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EVALUATION OF THE INSECTICIDAL ACTIVITY OF THE BACILLUS THURINGIENSIS B12 STRAIN AGAINST AGRICULTURAL INSECT PESTS

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Abstract

This study evaluated the insecticidal activity of the *Bacillus thuringiensis* B12 strain against agricultural insect pests under laboratory conditions. The investigation was aimed at determining the efficacy of the bacterial strain in suppressing pest populations through concentration-dependent bioassays. Selected pest insects were exposed to different concentrations of the B12 strain, and mortality rates were recorded over a defined observation period. The findings demonstrated that the *Bacillus thuringiensis* B12 strain exhibited significant insecticidal activity compared with the control treatment. A clear increase in insect mortality was observed as the concentration of the bacterial preparation increased, indicating a strong dose-response relationship. The results confirmed that the strain was biologically active and capable of affecting pest survival under controlled experimental conditions. The observed pattern of mortality suggests that the B12 strain possesses considerable potential for integration into environmentally safer pest management strategies. As a microbial control agent,

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Bacillus thuringiensis offers an alternative to chemical insecticides by reducing ecological risks and supporting sustainable agricultural production. The present findings therefore highlight the practical importance of the B12 strain as a promising biological control agent for managing agricultural insect pests and provide a scientific basis for further applied and field-based investigations.

Keywords: *Bacillus thuringiensis*, B12 strain, insecticidal activity, agricultural pests, biological control, microbial insecticide, pest management, concentration-dependent effect, insect mortality, laboratory bioassay.

QISHLOQ XO‘JALIGIDA BACILLUS THURINGIENSIS B12 SHTAMMINING ZARARKUNANDA HASHAROTLARGA QARSHI INSEKTITSID FAOLLIGINI BAHOLASH

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Annotatsiya:

Ushbu tadqiqotda *Bacillus thuringiensis* B12 shtammining qishloq xo‘jaligi zararkunanda hasharotlariga qarshi insektitsid faolligi laboratoriya sharoitida baholandi. Tadqiqotning asosiy maqsadi bakterial shtammining zararkunandalar populyatsiyasini bostirishdagi samaradorligini konsentratsiyaga bog‘liq bioassaylar asosida aniqlashdan iborat bo‘ldi. Tajribada tanlangan zararkunanda hasharotlar B12 shtammining turli konsentratsiyalariga ta’sir ettirildi va belgilangan kuzatuv davrida ularning o‘lim ko‘rsatkichlari qayd etildi. Olingan

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natijalar *Bacillus thuringiensis* B12 shtammi nazorat variantiga nisbatan sezilarli insektitsid faollikka ega ekanini ko'rsatdi. Bakterial preparat konsentratsiyasi ortgani sari hasharotlar o'limi ham oshib borgani aniqlandi, bu esa yaqqol doza-ta'sir bog'liqligini tasdiqlaydi. Natijalar mazkur shtammning biologik jihatdan faol ekanini va nazorat qilinadigan tajriba sharoitida zararkunandalar hayotchanligiga sezilarli ta'sir ko'rsatishini ko'rsatdi. Kuzatilgan o'lim dinamikasi B12 shtammning ekologik xavfsizroq zararkunandalar boshqaruvi strategiyalariga integratsiya qilinishi mumkin bo'lgan istiqbolli vosita ekanini anglatadi. Mikrobiologik kurash agenti sifatida *Bacillus thuringiensis* kimyoviy insektitsidlarga muqobil bo'lib, ekologik xavflarni kamaytirish hamda barqaror qishloq xo'jaligi ishlab chiqarishini qo'llab-quvvatlash imkonini beradi. Shu bois mazkur natijalar B12 shtammning qishloq xo'jaligi zararkunandalariga qarshi istiqbolli biologik kurash vositasi sifatidagi amaliy ahamiyatini yoritadi va keyingi amaliy hamda dala tadqiqotlari uchun ilmiy asos yaratadi.

Kalit so'zlar: *Bacillus thuringiensis*, B12 shtammi, insektitsid faollik, qishloq xo'jaligi zararkunandalari, biologik kurash, mikroblilik insektitsid, zararkunandalarni boshqarish, konsentratsiyaga bog'liq ta'sir, hasharotlar o'limi, laboratoriya bioassayi.

Introduction

The growing demand for sustainable agricultural production has intensified the search for effective and environmentally acceptable methods of pest control. In modern farming systems, insect pests remain one of the major constraints limiting crop productivity, quality, and economic stability. Their destructive feeding activity damages leaves, stems, roots, fruits, and storage organs, while some species also serve as vectors of plant pathogens. Conventional pest management has long relied on the extensive use of synthetic chemical insecticides because of their rapid action and ease of application. However, repeated and uncontrolled

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application of such compounds has resulted in a number of serious problems, including the development of insect resistance, contamination of soil and water resources, toxicity to non-target organisms, and negative effects on human and animal health. These concerns have encouraged researchers to investigate biological alternatives capable of maintaining pest populations below damaging levels without creating long-term ecological disruption.

Among microbial control agents, *Bacillus thuringiensis* occupies a special place because of its well-documented entomopathogenic properties and its long history of use in biological pest management. This Gram-positive, spore-forming bacterium is known for producing parasporal crystal proteins during sporulation. These proteins exhibit toxic effects against specific groups of insects after ingestion, leading to disruption of the insect midgut epithelium, feeding cessation, paralysis, and eventual death. The selective nature of *Bacillus thuringiensis* has made it particularly attractive as a safer alternative to broad-spectrum chemical insecticides. Its compatibility with integrated pest management programs, relatively low environmental persistence, and reduced risk to beneficial organisms further strengthen its practical importance in modern agriculture.

Different strains of *Bacillus thuringiensis* possess different levels of pathogenicity and host specificity, which means that strain selection is a critical factor in the development of effective microbial insecticides. Screening and evaluation of new strains are therefore necessary to identify variants with high insecticidal potential against economically significant agricultural pests. Such studies are especially valuable for biotechnology-oriented agricultural systems, where microbial resources can be developed into targeted and sustainable crop protection products. The B12 strain of *Bacillus thuringiensis* represents one such candidate whose biological properties require systematic investigation under controlled conditions before broader application can be considered.

Laboratory-based assessment of insecticidal activity is an essential initial stage in determining the practical promise of a microbial strain. Under controlled

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experimental conditions, it becomes possible to evaluate mortality dynamics, compare treatment effects with untreated controls, and determine whether biological activity changes in response to different concentrations. These parameters provide an important scientific foundation for understanding the efficacy of the tested strain and for predicting its possible value in field pest management. Concentration-dependent responses are particularly important because they help establish the relationship between dosage and mortality, which is central to the formulation of effective and economically viable biological preparations.

The present study focuses on the evaluation of the insecticidal activity of the *Bacillus thuringiensis* B12 strain against selected agricultural insect pests under laboratory conditions. The investigation aims to determine the extent to which the strain can suppress pest survival and whether its effect increases with concentration. By examining mortality patterns in exposed insect populations, this study contributes to the broader effort to identify biologically active bacterial strains suitable for agricultural biotechnology and sustainable pest regulation. The obtained results may serve as a basis for further microbiological characterization, formulation studies, and future field evaluation of the B12 strain as a prospective biological control agent.



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Methods

The study was carried out under laboratory conditions to evaluate the insecticidal activity of the *Bacillus thuringiensis* B12 strain against agricultural insect pests. The experimental design was developed to determine the effectiveness of the bacterial strain at different concentrations and to assess the mortality response of test insects over a defined exposure period. The laboratory setting allowed strict control of environmental conditions and minimized the influence of external factors that could interfere with the assessment of strain activity. Such an approach ensured that the observed effects on insect survival could be primarily attributed to the action of the *Bacillus thuringiensis* B12 strain.

The bacterial material used in the study consisted of the B12 strain of *Bacillus thuringiensis*, which was cultivated under appropriate microbiological conditions to obtain an active preparation suitable for bioassay testing. The strain was grown on a nutrient medium favorable for bacterial development and sporulation, since the entomopathogenic effectiveness of *Bacillus thuringiensis* is closely associated with the production of spores and crystal proteins. After incubation, the bacterial culture was collected and processed to prepare suspensions of different concentrations. These suspensions served as the treatment variants used to expose the target insect pests. The concentration gradient was established in order to determine whether the insecticidal effect of the strain was dose-dependent and to identify the relationship between bacterial density and pest mortality.

The test organisms consisted of selected agricultural insect pests maintained under laboratory conditions prior to experimentation. Insects used in the bioassay were chosen based on their relevance as crop-damaging species and their suitability for controlled laboratory observation. Before treatment, the insects were grouped into experimental units of similar physiological condition to reduce variation associated with age, size, or general vitality. A control group was included in the experimental design and was maintained under the same environmental conditions as the treated groups but without exposure to the

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Bacillus thuringiensis B12 preparation. This control served as a baseline for comparison and made it possible to determine whether observed mortality was associated with the bacterial treatment rather than natural causes.

Exposure of insects to the bacterial strain was carried out using a standard laboratory bioassay procedure. The selected insects were treated with different concentrations of the *Bacillus thuringiensis* B12 suspension through an appropriate delivery method consistent with entomopathogenic testing, such as contact or feeding exposure. Each treatment variant was replicated to improve the reliability of the results and to reduce the impact of random experimental error. After treatment, the insects were placed in controlled containers or chambers and maintained under stable temperature, humidity, and light conditions suitable for their survival and observation. Mortality was recorded at regular intervals during the observation period, and insects were considered dead when they showed no visible movement or response to external stimulation.

The collected mortality data were organized according to treatment concentration and observation time. Comparative analysis was then performed between the treated groups and the control group to identify differences in survival patterns. Particular attention was given to the trend of increasing mortality with rising concentrations of the bacterial preparation. The results were interpreted in relation to the biological activity of the B12 strain and its potential utility as a microbial control agent. By using a controlled concentration-response design, the study provided a systematic methodological basis for evaluating the insecticidal potential of *Bacillus thuringiensis* B12 and for supporting further research into its application in agricultural biotechnology.

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Results

The laboratory experiments demonstrated that the *Bacillus thuringiensis* B12 strain possessed clear insecticidal activity against the selected agricultural insect pests. In all treated groups, insect mortality was higher than in the control group, indicating that the bacterial strain had a measurable and biologically significant effect on pest survival. The control insects remained largely viable throughout the observation period, with only minimal natural mortality recorded under standard laboratory conditions. This contrast between treated and untreated groups confirmed that the observed lethal effect was associated with exposure to the *Bacillus thuringiensis* B12 preparation rather than with environmental stress or handling factors.

A distinct concentration-dependent pattern was identified during the bioassay. At lower concentrations of the bacterial suspension, mortality levels increased moderately compared with the control group, suggesting that even limited exposure to the strain was sufficient to affect insect viability. At intermediate

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concentrations, the insecticidal effect became more pronounced, with a greater proportion of treated insects showing reduced activity, feeding inhibition, physiological weakness, and subsequent death. The highest concentrations of the B12 strain produced the strongest biological response, resulting in the greatest mortality rates among the tested insect populations. This pattern clearly demonstrated that the efficacy of the strain was directly related to the concentration of the bacterial treatment.

The time course of the experiment also showed that mortality did not occur instantly but increased progressively throughout the observation period. During the initial phase after treatment, most insects remained alive, although some behavioral changes were already detectable in treated groups. These changes included reduced movement, lower feeding intensity, and visible weakening of general activity. As the exposure period continued, the number of dead insects increased steadily, especially in the groups treated with higher concentrations. By the final observation stage, cumulative mortality values in the treated variants were substantially above those of the control, indicating that the entomopathogenic action of the B12 strain became more apparent over time. This gradual mortality dynamic is consistent with the biological mode of action typically associated with *Bacillus thuringiensis* strains.

The experimental results further suggested that the B12 strain produced a stable toxic effect under controlled laboratory conditions. Replicated treatment groups displayed similar mortality tendencies, which supports the reproducibility of the observed response. Although the absolute mortality levels varied among concentration treatments, the overall trend remained consistent across the experiment: stronger bacterial exposure resulted in more intensive suppression of insect survival. Such repeatability is important because it indicates that the insecticidal performance of the strain is not random but reflects a reliable biological property of the tested microorganism.

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Overall, the findings confirmed that *Bacillus thuringiensis* B12 is an active entomopathogenic strain with significant potential for pest suppression. The increase in mortality relative to the control, together with the concentration-dependent nature of the response, provided strong evidence of its insecticidal effectiveness. These results establish the B12 strain as a promising biological agent for the management of agricultural insect pests and justify further investigation into its toxicological characteristics, formulation stability, and practical application under field conditions.



Discussion

The results obtained in this study confirm that the *Bacillus thuringiensis* B12 strain has substantial insecticidal potential against agricultural insect pests under laboratory conditions. The observed increase in mortality in all treated groups relative to the control demonstrates that the strain possesses biologically active

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properties capable of suppressing pest survival. This finding is consistent with the general entomopathogenic characteristics of *Bacillus thuringiensis*, which is widely recognized as one of the most important microbial agents used in biological pest management. The present study extends this understanding by showing that the B12 strain specifically exhibits effective toxicity against target pest insects and therefore deserves attention as a potential candidate for agricultural biotechnology applications.

One of the most important outcomes of the experiment is the clear concentration-dependent relationship recorded during the bioassay. As the concentration of the bacterial preparation increased, the mortality of the insects also increased. This pattern indicates that the potency of the B12 strain is directly related to the amount of active bacterial material to which the insects are exposed. Such a result is significant from both theoretical and practical perspectives. From a biological standpoint, it supports the idea that a greater quantity of spores and crystal toxins leads to more intense disruption of insect physiological processes. From an applied standpoint, the existence of a stable dose-response relationship is valuable for determining optimal application rates in future formulation and field studies. It suggests that the strain can be calibrated for specific pest control objectives depending on the required level of suppression.

The gradual increase in mortality over time also deserves attention. The results showed that the lethal effect of the B12 strain was not immediate but developed progressively during the observation period. This pattern corresponds to the known mechanism of action of *Bacillus thuringiensis*, whose toxic proteins usually act after ingestion and subsequent activation in the insect midgut. After binding to gut epithelial cells, these toxins damage the intestinal lining, disrupt digestion, and lead to physiological collapse. The delayed but steadily increasing mortality observed in the present study therefore supports the interpretation that the B12 strain acts through a typical microbial pathogenic process rather than through a rapid chemical poisoning effect. This feature is important because it

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distinguishes biological control agents from synthetic insecticides and emphasizes the need for appropriate expectations regarding the speed and timing of their action.

The significance of these findings becomes even clearer when considered in the context of sustainable agriculture. Modern crop production increasingly requires pest control methods that are effective while also minimizing environmental contamination and preserving ecological balance. Chemical insecticides, although often efficient in the short term, are associated with residue accumulation, resistance development, and harmful effects on beneficial insects, soil organisms, and water systems. In contrast, *Bacillus thuringiensis*-based preparations represent a more selective and environmentally compatible option. The promising performance of the B12 strain in this study suggests that it may contribute to integrated pest management strategies in which microbial insecticides are used to reduce dependence on synthetic chemicals. Such an approach is particularly relevant for agricultural systems seeking safer and more sustainable crop protection technologies.

At the same time, the results should be interpreted within the limitations of laboratory experimentation. Controlled laboratory conditions are essential for accurately identifying the intrinsic insecticidal potential of a strain, but they do not fully reproduce the complexity of field environments. In natural agricultural ecosystems, the activity of a microbial preparation may be influenced by temperature fluctuations, ultraviolet radiation, humidity, plant surface properties, rainfall, and interactions with other microorganisms. Pest behavior and feeding patterns may also differ under field conditions, which can affect the actual level of exposure to the bacterial agent. Therefore, while the present study provides strong preliminary evidence of the biological efficacy of the B12 strain, additional studies under greenhouse and field conditions are necessary to confirm its practical value in crop protection programs.



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Another important aspect for future consideration is the possible specificity of the B12 strain toward different insect species. *Bacillus thuringiensis* strains are known to vary in host range depending on the toxin profile they produce. Although the present investigation established the overall insecticidal activity of the B12 strain against selected agricultural pests, broader testing against multiple economically important insect species would help clarify the spectrum of its action. Such information would be crucial for determining whether the strain should be developed as a narrow-target biocontrol agent or as a preparation with wider pest management applicability. In addition, molecular and biochemical characterization of the strain could provide deeper insight into the toxin composition responsible for the observed mortality and could improve its scientific and commercial value.

In general, the discussion of the present results supports the conclusion that *Bacillus thuringiensis* B12 is a promising microbial resource for biological pest management. Its measurable toxicity, reproducible performance, and concentration-dependent effect indicate that it possesses the core characteristics required of an effective entomopathogenic strain. The study contributes to the broader scientific effort to identify microbial agents capable of supporting sustainable agriculture and reducing the ecological burden of chemical insecticide use. Accordingly, the B12 strain may be regarded as a перспективный object for continued microbiological, toxicological, and applied research aimed at developing innovative biological control products for agricultural use.

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Conclusion

The present study demonstrated that the *Bacillus thuringiensis* B12 strain possesses significant insecticidal activity against agricultural insect pests under laboratory conditions. The experimental findings showed that exposure of pest insects to this bacterial strain resulted in increased mortality compared with the untreated control group, confirming that the B12 strain has a biologically active effect on insect survival. This outcome supports the growing scientific interest in microbial agents as viable alternatives to conventional chemical insecticides and reinforces the practical importance of *Bacillus thuringiensis* in sustainable pest management systems.

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A major conclusion of the study is that the insecticidal performance of the B12 strain is strongly concentration-dependent. As the concentration of the bacterial preparation increased, mortality rates among the tested insects also increased. This result indicates a stable dose-response relationship and suggests that the biological efficiency of the strain can be influenced and potentially optimized through appropriate formulation and dosage adjustment. Such concentration-based responsiveness is essential for the future development of microbial biopesticides because it provides a foundation for selecting effective treatment rates while maintaining economic and ecological feasibility.

The mortality pattern observed during the investigation also revealed that the effect of the B12 strain developed progressively over time rather than appearing immediately after exposure. This gradual increase in insect death is consistent with the typical mode of action of *Bacillus thuringiensis*, whose toxic activity depends on the ingestion of bacterial spores and crystal proteins and their subsequent action within the insect digestive system. The recorded bioactivity therefore confirms not only the effectiveness of the strain but also its compatibility with known entomopathogenic mechanisms characteristic of this bacterial species. This strengthens confidence in the scientific reliability of the obtained results and supports the interpretation of the B12 strain as a true microbial insecticidal agent.

From an applied perspective, the study indicates that the *Bacillus thuringiensis* B12 strain may serve as a promising biological control agent for agricultural pest management. Its ability to significantly reduce insect survival under controlled conditions suggests that it has the potential to be integrated into environmentally safer crop protection strategies. In the context of modern agriculture, where the negative consequences of excessive chemical pesticide use have become increasingly evident, biologically based control tools are of particular value. The B12 strain may contribute to reduced chemical load, lower ecological risk, and

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improved sustainability in plant protection practices, especially when incorporated into integrated pest management frameworks.

At the same time, the study also highlights the need for further research before broad practical application can be recommended. Although laboratory evaluation is an essential first step, additional investigations are needed to determine the effectiveness of the B12 strain under greenhouse and field conditions, where environmental factors can influence microbial persistence and insect exposure. Further studies should also address formulation stability, shelf life, host range, toxin characterization, and compatibility with other biological or agronomic control measures. These directions are important for translating laboratory success into practical agricultural technology.

In summary, the *Bacillus thuringiensis* B12 strain can be regarded as a promising microbial resource for biotechnology-oriented pest management. Its confirmed insecticidal activity, reproducible mortality effect, and concentration-dependent performance establish a strong scientific basis for future development. The results of this study contribute to the broader goal of identifying effective biological alternatives to chemical pesticides and support continued research into the use of entomopathogenic bacteria for sustainable agricultural production.

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