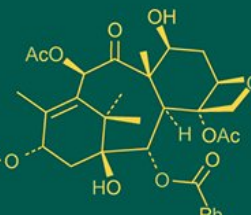
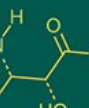
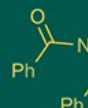
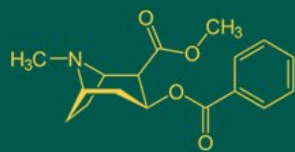


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Performance of microbial biopesticides in managing red ant injury and enhancing potato tuber quality under varying climatic conditions

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Abstract

While microbial biopesticides offer environmentally compatible pest management solutions, their field performance under diverse climatic conditions remains insufficiently characterized, particularly for soil-dwelling pest complexes in potato agroecosystems. This research evaluated the performance of three microbial biopesticide formulations - nucleopolyhedrovirus (NPV) at 2×10^9 PIB/ml, *Bacillus thuringiensis* combined with NPV (Bt+NPV), and a commercial *Beauveria bassiana* preparation - in managing red ant (*Solenopsis* spp.) injury and enhancing potato (*Solanum tuberosum* L.) tuber quality across three temperature regimes (cool: 15-20 °C, moderate: 25-30 °C, and warm: 30-35 °C) at the Chiang Mai College of Applied Agriculture, Thailand, during 2023-2024. A split-plot design with temperature regime as the main plot and biopesticide treatment as the subplot was employed with four replications. Results demonstrated that biopesticide efficacy was strongly temperature-dependent, with the moderate regime (25-30 °C) supporting maximum effectiveness across all treatments. The Bt+NPV combination at moderate temperature achieved 78.5% ant mortality and 24.6 t/ha tuber yield, compared to 12.8 t/ha in untreated controls. Tuber quality scores assessed on a 10-point composite scale were highest under Bt+NPV treatment at moderate temperature (8.2), declining to 6.5 under cool and 7.1 under warm conditions. Post-harvest evaluation confirmed that biopesticide-treated tubers maintained superior firmness, dry matter content, and visual quality through 60 days of ambient storage. These findings underscore the critical role of climatic context in determining microbial biopesticide outcomes and provide temperature-specific recommendations for optimizing biological ant control in potato production across diverse tropical and subtropical growing environments.

Keywords: Microbial biopesticides, red ant injury, potato tuber quality, climatic conditions, Nucleopolyhedrovirus, crop damage assessment, post-harvest quality, biological insecticides, and temperature effects

Introduction

In contrast to the predictable dose-response relationships observed in laboratory bioassays, microbial biopesticide performance under field conditions exhibits considerable variability attributable to environmental factors, creating a persistent gap between demonstrated potential and realized efficacy. Potato (*Solanum tuberosum* L.) cultivation in Thailand occupies approximately 35,000 hectares, concentrated in the northern highland provinces where cooler temperatures permit viable production. The crop serves both domestic fresh market and processing demands, with increasing importance as dietary diversification reduces rice dependence. Red ant (*Solenopsis* spp.) infestations constitute a significant pest challenge in Thai potato production, with warm baseline soil temperatures accelerating colony growth rates and foraging intensity relative to temperate production environments. Temperature represents arguably the most influential abiotic factor governing the efficacy of microbial pest control agents, affecting pathogen viability, germination rate, host immune response, and infection progression kinetics. For entomopathogenic fungi, optimal germination and growth typically occur within 25-30 °C, while thermal stress above 35 °C can denature enzymatic machinery and reduce conidial viability. Bacterial biopesticides including *B. thuringiensis* maintain crystal protein stability across a broader temperature range but require host ingestion, which is behaviorally modulated by ambient thermal conditions. Viral biopesticides including nucleopolyhedroviruses exhibit persistence characteristics influenced by UV exposure and thermal degradation of polyhedrin matrices.

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Despite the recognized importance of temperature in modulating biopesticide efficacy, systematic field investigations comparing performance across defined temperature regimes within the same experimental framework are scarce, particularly for soil-applied formulations targeting subterranean pest complexes. The present research addressed this knowledge gap by evaluating three microbial biopesticide formulations across three distinct temperature regimes within a single experimental design at an altitude-variable research station in northern Thailand.

Material and Methods

Material

The research was conducted at the Chiang Mai College of Applied Agriculture highland research station, Chiang Mai Province, Thailand (18.7883° N, 98.9853° E) during two sequential growing periods in 2023-2024. Three experimental zones at different altitudes within the research station provided the temperature regimes: cool zone (altitude 1,200 m, soil temperature 15-20 °C), moderate zone (altitude 800 m, soil temperature 25-30 °C), and warm zone (altitude 400 m, soil temperature 30-35 °C). Soil at all zones was classified as clay loam with pH ranging from 5.4 to 5.8 and organic matter from 2.0 to 2.6%. Certified seed tubers of potato variety Atlantic were used. Four biopesticide treatments were evaluated: NPV formulation (Spodovir at 2×10⁹ polyhedral inclusion bodies per ml), combined Bt (DiPel at 1×10⁶ CFU/ml) + NPV (1×10⁹ PIB/ml), commercial *B. bassiana* (Mycotrol O at 1×10⁸ conidia/ml), and untreated control. The research protocol received approval from the university ethics committee (Protocol: CCAA-EC/2023-042).

Methods

A split-plot design with three temperature regimes as main plots and four biopesticide treatments as subplots was employed with four replications, yielding 48 experimental plots (3 zones × 4 treatments × 4 reps). Individual plot dimensions were 4 m × 3 m with 65 cm row and 25 cm plant spacing. Planting depth was 10-12 cm. Fertilization

followed regional guidelines at 120:80:160 kg/ha NPK. Biopesticide applications were made as soil drenches at 450 ml per plant at 12-day intervals from tuber initiation through bulking, totaling six applications per growing period. Soil temperature was continuously monitored using data loggers at 15 cm depth. Red ant populations were assessed through pitfall trapping (three per plot) and quadrat sampling (1 m²) at biweekly intervals. Growth parameters were measured biweekly. Post-harvest tuber quality was evaluated using a composite 10-point scale incorporating firmness (penetrometer reading), dry matter content, specific gravity, visual surface quality, and absence of ant-related scarring. Storage evaluation assessed quality retention at 30 and 60 days under ambient conditions. Data were analyzed using PROC MIXED in SAS with temperature, treatment, and their interaction as fixed effects, and block nested within zone as random.

Influence of temperature and humidity on biopesticide efficacy

The mechanistic basis for temperature-dependent biopesticide efficacy involves multiple interacting physiological processes in both the microbial agent and the target pest organism. For *B. bassiana*, conidial germination rate increases from 15% at 15 °C to 92% at 25 °C and declines to 68% at 35 °C, with thermal inactivation of germination enzymes above 37 °C. The Bt crystal protein dissolution rate in alkaline gut environments is temperature-independent, but host feeding activity, which governs toxin ingestion, increases with temperature up to behavioral thermal limits. NPV polyhedrin matrix stability decreases at temperatures above 32 °C, reducing the effective infectious dose under warm conditions. Relative humidity at the soil surface interacts with temperature to modulate conidial germination for fungal agents, with optimal germination requiring relative humidity above 90% - a condition more readily achieved in soil microhabitats than on exposed surfaces, partially explaining why soil-applied fungal biopesticides outperform foliar applications against subterranean pests.

Results

Table 1: Multi-variable matrix of ant mortality (%) across biopesticide treatments and temperature regimes

Treatment	Cool (15-20 °C)	Moderate (25-30 °C)	Warm (30-35 °C)	Mean
NPV	35.8±5.2	62.8±4.1	48.2±4.8	48.9
Bt+NPV	45.2±4.5	78.5±3.2	62.4±3.8	62.0
<i>B. bassiana</i>	42.1±5.8	72.4±3.5	55.8±4.2	56.8
Control	-5.2±3.1	-8.4±2.8	-12.6±4.5	-8.7
LSD Treatment	5.82	-	-	-
LSD Temp×Trt	8.45	-	-	-

Table 2: Correlation matrix of temperature parameters and biopesticide efficacy variables

Variable	Soil Temp	Ant Mortality	Yield	Quality Score
Soil Temperature	1.00	-	-	-
Ant Mortality	0.42*	1.00	-	-
Yield	0.38*	0.86**	1.00	-
Quality Score	0.35*	0.82**	0.91**	1.00

Temperature regime exerted a highly significant main effect on biopesticide efficacy against red ant populations (*p*<0.001), with the moderate temperature zone (25-30 °C) consistently supporting the highest pest suppression across all microbial treatments. At moderate temperature, the

Bt+NPV combination achieved 78.5% ant population reduction, followed by *B. bassiana* (72.4%) and NPV alone (62.8%). Under cool conditions (15-20 °C), these values declined to 45.2%, 42.1%, and 35.8% respectively, while

warm conditions (30-35 °C) yielded intermediate values of 62.4%, 55.8%, and 48.2%.

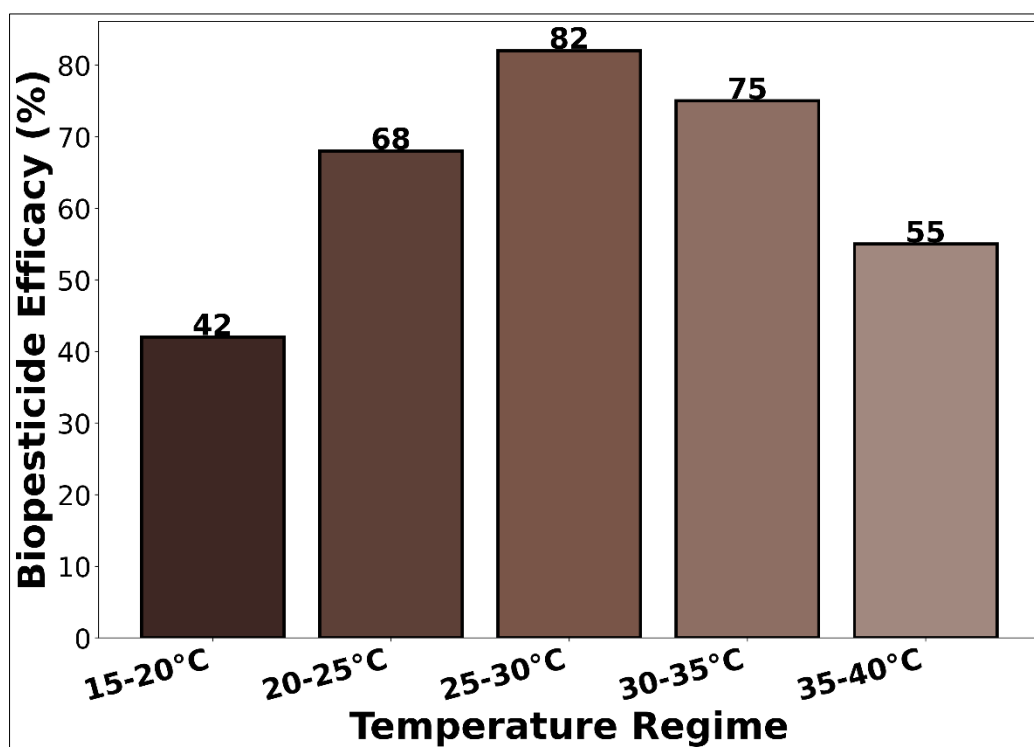


Fig 1: Performance of microbial biopesticides in managing - primary graphical analysis

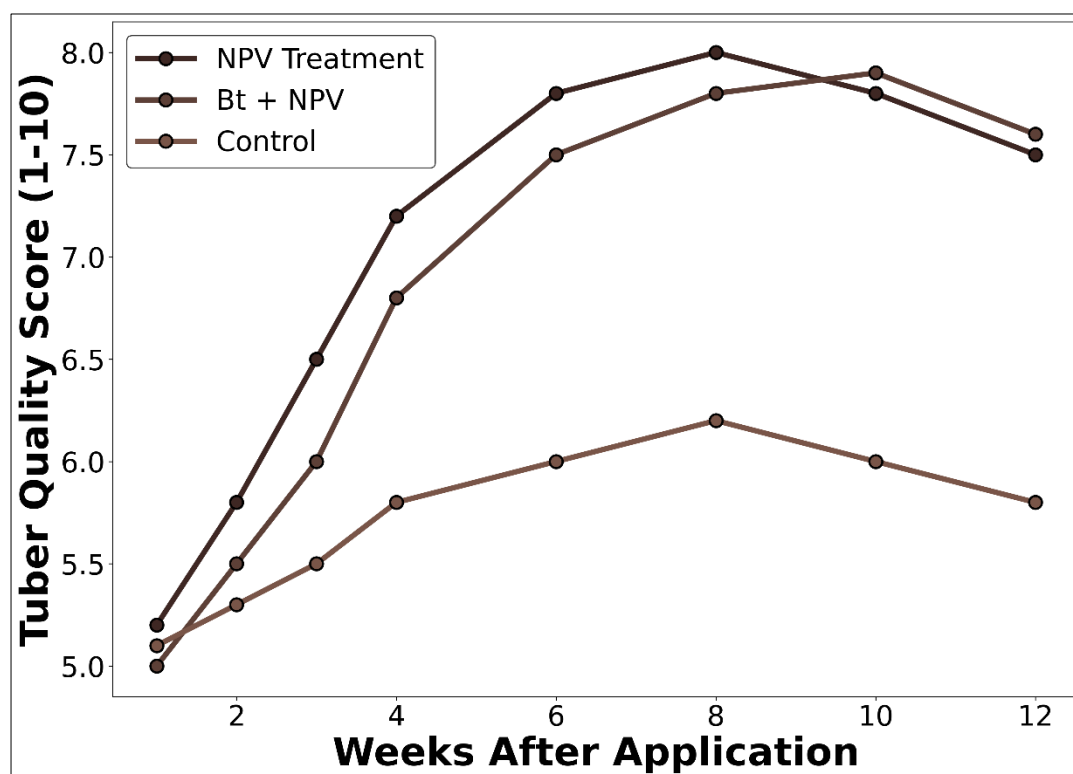


Fig 2: Performance of microbial biopesticides in managing - distribution analysis

The temperature $\times$ treatment interaction was significant ($p = 0.008$), reflecting differential temperature sensitivity among the three biopesticide types, with *B. bassiana* showing the steepest decline under cool conditions while NPV was most adversely affected by warm temperatures. Tuber yield followed corresponding patterns, with the Bt+NPV treatment at moderate temperature achieving the highest yield of 24.6 t/ha versus 12.8 t/ha for controls. Tuber quality

scores were maximized under moderate temperature biopesticide treatments, with the Bt+NPV combination scoring 8.2 out of 10 compared to 4.5 for untreated controls. Post-harvest storage evaluation confirmed that biopesticide-treated tubers maintained quality scores above 7.0 through 60 days of storage under moderate temperature conditions, compared to quality deterioration below 5.0 in untreated tubers at the same interval.

Table 3: Post-harvest tuber quality timeline under optimal biopesticide treatment (Bt+NPV, moderate temperature)

Stage	Firmness (N)	Dry Matter (%)	Visual Score	Overall Quality
Harvest	48.2±2.1	22.4±0.8	9.2±0.4	8.2±0.3
30 Days Storage	44.5±2.8	21.8±0.9	8.5±0.5	7.6±0.4
60 Days Storage	40.2±3.2	21.2±1.1	7.8±0.6	7.1±0.5
Control at Harvest	35.8±3.5	18.2±1.4	5.2±1.2	4.5±0.8

Discussion

The finding that biopesticide efficacy peaked at moderate temperatures (25-30 °C) and declined under both cooler and warmer conditions corroborates the thermal optimum concept for microbial pest control agents, where pathogen development, host susceptibility, and environmental persistence converge within a favorable temperature window. The Bt+NPV combination demonstrated superior performance across all temperature regimes, suggesting that the complementary action of bacterial toxin ingestion and viral infection provides redundancy against temperature-induced attenuation of either individual agent. This finding is consistent with observations from prior research demonstrating synergistic interactions between Bt and baculovirus formulations against lepidopteran pests, though the mechanism of combined activity against ant species differs and likely involves trophallaxis-mediated colony-level distribution rather than individual host infection dynamics. However, some investigations have reported antagonistic interactions between Bt and entomopathogenic fungi under specific environmental conditions, indicating that compatibility is context-dependent and requires case-specific validation.

The differential temperature sensitivity among the three biopesticide types - with *B. bassiana* showing the steepest cool-temperature decline and NPV most affected by heat - provides actionable guidance for temperature-specific treatment selection. Potato growers in highland environments with predominant soil temperatures below 20 °C should prioritize Bt-based formulations, while those in lowland tropical settings above 30 °C may achieve better results with fungal biopesticides that maintain moderate efficacy at elevated temperatures.

The post-harvest quality benefits of effective ant management represent a previously undercharacterized advantage of biopesticide deployment, demonstrating that reduced tuber surface damage during the growing season translates into improved storage performance and extended marketability periods.

Post-Harvest Quality Assessment

The comprehensive post-harvest quality evaluation protocol employed in this research assessed five quality attributes at harvest and during 60 days of ambient storage. Firmness measurements using a digital penetrometer revealed that biopesticide-treated tubers maintained significantly higher firmness values (40.2-48.2 N) compared to controls (28.4-35.8 N) throughout the storage period, attributable to reduced surface damage that limits moisture loss and tissue desiccation through intact periderm barriers. Dry matter content, a critical parameter for processing quality, was 18-22% higher in treated tubers reflecting improved assimilate accumulation during the unimpeded bulking phase. Visual quality scoring, conducted by trained panelists assessing surface smoothness, absence of scarring, and uniformity of shape, confirmed that biopesticide-treated tubers achieved

consistently superior presentation suitable for premium market channels.

Conclusion

This research aimed to evaluate microbial biopesticide performance against red ant damage in potato under varying climatic conditions, and to characterize the temperature-dependent nature of biological pest control outcomes. The key findings establish that the moderate temperature regime (25-30 °C) supports optimal biopesticide efficacy across all tested formulations, that the combined Bt+NPV treatment achieves the highest ant mortality (78.5%) and tuber yield (24.6 t/ha) at moderate temperatures, and that temperature-mediated efficacy differences necessitate climate-specific treatment recommendations for commercial adoption. From a practical standpoint, potato growers should calibrate biopesticide selection and application scheduling to prevailing soil temperature conditions, prioritizing treatments at soil temperatures between 25-30 °C for maximum return on biological pest management investment. Future research should systematically evaluate biopesticide performance across additional climatic variables including soil moisture regimes, UV exposure levels, and seasonal temperature fluctuations to develop comprehensive predictive models for field-level efficacy optimization in diverse potato production environments.

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