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Research Article
Photorhabdus and *Xenorhabdus* as Symbiotic Bacteria for Bio-Control Housefly (*Musca domestica* L.)

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Abstract

Background and Objective: The housefly poses a threat to the public health of humans and domestic animals since it can carry and transmit pathogens. Despite there are many attempts to control this insect, most of them depend on conventional pesticides. Thus, the current study aimed to evaluate the efficacy of whole-cell suspension, cell-free supernatant and crude cells of the symbiotic bacteria *Photorhabdus* sp. and *Xenorhabdus* sp., as bio-control agents for housefly stages. **Materials and Methods:** The *Photorhabdus* sp. and *Xenorhabdus* sp., were isolated from the entomopathogenic nematodes, *Heterorhabditis indica* and *Steinernema feltiae*, respectively. The phenotypic, as well as the enzymatic characterizations of both bacteria, were determined. In addition, histopathological changes of the alimentary canal of *M. domestica* adults treated with whole-cell suspensions (at 3×10^8 cells mL^{-1}) of both bacteria were carefully examined using transmission electron microscopy. **Results:** The results showed that both symbiotic bacteria significantly suppressed larvae, pupae and adults of *M. domestica*, particularly when they were applied as whole-cell suspensions. For example, the highest concentration of whole-cell suspension, cell-free supernatant and crude cells of *Photorhabdus* sp., induced larval mortalities by 94.7, 64.0 and 45.3%, while those of *Xenorhabdus* sp., induced larval mortalities by 58.7, 46.7 and 30.7% at 96 hrs, respectively. The results also showed that whole-cell suspensions of both symbiotic bacteria caused severe histopathological changes in the ultrastructure of the treated adults' alimentary canal. **Conclusion:** Both symbiotic bacteria can be effectively used, particularly the whole-cell suspension, as bio-control agents against the housefly either in the larval or adult stage.

Key words: Housefly, *Photorhabdus* sp., *Xenorhabdus* sp., whole-cell suspension, cell-free supernatant, crude cells, bio-control agents, histopathology

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The housefly, *Musca domestica* L. (Diptera: Muscidae) is considered one of the most nuisance and medical challenging insects, which is found in close association with human activities from tropical to temperate climes[1,2]. The main breeding sites of the housefly larvae are the cattle and chicken manure piles which are utilized as organic soil component[3]. Housefly adults used to visit garbage heaps and sewers and can feed on all the available food from different sources e.g., decaying foods, carrion, discharges from wounds, sores and sputum[4].

It can pose a threat to public health, since it can carry and transmit pathogens, including fungi, viruses, bacteria and parasites to humans and domestic animals[4]. These pathogens can cause serious diseases, such as cholera, typhoid, salmonella, dysentery, anthrax and tuberculosis[5-10]. Recently, the housefly has been documented as a mechanical vector of SARS-CoV-2[11]. Thus, controlling this insect is crucial for maintaining the public health of humans and domestic animals.

The strategies for controlling houseflies typically depend on using conventional pesticides. However, these pesticides are usually accompanied by negative impacts on the environment and human health[12]. Moreover, such insects can develop resistance to synthetic insecticides[13,14]. The development of insecticide resistance is a global issue that can limit the chemical control of pests[15,16]. An alternative and effective option to pesticides is the use of biological control agents, such as entomopathogenic nematodes (S*teinernema* spp. and *Heterorhabditis* spp.)[17,18], fungi (*Beauveria bassiana*)[19,20], bacteria (e.g., *Bacillus thuringiensis*)[21-23], essential oils and plant extracts[24-26]. Bio-control agents have been included in integrated pest management programs and are effective in controlling a wide range of insects, while remaining safe for humans and reducing the spread of resistant house fly strains.

Recently, symbiotic bacteria such as *Xenorhabdus* spp. and *Photorhabdus* spp., have been studied as biological control agents against some pests, e.g., mosquito larvae[27-29], sandfly larvae[30], cabbageworm larvae and the scarab beetle[31] and meadow spittlebug nymphs[32]. *Xenorhabdus* sp. and *Photorhabdus* sp. are genera in the family Enterobacteriaceae. Both bacteria are obligate mutualistic organisms with the entomopathogenic nematodes[33,34]. These symbiotic bacteria are sources for the production of bioactive compounds including antimicrobial, antiparasitic, cytotoxic compounds and potent insecticidal toxins that can be used against target hosts[35-37]. Different investigations used the symbiotic bacteria of *Xenorhabdus* and *Photorhabdus* for the insects' biological control, however, to our knowledge, the use of both bacteria as biological control agents for houseflies has not been documented in the literature.

Therefore, the current study aimed to investigate the efficacy of six concentrations of whole-cell suspension, cell-free supernatant and crude cells of *Photorhabdus* sp. and *Xenorhabdus* sp., as biological control agents against housefly stages at four exposure times. In addition, the histopathological changes of the alimentary canal of housefly adults treated with whole-cell suspensions of both bacteria were carefully examined using transmission electron microscopy (TEM).

MATERIALS AND METHODS

Study area: This study was conducted in the Entomology and Microbiology Laboratories, Faculty of Agriculture, Menoufia University, Egypt and the Entomology Laboratory, Faculty of Science, Tanta University, Egypt. The study was carried out in 2020 and 2021.

Housefly mass rearing: Larvae of houseflies were collected from manure piles at the Poultry Farm and specimens were transferred to the insectary of the Entomology Department, Faculty of Agriculture, Menoufia University, Egypt. Larvae were reared in glass cages (60×35×40 cm) under the laboratory conditions of 25±2°C and 60±5% relative humidity and 12 D:12 L photoperiod. Cages were covered by a mesh screen with a cloth sleeve opening at the top and provided with 20 watt electric lamps to control the temperature during the winter period. Housefly larvae were provided with a nutrient media in plastic cups, consisting of 9 g powder milk and 5 g yeast that was dissolved in 100 mL of distilled water and combined with 100 g fine bran as described in Abd El-Raheem and Eldafrawy[19].

Mass rearing of entomopathogenic nematodes: Two species of entomopathogenic nematodes (EPNs) (*Heterorhabditis indica* and *Steinernema feltiae*) were obtained from the Biological Control Department, Agricultural Research Center, Dokki, Giza, Egypt. The greater wax moth, *Galleria mellonella,* was used for mass culturing of both EPNs in the Entomology Laboratory, Faculty of Science, Tanta University. *S. feltiae* or *H. indica* was inoculated into *G. mellonella* larvae at a concentration of five infective juveniles per larva. Modified White traps were used to obtain sufficient numbers of infected juvenile nematodes. Rearing of EPNs was the first step for isolating symbiotic bacteria.

Isolation of bacterial symbionts and their metabolites: The method of Bussaman *et al.*[38] was used in the Microbiology Laboratory, Faculty of Agriculture, Menoufia University to isolate bacterial symbionts and their metabolites. Isolation was performed in a clean air Laminar Flow Cabinet sterilized by 70% alcohol with the fan-motor running at high speed for 15 min. Forty-eight hours post-exposure, dead *G. mellonella* larvae due to nematode infection were washed with distilled water, surface sterilized with 70% alcohol and air-dried. Larvae were placed in a Petri-dish and carefully opened using two mounted needles to allow hemolymph to ooze out. Some hemolymph was taken and spread on NBTA consisting of 37 g nutrient agar (Criterion, USA), 25 mg bromothymol blue powder (Lab-Chem UK) with 0.004% (w/v) 2,3,5-triphenyl tetrazolium chloride (Sigma-Aldrich, USA) in one litre of distilled water. Petri-dishes (five replicates) were sealed and incubated upside down at 28°C for 24 hrs. Pure single bacterial colonies were individually grown in 100 mL of Luria-Bertani (LB) broth (Sigma-Aldrich, USA) and placed in an incubator shaker (200 rpm) at 28°C for 48 hrs in complete darkness.

Phenotypic characterization of *Photorhabdus* sp. and *Xenorhabdus* sp.: Both genera were cultured on three different media (nutrient agar, NBTA and MacConkey). The media containing bacteria were incubated at 30°C for 72-96 hrs. Morphological properties of *Photorhabdus* sp. and *Xenorhabdus* sp., colonies were evaluated and compared as previously described by Shahina *et al.*[39] and Kazimierczak *et al.*[40]. Bioluminescence was checked in the darkroom after 10-15 min eye adaptation[41]. The optimum temperature for the growth of bacteria was selected by inoculating cultures in nutrient broth adjusted at calibrated temperatures in digital reciprocating shaker baths (shaking circulation) with cover and drain. Temperatures were adjusted to within ±0.1°C with a range up to 100°C. The shaking speed was 150 rpm. Temperatures from 28-42°C were evaluated in 1°C increment. Original strains were taken from cultures incubated at 28°C to avoid pre-selection as mentioned in Tailliez *et al.*[42].

Bacterial enzymatic test: The catalase tests were performed by placing 50 µL 3% (v/v) drops of H_2O_2 on bacterial colonies, catalase activity was indicated by the presence of gas bubbles[43]. Lipase activity was determined according to Richards and Goodrich-Blair[44]. Protease activity was evaluated on gelatin nutrient agar plates supplemented with 1.5% of gelatin or casein[45]. Chitinase activity was measured as previously described by Aggarwal *et al.*[46].

Preparation of whole-cell bacterial suspension: After incubation in LB broth, bacterial cell concentrations were determined by measuring optical density (OD) via a visible spectrophotometer (Model SPV-72® Germany) at 600 nm. The concentration of the whole-cell suspension was then adjusted to 3×10^9 cells mL^{-1} as a stock solution for the following concentrations (3×10^8, 3×10^7, 3×10^6, 3×10^5 and 3×10^4 cells mL^{-1})[47].

Preparation of cell-free bacterial supernatant (metabolites): The previously prepared whole-cell suspension was centrifuged at 2500×g for 10 min at -4°C and filtered through a 0.22 µm Millipore filter to obtain the cell-free supernatant. The filtrate was checked for sterility by placing 100 µL aliquots on LB agar plates[48]. Aliquots of filtrates were also cultured on NBTA plates to verify the absence of bacterial colonies. Six concentrations (100, 80, 60, 40, 20 and 5%) were prepared by diluting the stock filtrate in LB media.

Crude cells preparation: Bacterial broth suspension was centrifuged at 2500×g and -4°C for 10 min. The supernatant broth was drawn off and the cell pellet was re-suspended in sterile distilled water. The concentration of the bacterial cells was estimated and adjusted to 3×10^9 cells mL^{-1} as stock. The following concentrations were prepared as described above.

Larval bioassay: To evaluate the efficacy of bacteria against housefly larvae, *Photorhabdus* sp. and *Xenorhabdus* sp., were applied as whole-cell suspensions, cell-free supernatants and crude cells under laboratory conditions (28±2°C and 60±5% RH). Whole-cell suspensions and crude cells were applied at six concentrations, 3×10^4, 3×10^5, 3×10^6, 3×10^7, 3×10^8 and 3×10^9 cells mL^{-1}. While cell-free supernatant was applied at concentrations of 5, 20, 40, 60, 80 and 100% by diluting cell-free supernatants with distilled water on a v/v basis using 10 mL falcon tubes. Two milliliters of each concentration from each treatment was mixed well with 20 g of housefly larvae nutrient media in 9×3 cm plastic dishes containing 15 sec instar housefly larvae starved for 12 hrs before application, treatment was replicated five times. A parallel control per treatment with bacterial media or distilled water was included. The mortality of housefly larvae was checked daily for 96 hrs. After 96 hrs, all treatments were maintained and checked daily to record pupation. Developed pupae were separated in falcon tubes sealed with a mesh screen at the top and adult emergence percentages were recorded.

Adults bioassay: Six concentrations, 3×10^4, 3×10^5, 3×10^6, 3×10^7, 3×10^8 and 3×10^9 cells mL^{-1}, of each bacterial suspension, were used. Fifteen adult house flies were starved for 12 hrs before treatment and then kept in plastic cages ($20\times15\times10$ cm^2) under laboratory conditions (28 ± 2°C and 60 ± 5% RH). Cages were covered with mesh screens. Two milliliters of each concentration was placed on a piece of cotton for feeding. A parallel control treatment with bacterial media was included and each treatment was replicated five times. Mortality was checked and recorded every 24 hrs for 96 hrs.

Transmission electron microscopy: The alimentary canal of control and treated housefly adults (3×10^8 cells mL^{-1}) was removed and fixed for 3 h in 4% fixative glutaraldehyde phosphate buffer solution (pH = 7.2) at 4°C. Specimens were then postfixed for 2 hrs in 2% Osmium tetroxide buffer at 4°C. Fixed samples were washed in buffer and dehydrated in a graded series of acetone at 4°C. The samples were then embedded in resin and then sliced into the 90-angstrom-thick sections. Sections were placed on copper grids and stained with uranyl acetate for 5 min then with lead citrate for 2 min[49]. Finally, specimens were examined and imaged on a JEOL JSM-1400 PLUS TEM®.

Statistical analysis: The obtained data from the effect of different concentrations for the whole-cell suspensions, cell-free supernatants and crude cells of *Photorhabdus* sp. and *Xenorhabdus* sp., on the larval and adult stages of the housefly at four exposure times were subjected to the analysis of variance (ANOVA) using SPSS version 21) IBM Inc., Chicago, IL, USA). Means were compared by Duncan's Multiple Range Tests and data were presented as Mean±SE (standard error). The statistical significance was established at $p\leq0.05$ for the analyses of different parameters.

RESULTS

Phenotypic characterization of *Photorhabdus* sp. and *Xenorhabdus* sp.: Axenic bacterial cultures of *Photorhabdus* sp. and *Xenorhabdus* sp. were isolated from *H. indica* and *S. feltiae*, respectively. Both of the symbiotic bacteria were gram-negative, rod-shaped and motile by peritrichous flagella in Table 1. Also, the phospholipase, chitinase and protease assays were positive for both bacteria, meanwhile, the catalase assay was positive in *Photorhabdus* sp. and negative in *Xenorhabdus* sp. Results in Table 1 also showed that the *Photorhabdus* sp., bacterium displayed bioluminescence in the dark after 3-4 days from the culture on LB medium, no bioluminescence was observed from *Xenorhabdus* sp., colonies. Finally, the Maximum temperature for growth of *Photorhabdus* sp., in LB broth was 36°C, but 34°C for *Xenorhabdus* sp., in Table 1 and Fig. 1.

Both symbiotic bacteria were differentiated by colony characteristics on nutrient agar, NBTA and MacConkey in Fig. 2. After 4 days, the secondary bioformulation of *Xenorhabdus* sp., colonies on nutrient agar medium were convex, circular and off-white, while *Photorhabdus* sp., colonies were yellow in Table 1 and Fig. 2a. The *Xenorhabdus* sp., colonies formed on NBTA medium were blue and *Photorhabdus* colonies were dark green in Table 1 and Fig. 2b. On MacConkey medium, the *Xenorhabdus* colonies were brown and *Photorhabdus* colonies appeared pink-red in Table 1 and Fig. 2c.

Bio-efficacy of whole-cell suspensions, cell-free supernatants and crude cells of *Photorhabdus* sp., on larval mortality, pupation and adult emergence of the housefly: Figure 3 showed that the whole-cell suspension, cell-free supernatant and crude cell of *Photorhabdus* sp., significantly reduced the house fly larval population compared with control. The larval mortality rates were concentration and time-dependent, as the concentration and exposure time increased the mortality rates increased.

The whole-cell suspensions of *Photorhabdus* sp., significantly ($p\leq0.001$) reduced the second instar larval survival at all concentrations and time intervals in Fig. 3a. The highest mortality rate, 94.67%, was recorded at 96 hrs with a concentration of 3×10^9 cells mL^{-1} compared to the control treatment. On the other hand, at this concentration, pupation and adult emergence were completely inhibited in Fig. 4a.

Concerning the cell-free supernatant, the mortality percentages of the housefly second instar larvae showed that *Photorhabdus* sp., induced a moderate insecticidal activity ($p\leq0.001$), in Fig. 3b. With the highest concentration of the cell-free supernatant (i.e., 100% and 96 hrs), the larval mortality percentage was 64.0% compared with 0% for the control. Meanwhile, the pupation and adult emergence percentages were 26.7 and 5.3%, compared with 100 and 96% under the control treatment, respectively in Fig. 4b. However, the effects of the highest two concentrations (i.e., 80 and 100%) on the larval mortality were not significantly differed at 24, 72 and 96 hrs.

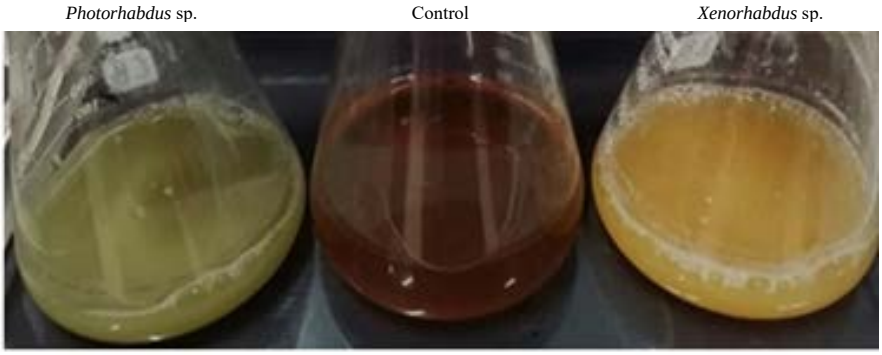

Fig. 1: Growth of *Photorhabdus* sp. and *Xenorhabdus* sp., in LB broth at the maximum temperature

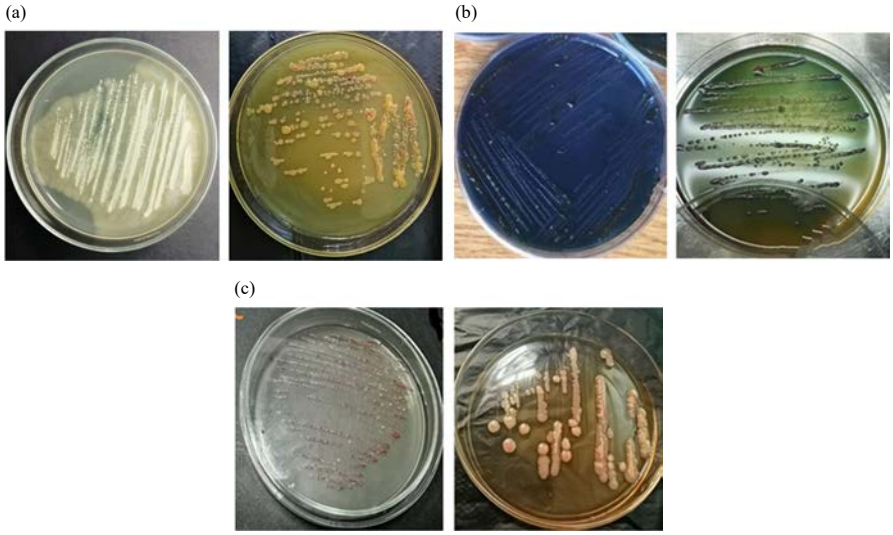

Fig. 2(a-c): *Xenorhabdus* sp., colony (left graph) and *Photorhabdus* sp. (right graph) colony on, (a) Nutrient agar, (b) NBTA and (c) McConkey

Table 1: Phenotypic characterization of *Photorhabdus* sp. and *Xenorhabdus* sp., bacteria

Characteristics	*Photorhabdus* sp.	*Xenorhabdus* sp.
Gram staining	(-)	(-)
Shape	Rod	Rod
Motility	+	+
Flagella type	Peritrichous	Peritrichous
Pigmentation on nutrient agar	Yellow	Off-white
Pigmentation on NBTA	Dark green	Blue
Pigmentation on MacConkey agar	Pink-red	Brown
Bioluminescence	+	-
Maximum temperature for growth [Luria-Bertani broth]	36°C	34°C
Catalase	+	-
Phospholipase	+	+
Chitinase	+	+
Gelatin/casein proteolysis	+/+	+/+

In addition, the different crude cells' concentrations of *Photorhabdus* sp., significantly induced ($p \leq 0.001$) the second instar larval mortality in Fig. 3c, For instance, the larval mortality under the highest crude cells concentration (i.e., 3×10^9 cells mL^{-1}) were 45.3 (at 96 hrs of exposure), while it was zero under the control treatment. Nevertheless, the effects of the highest two concentrations (i.e., 3×10^8 and 3×10^9 cells mL^{-1}) on the larval mortality were not significantly

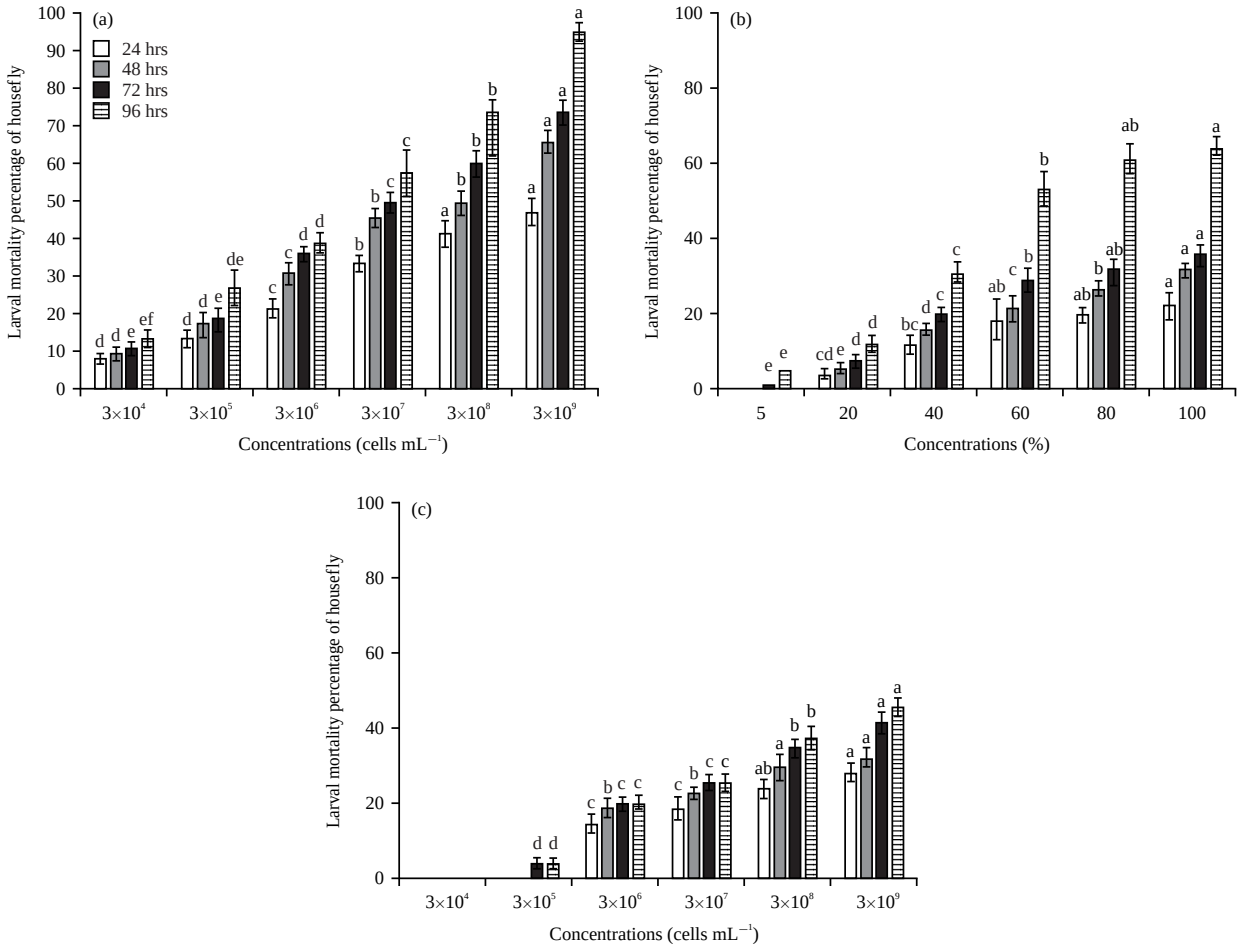

Fig. 3(a-c): Effect of *Photorhabdus* sp., on larval mortality of houseflies at different concentrations and time intervals, (a) Whole-cell suspension, (b) Cell-free supernatant and (c) Crude cells

The larval mortality percentage of houseflies under the control treatment was zero. Columns for each time at different concentrations followed by the same letter are not significant at the 5% level of significance.

differed at 24 and 48 hrs. Moreover, the pupation and adult emergence percentages were reduced upon the highest crude cells concentration treatment in Fig. 4c, as the pupation and adult emergence percentages were 22.7 and 5.3% (at 96 hrs of exposure), while they were 98.0 and 93.3% under the control treatment, respectively.

Bio-efficacy of *Xenorhabdus* sp., whole-cell suspensions, cell-free supernatants and crude cells on larval mortality, pupation and adult emergence of the housefly: Figure 5a and Fig. 6a shows, the treatment with the whole-cell suspensions *of Xenorhabdus* significantly ($p \leq 0.001$) reduced the housefly larval survival (at different time intervals), pupation and adult emergence compared with controls. For example, at the highest concentration (i.e., 3×10^9 cells mL^{-1}) the larval mortality increased from zero under the control treatment to 58.7% at 96 hrs (Fig. 5a), while the pupation and adult emergence percentages reduced from 100% to 17.3 and 4.0%, respectively.

On the other hand, treating the housefly second instar larvae with the cell-free supernatants of *Xenorhabdus* sp. induced a moderate insecticidal effect compared with whole-cell suspensions in Fig. 5b. In this respect, the larval mortality, pupation and adult emergence percentages were 46.7 (at 96 hrs), 25.3 and 17.3% at 100% concentration, respectively in Fig. 5b and 6b. Generally, the effects of the highest two concentrations of whole-cell suspensions and cell-free supernatants on the larval mortality were not significantly differed at 72 and 96 hrs.

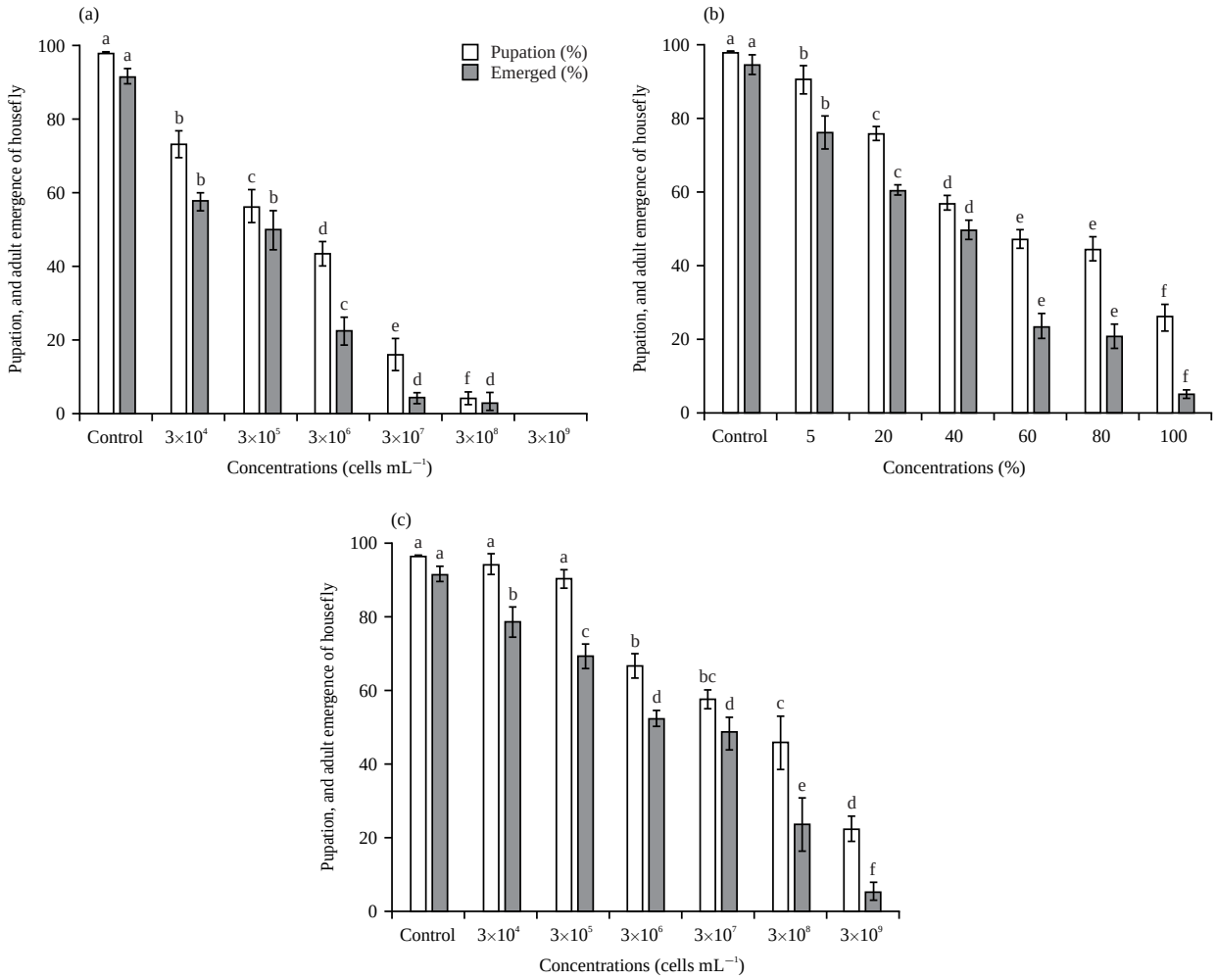

Fig. 4(a-c): Effect of *Photorhabdus* sp., on pupation and adult emergence of the housefly at different concentrations and time intervals, (a) Whole-cell suspension, (b) Cell-free supernatant and (c) Crude cells

Columns for each parameter at different concentrations followed by the same letter are not significant at the 5% level of significance

Regarding the crude cells of *Xenorhabdus* sp., the different concentrations result in the lowest insecticidal activity against the housefly larval compared to those obtained from whole-cell suspension and cell-free supernatant in Fig. 5c. At the highest concentration (3×10^9 cells mL^{-1}), the mortality was 30.7% at 96 hrs. However, this concentration slightly decreased the pupation and emergence percentages to 57.3 and 53.3%, respectively in Fig. 6c.

In conclusion, it was shown that the larval percentage mortality was increased by increasing both the bacterium concentrations and the time intervals, while vice versa was noted for the percentage of pupation and adult emergence. Furthermore, it was obvious that at the highest concentration and exposer time (96 hrs post-application), the most virulent bacterial form of *Photorhabdus* sp. and *Xenorhabdus* sp., was the whole-cell suspension, recording 94.67 and 58.67% larval mortality, respectively, followed by the cell-free supernatant giving 64 and 46.67 % larval mortality, respectively and finally, the crude cells which reduced the larval survival by 45 and 30.67%, respectively, Generally, the results showed that the *Photorhabdus* sp., was better than *Xenorhabdus* in terms of bio-controlling the housefly larvae in Fig. 7a-c.

Morpho-pathological changes in house fly larvae caused by *Xenorhabdus* and *Photorhabdus* symbiotic bacteria: Figure 8 shows the control house fly larvae have a cylindrical body with a greasy, cream-colour appearance in Fig. 8a, upon treatment with *Xenorhabdus* sp., bacterium, the colour turned to greyish in Fig. 8b. Meanwhile, the larvae acquired the reddish-brown colour when they were treated with *Photorhabdus* sp., bacterium in Fig. 8c.

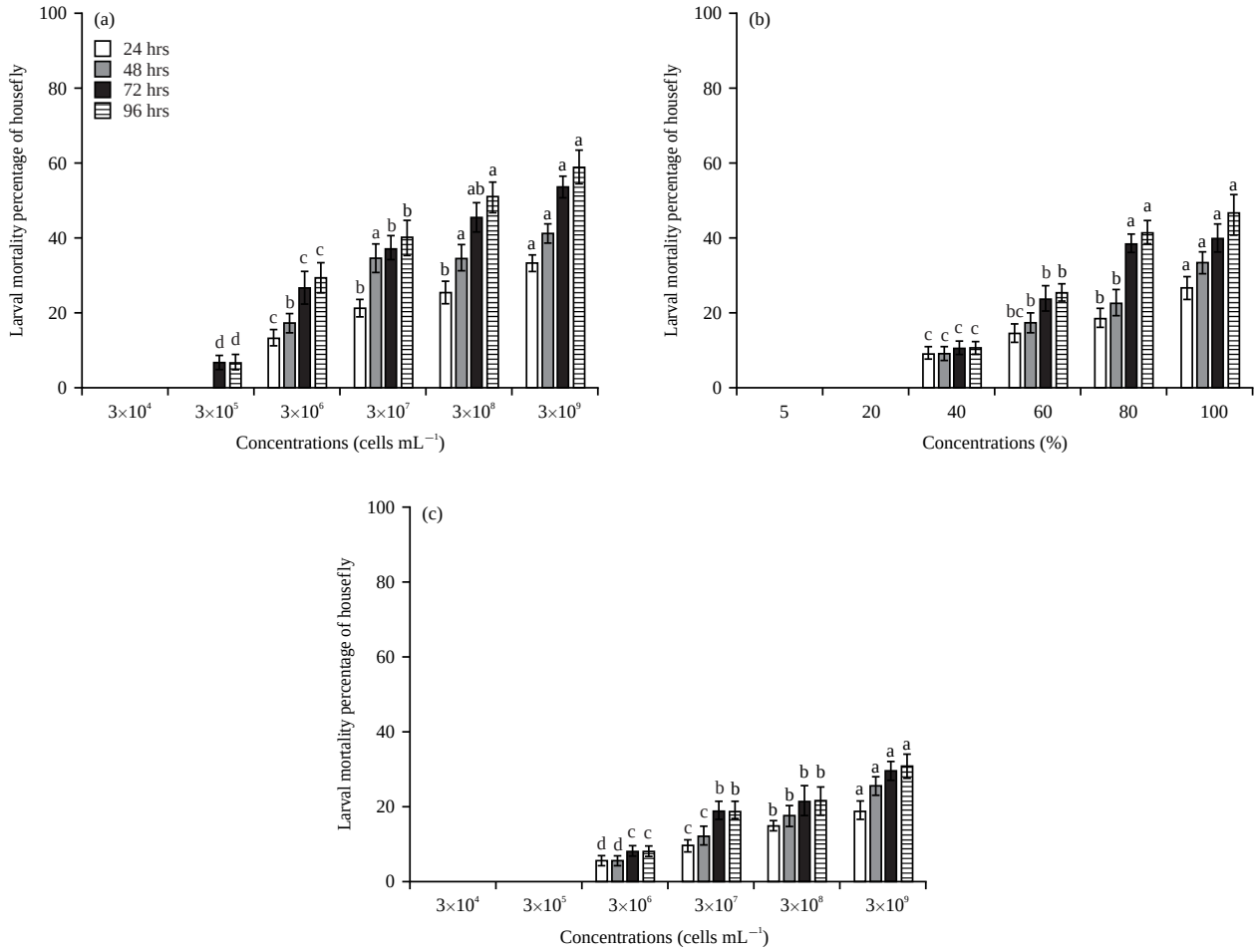

Fig. 5(a-c): Effect of *Xenorhabdus* sp., on larval mortality of houseflies at different concentrations and time intervals, (a) Whole-cell suspension, (b) Cell-free supernatant and (c) Crude cells

Larval mortality percentage of houseflies under the control treatment was zero, columns for each time at different concentrations followed by the same letter are not significant at the 5% level of significance

Bio-efficacy of whole-cell suspensions of *Photorhabdus* sp. and *Xenorhabdus* sp., against *M. domestica* adults: As the whole-cell suspension of both symbiotic bacteria was the most virulent form against larvae, it was tested against the housefly adults in Table 2. The whole-cell suspensions of both *Photorhabdus* sp. and *Xenorhabdus* sp., significantly ($p \leq 0.001$) increased the mortality of the adults in comparison to the control treatment. *Photorhabdus* sp., was more effective as it induced 100% mortality with the highest concentration (3×10^9 cells mL^{-1}) at 72 hrs of the exposure, while *Xenorhabdus* sp., induced 93.3% at 96 hrs (Table 2). Concerning the average adult mortality at different concentrations and time intervals, the whole-cell suspension of *Photorhabdus* sp. super passed *Xenorhabdus* sp., at 24, 48 and 72 hrs. However, the effect of both symbiotic bacteria was not significantly differed at 96 hrs.

TEM characterization of the pathological effect of *Xenorhabdus* sp. and *Photorhabdus* sp., whole-cell suspensions on the alimentary canal of housefly adults: The histoarchitecture of the alimentary canal of untreated adult houseflies in Fig. 9a shows epithelium consisting of columnar, goblet and regenerative cells bound externally by a basement membrane. These cells are separated from the lumen by a continuous peritrophic membrane.

Upon treatment with 3×10^8 cells mL^{-1} of *Xenorhabdus* sp., whole-cell suspension, extensive damage was detected in the alimentary canal of *M. domestica* adults in Fig. 9b, with some epithelial cells becoming detached from the gut wall and sloughed into the gut lumen. Others were destroyed, with the cytoplasm being vacuolated and emptied into the lumen, moreover the presence of dense bacterial cells inside the alimentary canal was observed in Fig. 9c.

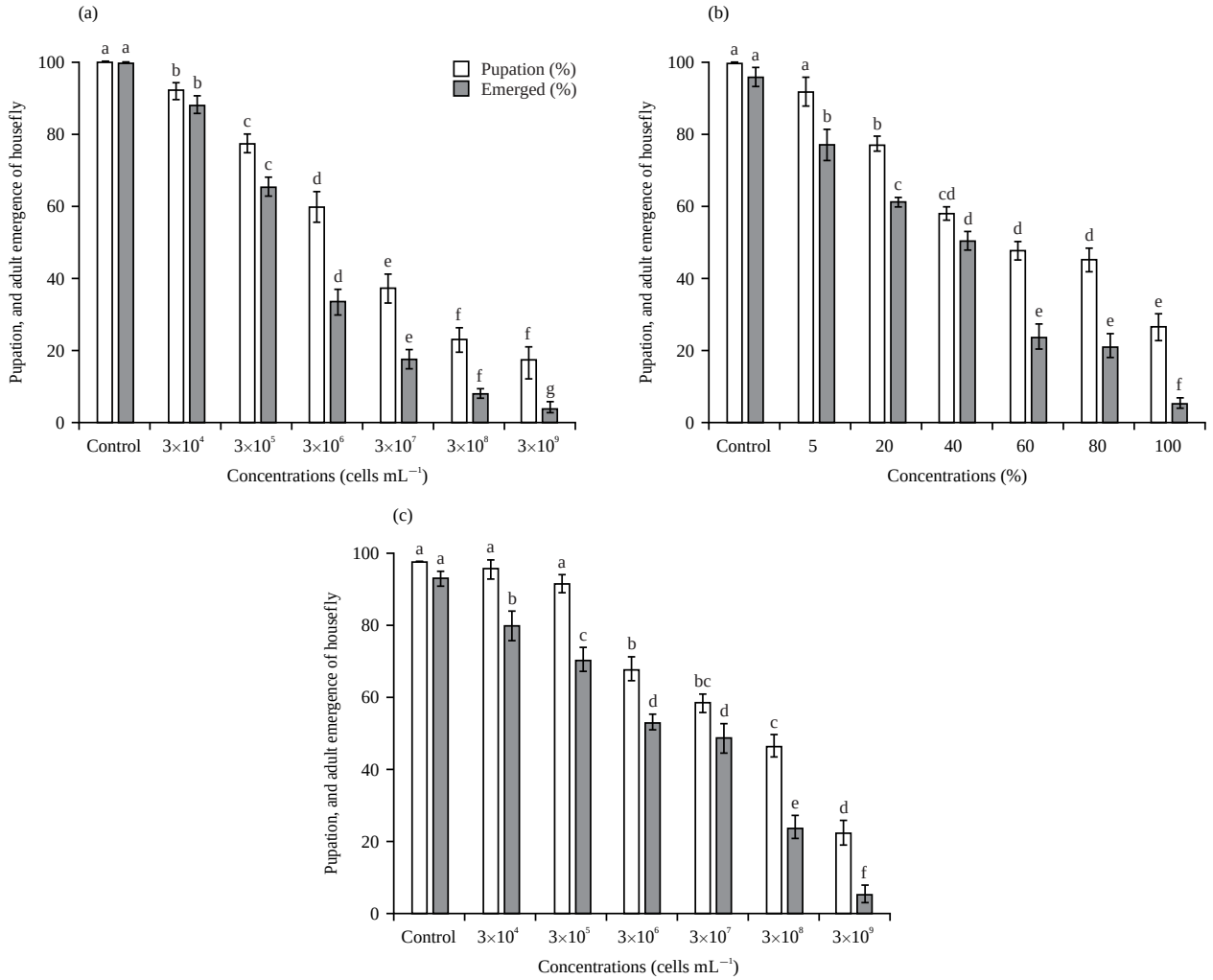

Fig. 6(a-c): Effect of *Xenorhabdus* sp., on pupation and adult emergence of the housefly at different concentrations and time intervals, (a) Whole-cell suspension, (b) Cell-free supernatant and (c) Crude cells

Columns for each parameter at different concentrations followed by the same letter are not significant at the 5% level of significance

Table 2: Mortality percentages (Mean±SE) of *M. domestica* adults caused by the whole-cell suspension of *Photorhabdus* sp., *Xenorhabdus* sp., bacteria at different concentrations and time intervals

		Mortality percentages (Mean±SE) of *M. domestica* adults at an exposure time			
Bacterium type	Concentrations (cells mL^{-1})	24 hrs	48 hrs	72 hrs	96 hrs
Photorhabdus sp.	3×10^4	6.67±2.11^{d}	9.33±1.63^{e}	22.67±3.40^{e}	25.33±2.49^{e}
	3×10^5	16.00±3.40^{c}	26.67±2.11^{d}	40.00±2.98^{d}	44.00±6.18^{d}
	3×10^6	24.00±3.40^{c}	38.67±4.90^{c}	49.33±4.00^{d}	52.00±6.11^{d}
	3×10^7	33.33±2.11^{b}	45.33±2.49^{c}	61.33±3.27^{c}	66.67±4.22^{c}
	3×10^8	44.00±3.40^{a}	54.67±3.89^{b}	74.67±5.33^{b}	81.34±3.89^{b}
	3×10^9	52.00±5.33^{a}	74.67±3.89^{a}	100.00^{a}	100.00^{a}
Average		29.33±3.29^{a}	41.56±3.15^{a}	58.00±3.16^{a}	61.56±3.82^{a}
Xenorhabdus sp.	3×10^4	2.67±1.63^{c}	6.67±2.11^{d}	12.00±2.49^{d}	28.00±3.89^{e}
	3×10^5	9.33±2.67^{c}	17.33±3.40^{c}	33.33±2.11^{c}	48.00±3.89^{d}
	3×10^6	24.00±5.81^{b}	34.67±4.42^{b}	49.33±6.18^{b}	61.33±2.50^{c}
	3×10^7	26.67±2.11^{ab}	38.67±4.42^{ab}	62.67±4.99^{a}	69.33±4.99^{bc}
	3×10^8	32.00±3.89^{ab}	42.67±3.40^{ab}	69.33±3.40^{a}	74.67±3.89^{b}
	3×10^9	36.00±3.40^{a}	48.00±3.27^{a}	73.33±7.60^{a}	93.33±2.11^{a}
Average		21.78±3.25^{B}	31.34±3.50^{B}	50.00±4.46^{B}	62.44±3.55^{A}

* In each column, means followed by the same letter are not significant at the 5% level of significance, the adult mortality percentage of houseflies under the control treatment was zero

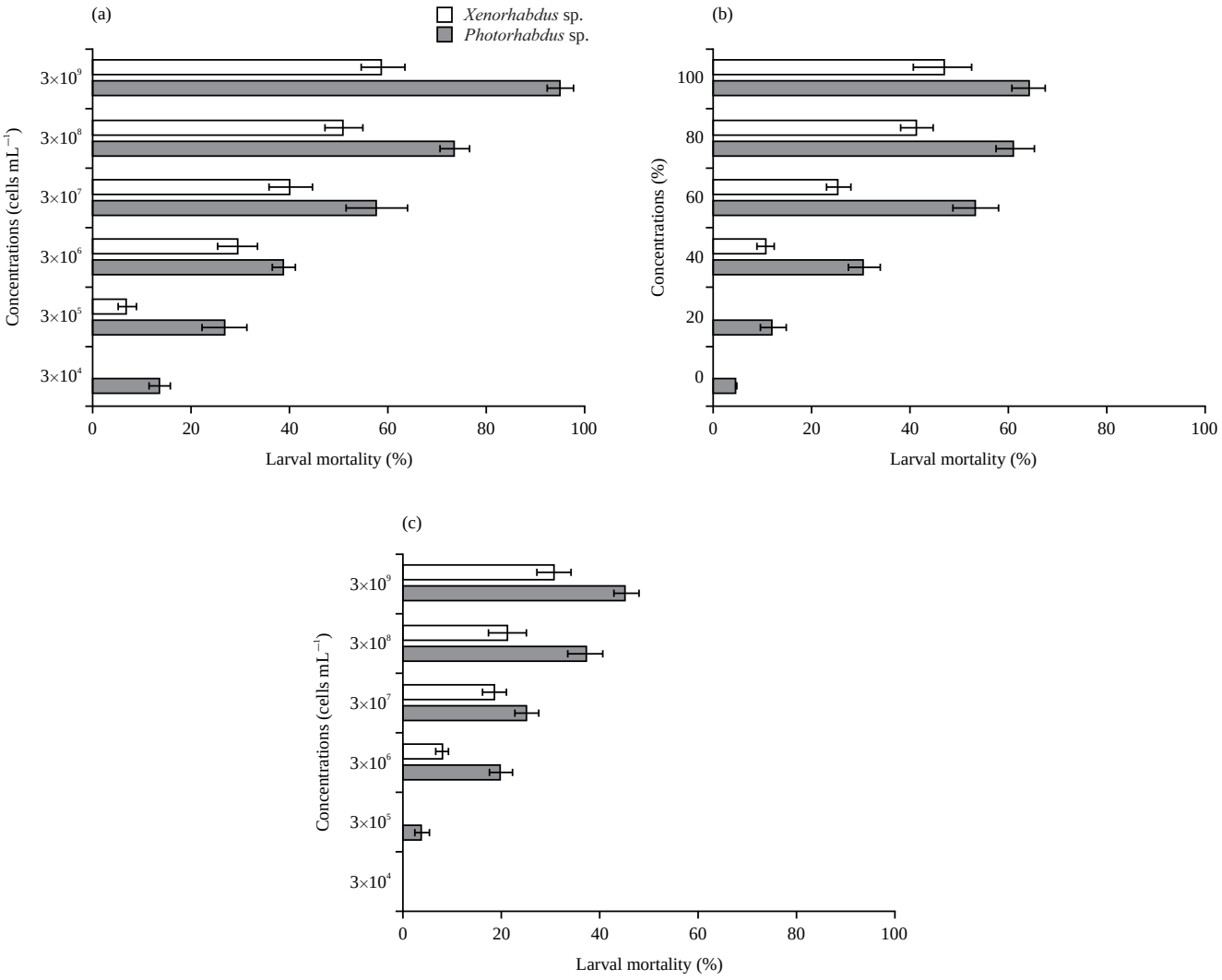

Fig. 7(a-c): Effect of different concentrations on *Photorhabdus* sp. and *Xenorhabdus* sp., on the mortality of *M. domestica* larval at 96 hrs post-application of, (a) Whole-cell suspension, (b) Cell-free supernatant and (c) Crude cells

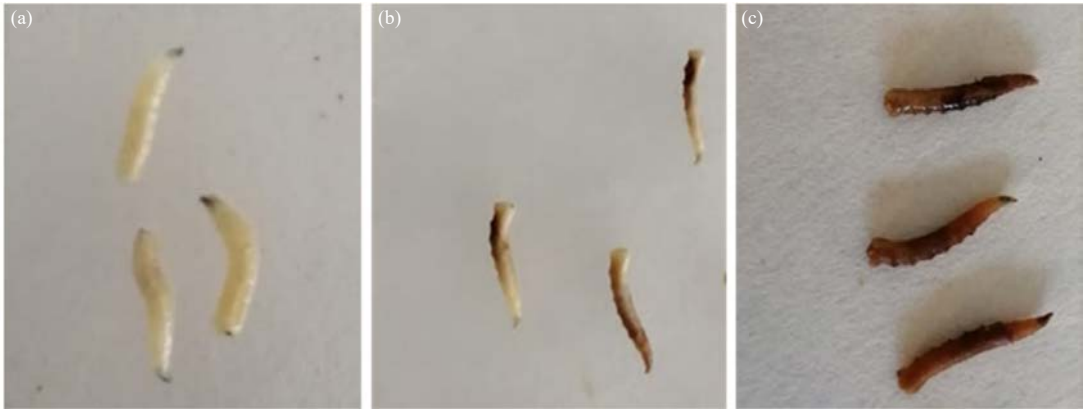

Fig. 8(a-c): Housefly larvae, (a) Control, (b) Treated with *Xenorhabdus* sp. and (c) Treated with *Photorhabdus* sp.

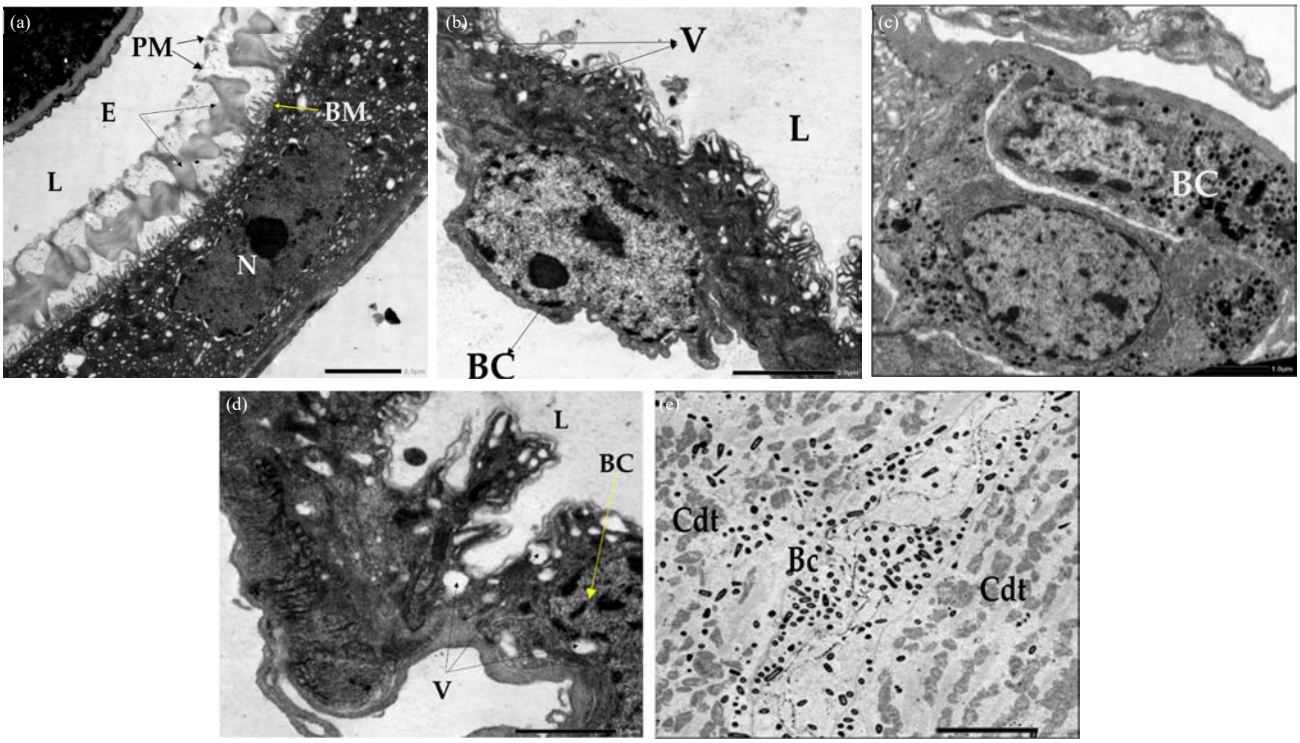

Fig. 9(a-e): (a) TEM of the alimentary canal of control *M. domestica* adults, showing the normal structure of Nucleus (N), Basement membrane (BM), Epithelium (E), Peritrophic membrane (PM) and Lumen (L), (b) TEM of the alimentary canal of adult *M. domestica* treated with 3×10^8 cells mL^{-1} of *Xenorhabdus* sp., whole-cell suspension showing extensive damage to epithelial cells, cytoplasmic vacuoles (V) and rod shape bacterial cells (BC), (c) *Xenorhabdus* bacterial cells (BC) inside the alimentary canal of adult *M. domestica*, (d) TEM of the alimentary canal of adult *M. domestica* treated with 3×10^8 cells mL^{-1} of *Photorhabdus* sp., whole-cell suspension showing disrupted tissues, cytoplasmic vacuoles (V) and rod bacterial cells (BC) and (e) *Photorhabdus* bacterial cells (BC) inside the alimentary canal of adult *M. domestica* with complete disrupted tissues (Cdt)

On the other hand, the treatment with 3×10^8 cells mL^{-1} of *Photorhabdus* sp., induced complete damage in multiple points along the alimentary canal with cytoplasmic vacuoles in Fig. 9d, all the tissues were destroyed and altered beyond recognition in coinciding with the presence of dense *Photorhabdus* bacterial cells and vacuolated cytoplasm in Fig. 9e.

DISCUSSION

Preserving human health is one of the most important priorities of peoples, so the elimination of any threat to public health rank first in interest. The housefly is one of the major threats to human health and domestic animals. Therefore, this study was designed to investigate the insecticidal activity of *Photorhabdus* sp. and *Xenorhabdus* sp., bacteria isolated from EPNs *Steinernema carpocapsae* and *Heterorhabditis indica*, respectively, on housefly larvae and adults. In addition, pupation and emergence percentages were recorded. This study was the first to evaluate the efficacy of *Xenorhabdus* sp. and *Photorhabdus* sp., bacteria on the survival of larval and adult stages of houseflies. However, many previous studies document the effectiveness of EPNs on *M. domestica*. For example, Arriaga and Cortez Madrigal[18], Sangjin *et al.*[50] and Mahmoud *et al.*[51] successfully applied different species of *Steinernema* and *Heterorhabditis* nematodes for the control of houseflies. Abd El-Raheem and Sweelam[17] showed that both *S. feltiae* and *H. bacteriophora* (Egyptian strains) successfully controlled houseflies under laboratory and field conditions. Likewise, Arriaga and Madrigal[18], Archana *et al.*[52] found that *H. indica* induced mortality in *M. domestica* larvae and adult stages. Additionally, the biological activity of both bacteria, *Photorhabdus* and *Xenorhabdus*, have been reported against many dipteran species. Vitta *et al.*[29] and

Yooyangket *et al*.[53] confirmed that both *Photorhabdus* and *Xenorhabdus* exhibit oral toxicity against larval mosquitoes *Aedes aegypti* and *Ae. albopictus*, larvae started to die 24 hrs after application. Furthermore, El-Sadawy *et al*.[30] reported toxicity of both *P. luminescens* and *X. nematophila* against larvae of the sandfly, *Phlebotomus papatasi*.

In the present study, both *Photorhabdus* and *Xenorhabdus* bacteria caused significant mortality in *M. domestica* larval and adult stages and *Photorhabdus* was more effective than *Xenorhabdus*. Mortality increased with increasing bacterial concentration and exposure time. Likewise, Ahantarig *et al*.[54] reported that *P. asymbiotica* was highly lethal to both *Ae. aegypti* and *Ae. albopictus*. Similarly, da Silva *et al*.[27] reported that *P. luminescens* caused 73% mortality of *Ae. aegypti* larvae via feeding and *X. nematophila* was less pathogenic, killing 52% of larvae. Additionally, *P. luminescens akhurstii*, a symbionts of *H. indica* caused 100% mortality of *Galleria mellonella* and *Spodoptera frugiperda* larvae 48 h after injection with 1×10^3 and 1×10^4 CFU/larva[55]. In addition, injecting *G. mellonella* larvae with 1×10^2 cells/larva of *P. luminescens* caused 100% mortality[56]. Finally, Elbrense *et al*.[31] reported that *Photorhabdus* caused significant mortality in cabbage worm, *Pieris rapae* and the scarab beetle, *Pentodon algerinus* and was more effective than *Xenorhabdus*. Conversely, Khandelwal and Banerjee-Bhatnagar[57] showed that the activity of naturally secreted outer membrane vesicles (OMVs) isolated from the *Xenorhabdus* strain was higher than the specific activity of OMVs isolated from *Photorhabdus* strain against neonatal larvae of *Helicoverpa armigera*. Likewise, Mahar *et al*.[47] indicated that *X. nematophila* was more effective than *P. luminescens* for control of locusts and seems to be the most suitable symbiotic bacterium for field application. Also, Vitta *et al*.[29] and Fukruksa *et al*.[58] showed that *X. ehlersii* isolates were likely to be effective in killing *Ae. aegypti* and *Ae. albopictus* larvae under both fed and unfed conditions with high mortality after 48-96 hrs after application, however, similar isolates of *P. luminescens* exhibited unsatisfied results.

The variable effectiveness of the EPB bacteria *Photorhabdus* sp. and *Xenorhabdus* sp., against insects can be attributed to several factors, including symbiotic bacterial tolerance to host immune defences, the sensitivity of insect stage to the bacteria and the failure of bacteria to kill the target host. Furthermore, mortality rates of treated insects could be linked to the quantity of bacterial cells encountered[34,54,59]. *Photorhabdus* sp. and *Xenorhabdus* sp., bacteria in the current study were used against housefly larvae in three distinct forms: Whole-cell suspensions, cell-free supernatants and crude cells. All the three forms were proved to be effective, however, the whole-cell suspensions were most effective, possibly because whole-cell suspensions contain both bacterial metabolites and bacterial cells. So the effect is an integrative way, if one of them fails, the other still kills the insect[60]. Current findings agreed with previous study[47,61] which demonstrated that cell suspensions of *X. nematophila* and *P. luminescens* were lethal to locusts and *G. mellonella*, respectively. Also, *P. luminescens* suspensions demonstrated substantial toxicity to *Drosophila suzukii* larvae and pupae, with 70-100% mortality after 10 days of treatment[49].

EL-Sadawy *et al*.[30] reported that treatment of sand fly larvae with *P. luminescens* bacterial toxin resulted in a mortality of 60%. Further, Vicente-Díez *et al*.[32] reported that after 5 days of exposure, cell-free supernatants from *P. laumondii* killed 64% of treated meadow spittlebug nymphs. Similarly, Ahmed *et al*.[28] reported that the *P. luminescens* bacterial toxin complexes showed promising larvicidal activities against the mosquito vector, *Culex pipiens*. Conversely, Shah *et al*.[62] found that *X. nematophila* bacterial supernatants and cell suspensions caused more than 91% larval mortality at a concentration of 50% and 48 h after treatment, while *P. laumondii* bacterial cell suspensions killed 75%, but bacterial supernatants were not effective at any tested concentration.

Current findings also demonstrated that crude cells are less effective, which we believe is due to the removal of the most critical metabolites during extraction. This assumption is supported by Bussaman *et al*.[38] who found that crude cells did not affect mushroom mite (*Luciaphorus* sp.) compared to cell-free supernatants and whole-cell suspensions. Likewise, El-Sadawy *et al*.[30] showed that the *P. luminescens* and *X. nematophila* crude cells were not effective against the second instar larvae of the sandfly, *P. papatasi*. The three forms of *Photorhabdus* and *Xenorhabdus* bacteria also significantly reduced pupation and adult emergence percentages. The whole-cell suspension of *Photorhabdus* sp. was most effective. Similarly, Park *et al*.[63] reported that culture fluids from *Xenorhabdus* and *Photorhabdus* spp., delayed pupation and emergence of adult mosquitoes of *Ae. albopictus* and *C. pipiens*.

The present study indicated that *M. domestica* adults were more susceptible than larvae to both symbiotic bacteria. This can be due to that the larvae feed on the nutrient medium mixed with the bacterial suspension, so the contents of nutrient media can affect the interaction of bacterial cells with the host. On the other hand, housefly adults fed directly on the bacterial whole suspension without any additions, consequently adults may have consumed a higher amount of the bacteria and response better than the larvae.

The specific mechanisms by which *Photorhabdus* and *Xenorhabdus* kill their insect hosts, whether larvae or adults are yet unknown. *Photorhabdus* and *Xenorhabdus* can resist cellular immune responses by killing hemocytes and preventing phagocytosis, resulting in hemolymph septicemia produced by several toxic metabolites[64-68]. Additional research is needed to understand how symbiotic bacteria, *Photorhabdus* and *Xenorhabdus* destroy the target hosts.

In the present study, the pathological effect of whole-cell suspension at the concentration of 3×10^8 cells mL^{-1} on the ultrastructure of the alimentary canal of *M. domestica* adults has been investigated by TEM. According to current findings, *Photorhabdus* sp., fully destroyed the alimentary canal tissue and the tissues were entirely altered beyond recognition. While the bacterium *Xenorhabdus* sp., has a mild effect on the tissues, causing detachment of the epithelial layer, as well as cytoplasm vacuolation. These findings are consistent with Khandelwal *et al*.[69], who reported histological impacts of *Xenorhabdus* and *Photorhabdus* bacteria on insects. In addition, Silva *et al*.[70] and Sicard *et al*.[71] reported that the primary site of action of *Xenorhabdus* Txp40 toxin in *Spodoptera littoralis* and *Manduca sexta*, respectively, is the alimentary canal. The injection of *Helicoverpa armigera* larvae with toxic protein (Txp40) from *Photorhabdus* sp. and *Xenorhabdus* sp. bacteria led to histological damage to midgut cells and fat bodies[72]. In addition, Ruiu *et al*.[73] observed ultrastructural abnormalities in the midgut of housefly larvae treated with *Brevibacillus laterosporus*, including cytoplasm vacuolization, changes in microvilli and mitochondria and deformation of the endoplasmic reticulum. Wang *et al*.[74] reported that the protein complex isolated from *X. nematophila* HB310 destroyed midgut tissues of *Plutella xylostella* larvae. A similar effect was observed in *P. papatasi* larvae treated with *H. bacteriophora* Hp88 nematode with its symbiotic bacterium *P. luminescens*[30].

CONCLUSION

The current findings proved that both *Xenorhabdus* and *Photorhabdus* bacteria effectively reduced the larval and adult populations of the housefly in all the three tested forms (whole-cell suspension, cell-free supernatant and crude cells). However, the whole-cell suspensions were the most effective ones. TEM recorded severe effects in the ultrastructure of the alimentary canal either treated with *Photorhabdus* sp., or *Xenorhabdus* sp. Such findings can enhance the empirical novelty in the field of housefly management since these bacteria are considered to be eco-friendly and effective alternatives compared to conventional chemical pesticides. To be more specific, we recommend the whole-cell suspension of *Photorhabdus* bacteria to be used for controlling housefly larvae and adults.

SIGNIFICANCE STATEMENT

This study discovered that both symbiotic bacteria significantly suppressed larvae, pupae and adults of the housefly, particularly when they were applied as whole-cell suspensions, Also, *Photorhabdus* sp., was more effective than *Xenorhabdus* sp. and housefly adults were more susceptible than larvae, that can be beneficial for integrated management programs for houseflies and highlights new ideas that can be used as an alternative to pesticides. This study will help the researchers to uncover the critical areas of finding unconventional methods to control the housefly as a global vector of diseases.

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