed on the 3rd day and 100% on the 5th day (Table 2).



Only 1.0% concentration of *B. thuringiensis* strains against L₄ larvae of *H.armigera* was treated. The efficiency of Bt-18fo strain was 13.3% on 5th day, 21.4% on 7th day, 35.7% on 10th day and death. In strain Bt-31, 5th day was 6.6%, 7th day was 7.1%, 10th day was 28.7%. The Bt-84 strain showed 7.1% insecticidal activity only on the 10th day, while the Bt-93 strain showed 7.0% efficiency on 5 days, 21.4% on the 7th day, and 42.8% on the 10th day. did When 0.001 ml of the T-34 insecticide used as a model was used, it was observed that 73.3% died on the 3rd day, 92.8% on the 5th day, and 100% on the 7th day (Table 3).



Conclusion.

Our present study revealed that when the efficacy of different local strains of *Bacillus thuringiensis* was studied as an alternative to control *H.armigera*, two-year-old (L₂) larvae at 1.0% concentration had higher insecticidal efficacy than four-year-old (L₄) larvae. it has been. Therefore, the use of *Bacillus thuringiensis* strains against two-year-old (L₂) larvae of *H. armigera* shows high insecticidal activity.

REFERENCES

1. Abbot W.S. A method of computing the effectiveness of an insecticide // J. Econ. Entomol. – 1925. – V.18. - №3. – P. 265-267.
2. Cui J, Chen H, Zhao X, et al. Research course of the cotton IPM and its prospect. Cotton Sci. 2007;19(5):385–90
3. Chen P, Xiao Q, Zhang J, et al. Occurrence prediction of cotton pests and diseases by bidirectional long short-term memory networks with climate and atmosphere circulation. Comput Electron Agric. 2020; 176: 105612.
4. Dean, D.H. *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiol. Mol. Biol. Rev. 1998, 62, 775–806.
5. Fitt G. P., Wilson L. J. Genetic engineering in IPM: Bt cotton // In: Kennedy G. G., Sutton T. B. Emerging technologies in integrated pest management: concepts, research and implementation. USA: St. Paul, MN, APS Press, 2000. P. 108–125.
6. Flachs A. Transgenic cotton: high hopes and farming reality. Nat Plants. 2017;3(1): 1–2
7. Gunning R. V., Dang H. T., Kemp F. C., Nicholson I. C., Moores G. D. New resistance mechanism in *Helicoverpa armigera* threatens transgenic crops expressing *Bacillus thuringiensis* Cry 1 Ac toxin // Applied Environmental Microbiology. 2005. Vol. 71. No. 5. P. 2558–2563. DOI: 10.1128/AEM.71.5.2558–2563.2005.
8. Ma XM, LiuXX, NingX, Zhang B., Han F., Guan X.-M. and others. (2008). Effects of *Bacillus thuringiensis* toxin Cry1Ac and *Beauveria bassiana* on Asian corn borer (*Lepidoptera: Crambidae*). J. Inverteb. Pathol. 99, 123–128. doi: 10.1016/j.jip.2008.06.014
9. Marzban R., He Q., Liu X., and Zhang Q. (2009). Effects of *Bacillus thuringiensis* toxin Cry1Ac and cytoplasmic polyhedrosis virus of *Helicoverpa armigera* (Hübner) (HaCPV) on the bollworm (*Lepidoptera: Noctuidae*). J. Inverteb. Pathol. 101, 71–76. doi: 10.1016/j.jip.2009.02.008
10. Naranjo SE. Impacts of Bt transgenic cotton on integrated pest management. J Agric Food Chem. 2011;59(11):5842–51.
11. Qayyum, M.A., W. Wakil, M.J. Arif and S.T. Sahi, 2015. *Bacillus thuringiensis* and nuclear polyhedrosis virus for the enhanced biocontrol of *Helicoverpa armigera*. Int. J. Agric. Biol., 17: 1043–1048.
12. Roh, J.Y.; Choi, J.Y.; Li, M.S.; Jin, B.R.; Je, Y.H. *Bacillus thuringiensis* as a specific, safe, and effective tool for insect pest control. J. Mol. Biol. 2007, 17, 547–559.
13. Sanchis, V. From microbial sprays to insect-resistant transgenic plants: History of the biopesticide *Bacillus thuringiensis*. A review. Agron. Sustain. Dev. 2011, 31, 217–231.
14. Schnepf, E.; Crickmore, N.; van Rie, J.; Lereclus, D.; Baum, J.; Feitelson, J.; Zeigler, D.R.; Dean, D.H. *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiol. Mol. Biol. Rev. 1998, 62, 775–806.
15. Tarazi R, Jimenez JLS, Vaslin MF. Biotechnological solutions for major cotton (*Gossypium hirsutum*) pathogens and pests. Biotechnol Res Innov. 2019;3(1): 19–26.
16. Wakil, W., M.U. Ghazanfar, Y.J. Kwon, M.A. Qayyum and F. Nasir, 2010. Distribution of *Helicoverpa armigera* Hübner (*Lepidoptera: Noctuidae*) in tomato fields and its relationship to weather factors. Entomol. Res., 40: 290–297
17. Wu KM, Lu YH, Feng HQ, et al. Suppression of cotton bollworm in multiple crops in China in areas with Bt toxin-containing cotton. Science. 2008;321(5896): 1676–8

18. Zhang H., Tian W., Zhao J., Jin L., Yang J., Liu C., Yang Y., Wu S., Wu K., Cui J., Tabashnik B. E., Wu Y. Diverse genetic basis of field-evolved resistance to Bt cotton in cotton bollworm from China // PNAS (Proceedings of the National Academy of Science of the USA). 2012. Vol. 109. No. 26. P. 10275–10280. DOI: 10.1073/pnas.1200156109.
19. Бей-Биенко Г.Я. Определитель насекомых Европейской части СССР первая часть II том двукрылые, блохи. М.Л.: Наука, 1969.-С.80-421.
20. Бей-Биенко Г.Я. Определитель насекомых Европейской части СССР том II жесткокрылые и веерокрылые. М.Л.: Наука, 1965. – С. 356
21. Бондаренко Н.В. “Биологическая защита растений”. Колос 1978 й. -С.176-178.
22. Волков С.М., Зимин Л.С., Руденко Д.К. и др. Альбом вредителей и болезней сельскохозяйственных культур. - М.: Ленинград, 1955.-С.57-295.
23. Илхом Халилов, Гулчехра Кадилова, Феруза Халилова. (2020). Многофункциональные свойства штаммов бактерий *Bacillus thuringiensis* и новый подход в борьбе с хлопковым червем. Международный журнал передовой науки и техники, 29(05), 1652 - 1658.
24. Татьяна Васильевна Долженко, Бактериальные инсектоакаритсиды для защиты растений: Изучение и перспективы применения. Plant Biology and Horticulture: theory, innovation. 2021. №3 (160) С.50-62.
25. Хо‘jayev Sh.T. Insektitsid, akaritsid va biologik faol moddalar va fungitsidlarni sinash bo‘yicha uslubiy ko‘rsatmalar// Toshkent.-2004. –В.37.
26. Хужамшукуров Н.А. Влияние биопрепарата Antibas_Uz на хлопковую совку (*Helicoverpa armigera* Hb.) хлопчатника в условиях Узбекистана // Вестник Алтайского государственного аграрного университета. 2016. № 12 (146). С. 18–25