Egypt. J. Microbiol. **60**, pp. 25 - 31 (2025)

Egyptian Journal of Microbiology
http://ejm.journals.ekb.eg/

The effectiveness of Neem seed oil, Spirulina, Curcumin and Nano-Graphene Oxide against the Cotton leafworm *Spodoptera littoralis* (J. E. Smith) (Lepidoptera: Noctuidae)

Abeer M. Mohammad [1*] and **A. M. Shaker** [2]
[1] *Cotton leafworm Department, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt*
[2] *Sids Agric. Res. Station, Beni Suef, Plant Protection Research Institute, ARC, Giza, Egypt*

BY FEEDING on young leaves, undermining growth, and diminishing agricultural output, *Spodoptera littoralis*, commonly known as the cotton leafworm, significantly impacts over 90 plant species. Eco-friendly techniques to lessen harm to non-target organisms are given priority in integrated pest management systems. Among these, biological management techniques, such as the application of plant extracts and nanoparticles, have demonstrated significant effectiveness in managing the cotton leafworm. This study evaluated how neem seed oil, spirulina, curcumin, and nano-graphene oxide affected the biological traits of *Spodoptera littoralis*. Larvae in their fourth instar were given fresh castor leaves that had been individually treated with each of the four compounds.Using the fourth instars, the calculated LC_{50} values for Neem seed oil, Spirulina, Curcumin and Nano-Graphene Oxide were84.77, 54.3, 102.2 and 47.717 ppm respectively, using the fourth instars. All treatments influenced larval and pupal durations, extending them by 24 % and 41% with neem seed extract, 23 and 87 % by Spirulina, by 7% and 54% with Curcumin and by 27 and 31 % with Nano-Graphene Oxide. Adult longevity also affected negatively by 29, 85, 41 and 35 % with Neem seed oil, Spirulina, Curcumin and Nano-Graphene Oxide, respectively. Exposure to LC-values from all treatment formulations substantially modified enzymatic processes within the insects' digestive systems.The study concludes that Neem seed oil, Spirulina, Curcumin and Nano-Graphene Oxide have strong larvicidal effects against *Spodoptera littoralis*. At low doses, all treatments successfully extended the duration of larval and pupal stages and decreased the success of adult emergence. Additionally, these treatments adversely affected the insects' enzyme functions, indicating their potential as natural insecticides in managing the fall armyworm.

Key words: *Spodoptera littoralis*, *Spirulina*, Curcumin, Graphene Oxide.

1. Introduction

The moth, *Spodoptera littoralis*, belongs to the Noctuidae family, which is better known by its common name, the cotton leafworm. It is a significant agricultural pest that has a bad reputation for wreaking havoc on a variety of crops all over the world. According to **Abdul El-Rahman et al. (2020)**, this species is native to regions with temperate and subtropical climates. These caterpillars can eat a wide variety of plant species, including economically significant crops like sorghum, rice, cotton, and maize (**FAO, 2019**). Chemical pesticides are frequently used to manage *Spodoptera littoralis*, raising concerns for both human health and the environment. Additionally, the pest's swift adaptation and resistance to a range of pesticides necessitate ongoing innovation in management techniques, increasing the costs to the environment and economy (**Taufika et al., 2021**). **Nawaz et al. (2021)** highlight that sustainable pesticides are valued for their biodegradability and minimal impact on non-target organisms. The integration of these pesticides into pest management initiatives is made easier by this **(Khursheed et al., 2022; Usama et al., 2023; Kamaraj et al., 2018; Amin & Mohammad,2022)**. As the need for safe insecticides has grown, a lot of research has been done to identify alternative sources. In this regard, the substantial benefits and potential applications of nanotechnology cannot be overstated. Nanotechnology is crucial for pest management, especially in agriculture, as it makes it possible to use fewer dangerous chemical pesticides. Several studies on green algae have also shown that they have insecticidal qualities (**Rahman et al., 2022; Hasanin et al., 2022 & Shabir et al., 2023**). The important role that plant extracts play as biocides against insect pests must also be acknowledged (**Nurmaisah et al., 2023**). The purpose of this work was to examine the insecticidal effects of spirulina, curcumin, and nano-graphene oxide on the larval stages of Spodoptera littoralis, the cotton leafworm, with a particular emphasis on the second and fourth instars.

*Corresponding author e-mail: abeer.yousuf.yy@gmail.com
Received:12/04/2025; Accepted: 13/07/2025
DOI: 10.21608/EJM.2025.374993.1283

2. Material and Methods

2.1. Insect rearing

Utilizing fresh *Ricinus communis* (L.) leaves, the cotton leafworm *Spodoptera littoralis* was raised in a lab setting (28 ± 2 °C, RH 60 ± 5%) according to **Rashwan and Hamed (2020)** methods. Colonies of *S. littoralis* were gathered from different parts of the governorate of Bani-Suef and were routinely raised in lab settings for a maximum of three generations. Larvae were maintained in large glass containers and provided with castor bean leaves until they developed into pupae; adults emerged a week later (**Adamczyk et al., 1999**). In glass jars, the newly emerging adults were allowed to mate after being fed cotton pads moistened within a 10% sugar solution and given castor oil plant leaves to encourage egg-laying. Masses of eggs were then stored in plastic jars until they hatched.

2.2. Tested compounds

Extract of neem seeds

The seeds were extracted by gathering mature neem fruits and grinding them. Then, to maximize the extraction's effectiveness, the seeds were let to dry in the open for five days. Neem seed was ground in a blender (Kenwood Limited, Havant, UK) and heated to 55 °C for 10 minutes immediately prior to extraction to achieve optimal dehydration in order to form neem seed kernel cake (**Paragas et al., 2018**). The Soxhlet extraction method was employed to derive oil from neem seeds. Neem oil was concentrated, then placed in an evaporating dish and kept within a desiccator for later application. (**Oyekanmi et al. 2021**).

Algal specimen

The Phycology Unit's algal culture collection at the Botany Department, Faculty of Science, Ain Shams University, Cairo, Egypt, provided the algae specimens. After being cut, gathered, and stripped of their reproductive organs, newly created thalli were left to dry within room temperature and kept at –20 ºC until the next experimental stage **(Saber *et al.* 2018).**

Nano Graphine oxide

The method to prepare Graphene Oxide is described by **Marcano et al., 2010**. In short, graphite flakes (3.0 g) were combined with a chilled 9:1 H_2SO_4/H_3PO_4 mixture (360:40 mL) under ice-bath conditions. After adding 18 g of $KMnO_4$ to the mixture very gradually, the reaction was maintained at 50 °C under continuous stirring for 12 hours. Once cooled to ambient temperature, the reaction (3 ml 30% H2O2 and 400 ml deionized water) was dumped onto ice. The purifying process of GO suspension was performed according to **Chen et al., 2009**. Filter paper and a funnel were used to wash the GO suspension with a 1:10 HCl solution (5 L). After that, the GO paste was removed and allowed to solidify at 60 degrees Celsius. A glass bar is used to gently swirl the solid after it has been disseminated in deionized water and left undisturbed for two to three hours. After filtering, the suspension is rinsed for two days with a lot of deionized water until the PH reaches almost 7. The GO powder is acquired by vacuum dehydration at 60^{o}C for 6 hrs. The GO powder is dispersed into water by ultra-sonication. The resulting brown solution undergoes centrifugation at 4000 rpm for half an hour to eliminate any un-exfoliated GO.

Curcumine extract

Turmeric rhizomes met the following requirements: they had to be yellow, ±4.5 cm thick, and ±3.5 cm long. After being cleaned under running water, the turmeric rhizomes were drained, chopped, and spread out on paper to absorb the water. Two weeks were spent drying the turmeric. After that, a blender was used to powder it, and it was kept in a glass jar. The simplicia was soaked in ethanol for three days in order to extract it. A rotary evaporator was then used to filter and concentrate the extract solution, yielding a crude extract. Cut cabbage leaves measuring 25 cm2 were dipped in the treatment for 60 minutes, and they were then allowed to dry for 10 to 20 minutes at room temperature. Disposable petri dishes were used to hold the leaves. Each repetition contained five test larvae, with one larva in each dish. Every six hours, observations were made. **Nurmaisah et al., 2023.**

2.3. Bioassay

To prepare the leaves of the castor oil plant, they were submerged in solutions with varying component concentrations (1000, 500, 250, 125, and 62.5 ppm). Each of the four substances was used for a distinct treatment. Controlled leaves were not given any treatment. Each treatment consisted of three copies, each carrying 10 larvae in their fourth instar. The bioassays were conducted at 65 ± 5% relative humidity and 25 ± 2°C. The insect fatality data was statistically evaluated using Ldpline software for Probit analysis. The fourth-instar larvae underwent treatment for twelve days after hatching. The entire life cycle of the experiment was conducted, and it ended when the eggs hatched. Leaves of the castor oil plant were submerged in each test

concentration for five minutes, and then they were let to air dry. After the larvae had been permitted to consume the leaves for two days before being switched to untreated ones. Any leftover solution from the treatment was carefully disposed of.

2.4. Biological Aspects

The effect on S of each compound's LC50. The biological activities of Littoralis were investigated, encompassing the lengths of larval and pupal stages, adult longevity, and deformities of larval, pupal, and moth stages. Ten larvae in their fourth instar were used in the bioassay test. Fresh castor leaves treated with each chemical were given to the larvae; the trials were conducted three times. As controls, untreated leaves were employed (**Tulashie et al., 2021**).

2.5. Determination of Carbohydrate Hydrolyzing Enzymes Activities

This study aimed to assess how the tested materials influenced the physiological processes of the larvae's digestive system. The method described by **Ishaaya and Swirski (1976)** was followed to measure enzyme activity, using 3,5-dinitrosalicylic acid to determine glucose levels released during starch and sucrose digestion. Different reaction solutions were formulated for individual enzymes, and the enzymatic activity was quantified in µg glucose released/min/g body weight. The activity of three enzymes that hydrolyze carbohydrates—trehalase, invertase, and α-amylase—was assessed. After reaching the sixth instar stage, three treated larvae were individually weighed, transferred into freezer-safe Eppendorf tubes, and then submitted to a laboratory for further testing. The extracts listed below were utilized for the enzyme activity test: Every study was conducted at the Plant Protection Research Institute's (PPRI) Microchemical Analysis Lab (MCAL) at the Agricultural Research Center (ARC) in Giza, Egypt. Three larvae were transferred into Eppendorf tubes and kept in the freezer after being fed with each specific enzyme until they reached the sixth instar. After that, these samples were delivered to the lab for analysis.

2.6. Statistical Analysis

The percentage of larval mortality was computed and adjusted using the Abbott formula (**Abbott, 1925**). Probit analysis was used to estimate the LC50 values for the three treatments. These LC50 values were used to examine various biological consequences. The P value, F-value, and L.S.D. at 0.05 degrees of freedom were obtained by data analysis using Excel. Tukey's test for mean comparison ($P < 0.01$) and an ANOVA were performed on enzymatic activity data using the SISVAR program (**Ferreira, 2011**).

4. Results & Discussion

4.1. Laboratory toxicity bioassay

On the fourth instars of the cotton leafworm, *S. littoralis*, the toxicity of five concentrations (1000, 500, 250, 125, and 62.5 ppm) of each compound was assessed compared with a control. After 48 hours, larval mortality was initially noticed and then every day following that. Longer exposure times and greater extract concentrations led to a proportionate increase in the larval death rate. When Nano-Graphene Oxide was used at 250 ppm, the mortality rate was 90%, but curcumin only produced a 30% mortality rate for the fourth instars at the same concentration. Furthermore, 100% of patients died at 1000 ppm following the exposure time for all therapies. Lethal toxicity values for each of the four chemicals were also established for the fourth instar. Tables 1 display the LC50 values, which represent overall mortality. The values for graphene oxide (47.717 ppm), neem seed oil (84.775 ppm), curcumin (102.167 ppm), and 54.27 ppm were obtained for the fourth instars.

Table 1. Susceptibility of *Spodoptera littoralis* 4nd instars.

Treated compound	LC_{50} (ppm)	Confidential limit (95%)		Slope ± S.E.
		Lower	Upper	
Spirulina	54.27	24.8344	91.8273	0.6772 ± 0.1329
Curcumin	102.167	55.8629	175.4586	0.6664 ± 0.1312
Nano-Graphene Oxide	47.717	16.4492	76.709	0.8249 ± 0.1363
Neem seed oil	84.775	39.3	108.07	0.8854 ± 0.1369

4.2. Biological aspects

The impacts on the biological characteristics of *S. littoralis* larvae in their fourth instar, using Spirulina curcumin, nanographene oxide, and spirulina in at sub-lethal concentrations,are detailed in Tables 2, and by Figures 1 and 2. When compared to the control, the data in Table 2 show a considerable expansion in both the

larval and pupal periods. In particular, compared to the control, which lasted 22.55 days, the average larval lengths for the neem oil treatment were 27.85 days, 29.15 days for the spirulina, 24.15 days for the curcumin, and 28.65 days for the graphene oxide. In contrast to the control, which had a pupal duration of 3.9 days, the mean pupal durations for neem seed oil, spirulina, and graphene oxide were 5.5 days, 9 days, 5.95 days, and 5.1 days, respectively. Table (2) also presents the adult longevity with all mixtures treatment. The calculated values were 5.5, 7.95, 6.0, 5.75 and 4.25 days with using Neem seed oil, Spirulina, Curcumin and Nano-Graphene Oxide and control treatment, respectively.

Table 2. Duration of the larval and pupal stages & Adult longevity of *S. littoralis* treated with Spirulina, Curcumin and Nano-Graphene Oxide. (2^{nd} instars).

Treatment	Mean larval duration (days ±SE)	Mean pupal stage (days ±SE)	Adult longevity (days ±SE)
Control	22.55 ^{b} ±1.089	3.9^{d} ± 0.29	4.25 ^{c} ± 0.15
Neem seed oil	27.85 ^{a} ± 1.65	5.5 ^{bc} ± 0.17	5.5^{b} ± 0.29
Spirulina	29.15 ^{a} ± 1.19	7.3^{a} ± 0.18	7.95^{a} ± 0.15
Curcumin	24.15 ^{b} ± 1.15	5.95^{b} ± 0.13	6.0^{b} ± 0.18
Nano-Graphene Oxide	28.65 ^{a} ± 1.65	5.1^{c} ± 0.06	5.75^{b} ± 0.16
F value	0.0009***	0.0000***	0.0000***
LSD	2.7333	0.570	0.5909

Means with the same letters have no significant differences; *: significant ***(p<0.01).***

Neem derivatives present in seed oil extract are responsible for neem extract's effects, which include repellence, developmental delays, decreased fecundity and fertility, and behavioral changes that may result in death. These findings are consistent with previous research which stated that, this substance causes changes in the pest's physiology and behavior [**Prates et al., 2003; Roel et al., 2010; Shukla et al., 2015**]. **El-Bendary and El-Helaly (2013) and Lavicoli et al. (2019**) also discovered that nanoparticles have an impact on *Spodoptera* fecundity and fertility. **Pittarate et al. (2023**) reported similar results, stating that nanoparticles impact insect pests' respiration and metabolism in addition to suppressing substrate migration across the cellular membrane. According to reports from other researchers, turmeric extract kills cotton leafworms and affects nutritional activity. According to **Nurmaisah et al. (2023**), the color and texture of the affected larvae had changed. This is because the active ingredients in the extract from the rhizome of turmeric affect the larvae's ability to feed and their mortality rate. When applied to cabbage leaves, a plant pesticide containing turmeric extract has the potential to kill the target insect directly and indirectly. The death rate of nanographene oxide composite against the cotton leafworm *S. littoralis* was investigated by **Abd El-Rahman et al in 2020**. They discovered that, the mortality rate rose as the nanocomposite's concentration rose.

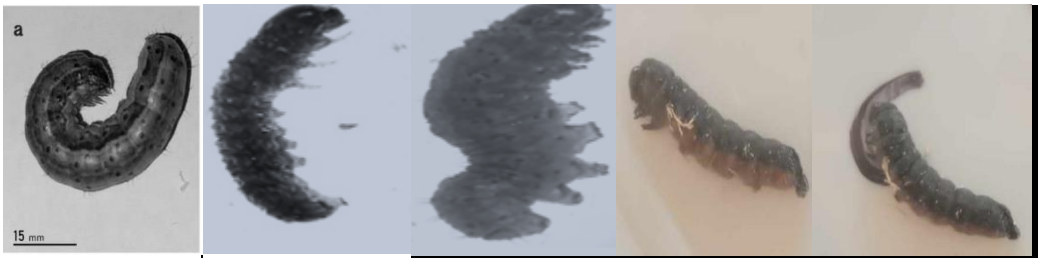

Fig. 1. Malformed 6^{th} instar larvae treated as 4^{th} instars with:
a: Control; b: Spirulina; c:Curcumin; d:Nano-Graphene Oxide e-Neem extract

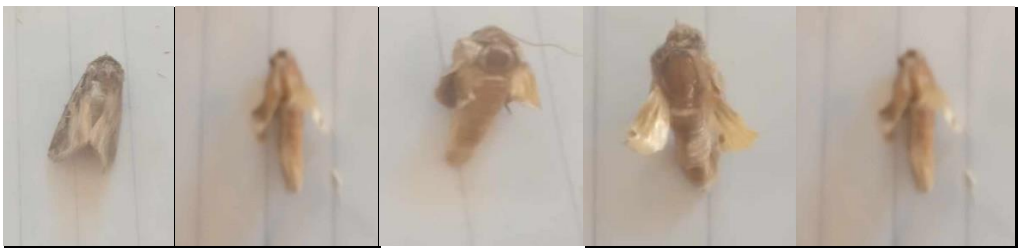

Fig. 2. Malformed adults treated as 4^{th} instars with:
a: Control; b: Spirulina; c:Curcumin; d:Nano-Graphene Oxide e-Neem extract

4.3. Enzymatic activity

The effects of neem extract, spirulina, curcumin, and nano-graphene oxide on the digestive systems of *S. littoralis* were detailed in Table 3. The digestive enzymatic activity of *S. littoralis* was considerably affected by the ingestion of 54.27 ppm of spirulina, 102.167 ppm of curcumin, 47.717 ppm of nano-graphene oxide, and 84.775 ppm of neem extract. According to the data, the larvae fed with Spirulina, Curcumin, Neem extract, and Nano-Graphene Oxide showed increases in α-amylase activity levels by 15%, 8%, 37%, and 7%, respectively. Additionally, trehalase activity rose by 14% when neem seed extract was used, while it decreased by 37%, 15%, and 42% when utilizing spirulina, curcumin, and nano-graphene oxide, respectively. When applying Spirulina, Curcumin, Neem extract, and Nano-Graphene Oxide, the activity levels of the enzyme invertase were 46.57, 64.67, 35.53, and 61.73 µg of glucose released per minute per mg of protein, respectively, in contrast to the control treatment, which demonstrated 162.73 µg of glucose released. These results are consistent with recent studies on the biological characteristics of *S. littoralis*, indicating a decline in trehalase enzyme activity. Trehalose catabolism is a crucial step in the management of energy and the synthesis of glucose [**Rahman et al., 2022; Shukla et al., 2015**]. Additionally, it has been observed that the active components in neem seed extract have an impact on the digestive enzyme activity of *S littoralis* larvae (**Alzohairy, 2016; Foudal et al., 2022).** The results demonstrated that oxidative stress intensified following GO injection, as seen by elevated enzyme activity. Additionally, exposure to AgNPs led to a reduction in the activities of amylase, protease, lipase, and invertase in *S. litura* (**Bharani & Namasivayam 2017**). In general, all compounds represent an environmentally sustainable approach and may serve as viable alternatives to conventional chemical pesticides, which are known to harm both ecosystems and living organisms [**Tulashie et al., 2021& Shabir et al., 2023**].

Table 3. Specific activity of the selected enzymes of *S. littoralis* (µg /min/ mg protein).

Treatment	Amylase ± SE	Trehalase ± SE	Invertase ± SE
Spirulina	246.5^{b} ± 3.55	119.1^{b} ± 2.76	46.57^{b} ± 5.17
Curcumin	231.5^{c} ± 2.0	105.1^{c} ± 1.73	64.67^{a} ± 5.07
Nano-Graphene Oxide	228.1^{d} ± 4.22	129.4^{a} ± 3.29	35.53^{c} ± 5.17
Neem seed extract	293.1^{a} ± 6.5	78.63^{e} ± 2.452	61.73^{a} ± 5.17
Control	214.2^{d} ± 1.99	91.03^{d} ± 1.412	57.17^{a} ± 5.17
F value	0.0000***	0.0000***	0.0001***
L.S.D	12.528	7.5136	8.0179

Means with the same letter are not significantly different ($p \leq 0.05$).
**Highly significant, ***Very highly significant

5. Conclusion

This research highlights the use of Spirulina, Curcumin, neem extract and Nano-Graphene Oxide as environmentally benign insecticidal agents for the control of *Spodoptera littoralis*. The tested compounds displayed strong larvicidal effects, particularly against fourth-instar larvae, by prolonging developmental stages and reducing adult survival rates even at minimal concentrations. Furthermore, exposure to lethal concentrations of these substances significantly disrupted the insect's enzymatic functions. The findings offer foundational insights for future studies on eco-friendly pest management using plant and algal-based solutions, which are both non-toxic and biodegradable. Such methods align with the principles of sustainable farming and eco-conscious biological pest control strategies.

Acknowledgments: We extend our gratitude to Plant Protection Research Institute - Agricultural Research Center for providing the devices utilized in the measurements conducted for this study.
Funding statements: No funding was received
Conflicts of interest: Authors have declared that no competing interests exist.

6. References

Abd El-Rahman, S.S., Ahmed, S.S. and Abdel Kader M.H. 2020. Toxicological, biological and biochemical effects of two nanocomposites on cotton leaf worm, *Spodoptera littoralis* (Boisduval, 1833). Polish Journal of Entomology 89(2): 101–112.

Abbott W.S. 1925. A Method of Computing the Effectiveness of an Insecticide. *Journal of Economic Entomology* 18 2 265–267.

Adamczyk J. J.; Leonard B. R. and Graves J. B. 1999. Toxicity of Selected Insecticides to Fall Armyworms (Lepidoptera: Noctuidae) in Laboratory Bioassay Studies. The Florida Entomologist, 82, No. 2, pp. 230-236.

Alzohairy M. A. 2016 Therapeutics role of *Azadirachta indica* (neem) and their active constituents in diseases prevention and treatment. *Evidence-Based Complementary and Alternative Medicine* 2016 Article ID 7382506, 11.

Amin, N.S., Mohammad A.M. 2022. The biological and Histopathological aspects of the black Cutworm, Agrotis ipsilon (Huufnagel) (Lepidoptera: Noctuidae) treated with flufenoxuron. Egyptian Academic Journal of Biological ciences, F. Toxocology & Pest Control. 6 (12): 1883-1670.

Bharani RA, Namasivayam SKR. 2017. Biogenic silver nanoparticles mediated stress on developmental period and gut physiology of major lepidopteran pest *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae) An eco-friendly approach of insect pest control. *Journal of Environmental Chemical Engineering*, 5(1): 453–467.

Chen T., Zeng B., Liu J. L., Dong J. H., Liu X. Q., Wu Z., Yang X. Z., Li Z. M. (2009). Journal of Physics: Conference Series 188,012051. **DOI** 10.1088/1742-6596/188/1/012051.

El-bendary H. M. and El-Helaly A. A. 2013 First record nanotechnology in agricultural: Silica nanoparticles a potential new insecticide for pest control. *App. Sci. Report.* 4 3 241-246.

FAO, CABI, 2019 Community-Based Fall Armyworm Monitoring, Early Warning and Management: Training of Trainers Manual.

Ferreira D.F., 2011 SISVAR: A Computer Statistical Analysis System. *Ciência e Agrotecnologia* 35 1039-1042.

Foudal A., Eid A. M., Abdel-Rahman M. A., EL-Belely E. F., Awad M. A., Hassan S., AL-Faifi Z. E., Hamza M. F. 2022 Enhanced Antimicrobial, Cytotoxicity, Larvicidal, and Repellence Activities of Brown Algae, Cystoseira crinita-Mediated Green Synthesis of Magnesium Oxide Nanoparticles. *Frontiers in Bioengineering and Biotechnology* 10 849921.

Hasanin M., Al Abboud M. A., Alawlaqi M. M., Abdelghany T. M. & Hashem A. H. 2022 Ecofriendly Synthesis of Biosynthesized Copper Nanoparticles with Starch-Based Nanocomposite: Antimicrobial, Antioxidant, and Anticancer Activities. *Biological Trace Element Research.* 200 2099–2112

Ishaaya I. and Swirski, E. 1976 Trehalase, invertase and amylase activities in the black scale, Saissetia *oleae* and their relation to host adaptability. *Journal of Insect Physiology* 22 1025-1029.

Kamaraj C., Gandhi P.R., Gandhi E.; Karthi,S. Chung M. and Rajakumar G. 2018 Novel and environmental friendly approach; Impact of Neem (*Azadirachta indica*) gum nano formulation (NGNF) on *Helicoverpa armigera* (Hub.) and *Spodoptera litura* (Fab.). *International Biological Macromolecules* 107 59-69.

Khursheed A., Rather M.A., Jain,V. Wani A.R., Rasool S., 2022. Plant based natural products as potential ecofriendly and safer biopesticides: A comprehensive overview of their advantages over conventional pesticides, limitations and regulatory aspects. *Microbial Pathogenesis* 173 105854.

Lavicoli I.; Leso V.; Beezhold D. and Shvedovo A.A. 2019 Nanotechnology in agriculture: Opportunities, toxicological implications, and occupational risks. *Toxicol. Appl. Pharmacol*. 329 96-111

Marcano D. C. , Kosynkin D. V., Berlin J. M., Sinitskii A., Sun Z., Slesarev A., Alemany L. B., Lu W., Tour J. M. 2010 Improved Synthesis of Graphene Oxide. ACS Nano, 4, 8: 4806–4814. https://doi.org/10.1021/nn1006368

Nurmaisah, Mau M W, Abdul Rahim (2023). Application of Turmeric Extract Botanical Insecticide On Feeding Activity And Mortality Of Spodoptera Litura Larvae. Jurnal Pertanian Tropik, 10 (4) :20- 29

Nawaz A.; Ali H.; S. Fiaz; M. Sufyan; M. Abdul Qayyum; R. Azad; Y. Niaz; M. A. Badlah and K.A. Khan. 2021 Analysis of food resources, host availability and insecticidal impacts on the fecundity, longevity and parasitism efficiency of *Diaertiella rapae* (M'intosh). International Journal of Tropical Insect Science, 41, 2883–2896.

Oyekanmi, A. A. U, Kumar S. U. and Khalil. A. 2021 Functional Properties of Antimicrobial Neem Leaves Extract Based Macroalgae Biofilms for Potential Use as Active Dry Packaging Applications. *Polymers* 13 1664.

Paragas D.S., Fiegalan E.R., Cruz K. D. 2018 Assessment of Green Solvents and Extraction Methods for Biopesticide Preparation from Neem (*Azadirachta indica)* Leaves against Oriental Fruit Fly Bactrocera Dorsalis (Hendel). *Preprints*. https://doi.org/10.20944/ preprints 201805.0179.v1

Pittarate S., Perumal V., Kannan S., Mekchay S., Thungrabeab M., Suttiprapan P., Senthil-Nathan S., Patcharin K. 2023 Insecticidal efficacy of nanoparticles against Spodoptera frugiperda (J.E. Smith) larvae and their impact in the soil. Heliyon, 9: e16133. doi.org/10.1016/j.heliyon.2023.e16133

Prates H.; Viana P, Waquil J. 2003 Activity of neem tree (*Azadirachta indica*) leaves aqueous extract on *Spodoptera fruiperda*. *Pesquisa Agropecuária Brasileira* 38 437-439.

Rahman A., Pittarate S., Perumal V., Rajula J. 2022 Larvicidal and Antifeedant Effects of Copper Nano-Pesticides against *Spodoptera frugiperda* (J.E. Smith) and Its Immunological Response. *Insects* 13 1030.

Rashwan R S and. Hammad D M. (2020). Toxic effect of *Spirulina platensis* and *Sargassum vulgar* as natural pesticides on survival and biological characteristics of cotton leaf worm *Spodoptera littoralis*. Scientific African, 8 (2020) e00323.

Roel A. R., Dourado D. M., Matias R., Porto K. R. A., Bednaski A. V. and Reginaldo B. da Costa 2010 The effect of sub-lethal doses of *Azadirachta indica* (Meliaceae) oil on the midgut of *Spodoptera frugiperda* (Lepidoptera, Noctuidae). *Revista Brasileira de Entomologia* 54 3 505–510.

Saber AA, Ballot A, Schneider SC, Cantonati M 2018. Morphological and molecular features of a *Chara vulgaris* population from desert springs on the Sinai Peninsula (Springs of Moses, Egypt). *Bot Lett* 165(1): 77-89.

Shabir A., Sarwar Z.M. and Ali H. 2023 Eco-friendly approaches of zinc oxide and silver nitrate nanoparticles along with plant extracts against Spodoptera litura (Fabricius) under laboratory conditions. Science Progress, 106 (4) 1–16. doi :10.1177/00368504231219171

Shukla E.; Thorat L. J.; Nath B. B. and Gaikwad S. M 2015 Insect trehalase: Physiological significance and potential applications. *Glycobiology* 25 4 357–367.

Taufika, R., Nugroho, S. A., & Nuraisyah, A. (2021). Efektivitas campuran ekstrak daun srikaya (*Annona squamosal* L.) dan rimpang kunyit (*Curcuma domestica* Val.) pada mortalitas larva *Spodoptera litura* F.(Lepidoptera: Noctuidae). *Jurnal Ilmu Pertanian Indonesia*, *26*(1), 32–41.

Tulashie S., Adjei, F., Abraham. J. and Addo, E. 2021 Potential of neem extracts as natural insecticide against fall armyworm (*Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) Case Studies. *Chemical and Environmental Engineering* 4 100130.

Usama M. A., Salah A. M., Ayashi M.M., El-Sakhawy M.A., Ateya A.A.E., Waggiallah H.A.. 2023 Characterization of Bacillus thuringiensis isolated from soils in the Jazan region of Saudi Arabia, and their efficacy against Spodoptera littoralis and Aedes aegypti larvae. *Saudi Journal of Biological Sciences* 30 8 103721.