VNU Journal of Science: Natural Sciences and Technology

Journal homepage: https://js.vnu.edu.vn/NST

Original Article

Development and Testing of Multi-Microbial Strain Biopesticides Against *Spodoptera litura* F. and *Plutella xylostella* L. under Laboratory Conditions

Tran Thi Lan[1], Pham The Hai[1], Nguyen Hong Minh[2], Ho Thi Thu Giang[3], Nguyen Duc Khanh[3], Nguyen Kim Nu Thao[1,*]

[1]*VNU University of Science, 334 Nguyen Trai, Thanh Xuan, Hanoi, Vietnam*

[2]*Bioresource Research Center, Phenikaa University (PU-BRC), Yen Nghia, Ha Dong, Hanoi, Vietnam*

[3]*Vietnam National University of Agriculture, Gia Lam, Hanoi, Vietnam*

Received 09th September 2024
Revised 12th May 2025; Accepted 16th May 2025

Abstract: In Vietnam, there is a growing demand for safe vegetable production, leading to the need for biological agents as alternatives to pesticides. As a result, biopesticides have gained significant attention in research worldwide. Most microbial solutions for pest control developed so far have been based on single cultures, which limits their effectiveness. In this study, various mixed cultures of *Bacillus thuringiensis* (*Bt*) strains TN0017 and TN0020, *Metarhizium anisopliae* strain TN0024, and *Beauveria bassiana* strain PAM21, were developed and investigated their potential for controlling *Spodoptera litura* F. and *Plutella xylostella* L. on vegetables. The tested mixed cultures included combinations of two strains: one *Bt* strain and one fungal strain, as well as combinations of three strains: one *Bt* strain and two fungal strains. The results demonstrated that the two most effective combinations were HH3-SK (TN0020 + TN0024 + PAM21) and HH3-ST (TN0017 + TN0024 + PAM21) at a concentration of 10^8 spores/g under conditions of 25 ± 1 °C and 70 ± 5% humidity. At 96 hours post-treatment, HH3-SK achieved an 85% mortality rate for *S. litura*, while HH3-ST achieved an 83.33% mortality rate for *P. xylostella*. Additionally, both combinations reached a mortality rate of 91.67% for *S. litura* and *P. xylostella* after 120 hours of treatment. This result demonstrates that utilizing multi-strain microbial products can promote synergistic effect and complementary interactions among the strains to enhance overall insecticidal effectiveness, representing a promising novel approach for developing biopesticides.

Keywords: Biopesticides, mixed culture, *Bacillus thuringiensis*, *Metarhizium anisopliae*, *Beauveria bassiana.*

* Corresponding author.
E-mail address: thaonkn@hus.edu.vn

https://doi.org/10.25073/2588-1140/vnunst.5814

1. Introduction

In current commercial vegetable production models, relying solely on plant protection chemicals is often necessary to combat pest attacks. However, the use of chemical pesticides poses significant threats to the ecosystem by contaminating air, soil, and both surface and groundwater. Consequently, the widespread misuse of chemical pesticides globally directly endangers human health and affects non-target species. The frequent overuse of chemicals in the production and processing of commercial vegetables has resulted in critical issues: i) Pollution of water and soil from chemical residues; ii) The prevalence of unsafe vegetable products that seriously endanger consumer health; and iii) A decrease in the value of agricultural products, negatively impacting farmers' income and livelihoods. Pesticides have polluted our environment, significantly impacting human health and biodiversity. They disrupt food chains and destroy habitats, posing risks to the environment and threatening both human health and non-target species [1]. This pressing situation necessitates a fundamental shift in the approach to safe commercial vegetable production, with a particular emphasis on using biological products as alternatives to chemicals. To address these challenges, biocontrol methods based on microbial strains have been assessed for their effectiveness in controlling insect pests [2]. Research on biological products for plant protection, including biological pesticides, mainly focuses on microorganisms due to their diverse antagonistic properties and safety.

Additionally, microbial biopesticides can be derived from a wide variety of organisms, which helps to tackle resistance issues and enhance sustainability [3]. *Bacillus thuringiensis* (*Bt*) is a widely recognized bacterial pesticide and has been developed into commercially available products [4]. The insecticidal properties of this bacterium are based on the production of crystal toxins (Cry and Cyt) associated with parasporal bodies formed during sporulation, as well as other toxins and virulence factors, some of which are synthesized and released by the bacterium during the vegetative growth phase [5]. Meanwhile, pathogenic fungi encompass a range of genera and species targeting various hosts with different levels of specificity. The infection process typically begins when conidia or spores that have contacted the host's cuticle germinate. Through a combination of enzymatic and mechanical actions, the fungus penetrates the host's body, and the mycelium grows internally, often producing different types of conidia or spores that colonize the host [4]. The ascomycetes *Metarhizium anisopliae* and *Beauveria bassiana* are the most well-studied entomopathogenic fungi and are the most commonly used in biological control programs compared to other described species [6]. The biopesticide mechanism of *Metarhizium* and *Beauveria* involves the infection of insect hosts through the adhesion of fungal spores to the cuticle, followed by germination, penetration, and subsequent proliferation within the host, leading to the production of toxic compounds that ultimately result in the insect's death [7]. However, the most bioproducts rely on the activity of individual microorganisms, which leads to a relatively narrow spectrum of effectiveness. Therefore, to achieve high effectiveness and a broader impact, the focus of research and development should be on multi-strain products. Members of our research groups have conducted a study that showed mixed cultures of *Bt*, *M. anisopliae*, and *B. bassiana* effectively controlled harmful insects on vegetables, achieving high insecticidal efficacy against pests like *Helicoverpa armigera* and *Liriomyza sativae*. The results indicated that utilizing these microbial mixtures can enhance synergistic interactions, thereby improving overall pest control effectiveness compared to single-culture applications [2]. In this study, multiple formulations combining highly active insecticidal fungi and bacterial strains have been produced in a simple and cost-effective

manner, and evaluated their effectiveness on *Plutella xylostella* L. and *Spodoptera litura* F. The goal of the study is to explore and identify a formula of multi-strain microorganisms that can effectively prevent numerous species of vegetable pests, laying the groundwork for further studies aimed at developing a mixed microorganism product with high efficiency and a broad spectrum, to support safe vegetable production.

2. Materials and Methods

2.1. Materials

The microorganisms utilized include *Bt* strain TN0016, TN0020, and TN0017; *M. anisopliae* strain TN0024; and *B. bassiana* strain PAM21, which are part of the microbial collection at the GreenLab, Center for Life Science Research (CELIFE) at the University of Natural Sciences, Vietnam National University, Hanoi.

Pupa and larvae of *S. litura* and *P. xylostella* were obtained from brassica vegetable field in Gialam, Hanoi. Insects then were continuously reared in laboratory of Department of Entomology, Vietnam National University of Agriculture for at least 2 generations prior to conduct assays. Larvae of both two species were reared in cylindrical plastic containers, which the top was covered with a cloth mesh gauze for ventilation and a filter paper disc (9-cm diameter) moistened with distilled water were placed at the bottom under the following conditions: 25 ± 1 °C with 75 ± 5% relative humidity and a light: dark period of 14:10 hours.

S. litura larvae were reared on artificial diet based on beans, wheat germ and agar. Collected pupae were kept in plastic box with top covered by clean sawdust and were transfered to ovipositor cage. Adult armyworms were kept in ovipositor cage and fed with a piece of cotton soaked in 10% honey water solution. Their egg masses were collected and kept in Petri dishes for hatching.

P. xylostella larvae were fed daily by fresh cabbage leaves (*Brassica oleracea* var. capitata - KK. Cross F1). The pupae were placed in a Petri dish then were transferred into ovipositor cage. After emergence, adults diamondback moth were allowed to mate and oviposit in the ovipositor cage, which contained a piece of cotton filled with 10% honey water solution and a potted cabbage plants cabbage inside as ovipositor site. Their eggs then were collected and kept in cabbage leaf dishes for hatching.

2.2. Methods

Preparation of microbial samples

After 9 days of cultivation of *M. anisopliae* TN0024 or *B. bassiana* PAM21 on a solid fermentation medium composed of 200 g rice, 100 g potatoes, and 400 g corn grits, with 30% water and 0.1% trace elements added, at a temperature of 26 °C with, fungal spores were collected. To facilitate separation, the conidia and spores were vortexed for 15 min using sterilized distilled water containing 0.001% Tween-80. Spore density was measured with an Neubauer hemocytometer. Sterile water was then used to adjust the spore concentrations to 2×10^9 cfu/ml.

The bacterial biomass of *Bt* strains was harvested from T3 medium (0.005 g $MnCl_2$, 1.5 g yeast extract, 3 g tryptone, 6.9 g NaH_2PO4, 8.9 g Na_2HPO4 per one liter) after 72 hours of submerged fermentation at (30 ± 1 °C) using the tangential flow filtration method. To obtain spores, the biomass solution was diluted with 0.9% NaCl, vortexed thoroughly, and then heated for 5 min at 90 °C. Spore density was determined by spread plate method on LB agar plate, while the density of toxic crystals was measured using an Neubauer hemocytometer. To adjust spore concentrations, sterile water was used to achieve 2×10^9 cfu/ml.

The spore solutions were diluted to their final concentrations for the bioassay. For multi-strain formulations, spore solutions of each strain were combined in a 1:1 ratio for two-strain combinations and in a 1:1:1 ratio for three-strain combinations, before being diluted to the final concentration.

The formulations HH2-SK (TN0020 + TN0024), HH3-SK (TN0020 + TN0024 +

PAM21), HH2-ST (TN0017 + PAM21), and HH3-ST (TN0020 + TN0024 + PAM21) were prepared using a ratio of 10% biomass to 90% carrier, consisting 10% glucose, 40% talc, 20% skim milk, 10% mannitol, 10% cassava starch. The samples were then dried at 45 °C for 2 hour, resulting in a final microbial density of 2×10^8 cfu/g for the bioproducts.

Determination of the LC_{50} values of microbial samples against *S. litura* and *P. xylostella*

The 2nd and 3rd larvae of *S. litura* and *P. xyllostella* with the same size were selected for the assays and were maintained under laboratory controlled conditions (temperature of 25 ± 1 °C with 70 ± 5% relative humidity and 14 hours photoperiod).

Dose-response bioassays were performed with 3rd *S. litura* and *P. xyllostella* larvae using the leaf-dip method for determination of median lethal concentration 50 (LC_{50}). From the stock concentration (2×10^9 cfu/ml), six different concentrations (2×10^3 to 2×10^9 cfu/ml) were obtained by diluting of this suspension in distilled water. Cabbage leaves were washed with distilled water and then kept air dried for about 1 hour. Cabbage leaf discs (diameter, 5 cm) were cut and dipped in each suspension solution for 10 s and kept at room temperature for 2 hour for drying. For each leaf disc, ten 3rd-instar larvae were introduced and transfered to a transparent plastic container with net cloth cover up. Three replications were carried out with total of 240 insects per treatment. Control leaf discs were dipped in 0.1% Triton X-100 solution only. Mortality was recorded at 24 and 48 hours larvae were considered dead if they did not respond when probed with a fine brush. The LC_{50} was obtained by a Probit analysis using PC POLO Plus program (Leora Software 2002).

Efficacy assessment of various multi-strain mixtures against *S. litura* and *P. xylostella*.

Selective assays were carried out using leaf dipping method to determine controlling efficacy of the 24 spore suspensions, 10 of which against *S. litura* and 14 against *P. xylostella*. Bioassays was performed with the same protocol as above, in which single strain spore solutions and combination of two- and three-strains spore suspensions at 2×10^8 cfu/ml were tested instead. The mortality data was recorded daily till 7 days after inoculation. Insect mortality rate was corrected according to Abbott's formula (Abbott, 1925), final data were converted to $\arcsin(x)^{1/2}$ and were compared by ANOVA technique, Duncan's multiple range test (DMRT) using IBM SPSS Statistics 20 Software.

Efficacy assessment of multi-strain bioproducts against *S. litura* and *P. xylostella*.

For evaluating the efficacy of multi-strain bioproducts against tested insects, laboratory spray apparatus modified from an airbrush coupled to an air compressor was used to spray the wettable powder suspensions. For bioassay, conventional spray nozzle was used and the outlet pressure of insecticide was maintained at 0.5 kgf/cm^2. The multi-strain bioproducts were tested to target *S. litura* and *P. xylostella* and a *Bt*-based commercial insecticide AZ Tron WG and a water treatment application were used as control. Firstly, all products were diluted in distilled water which equivalent to 2×10^8 cfu/ml. Then, ten 3rd-instar larvae were transferred into each prepared free-insecticide cabbage plant, composing one replicate, three replicates per treatment in total. After that, 2 ml of each solutions were injected in airbush paint tank for spraying wet onto the prepared plants. Larval mortality for all bioproducts were recorded at 24 hours intervals for 168 hours to ensure that the maximum toxic effect for all tested products. Insect mortality rate was corrected and statistical analyzed follow above procedure.

3. Results and Discussion

3.1. Effect of Microbial Density on Controlling of S. litura and P. xylostella

The insecticidal efficacies against *S. litura* and *P. xylostella* of five potential strains, including *Bt* strains TN0016, TN0020, and TN0017; *M. anisopliae* TN0024; and *B. bassiana* PAM21, were tested at various

concentrations to determine the median lethal concentration (LC_{50}). The data were presented in Figures 1 and 2, with detailed information provided in Figures S1-S2 (Supplemental data). Generally, the insecticidal activity of the microbial strains, continuously monitored over 120 hours, exhibited a linear increase with the rise in microbial density (cfu/ml).

The *Bt* TN0020 exhibited the strongest activity against *S. litura*, with the lowest LC_{50} value recorded at 7.1×10^4 cfu/ml. Between the two fungal species, *M. anisopliae* TN0024 displayed lower LC_{50} value than *B. bassiana* PAM21 (Figure 1). The results also suggested the potential for combining bacterial strains (TN0016, TN0020) with fungal strains (TN0024, PAM21) to effectively control *S. litura*.

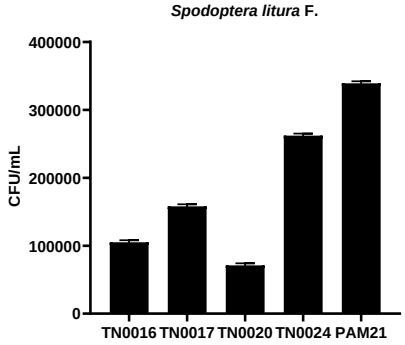

Figure 1. Median lethal concentration LC_{50} (CFU/mL) of microbial strains against *Spodoptera litura*.

For *P. xylostella*, the *Bt* strains TN0017 and TN0020 demonstrated strong toxicity, with LC_{50} values of 3.9×10^4 cfu/ml and 4.1×10^4 cfu/ml, respectively. Meanwhile, *B. bassiana* PAM21 exhibited lower LC_{50} value than *M. anisopliae* TN0024 (Figure 2). The results also indicated the potential for combining bacterial strains (TN0016, TN0017) with fungal strains (TN0024, PAM21) to effectively control *P. xylostella*.

This study demonstrated that the *Bt* strains TN0017 and TN0020 exhibited strong insecticidal activities against *P. xylostella* and *S. litura*, both of which are significant pests within Lepidoptera order, posing a serious threat to numerous economically important crops worldwide. Therefore, significant efforts have been made to isolate new *Bt* strains with high toxicity to these pests [8, 9]. However, most *Bt* strains do not exhibit high activity against mixed pest larvae over extended periods [10]. Our findings also highlight the high efficacy in both tested fungal strains, leading to the possibility to develop multi-strain formula with improved pest control performance.

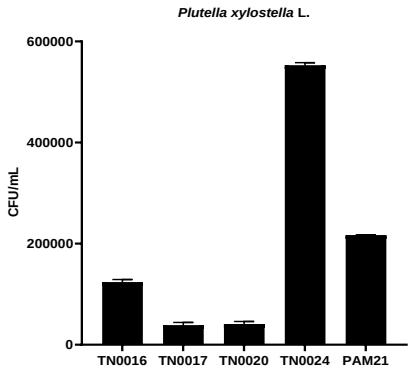

Figure 2. Median lethal concentration LC_{50} (CFU/mL) of microbial strains against *Plutella xylostella.*

3.2. Efficacy Assessment of Various Multi-strain Mixtures against S. litura and P. xylostella

Based on the LC_{50} results, experiments were conducted to evaluate the efficacy of several multi-strain mixtures with the aim to determine the optimal combination that effectively controls *S. litura* and *P. xylostella*.

The results presented in Figure 3 indicated that the combinations TN0020+PAM21+TN0024 and TN0020+TN0024 had the highest efficacy against *S. litura*. At 96 h after treatment, the efficacy of single strains ranged from 46.7% to 63.3%, with TN0020 showing the highest effect was 63.3%, followed by TN0016, TN0024, and PAM21 with 60%, 46.7%, and 56.7%, respectively. The two combinations exhibited the highest efficacy were TN0020+TN0024

(66.7%) and TN0020+PAM21+TN0024 (56.7%). The remaining combinations showed low to medium effectiveness, suggesting that while combining strains can enhance efficacy, not all combinations are optimal.

After 168 hour of treatment, the insecticidal efficacy of the fungal and bacterial strains ranged from average to high. The TN0024 strain recorded the highest efficacy at 83%, followed by TN0016, TN0020, and PAM21 with effectiveness rates of 79.6%, 75.6%, and 68.9%, respectively. Among the multi-strain mixtures, TN0020+TN0024 achieved the highest efficacy at 93%, followed by TN0020+PAM21+TN0024 at 89.3%. The remaining combinations showed only average to low effectiveness. Optimizing multi-strain mixtures and understanding the mechanisms of each strain are essential for achieving the best pest management outcomes.

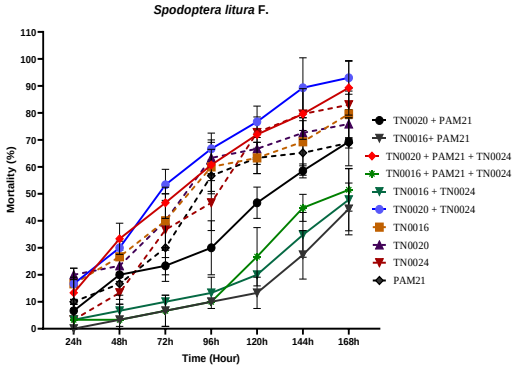

Figure 3. Mortality rates of different multi-strain mixtures against *S. litura* larvae.

The research results presented in Figure 4 indicated that the insecticidal efficacy of the two combinations TN0017+PAM21+TN0024 and TN0017+PAM21 was the highest. After 24 hour of treatment, the efficacy of the multi-strain mixtures ranged from 36.7% to 56.7%. The highest efficacy was observed with TN0017+TN0024+PAM21 at 56.7%, followed by TN0017+PAM21 and TN0020+TN0024 at 53.3%. Meanwhile, single strain samples demonstrated lower efficacy against *P. xylostella*, with TN0020 reaching 53.3%. TN0024 and PAM21 followed with 33.3%, respectively, while TN0017 recorded the lowest efficacy at 26.7%.

At 120 hour into the experiment, the effectiveness of combinations TN0017+TN0024+PAM21, along with other combinations, exhibited very high insecticidal activity, with TN0017+PAM21 and TN0017+TN0024 exceeding with effectiveness rates of 96.7% and 93.3%. Single strain samples with TN0017 and TN0020 also demonstrated high effectiveness, surpassing 90%.

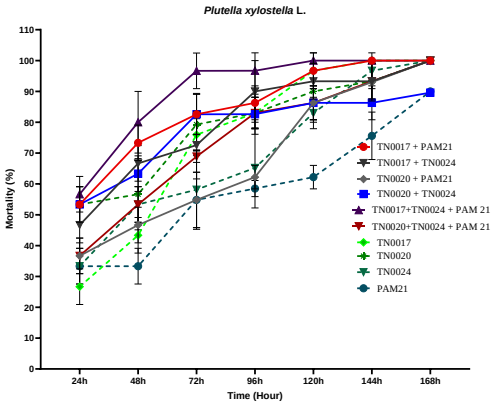

Figure 4. Mortality rates of different multi-strain mixtures against *P. xylostella* larvae.

In this study, the increasing insecticidal effect over time suggests that the active components in the strains become more effective as they interact with the pest. This might be due to the accumulation or enhanced activity of the toxins, or the progressive effect of these formulations on the pest's physiology. The single strain formulas containing TN0017 and TN0020 also showed impressive effectiveness, with mortality rates above 90% after 144 hours treatment. This highlights the potency of individual strains and their potential for use as standalone treatments. However, the combined formulas still achieved high efficacy, suggesting that there may be additional benefits to using multiple strains together.

Unlike synthetic pesticides, biological agents provide the advantage of specificity and reduced risk to non-target species. Our findings support this narrative, demonstrating that the

tested biological preparations not only effectively control *S. litura* and *P. xylostella* but are also safe and ecologically friendly. This highlights the strategic role of biopesticides in sustainable agriculture, particularly in farming systems where chemical usage must be minimized.

3.3. Efficacy Assessment of Multi-strain Bioproducts against S. litura and P. xylostella

Based on the result above, four multi-strain biological preparations were produced and tested. Two formulations targeting *S. litura* include HH2-SK, which contains strains TN0020+TN0024, and HH3-SK, which contains strains TN0020+TN0024+PAM21. The two formulations aimed to control *P. xylostella* are HH2-ST, containing strains TN0017+PAM21, and HH3-ST, which includes strains TN0017+TN0024+PAM21. The commercial product AZ Tron WG containing *Bt* was utilized as control. The results were shown in Figures 5 and 6.

The results showed that all treatment (bioproducts + control) have significant impact on mortality percentage of tested larvae. Figure 5 demonstrated that maximum efficacy was observed at 96 hours after treatment, of which *S. litura* mortality rate reached more than 80%.

After 24 hours, HH3-SK attained highest mortality efficacy at 33.33%, while HH2-SK and AZ Tron WG control reached 22.67%. After 48 hours of treatment, both HH3-SK and AZ Tron WG control exhibited effectiveness against the cavity pest at 55.33%, while the HH2-SK formula corresponded to 44.33%.

At 72 hours post-treatment, the insecticidal effects of the three formulas shifted, with HH3-SK showing the highest efficacy at 77.33%, HH2-SK at 66.33%, while AZ Tron WG control showed efficacy at 77.67%. The order of effectiveness continued to change at 96 hour after treatment, with the rankings being HH2-SK then HH3-SK, and this trend persisted until the end of the experiment at 120 hours post-treatment. Overall, the most effective formula was HH3-SK, achieving 91.67%, followed by HH2-SK at 85%, and AZ Tron WG at 88.33%.

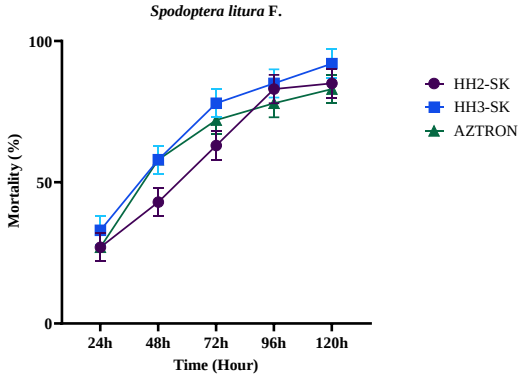

Figure 5. Efficacy of different bioproducts against *S. litura* larvae.

Our study evaluating the effectiveness of multi-strain bioproducts against *S. litura* yielded promising results, aligning with existing research on the use of biological agents for pest control. Our findings demonstrate that HH3-SK and HH2-SK significantly reduced *S. litura* infestation. Specifically, the HH3-SK formula achieve over 90% efficiency after 120 hours of exposure. Ullah et al., [11] reported that *I. fumosorosea* could significantly reduce the efficiency of conversion of ingested food (ECI) in *S. litura* larvae, with a mortality rate of 58.0%, whereas that of *B. bassiana* was 33.3%. Specifically, in this study, the authors mentioned that *B. bassiana* significantly delayed the development of *Spodoptera* larvae and adults compared to untreated controls. The mortality rate of the larvae increased markedly when exposed to *B. bassiana*, demonstrating its potential for pest control. Vinayaga *et al.*, [12] also observed a similar reduction in pest incidence on vegetable crops, with an approximate 80% increase in mortality rates with biological preparations, which aligns with our findings.

Figure 6 illustrated the effect of bioproducts on *P. xylostella*. After 24 hours, the most effective biological product was HH3-ST at 33.33%, same as AZ Tron WG control, while HH2-ST recorded 20%. After 48 hours of treatment, HH3-ST and AZ Tron WG control demonstrated effectiveness at 44.83%, while HH2-ST corresponded to 44.67%.

At 72 hours post-treatment, the effectiveness of the two formulas ranked from high to low as follows: HH3-ST then HH2-ST. At 96 hours after treatment, the effectiveness of the three formulas remained consistent with the trend observed at 72 hours, with HH3-ST achieving the highest effectiveness at 99.67%, while HH2-ST at 77.83%.

After 120 hours of experimentation, the most effective formula was HH3-ST, at 91.67%, followed by HH2-ST at 88.50%. The differences in effectiveness between the formulas were statistically significant. Based on these results, we observed that HH3-ST demonstrated effectiveness against *P. xylostella* by over 90%, which is promising for further experiments to assess its insecticidal potential.

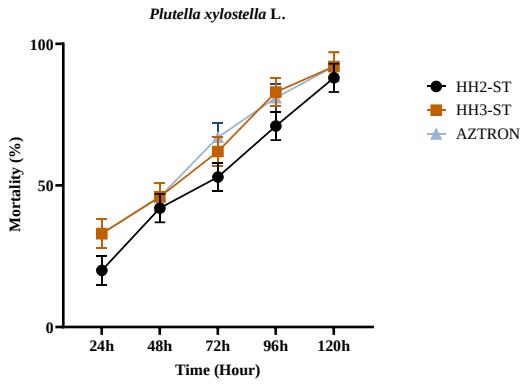

Figure 6. Morality rates of different formulations against *P. xylostella* larvae.

It can be observed that the synergistic interaction between the biological insecticides derived from *Bt* strains TN0017, TN0020; *M. anisoplae* TN0024, *B. bassiana* PAM21, results in promising efficacy in controlling of larvae in the field for both *S. litura* and *P. xylostella* larvae. Wraight *et al.*, [13] examined the interactions between *B. bassiana* and *Bt* var. *tenebrionis*-based biopesticides when applied as mixtures against field populations of *L. decemlineata* during three field seasons and found that combining multiple biological agents resulted in remarkable efficacy. Nguyen et al., [14] isolated two parasitic fungi strains, *Metarhizium anisopliae* AS2 and *Beauveria bassiana* AS1, which exhibited high cellulase and chitinase activities, and found that combining these fungi with *Bacillus thuringiensis* BA3 resulted in higher efficiency in controlling mango stem borers compared to using individual strains, providing a scientific basis for further biological control testing.

In conclusion, the results of this study provide strong evidence for the effectiveness of multi-strain bioproducts in controlling of *S. litura* and *P. xylostella* in laboratory conditions. In particular, the two most effective combinations were HH3-SK and HH3-ST, both containing one *Bt* strain, one *M. anisoplae* strain and one *B. bassiana* strain. Although biological products require more time to reach maximum effectiveness, they are still a promising choice for safe and sustainable pest control. These findings support the integration of biological products into pest management strategies. However, further research is needed to optimize fermentation and downstream processing as well as access long-term impacts on a larger scale.

Acknowledgements

This research was funded by the Hanoi Department of Science and Technology, Hanoi People's Committee, under grant number 01C-06/02-2020-3.

References

[1] R. Leoci, M. Ruberti, Pesticides: An Overview of the Current Health Problems of Their Use, *Journal of Geoscience and Environment Protection*, Vol. 9, No. 8, 2021, pp. 1-20.

[2] L. T. Dat, N. T. T. Thuy, N. H. Hoan, P. T. Det, T. T. A. Dao, V. M. Tien, P. T. Hai, N. T. Giang, Effects of Mixed Microorganisms Cultures in Controlling Harmful Insects on Vegetables, *Hue University Journal of Science: Agriculture and Rural Development*, Vol. 130, No. 3D, 2021, pp. 117-130 (in Vietnamese).

[3] M. S. Ayilara, B. S. Adeleke, S. A. Akinola, C. A. Fayose, U. T. Adeyemi, L. A. Gbadegesin, R. K. Omole, R. M. Johnson, Q. O. Uthman and

O. O. Babalola, Biopesticides as a Promising Alternative to Synthetic Pesticides: A Case for Microbial Pesticides, Phytopesticides, and Nanobiopesticides, *Frontiers in Microbiology*, Vol. 14, 2023, pp. 1-16.

[4] L. Ruiu, Microbial Biopesticides in Agroecosystems, *Agronomy*, Vol. 8, No. 11, 2018, pp. 1-12.

[5] J. L. J. Fuentes, T. A. Jackson, Bacterial Entomopathogens, *Insect Pathology*, 2nd Edition, 2012, pp. 265-349.

[6] A. Schrank, M. H. Vainstein, *Metarhizium anisopliae* Enzymes and Toxins, *Toxicon*, Vol. 56, No. 7, 2010, pp. 1267-1274.

[7] C. B. Mendoza, M. D. R. R. Montes, S. Moonjely, M. J. Bidochka, C. Toriello, A Review on the Genus *Metarhizium* as an Entomopathogenic Microbial Biocontrol Agent with Emphasis on Its Use and Utility in Mexico, *Biocontrol Science and Technology*, Vol. 29, No. 1, 2019, pp. 83-102.

[8] R. G. Monnerat, A. C. Batista, P. T. De Medeiros, E. S. Martins, V. M. Melatti, L. B. Praça, V. F. Dumas, C. Morinaga, C. Demo, A. C. M. Gomes, Screening of Brazilian *Bacillus thuringiensis* Isolates Active Against *Spodoptera frugiperda*, *Plutella xylostella* and *Anticarsia gemmatalis*, *Biological Control*, Vol. 41, No. 3, 2007, pp. 291-295.

[9] M. G. Park, J. Y. Choi, J. H. Kim, D. H. Park, M. Wang, H. J. Kim, S. H. Kim, H. Y. Lee, Y. H. Je, Isolation and Molecular Characterization of *Bacillus thuringiensis* subsp. *kurstaki* Toxic to Lepidopteran Pests *Spodoptera* spp. and *Plutella xylostella*, *Pest Management Science*, Vol. 78, No. 7, 2022, pp. 2976-2984.

[10] D. I. Chandrasena, A. M. Signorini, G. Abratti, N. P. Storer, M. L. Olaciregui, A. P. Alves, C. D. Pilcher, Characterization of Field-evolved Resistance to *Bacillus thuringiensis*-derived Cry1F δ-endotoxin in *Spodoptera frugiperda* Populations from Argentina, *Pest Management Science*, Vol. 74, No. 3, 2018, pp. 746-754.

[11] M. I. Ullah, N. Altaf, M. Afzal, M. Arshad, N. Mehmood, M. Riaz, S. Majeed, S. Ali, A. Abdullah, Effects of Entomopathogenic Fungi on the Biology of *Spodoptera litura* (Lepidoptera: Noctuidae) and Its Reduviid Predator, *Rhynocoris marginatus* (Heteroptera: Reduviidae), *International Journal of Insect Science*, Vol. 11, 2019, pp. 11795433-19867116.

[12] P. V. Moorthi, C. Balasubramanian, S. Selvarani, A. Radha, Efficacy of Sub Lethal Concentration of Entomopathogenic Fungi on the Feeding and Reproduction of *Spodoptera litura*, *SpringerPlus*, Vol. 4, 2015, pp. 1-12.

[13] S. Wraight, M. Ramos, Synergistic Interaction Between *Beauveria bassiana* and *Bacillus thuringiensis tenebrionis* Based Biopesticides Applied Against Field Populations of Colorado Potato Beetle Larvae, *Journal of Invertebrate Pathology*, Vol. 90, No. 3, 2005, pp. 139-150.

[14] T. H. M. Nguyen, T. Q. Nguyen, T. T. H. Dao, Q. B. Trinh, D. T. Nguyen, T. K. L. Pham, V. T. Vo, D. H. Chu, T. L. T. Pham, Isolation and Selection of Entomopathogenic Fungi Combined with *Bacillus thuringiensis* for Controlling Mango Stem Borers, *Vietnam Journal of Agricultural Science and Technology*, Vol. 03, No. 136, 2022, pp. 87-91 (in Vietnamese).