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Research Article

Efficacy of *Xenorhabdus* sp. (X1) as biocontrol against for controlling mushroom mites (*Luciaphorus* sp.)

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Abstract

We investigated the efficacy of symbiotic bacteria to control the mite (*Luciaphorus* sp.) which is a pest of several mushroom species. Six species of symbiotic bacteria, *Xenorhabdus* sp. (X1), *X. nematophila* (X2), *X. poinarii* (X3), *Xenorhabdus* sp. (X4), *Photorhabdus luminescens* (P1) and *P. luminescens* akhurstii (P2), were evaluated. A cell suspensions ($1x10^8$ cells/ml) of *Xenorhabdus* sp. (X1) caused 82.5% mite mortality. Mortality varied with bacterial culture age, 2-day old culture had 82.5% mortality and 3- day old X1 bacterial cultures had 74.17%. In addition, the bacterial culture when grown at an optimal temperature (30°C) killed mites at a mortality rate of 83.33%. The cell-free supernatant of X1 was also found to effectively kill the mushroom mites at a rate as high as 90.83%. Furthermore, the cell-free supernatant of 2- and 3- day old X1 culture resulted in mites mortality of 87.5 and 78.33%, respectively. In conclusion, this study has shown that all symbiotic bacteria tested appeared to have miticidal activity. Both the cell suspensions and the cell-free supernatant of *Xenorhabdus* sp. (X1) bacteria has miticidal properties and has the potential as a biocontrol agent for controlling mites infesting the cultivated mushrooms.

Keywords : *Xenorhabdus* sp., *Luciaphorus* sp. and biological control

Introduction

Luciaphorus sp.(Acari: Pygmephoridae) is considered to be one of the most destructive pests of several commercial mushrooms species, including *Lentinus squarrosulus*, *L. polychrous*, *Auricularia auricula* and *Felmularia velutipes*, These mushrooms are widely cultivated in the North and the Northeast of Thailand (Bussaman *et al*., 2004). This mushroom mite can be

controlled by several chemical pesticides but in the recent years resistance has developed. Pesticide-resistance and concerns about the risks of using chemical pesticides for human health and environment has increased interest in searching for alternatives that are effective for controlling this mite (Bussaman *et al*., 2009).

Photorhabdus sp. and *Xenorhabdus* sp., the bacteria symbiotically associated to heterorhabditid and steinernematid nematodes, are known to be highly virulent to several insects (Forst *et al*., 1997). Both bacterial culture and cell-free supernatant of *Xenorhabdus* and *Photorhabdus* bacteria have been found to be toxic to several insect pests, including the cabbage butterfly *Pieris brassicae* (L.) and the diamondback moth *Plutella xylostella* (L.) (Abdel-Razek, 2003; Mohan *et al*., 2003). Morever, the bacterial culture of *P. luminescens* subsp. *laumondii* (strain GPS12) and *X. nematophila* (X1) have been found to be highly virulent to against *Luciaphorus* sp., resulting in 76 and 85% mortality of female mites within 3 day after treatment, respectively (Bussaman *et al*., 2006 ; Bussaman *et al*., 2009). The objective of this study is to investigate the effect of *Xenorhabdus* sp. (X1) and its metabolites on mushroom mite (*Luciaphorus* sp.) infesting mycelium of *Lentinus squarrosulus.*

Materials and Methods

Luciaphorus sp. mites were collected from *L. squarrosulus* basidiocarps and composts obtained from Rapeephan mushroom farm in Khon Kaen province in the Northeast of Thailand. A male and female mite were placed in each glass bottle containing *L. squarrosulus* spawn and maintained at 28°C for reproduction. These in-house bred mites were used in all experiments.

Lentinus squarrosulus, was obtained from the Mushroom Researchers and Growers Society of Thailand and was sub-cultured using 90-mm plastic Petri-dish containing Potato Dextrose Agar (PDA, Sigma Ltd, USA.) and incubated at 25°C in the dark. The mushroom mycelium was then inoculated into a sawdust and sorghum grain mixture to grow fresh spawn (Bussaman, 2005).

Isolation of the bacterial symbiont. *Galleria mellonella* larvaes that were infected with IJs of *Steinernema siamkayai, S. carpocapsae, S. glaseri, S. riobrave, Heterorhabditis bacteriophora* and *H. indica* (Table 1) and died within 24-48 h were surface-sterilised using 70% alcohol for 10 min. The larvaes were carefully dissected using sterile needles and scissors to avoid damage to the gut epithelium, and then a drop of the oozing haemolymph was collected. The haemlymph was streaked on the nutrient agar [37 g nutrient agar (Criterion, USA.); 25 mg bromothymol blue powder (Labchem, England.); 4 ml of 1% 2, 3, 5 triphenyl-tetrerzolium chloride (Sigma chemical, USA.); 1000 ml distilled water]. The nutrient agar Petri-dishes were sealed using parafilm and incubated at 28 °C in the dark for 24 h. The bacteria single colonies were selected and streaked on separate nutrient agar plates, and they were subcultured continually until the colonies appear to have the same size and morphology as previously described by Kaya and Stock (1997). Each single colony was selected and inoculated to a tryptic soy broth (containing 15 g of tryptic soy broth (Criterion, USA.) in 500 ml distilled water). The flask was then placed in a shaking incubator adjusted at 200 rpm for 24 h at 28 °C. The concentration of bacterial cells in the broth suspension was determined by the plate-count technique (Klement *et al*., 1990). For all experiments, the concentration of bacterial suspension was adjusted to be $1x10^8$ cells/ml. To obtain cell-free supernatant of the bacterial culture, the cell suspension was filtered using 0.22 μm filter.

Table 1 *Heterorhabditis* and *Steinernema* species and their symbiotic bacteria

Nematode species	Bacterial species	Code
Steinernema siamkayai	*Xenorhabdus* sp.	X1
S. carpocapsae	*X. nematophila*	X2
S. glaseri	*X. poinarii*	X4
S. riobrave	*Xenorhabdus* sp.	X5
Heterorhabditis bacteriophora	*Photorhabdus luminescens*	P1
H. indica	*P. luminescens akhurstii*	P2

Virulence of *Photorhabdus* and *Xenorhabdus* cultures on *Luciaphorus* sp. The effect of cells suspension and cell-free supernatant of *Photorhabdus* and *Xenorhabdus* on *Luciaphorus* sp. were investigated using plastic Petri-dishes containing mushroom mycelia, as previously described by Bussaman *et al*. (2006). One hundred adult female mites were transferred to each 50-mm diameter plastic Petri-dishes , containing mushroom mycelia grown on PDA. Then 500 µl of bacterial culture ($1x10^8$ cells/ml) and cell-free supernatant were sprayed to the mushroom colony. The same volume of TSB broth was used as a negative control. Each bacterial strain was tested in quadruplicates. All plates were covered and placed in growth chamber at 28°C and 80% RH in the dark. The mite mortality was monitored every 24 h for 5 days after treatment.

Effects of cell suspensions and cell-free supernatant of *Xenorhabdus* sp. (X1) at different concentration on the female mites. Cell suspensions and cell-free supernatant of *Xenorhabdus* sp. (X1) were prepared as described above and serially diluted to be $1x10^2$, $1x10^4$, $1x10^6$ and $1x10^8$ cells/ml. One hundred adult female mites were transferred into 50-mm each plastic Petri-dish which containing mushroom mycelium grown on PDA medium. Then 500 µl of each bacterial suspension and cell-free supernatant were sprayed to the mushroom colony. An equal volume of TSB broth was used as a negative control. Each treatment was performed in quadruplicates. All plates were covered and placed in the growth chamber at 28°C and 80% RH in dark. The mite mortality was monitored every 24 h for 5 days after treatment.

Effects of growth phase of *Xenorhabdus* sp. (X1) on mite mortality. *Xenorhabdus* sp. (X1) was cultured in TSB broth for 1, 2, 3, 4 and 5 days, and bacterial cell suspensions were harvested and adjusted to a concentration of $1x10^8$ cells/ml, as described above. Then 500 µl of bacterial cell suspension and cell-free supernatant at different growth phases were sprayed to the mushroom mycelium grown on PDA medium. One hundred adult female mites were transferred to each 50-mm diameter plastic Petri-dish containing mushroom mycelium grown on PDA medium. Each bacterial culture was tested in quadruplicates. An equal volume of TSB broth was used as a negative control. The mite mortality was monitored every 24 h for 5 days after treatment.

Effects of cell suspensions and cells-free supernatant of *Xenorhabdus* sp. (X1) against female mites at different temperatures. For this experiment, four different temperatures for growing bacterial culture were selected for testing mite mortality. The protocol for testing cell suspensions and cell-free supernatant on mite mortality was performed as described above. One hundred adult female mites were added to each Petri-dish and incubated at 20, 25, 30 and 35°C. Mite mortality was recorded every 24 h for 5 days after treatment.

Statistical analyses. All data were subjected to analysis of variance using the general linear models procedure (SAS Institute, Cary, NC, USA.). Data on the percentage of mite mortality were arcsine transformed before analysis. Significant differences between treatment means were determined using the Sheffe test at $p < 0.05$. As no differences were observed in mite mortality between the repeated experiments, data from the two repeats were pooled for each experiment.

Results

All symbiotic bacteria in this study appeared to have miticidal activity against female *L. perniciosus* (Fig. 1). For all treatments, the percentage of mite mortality increased gradually and remained steady from day 3. The cell suspensions and cell-free supernatant of *Xenorhabdus* sp. (X1) were found to be highly virulent to the female mites when compared to the control with a maximum mortality 82.5 and 83.33%, respectively at day 3. The cell suspensions and cell-free supernatant of *Xenorhabdus* sp. (X1) appeared to cause mite mortality in a dose dependent manner (Fig. 2). Interestingly, the mortality rate of mites treated with bacterial culture at a concentration of $1x10^8$ cells/ml was not significantly different from those obtained when using the commercial miticide (Propargite). These results indicated that *Xenorhabdus* sp. (X1) at a concentration of $1x10^8$ cells/ml caused the highest mortality to female mites (Fig. 2). The cell suspensions and cell-free supernatant of the lowest bacterial concentration ($1x10^2$ cells/ml) was less effective against *Luciaphorus* sp., resulting in 35.83 and 45% mite mortality, respectively; where as the cell suspensions and the cell-free supernatant of the highest bacterial concentrations ($1x10^8$ cells/ml) were capable of causing about 82.5 and 90.83% mortality, respectively. The growth phase of *Xenorhabdus* sp. (X1) significantly altered their virulence to *Luciaphorus* sp. female mites (Fig. 3). The results indicated that the 2-day old cell suspensions and its cell-free supernatant of *Xenorhabdus* sp. (X1) could cause the highest mite mortality (82.5 and 87.5%, respectively), however this not significantly different from the 3-day-old culture. The mortality of mites induced by all treatments increased gradually until day 3. The incubation temperature were shown to have a significant ($p<0.05$) effect on the mortality of mite (Fig. 4). Treatment using either *Xenorhabdus* sp. (X1) cell suspensions or cell-free supernatant at 30°C temperature resulted in higher mortality (83.33 and 87.5%, respectively) than those grown at 20°C which caused only 46.67 to 50.83% mortality. Interestingly, there was no significant difference between treatments using *Xenorhabdus* sp. (X1) and those using the commercial miticide (Propargite).

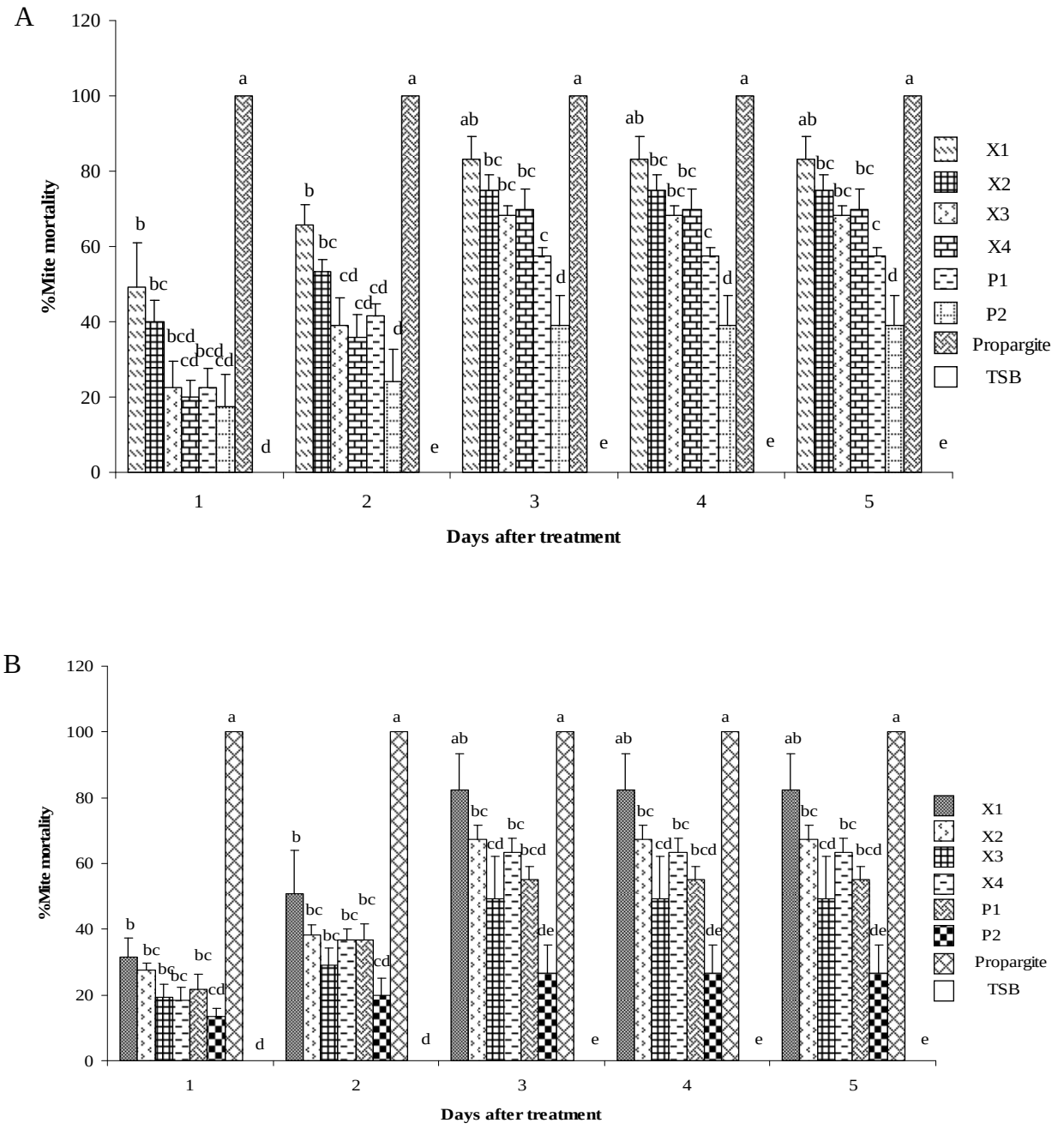

Figure. 1 The mortality of *Luciaphorus* sp. female mites treated with cell suspensions (A) and cell-free supernatant (B) of bacteria (1.0 x 10^8 cells/ml) and incubated at 28°C, 80 % RH in the dark for 5 days**.** The bars have standard error bars??? And the bar with the same letter are not significantly different.???

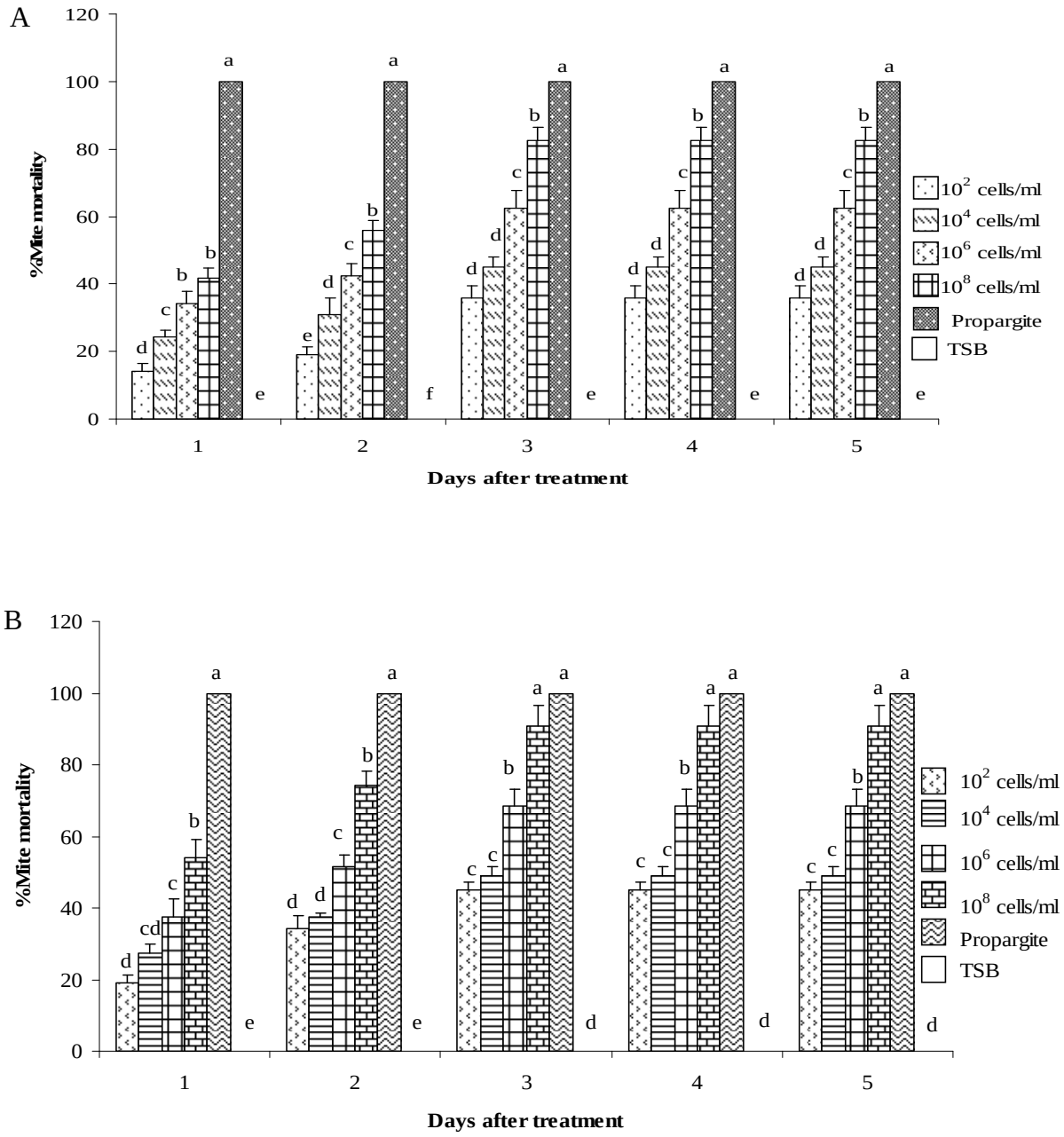

Figure. 2 The mortality rates of *Luciaphorus* sp. female mites when being treated with cell suspensions (A) and cell-free supernatant (B) of *Xenorhabdus* sp. (X1) at different concentrations and incubated at 28^{o}C, 80 % RH in the dark for 5 days The bars have standard error bars??? And the bar with the same letter are not significantly different.????.

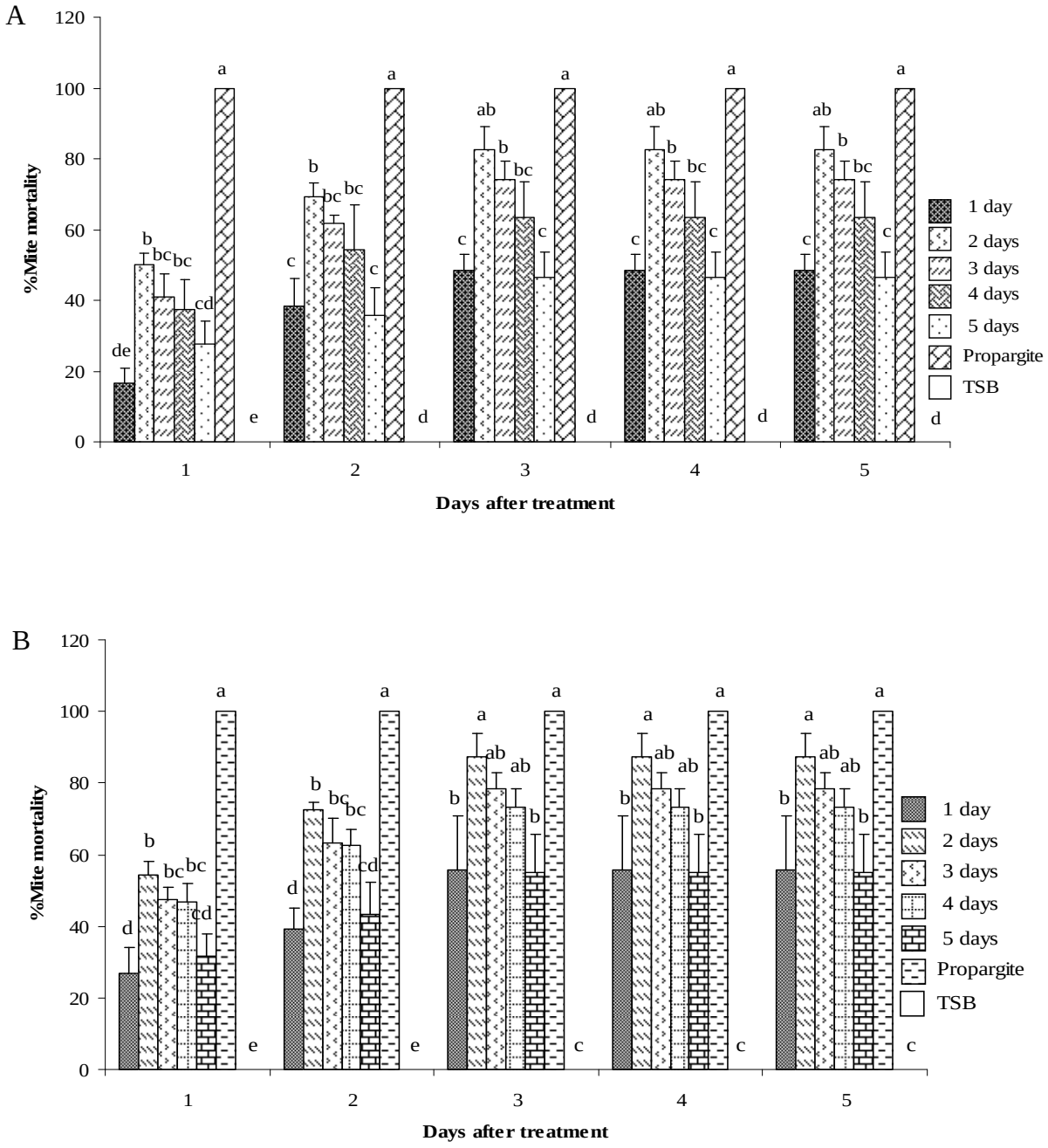

Figure. 3 The mortality rates of *Luciaphorus* sp. female mites when being treated with cell suspensions (A) and cell-free supernatant (B) of *Xenorhabdus* sp. (X1) prepared from different growth phase at concentration of 1.0 x 10^8 cells/ml. The bars have standard error bars??? And the bar with the same letter are not significantly different.????

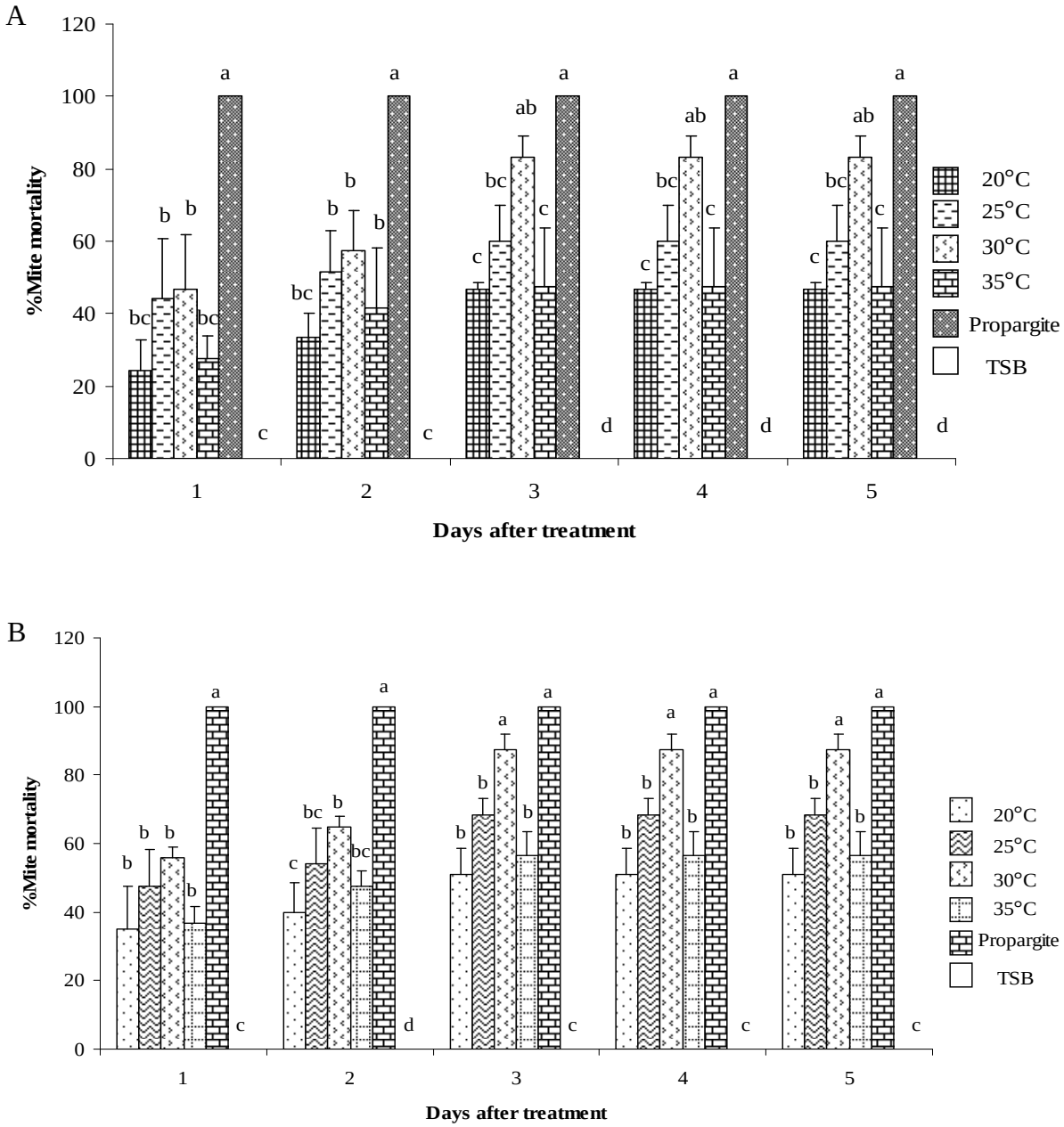

Figure. 4 The mortality rates of *Luciaphorus* sp. female mites when being treated with cell suspensions (A) and cell-free supernatant (B) of *Xenorhabdus* sp. (X1) grown at different temperatures. The bars have standard error bars??? And the bar with the same letter are not significantly different.????

Discussion

The results of this study have shown that different bacterial strain displayed different levels of virulence to mushroom mites. The bioassays for measuring the virulence of six symbiotic bacterial strains indicated that *Xenorhabdus* sp. (X1) at a concentration of $1x10^8$ cells/ml was capable of causing the highest mortality of *Luciaphorus* sp. Bussaman *et al*. (2009) reported that the culture of *X. nematophila* strain X1, which was isolated from *S. siamkayai* nematode, was virulent to mushroom mite. The cell suspension of *X. nematophila* or cell-free supernatant (containing its metabolites) have been shown to be capable of controlling the larvae of *P.*

xylostella (Mohar *et al*, 2004). Bussaman *et al*. (2006) are the first to report the miticidal activity of *Photorhabdus* and *Xenorhabdus* bacteria, and they showed that bacterial culture of the X1 strain of *X. nematophila* is more effective than the previously tested Thai strains. At day 1 and day 2 after treatment. *X. nematophila* X1 could cause 65 and 72% mortality of the mushroom mites which were higher than those caused by Thai strain (30 and 60% mortality). The results shown here may reveal the potential of *Xenorhabdus* sp. (X1) bacterial suspension for controlling the mushroom mites. Our results show that bacterial culture of *Xenorhabdus* sp. (X1) at a concentration of $1x10^8$ cells/ml could resulted in the mite mortality at the levels similar to those caused by *P. luminescens* strain GPS12 and *P. temperate* strain GPS11 as previously reported by Bussaman *et al*. (2006).

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