Review Form 1.6

Journal Name:	**Biotechnology Journal International**
Manuscript Number:	**Ms_BJI_93122**
Title of the Manuscript:	**Laboratory Evaluation of the Insecticidal Toxins from Entomopathogenic Nematode Symbiotic Bacteria to Control Vegetable Diseases and Pests**
Type of the Article	**Short Research Article**

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty'**, provided the manuscript is scientifically robust and technically sound.
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PART 1: Review Comments

	Reviewer's comment	**Author's comment** *(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*
Compulsory REVISION comments	**Comments & Corrections** **The red colour is used for corrections, and the blue is for deleting.** **Abstract** **Aims:** Lines 2-4, worldwide used as microbial control agents. We isolated toxins from EPN bacteria and provided basis for using this potential resource to biological control vegetable diseases and pests. Please modify to: Toxins from EPN symbiotic bacteria were isolated and provided basis for using this potential resource as biocontrol agent against vegetable diseases and pests. **Results:** Lines 2 & 3, and strain SY5 had the most obvious insecticidal activity and antifungal activities to *Trichothecium roseurn* and *Fusarium oxysporum*. Please modify to: and strain SY5 showed the most obvious insecticidal and antifungal activities against *Trichothecium roseurn* and *Fusarium oxysporum*. **Conclusions:** Lines 2 & 3, Therefore, it had potential on applying to vegetable diseases and pest biocontrol. Please modify to: Therefore, it had the potential for applying to control vegetable diseases and pests. 1. INRTODUCTION Please write in bold: **INRTODUCTION** Line 2, vegetables will be greatly reduced by diseases and pests. Please modify: vegetables will be greatly reduced by plant diseases and pests. Line 6, cabbage, cabbage. Is it repeated?? Line 10, vegetable production. *Trichothecium roseurn* is the disease in tomatos, Please pay attention: *Trichothecium roseurn* is a fungus in the division Ascomycota, cause plant diseases, **it is not a disease, it is a causal for diseases.** So, correct: *Trichothecium roseurn* is the causal for many diseases of tomatoes, [tomatoes not tomatos]. **The second paragraph in the INTRODUCTION** Line 1, Entomopathogenic nematodes (EPN), natural enemy of insect, exist widely in the ... Please add "s" for plural and "a"- as shown: Entomopathogenic nematodes (EPNs), a natural enemy of insect,	

Line 9,

In this study, the toxins were extracted from entomopathogenic nematodes bacteria.

Please modify: the toxins were extracted from entomopathogenic nematode symbiotic bacteria.

Last two lines,

microbial insecticides, insecticides toxins and will provide new ways and methods for vegetable pest and fungal plant disease biological control.

Please modify: will provide new ways and methods for biological control of vegetable against pests and fungal-plant diseases.

In the INRTODUCTION

The first paragraph is okay.

The second paragraph: Please modify as follows:

Application of entomopathogenic nematode–symbiotic bacteria (EPNs) with their bacterial endosymbionts become a prime approach in the biocontrol sector as an ecologically safer tool in a sustainable agriculture perspective as well as in integrated pest management (Tomar et al. 2022). Since the early 1970s, there has been a tremendous research and commercial interest in entomopathogenic nematodes and their associated bacteria (Kaya et al. 2006). In the 1990s and twentieth century's vast studies on EPNs were carried out, and it was reported that EPNs were distributed worldwide (Tomar et al. 2022). The EPNs exist widely in the soil. They are non-toxic and harmless to plants, humans, animals and the environment [6]. EPN (genera Steinernema and Heterorhabditis) kill insects with the aid of a mutualistic symbiosis with a bacterium (*Xenorhabdus* spp. and *Photorhabdus* spp. for Steinernematidae and Heterorhabditidae, respectively). *Xenorhabdus* and *Photorhabdus* secrete a wide variety of substance into the culture medium including toxins, lipases, proteases, antibiotics and lipopolysaccharides. EPN and its symbiotic bacterium are worldwide used as microbial control agents in agriculture [7-10]. Entomopathogenic nematode–bacterium complex research is being conducted in many parts of the world. Many countries and regions working on these important biological control agents of soil pests. In Central America, initial attempts to control insect pests and mass production research are reported (Kaya et al. 2006). In North America and Europe, emphasis on the status of commercially available nematodes was placed. In China, Korea, and India, research activities in the use of nematode for controlling insect pests or soil plant pathogens was emphasized (Tomar et al. 2022), as well as in Japan, where the development of commercial nematodes was available. Overall, the intensity of research varies by country or regions. In most cases, the research in developing countries shows that the emphasis is to demonstrate the usefulness of the entomopathogenic nematodes or their symbiotic bacteria against various pests. In this study, the toxins were extracted from entomopathogenic nematodes bacteria. The insecticidal activities and antifungal activities to vegetable diseases and pests were determined by bioassay.

The results of the present study will be helpful for the development of new microbial insecticides, insecticides toxins and will provide new ways and methods for vegetable pest and fungal plant disease biological control. [This part should be added to the **CONCLUSION**].

And, Please, add these two references:

Kaya H, Aguillera M, Alumai A, *et al*. Status of entomopathogenic nematodes and their symbiotic bacteria from selected countries or regions of the world. Biolog. Control, 2006; 38: 134–155.

Tomar P, Thakur N. and Yadav A. Endosymbiotic microbes from entomopathogenic nematode (EPNs) and their applications as biocontrol agents for agro‑environmental sustainability. Egy. J. Biological Pest Control, 2022; 32:80-98.

3. RESULTS AND ANALYSIS

3.1 Insecticidal activity of toxins against *P. xylostella*

2.2 Insecticidal activity of toxins against *L. exigua*

Please be careful with the serial number, it is **3.2 not 2.2**

2.3 Antifungal activities of the toxin to *T. roseurn* and *F. oxysporum*

Again, it is **3.3 not 2.3**

The second paragraph in the RESULTS

Lines 4-6,

The insecticidal activity of all toxin had no significantly different at 3rd day (Fig. 1A), and had significantly different at 4th day and 5th day. Among these strains, the toxin of SY5 had the highest oral insecticidal activity to *P. xylostella*,
Please modify:

The insecticidal activity of all toxin had no significant difference at 3rd day (Fig. 1A), while it had significant difference at 4th day and 5th day. Among these strains, the toxin of SY5 showed the highest oral insecticidal activity to *P. xylostella*, ...

Figure 1
Fig.1 Insecticidal activity of toxins against *P. xylostella*

A. The average corrected mortality of 3d

B. The average corrected mortality of 4d

C. The average corrected mortality of 5d

D. The average inhibiting rate of larval weight

Please modify as follows:

Fig.1. Insecticidal activity of toxins against *P. xylostella*: A) The average corrected mortality of 3d; B) The average corrected mortality of 4d; C) The average corrected mortality of 5d; D) The average inhibiting rate of larval weight.

Also, Fig.1A: The insecticidal activity of all toxin had no significantly different at 3rd day (Fig. 1A),
No significant difference at all among toxins??, Please clarify.
Figure 2
Please modify as follows: [as clarify above].

Fig.2 Insecticidal activity of toxins against *L. exigua*: A) The average corrected mortality of 3d; B) The average corrected mortality of 4d; C) The average corrected mortality of 5d; D) The average inhibiting rate of larval weight.

Figure 3
Please modify as follows:

Fig3. Antifungal activities of the toxin to *T. roseurn* and *F. oxysporum*: A) The inhibiting rate against *T. roseurn*, and B) The inhibiting rate against *F. oxysporum*.

The last paragraph in the RESULTS

The inhibiting rate against *T. roseurn* and *F. oxysporum* at 5th day were 33.82% and 39.66%, which significantly raised compare to the inhibiting rate of 3d and 4d.

But, however, the inhibiting rate is low.

4. DISCUSSION

The second paragraph:

Line 3,

Leeks, and cutworms [17-19]. EPNs and their bacteria have a wide range of parasitic ....

Please modify: EPNs and their symbiotic bacteria have ....

Line 4,

pests, and can produce different types of insects toxins. Study on such bacteria ...

Please correct: pests, and can produce different types of insect's toxins. Studying on such bacteria ....

	Line 12, symbiotic bacteria SY5 toxin against the two pest were different, which .... Please correct: symbiotic bacteria SY5 toxin against the two pests were different, which .... **The last paragraph:** At present, there have been many reports on the toxins and genes of the symbiotic bacteria of entomopathogenic nematodes [25-28]. First: the references are only 27 not 28. Please check. Second: Regardless of reference 25 [2014], the other two references are 1998, are too old references. How can we say: At present, there have been many reports?? Please add recent references. **5. CONCLUSION** Last two lines, The study provided biocontrol resource for vegetable pest and disease control. Please modify: The study provided an alternative resource for controlling pests and diseases of vegetables. The results of the present study will be helpful for the development of new microbial insecticides, insecticides toxins and will provide new ways and methods for vegetable pest and fungal plant disease biological control. [This part should be added to the **CONCLUSION**].	
Minor REVISION comments	**The REFERENCES could be updated.** It is assumed that no references should be added in the section of RESULTS.	
Optional/General comments	In the INTRODUCTION, the two added paragraphs are optional, but important for the article. And subsequently, two references should be added.	

PART 2:

	Reviewer's comment	**Author's comment** *(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*
Are there ethical issues in this manuscript?	*(If yes, Kindly please write down the ethical issues here in details)*	

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