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ORIGINAL ARTICLES

Use Of The Entomopathogenic Nematode Symbiont *Photorhabdus Luminescens* As A Biocontrol Agent B- Factors Affecting The Cell-Free Filtrates From The Bacterium

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ABSTRACT

Four entomopathogenic bacterial isolates of *Photorhabdus luminescens* designated as A, C, D, and E were separately obtained from the haemolymph of *Galleria melonella* infected with the corresponding indigenous nematode isolates of *Heterorhabditis bacteriofora* and the fifth *P. luminescens akhurstii* B from Egyptian *H. indica* isolate B. The effect of metabolite concentration for each of these bacterial isolates and some physical parameters (i.e., temperature, pH, and sodium chloride) on the metabolite-induced mortality for *G. mellonella* larvae as well as the magnitude of the bacterial growth under different concentrations of NaCl and pH values was investigated. This mortality differed ($P \leq 0.05$) among the tested isolates at the assigned ranges of these physical parameters. The cell-free filtrates of 4×10^7 cells/ml broth of the bacterium *P. luminescens akhurstii* isolate B could induce 100% insect mortality after one day of exposure, showing the highest virulence against *G. mellonella* larvae. Growth of this bacterium was superior to that of all others in broth with 0.3% NaCl. The higher the metabolite dose was the more the percentage mortality of *G. mellonella* recorded up to ten days of exposure. The knowledge gained from physical parameters tested herein may be implemented to develop and optimize the use of these bacteria and their secretions as a bio-pesticide. The potential for biological control of these indigenous bacterial isolates against insect pests are discussed and further comparisons are warranted.

Key words: Biological control, *Galleria mellonella*, entomopathogenic nematodes, *Photorhabdus*.

Introduction

Photorhabdus spp. are entomopathogenic enterobacteria that exist in a state of mutualistic symbiosis with nematodes of the family Heterorhabditidae. The bacterium species and subspecies associations are *Heterorhabditis bacteriophora* subgroup Brecon (*Photorhabdus luminescens luminescens*), *H. bacteriophora* subgroup HP88 (*P. luminescens laumondii*), *H. indica* (*P. luminescens akhurstii*), *H. zealandica* , *H. bacteriophora* subgroup NC, *H. megidis* Nearctic group of Ohio and Wisconsin (*P. temperata*), and *H. megidis* Palaearctic group (*P. temperate temperata*). Another species that has clinical opportunistic strains is *P. asymbiotica* (Fisher-Le Saux *et al*., 1999, Boemare, 2002, Plichta *et al*., 2009). The bacteria are mainly located in the anterior part of the entomopathogenic nematode (EPN)-free-living, infective juvenile (Ij) guts (Boemare *et al*., 1996). Upon entering an insect host, the Ijs release the bacteria by regurgitation directly into the insect's hemocoel. The bacteria once expelled from the Ijs enter the hemocoel of the insect, actively multiply avoiding the host defenses and causing an acute disease conditions that is followed by insect death usually within 48 hours. *P. luminescens* can directly and rapidly kill the insect host by producing toxins such as TcA (Rodou *et al*., 2010). It also secretes enzymes which break down the body of the infected insect and bioconvert it into nutrients which can be utilized by both nematode and bacteria. Bioconversion of the insect's body by *P. luminescens* produces a rich food source for the bacteria and nematode development and reproduction for numerous cycles; depending on the available food and environmental conditions. The bacteria continue to replicate within the insect cadaver and the nematodes feed off the bacterial–insect medium. The bacteria enter the nematode progeny as they develop. *P. luminescens* also secretes antibiotics to protect against competition from other bacteria. *Photorhabdus* species exist in two forms, primary and secondary variants, which differ in morphological and physiological traits. It is hypothesized that primary variants correspond to the nematode-associated and insect-infective form, and secondary variants correspond to late stationary phase cells in infected insects and the re-associative form of the bacteria (Turlin *et al*., 2006, Shahina *et al*., 2011). Bacterial culture and cell-free supernatant of both *Xenorhabdus spp.,* symbionts of the EPNs *Steinernema* spp., and *Photorhabdus* spp. have been found to be toxic to several plant pests (Bussaman *et al*., 2009).

Genetic improvement of these entomopathogenic nematodes and their symbiotic bacteria to enhance their biological control potential through selective breeding is likely only if the desired alleles are present in the

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genepools of different nematode populations. For example, insufficient genetic variation in resistance to ultraviolet light resulted in rejecting selective breeding as an option to improve UV tolerance in *Steinemema carpocapsae* (Gaugler *et al*., 1989). Ongoing research programs have recorded new additional populations of EPN in Egypt (e.g., Shamseldean and Abd-Elgawad, 1994; Salama and Abd-Elgawad, 2001; Abd-Elgawad and Nguyen, 2007; Shamseldean, 2010; Abu-Shady *et al*., 2011) and worldwide (e.g., Plichta *et al*., 2009). The diversity and natural occurrence of EPN in Egypt led a recent study (Abu-Shady *et al*., 2011) to consider four natural geographic regions to be surveyed for EPN isolation in the country. New isolates provide the possibility to increase the genetic variation and consequently develop new nematode/symbiont strains. In a previous study (Abd El-Zaher *et al*., 2008), we determined the effect of different *Photorhabdus* isolates on mortality of the greater wax moth, *Galleria mellonella* larvae under different substrates; two kinds of media (wheat bran and fine sand) were compared to broth alone (control). They showed that the highest mortality occurred on *Photorhabdus*-inoculated sand. Here, we further report the direct effect of applying cell-free metabolites of indigenous *P. luminescens* isolates on the mortality of *G. mellonella* larvae under a range of different physical parameters. In this study, the effect of bacterial metabolite at different concentrations, temperatures, pH conditions, and sodium chloride concentrations on the metabolite-induced mortality for *G. mellonella* larvae as well as *P. luminescens* growth under different concentrations of NaCl and pH values was investigated.

Materials and Methods

Nematode extraction and identification:

Five heterorhabditid nematode isolates used herein were extracted from Egyptian soil at different geographical localities (e.g., Salama and Abd-Elgawad, 2001; Abd-Elgawad and Nguyen, 2007; Abd-Elgawad *et al*., 2009). These isolates, designated as A, B, C, D, and E, were separately processed through last-instar larvae of the greater wax moth, *Galleria mellonella,* obtained from The National Research Center (NRC) insectary at nematology laboratory of NRC (Abd-Elgawad, 2001), several times to obtain pure cultures. Then, they were identified by gene sequence analysis as *Heterorhabdits bacteriophora* except isolate B as *H. indica* (Abd-Elgawad and Nguyen, 2007; R. Campos–Herrera and L.W. Duncan, pers comm.).

Isolation of bacterial symbionts and their metabolites:

Five separate isolates of *Photorhabdus* bacteria were obtained from the haemolymph of last instar *G. mellonella* larvae infected with Ijs of their corresponding nematode isolates. Bacterial colonies were established on nutrient agar by streaking haemolymph from these insects (Kaya and Stock, 1997). Since *Photorhabdus* spp. occur as two phase variants (primary and secondary), but usually only the primary phase that produces antibiotics (Akhurst 1980; Forst and Clarke 2002), we used bacteria in the primary form. A selective medium (NBTA or MacConkey Agar) was used to indicate primary variant characteristics during bacterial isolation and culturing according to Kaya and Stock (1997). A loopful of bacteria was obtained from the nutrient agar and streaked onto NBTA or MacConkey Agar plates (Akhurst, 1980). The plates, sealed with Parafilm, were incubated at 28°C in the dark for 24-48 h, and then single colony of bacterium was selected and streaked onto new plates of nutrient agar. Sub-culturing was continued until bacterial colonies of uniform size and morphology were obtained. Based on the above-mentioned nematode identification, all the bacterial isolates were designated as *Photorhabdus luminescens* A, C, D, and E except the bacterium isolated from *H. indica* isolate B which was identified as *Photorhabdus luminescens akhurstii* B (Boemare, 2002). After obtaining pure colonies, the pathogenicity of the isolates was confirmed by injecting the bacterial cells into the body of *G. mellonella* larvae and streaking the haemolymph of the infected larvae on NBTA plates. A single colony of the bacterium was selected and inoculated into 500 ml of nutrient broth solution (15 g nutrient broth and 500 ml of distilled water in a flask plugged by sterile cotton) and placed in a shaking incubator at 150 rpm at 28 °C for 4 days (Kaya and Stock, 1997). The concentration of the bacterial broth suspension was determined by measuring the optical density using a spectrophotometer adjusted to 600 nm wave length. The concentration of the bacterial cells used in the present experiments was adjusted to 4×10^7 cells/ml as described by Elawad (1998) and an aliquot of 3% Tween 80 was added as an emulsifier. The bacterial broth suspension was then centrifuged at 10 000 rpm for 20 minutes and then filtered using a Whatman 25 Mm GD/X paper with pore size of 0.2 µm to obtain cell-free supernatants (bacterial metabolites).

Effect of different doses of the bacterial metabolites on G. mellonella larval mortality:

Fresh cell-free filtrates of each bacterial isolate prepared in broth as previously mentioned from suspensions with 4×10^7, 4×10^5, and 4×10^3 cells/ml and few drops of 3% Tween-80 were added (Mahar *et al*., 2005). The broth check did not contain bacterium. Twenty five gram of fine sterilized sand was put into 9-cm-

diameter Petri-dishes and the moisture contents were adjusted to 10% by sterilized distilled water. Five last instar *G. mellonella* larvae of similar size, age and color were placed in each Petri-dish. The larvae were then sprayed with 2 ml of each filtrate by a micropipette. Each treatment was replicated thrice. Control was treated with only broth media. All Petri-dishes were covered, sealed with Parafilm and incubated at 25ºC. Mortality in each dose/isolate was recorded daily for ten days.

Effect of different temperatures and pH conditions on the efficiency of the bacterial metabolites:

The effect of temperature on the metabolites was done by incubating in water bath the cell-free supernatants of five bacterial isolates, designated as A, B, C, D, and E, at 30, 40, 50, 60, 70 and 80°C for half an hour followed by cooling at ambient temperature then added to Petri-dishes as described before. Insect mortality was recorded daily for five days. Untreated control with only broth media was set under the same temperatures. Such a mortality was similarly recorded when the broth was set, before inoculating and filtering the bacterium, at different pH conditions between 4.8 to 9 using 100 mM of sodium phosphate buffer for pH values 4.8, 5.5 and 7, but Tris-HCl buffer for pH values 8 and 9.

Effect of different doses of NaCl on the bacterial metabolites:

Instead of different pH conditions, NaCl was added before inoculating and filtering the bacterium at doses 0.5, 2, 3. 4 and 5 g NaCl /liter nutrient broth and examined for its effect on the ability of the cell-free filtrates to induce *G. mellonella* larval mortality. Untreated controls without the bacteria was set under the same NaCl concentrations.

Effect of different pH conditions or NaCl doses on the bacterial replication:

The broth was set at different pH conditions or NaCl doses before inoculating the bacterium as mentioned above and the bacterial replication in the cell suspensions was recorded after 3 days.

Statistical analyses:

All data were subjected to analysis of variance followed by mean separation using Duncan's New Multiple Range Test at $P \leq 0.05$. Data on the percentage of insect mortality were arcsine transformed before analysis.

Results:

Effect of different doses from the metabolites:

Cell-free suspensions for each of the five *P. luminescens* isolates were toxic to the *G. mellonella* larvae inflicting significant ($P \leq 0.05$) mortality. Also, significant ($P \leq 0.05$) differences in the induced mortality among the bacterial metabolites were frequently observed (Figure 1) probably depending on the toxicity of such metabolites. For all isolates (A-E) the mortality percentages were more developed by time increasing particularly after 5 and 7 days and reached their maximal effect after 10 days (Figure 1). Yet, such a development of mortality was not equal among the different isolates as time elapsed. Yet, while the lowest dose of metabolites for isolate D did not induce insect mortality after one day of exposure (Figure 1), such a mortality caused by the medium dose ranged 20-50% (Figure 1b) for the same period. The general trend of mortality was gradually increased with the increase in exposure time; probably after feeding on metabolites containing media. The induced rate of insect mortality with the highest dose (4×10^7) was always the strongest (Figure 1). Isolate (B) had very strong effect; 100% mortality occurred to the larvae when treated with its secretion after one day only. The mortality percentage by this dose was fast and gradually increased to reach 100% in most isolates after 5 days. Eventually, the higher the dose was the more the percentage mortality of *G. mellonella* recorded (Figure 1).

Effect of different temperatures on the efficiency of the metabolites to kill Galleria mellonella larvae:

The bacterial metabolites were incubated at six different temperatures for half an hour then cooled before inoculation. The incubation temperatures were shown to have a pronounced effect on the mortality of *G. mellonella* larvae (Figure 2). Treatment using cell-free suspension at 30 - 40^0C resulted in full mortality two days after exposure (Figure 2). At higher temperatures, the metabolites appeared to cause larval mortality in a temperature dependent manner. The metabolites of the bacterium *P. luminescens* isolate C demonstrated resistance to high temperature since they could induce 90% insect mortality five days after pre-incubation at

60^0C. The percentage mortality caused by the metabolites of all isolates decreased drastically when pre-incubated at 70^0C and the metabolite effect on insect toxicity was negated when pre-incubated at 80^0C.

Effect of pH on bacterial growth and insect mortality:

Effects of different pH values on the metabolite-induced mortality for *Galleria mellonella* larvae are shown in Figure (3). Apparently, none of the tested pH conditions permitted toxic secretions of the tested bacterial isolates since all of them could induce insect mortality. Generally, with the increase in the initial pH (Figure 3), cell concentration and production of secretion increased, and the maximum secretion was attained at pH = 7.0; after that the cell density and production of secretion decreased modestly up to pH 9. Significant ($P \leq 0.05$) differences in the induced mortality among the bacterial metabolites were found at pH range of 4.8–9. Based on the induced insect mortality, the metabolite of bacterial isolate A was superior ($P \leq 0.05$) to the others at pH 4.8 where it could attain full mortality after 3 days, while the bacterial isolates B and C were more tolerant to high pH range 8-9.

Effect of adding NaCl on the efficiency of the metabolites to kill Galleria mellonella larvae:

The effect of NaCl on the efficiency of the metabolites to kill *G. mellonella* larvae differed ($P \leq 0.05$) from one bacterial isolate to another (Figure 4). Based on insect mortality, NaCl at 0.05 concentration in the broth seemed superior ($P \leq 0.05$) to other tested concentrations especially at the first day of exposure. Full insect mortality was achieved by metabolites with most tested NaCl doses by the third day of exposure. Yet, the percentage insect mortality increased by time elapsed after insect exposure to the metabolites where full insect mortality was achieved by any metabolite isolate after four days. Also, the results demonstrated the necessity to differentiate ($P \leq 0.05$) between the metabolites based on their NaCl-dose-efficacy since the larval mortality appeared to occur in a bacterial isolate dependent manner.

Effect of different NaCl concentrations or pH conditions on the bacterial replication:

Results of *P. luminescens* growth in the flask experiment containing broth set at different NaCl concentrations are shown in Table (1). The bacterial isolates varied in their growth response to different NaCl concentration. The highest growth was attained by the bacterium isolate B which has mutualistic relationship with *H. indica* compared to the other four *H. bacteriophora*-symbiotic bacterial isolates. Growth of this bacterium was superior to that of all others in broth with 0.3% NaCl. None of the tested bacterial isolates showed a linear growth correlation with NaCl concentration.

The bacterial growth varied greatly under different pH conditions within and among the five isolates tested (Table 2). The highest growth was attained by the bacterium isolate B at pH 4.8 followed by isolate A at pH 9 and isolate E at pH 4.8, respectively. None of the tested bacterial isolates showed limited replication at pH range of 4.8–9.

Discussion:

Our *in vitro* assays indicated that the cell-free filtrates of all bacterial isolates tested could induce insect mortality, yet virulence varied among the different bacterial subspecies and isolates. Similarly, substantial variation in virulence among cell suspensions and metabolites of different *Photorhabdus* and *Xenorhabdus* species and strains to different pests and pathogens has been observed in other studies (Abdel-Razek, 2002 and 2003; Abd El-Zaher *et al*., 2008; Shapiro-Ilan *et al*., 2009; Bussaman *et al*., 2009). For example, Abdel-Razek (2002) found differences in insect mortality caused by *Xenorhabdus* and *Photorhabdus* strains to *G. mellonella*. Shapiro-Ilan *et al*. (2009) reported different suppressive abilities of bacterial metabolites derived from *Xenorhabdus* and *Photorhabdus* spp. on *Glomerella cingulata*, *Phomopsis* sp., *Phytophthora cactorum*, and *Fusicladosporium effusum*, which are fungal or oomycete pathogens of pecan, and *Monilinia fructicola*, a fungal pathogen of peach. Various toxins have recently been identified and characterized in this bacterium which indicates that *P. luminescens* has all the necessary means for the secretion of virulence factors capable of establishing a microbial infection (Rodou *et al*., 2010).

In addition to virulence, other characteristics important for biological control that are known to differ among strains/isolates within species of entomopathogenic nematodes/their symbionts include reproductive potential (or replicate in the case of the bacteria), and environmental tolerance (Somasekhar *et al*., 2002, Stuart *et al*., 2004; Bussaman *et al*., 2009). So, major objectives of the present investigation were to determine the effects of cell-free metabolites of different *P. luminescens* isolates on mortality of *Galleria mellonella* larvae under different temperatures, metabolite concentrations, pH values and sodium chloride additions as well as the bacterial replication under different concentrations of NaCl and pH values. Other valuable aspects of these

bacteria such as their virulence mechanisms has been discussed and documented elsewhere (Dowds and Peters, 2002). Yet, our study is the first to report the insecticidal activity of indigenous *Photorhabdus* under a reasonable range of several physical parameters. It is assumed that with such tools and research knowledge to optimize their usage, these bacteria and their secretions can be developed as a bio-pesticide. Recent evidence indicated that when filter, bran, wheat and sand substrates were compared, the later one was observed as appropriate (Mahar *et al*., 2005; Abd El-Zaher *et al*., 2008). Clearly, understanding of such factors affecting microbial colonization and persistence factors that can bring about fitness outcomes in terms of enhancing pest mortality is of fundamental importance. Moreover, An and Grewal (2010) found that *P. temperata* growth was normal in the medium within a moderate pH range of 6–9, limited at pH 5, and no longer permitted at pH 4 or 10. It is clear that pH plays an important role on the metabolite-induced mortality for *Galleria mellonella* larvae (Figure 3; Table 2).

Table 1: Growth of five isolates (A-E) of *Photorhabdus luminescens* in broth with different NaCl concentrations after 3 days.

NaCl concentration	A	B	C	D	E
0.05%	$20x\ 10^{5}$	$30x10^{5}$	$35x10^{5}$	$20x10^{6}$	$55x10^{6}$
0.2%	$45x10^{5}$	$70x10^{7}$	$40x10^{5}$	$45x10^{7}$	$45x10^{5}$
0.3%	$15x10^{5}$	$77x10^{7}$	$35x10^{5}$	$20x10^{5}$	$30x10^{5}$
0.4%	$15x10^{5}$	$30x10^{5}$	$35x10^{5}$	$20x10^{5}$	$25x10^{5}$
0.5%	$45x10^{5}$	$30x10^{5}$	$45x10^{5}$	$25x10^{7}$	$50x10^{5}$

Table 2: Growth of five isolates (A-E) of *Photorhabdus luminescens* in broth with different pH values after 3 days.

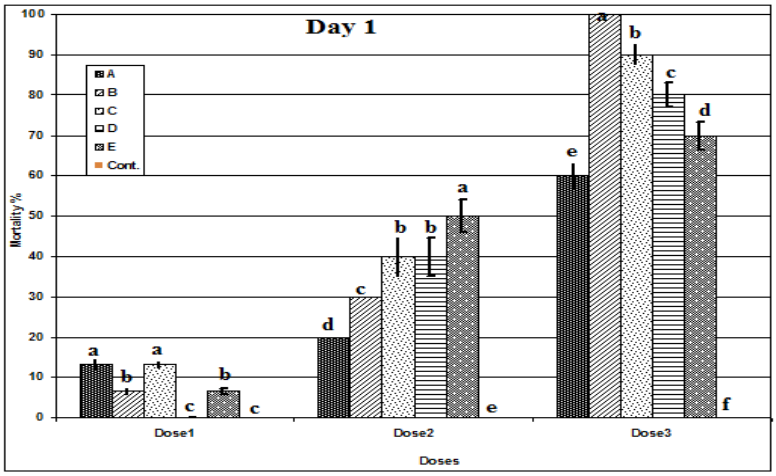
pH value	A	B	C	D	E
4.8	$75x10^{7}$	$100x10^{9}$	$25x10^{7}$	$50x10^{5}$	$30x10^{8}$
5.5	$20x10^{6}$	$20x10^{5}$	$80x10^{7}$	$40x10^{5}$	$10x10^{2}$
7.0	$20x10^{6}$	$10x10^{8}$	Not detected	$20x10^{8}$	$10x10^{2}$
8.0	$30x10^{6}$	$55x10^{7}$	$30x10^{7}$	$25x10^{7}$	$40x10^{7}$
9.0	$40x10^{9}$	$55x10^{7}$	$10x10^{7}$	$25x10^{7}$	$20x10^{7}$

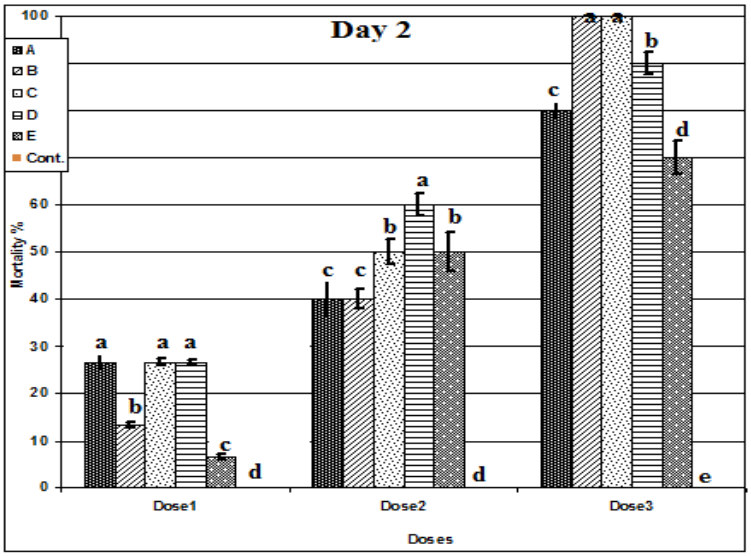
Admittedly, based on virulence, reproduction potential and environmental tolerance, the overall goal of such biocontrol agents is the identification and deployment of highly effective strain(s) against insect pests before their development into registered, ready-for-sale plant protection products. So, pronounced variations among the bacterial isolates tested herein may be economically exploited. Yet, efficacy gap between field and laboratory application of these metabolites should be considered. Also, even if new products of such biocontrol agents are available, local ones may be more adaptive and less expensive without any risk to Egyptian fauna and flora than imported strains. So, these factors as well as the relatively high efficacy demonstrated herein by some of the tested isolates may nominate them for further experimentations and development.

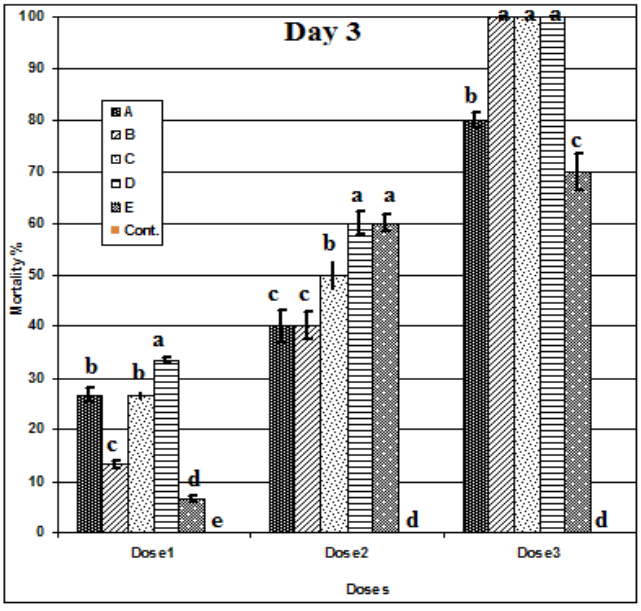
Most of the insecticidal toxins used in agriculture come from a single bacterium *Bacillus thuringiensis* or 'Bt'. Yet, ffrench-Constant *et al*. (2007) reviewed the array of toxins produced by *Photorhabdus* and *Xenorhabdus* bacteria that are symbiotic with entomopathogenic nematodes, and discussed their potential for use in agriculture as alternatives to Bt. It has been suggested that the genes encoding for insecticidal toxins could be added to plants to make them resistant to insect herbivores, in a similar manner to those expressing Bt toxin (Bowen *et al*., 1998) or inserting of both toxin genes (from Bt and *Photorhabdus*) to counter resistance development in target insects consuming these transgenic 'Bt' plants (ffrench-Constant and Bowen, 1999). In this respect, numerous studies have recently shown the importance of the bacterium *Photorhabdus luminescens* as a biological control agent against insect pests (Forst *et al*., 1997 Abdel-Razek, 2003; Mohan *et al*., 2003; Shahina *et al*., 2011), mushroom mites (Bussaman *et al*., 2006; Bussaman *et al*., 2009) and plant pathogens (Webster *et al*., 2002; Shapiro-Ilan *et al*., 2009). The nature of insecticidal activity of *P. luminescens* was investigated for its potential application as an insect control agent (Lining *et al*., 1999). Also, despite the fact that both *Photorhabdus* and *Xenorhabdus* are introduced directly into the insect blood stream by their nematode vectors, they can produce a range of toxins with both oral and inject insecticidal activity (ffrench-Constant *et al*., 2007). If so, research is necessary to find new and more efficacious bacterial strains/isolates or to develop other improvements to maintain efficacy at reasonable levels. It is interesting that the cell-free filtrates of 4×10^{7} cells/ml broth of only the bacterial isolate B, *P. luminescens akhurstii*, could induce 100% insect mortality after just one day of exposure, showing the highest virulence against *G. mellonella* larvae (Figure 1).

These bacteria are non toxic to human. Opportunistic infections by *Photorhabdus* spp., was reported by Farmer *et al*. (1989), but early reports indicated that these bacteria were not associated with nematodes (Boemare, 2002). Both the bacterium alone and its symbiotic *Photorhabdus*–nematode complex are known to be highly pathogenic to insects (Shahina *et al*., 2011). So, despite the high pathogenicity of EPN and their symbionts to insects, their lack of threat to the environment led the U.S. Environmental Protection Agency to exempt EPN from registration requirements; nearly all countries provide a similar exemption. This high degree of safety means that unlike chemicals, or even the bacterial biocontrol agent *Bacillus thuringiensis*, their applications do not require masks or other safety equipment and re-entry time, residues, pollution, and pollinator toxicity are not issues. Even if it happens that such biocontrol agents might have some minor side effects against

certain natural enemies, we will be using indigenous nematodes, neither imported nor introduced (Abd-Elgawad, 1998 and 2001).







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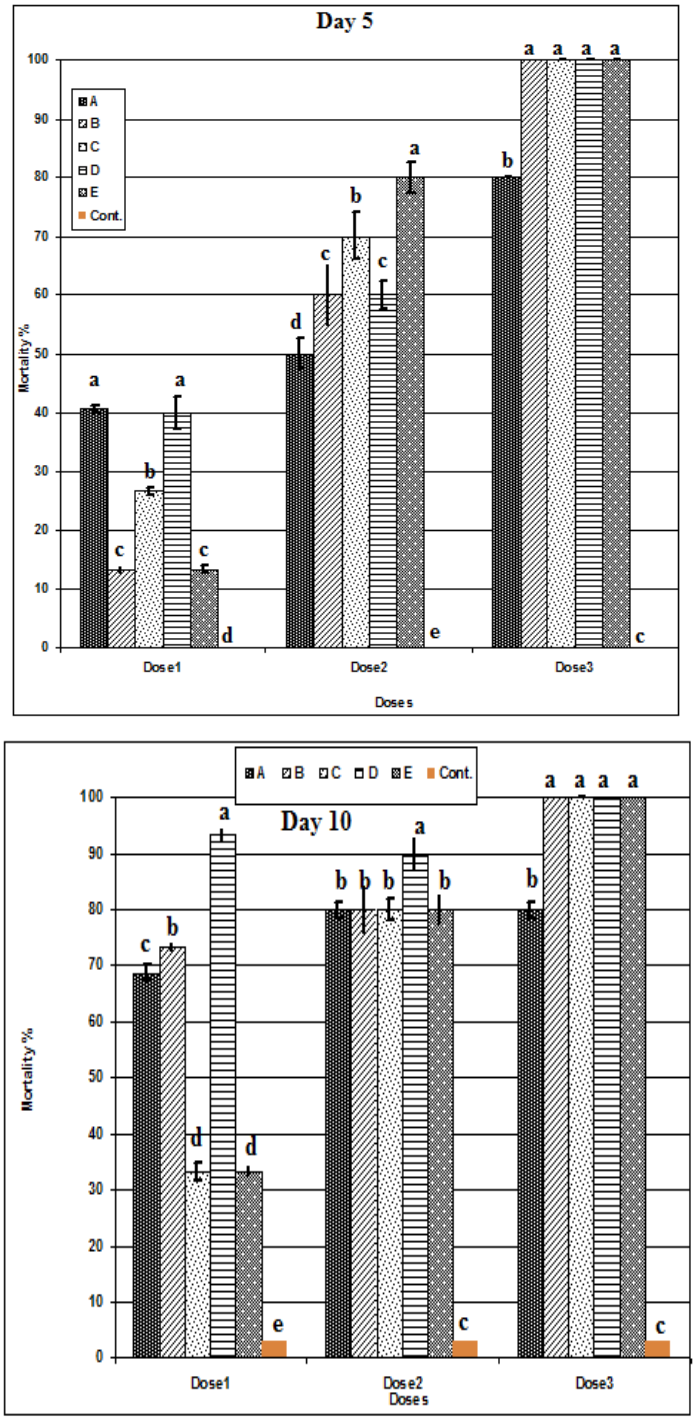

Fig. 1: Mortality rates of *Galleria mellonella* larvae treated with metabolites of three doses (dose 1 = 4 x 10^3, dose 2 = 4 x 10^5 and dose 3 = 4 x 10^7 cells/ml broth) of five Egyptian *P. luminescens* isolates (A, B, C, D, and E) and control (cont) without metabolite recorded after 1, 2, 3, 5, 7, and 10 days. Bars (mean ± SE) in the same time interval and dose with the same letter are not significantly different ($P \leq 0.05$) using DNMR test.

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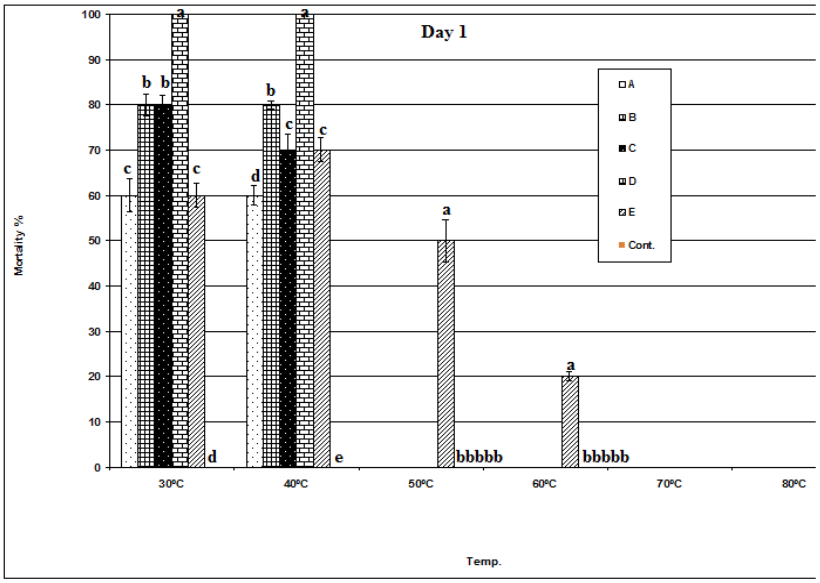

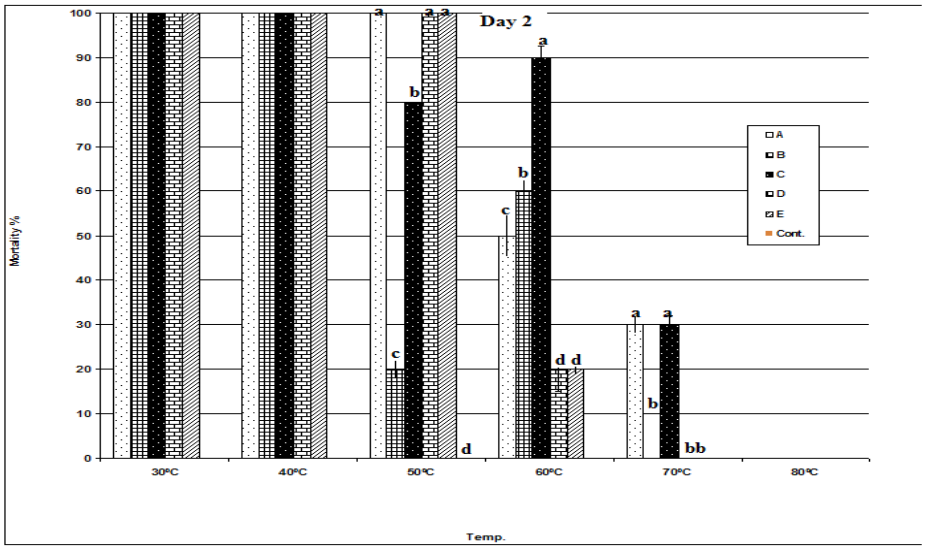

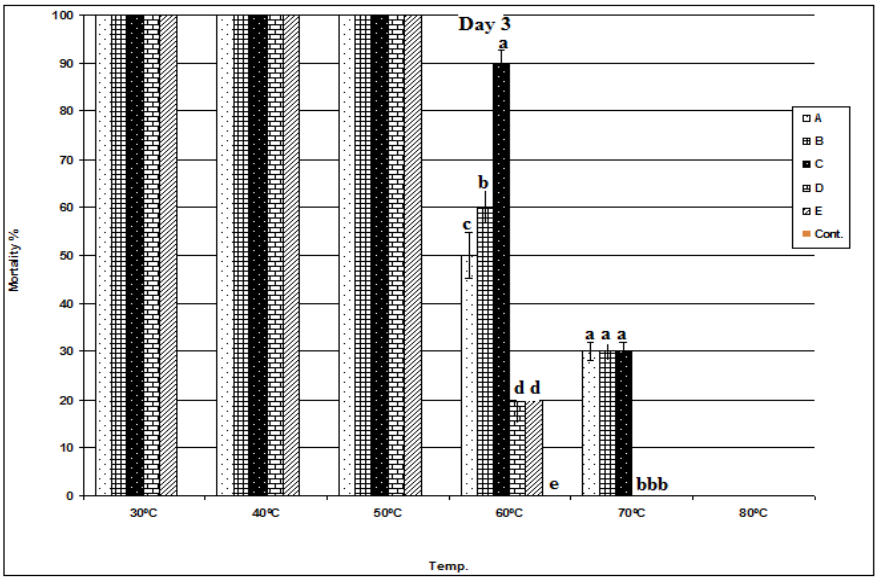

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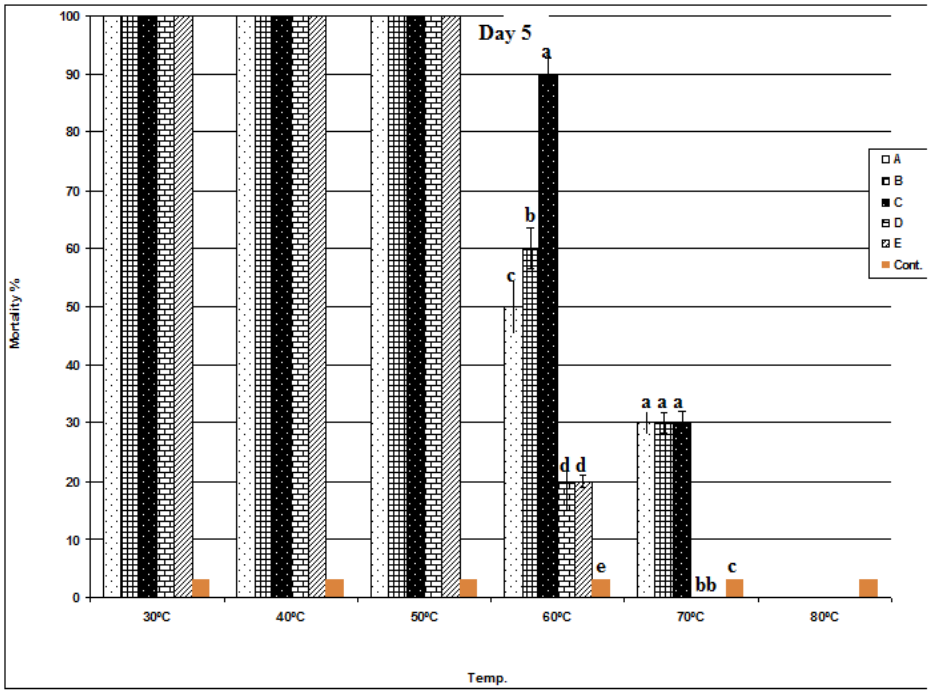
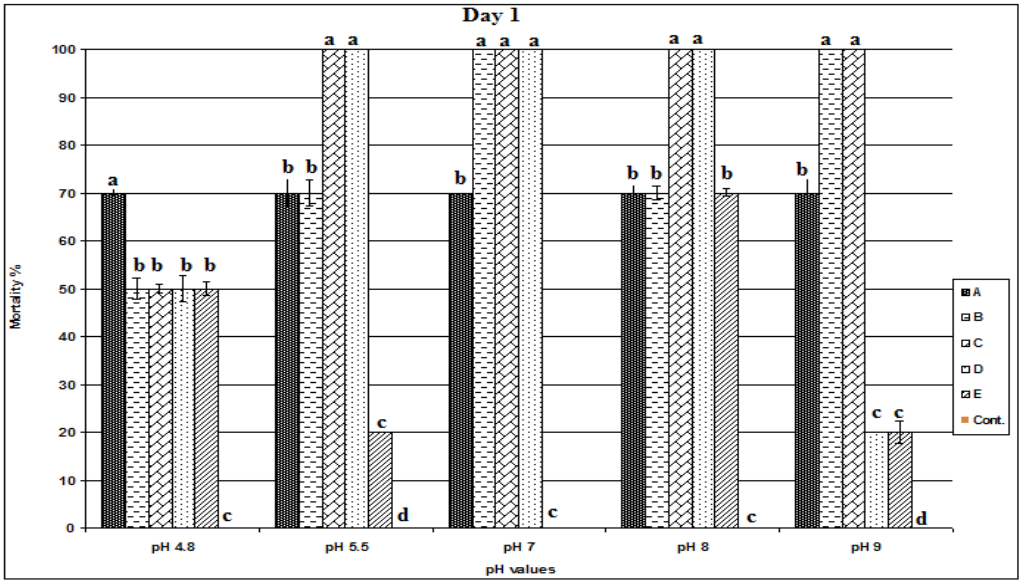


Fig. 2: Mortality rates of *Galleria mellonella* larvae treated with metabolites, initially incubated at 6 different temperatures for 0.5 hour, of five Egyptian *P. luminescens* isolates (A, B, C, D, and E) and control (cont) without metabolite recorded after 1, 2, 3 and 5 days. Bars (mean ± SE) in the same time interval and temperature with the same letter are not significantly different ($P \leq 0.05$) using DNMR test.



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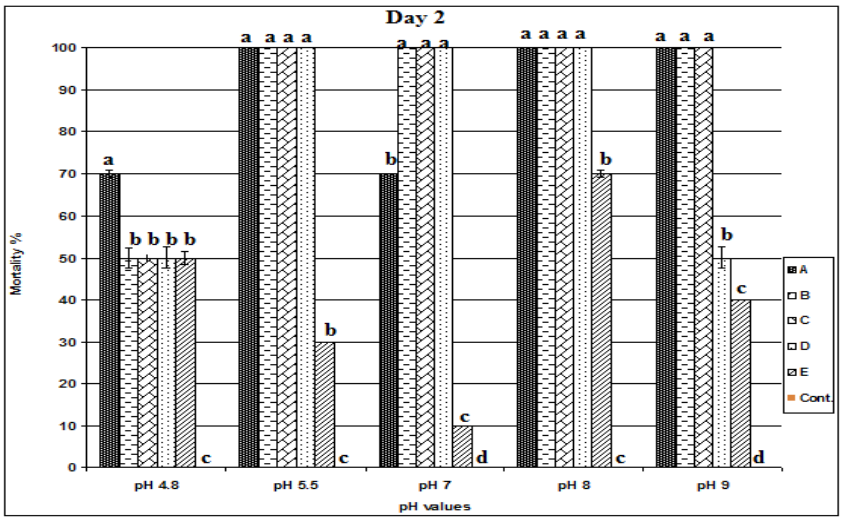

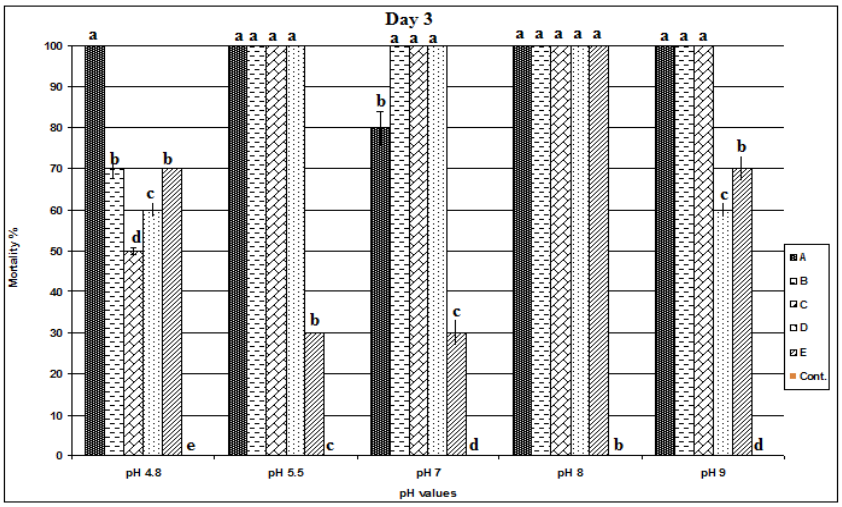

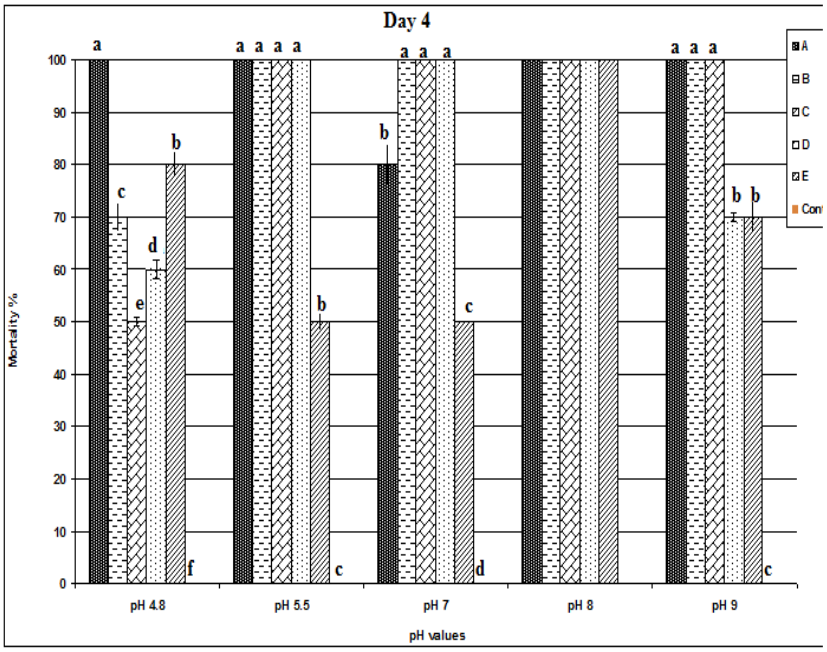

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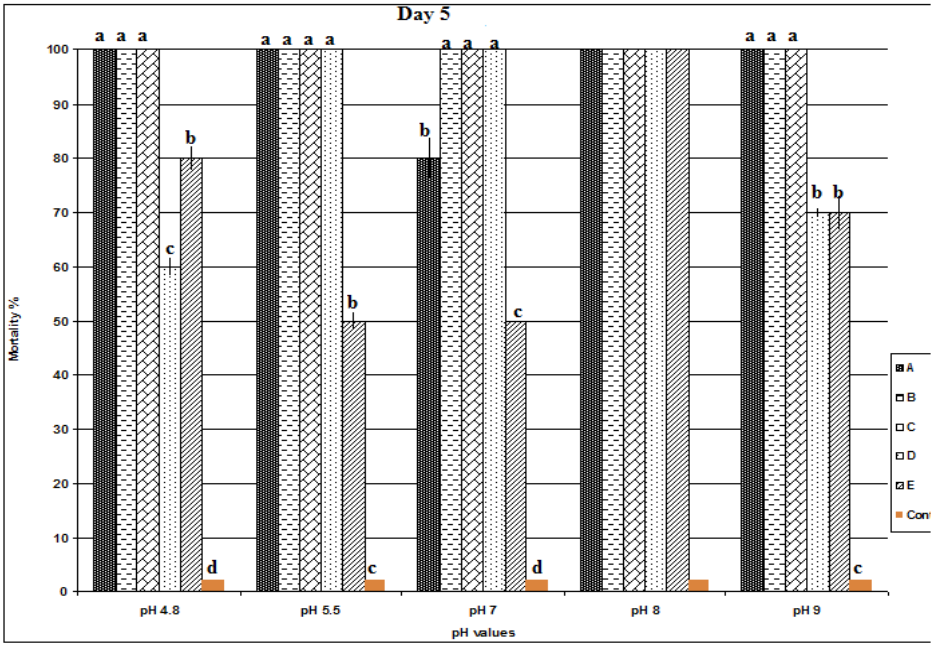
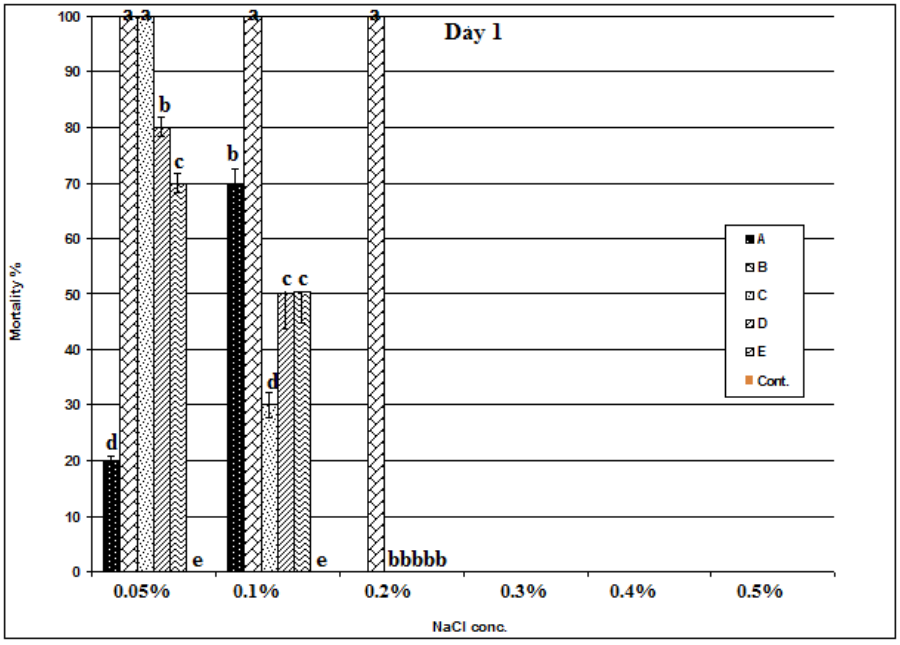
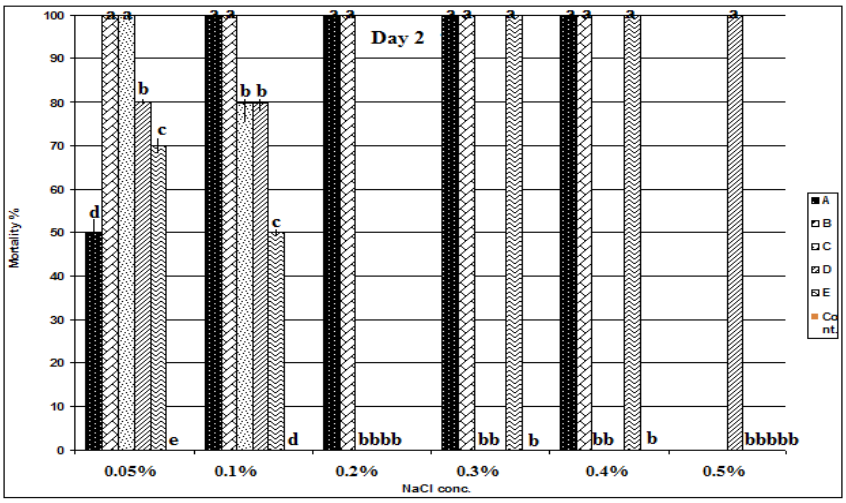
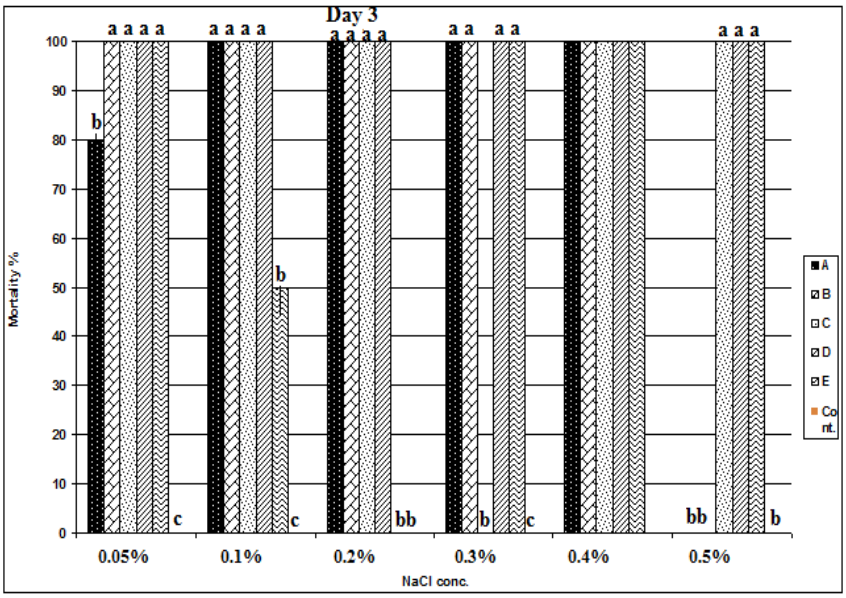


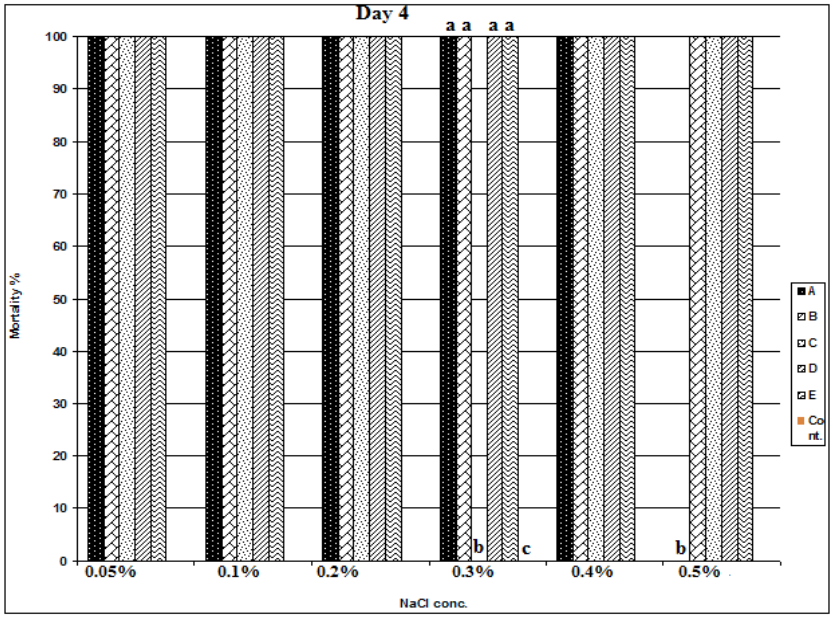
Fig. 3: Mortality rates of Galleria mellonella larvae treated with metabolites, incubated at different pH conditions, of five Egyptian P. luminescens isolates (A, B, C, D, and E) recorded after 1, 2, 3, 4, and 5 days. Bars (mean ± SE) in the same time interval and pH value with the same letter are not significantly different ($P \leq 0.05$) using DNMR test.



J. Appl. Sci. Res., 8(8): 4600-4614, 2012







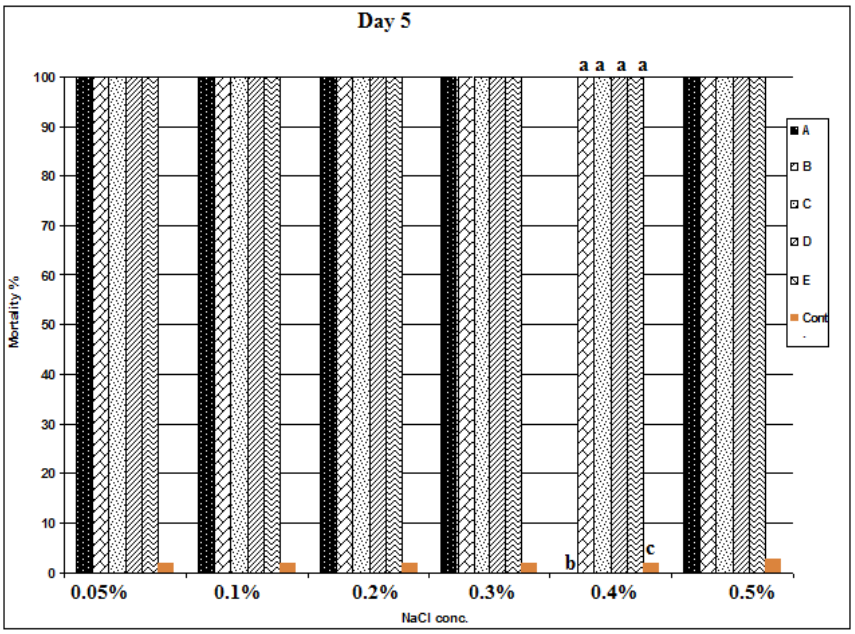


Fig. 4: Mortality rates of *Galleria mellonella* larvae treated with metabolites, with different concentrations: 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5% of NaCl, of five Egyptian *P. luminescens* isolates (A, B, C, D, and E) recorded after 1, 2, 3, 4, and 5 days. Bars (mean ± SE) in the same time interval and NaCl concentration with the same letter are not significantly different ($P \leq 0.05$) using DNMR test.

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