Efficiency of lactic acid in mortality Aphis gossypii nymphs on okra plants under laboratory conditions.

Sajjad Alaa Hassan Al Kamil1*, Israa Abed Ali Hassan Al Hawani2 , Hadi AbdulJalil Naas3 1,2,3Biological Control Technologies Department, Technical College / Al - Mussaib, Al-Furat Al-Awsat Technical University, Babylon, Iraq. *Corresponding author: sajjad.alaa.tcm.69@student.atu.edu.iq 2Email: IsraaAbedali@atu.edu.iq

Abstract

Lactic acid, classified as GRAS (generally regarded as safe) by the FDA, It is widely used in the agricultural, industrial, medical and food sectors. In this study, the efficiency of lactic acid in mortality Aphis gossypii nymphs on okra plants was evaluated under laboratory conditions and in three replicates according to C.R.D. statistical analysis. The results showed the occurrence of mortality amounting to (82.68) % after 24 hours of treatment with lactic acid at a dilution of (10-1) compared to (0.00) % in the comparison treatment, and after 48 hours the percentage of nymph mortality reached (100)% for the same dilution in comparison with (0.00) % in the comparison treatment, while the percentage of nymph mortality was (85.32)% when treated with (10-3) dilution after 72 hours, compared to (0.00)% in the comparison treatment. It is clear from the above that there is a direct proportion relationship between the rate of mortality and time periods, as the highest rate of mortality reached (60) % after 72 hours, while the lowest rate of mortality reached (25.67) % after 24 hours. There is also an inverse relationship between dilution and the rate of mortality, as the highest rate of mortality rate of nymphs was (94.22) % at the lowest dilution (highest acid concentration) (10-1), while the lowest rate of destruction was (27.56) % at the highest dilution (lowest acid concentration) (10-5). Based on these results, lactic acid can be used as a highly safe and environmentally friendly organic pesticide instead of synthetic chemical pesticides in combating A. gossypii.

Keywords: Aphis gossypii, Okra, Lactic acid, GRAS Introduction

Okra (Abelmoschus esculentus L.) is an annual plant of the Malvaceae family [1]. It is widely cultivated all over the world, especially in tropical, subtropical and warm temperate regions, and is native to Ethiopia [2]. People consume young okra fruits as cooked vegetables due to their immature fruits and soft leaves, which makes them an important vegetable in terms of their nutritional value, medicine and industry [3]. Moreover, okra is rich in many beneficial nutrients, such as insoluble and soluble fibres, mucilage, and pectin, which play an important role in maintaining the overall health of the consumer [4]. Okra cultivation in many countries faces many problems; One of the most important challenges faced by different okra varieties is the spread of insect pests in the fields [5]. The cotton aphid Aphis gossypii (Glover) is one of the most common sucking insect pests of okra [6]. It is a widespread pest in many agricultural crops [7]. The damage is represented by the absorption of plant sap and the secretion of honeydew, which encourages the growth of smut mold fungi and also transmits many viruses that are harmful to plants [8.[

On the other hand, chemical control methods are becoming increasingly ineffective and have a negative impact on the environment, human and animal health. [9] The recent development of various molecular methods has led to the discovery of active chemicals produced by many microbial pathogens and their mechanisms of action against target pests [10]. Bacteria produce a variety of secondary products, many of which are antagonistic and inhibitory to insect pests and plant diseases [11]. Lactic acid is considered the final and main metabolic product of the fermentation of carbohydrates and sugars by lactic acid bacteria LAB [12]. Generally recognized as safe (GRAS) [13]. Lactic acid (2 hydroxypropionic), a natural organic acid was first discovered in sour milk by Scheele in 1780 [14]. Lactic acid bacteria (LAB) are considered one of the most common bacterial families that produce lactic acid, and their name comes from their ability to form this acid as a primary and individual result of sugar fermentation [15]. Lactic acid is an important from a biotechnological point of view because it is widely used in the medical, chemical, food, pharmaceutical and cosmetic sectors [16] [17]. Lactic acid has been used in the production of cellophane, resins, and some herbicides and insecticides, and lactic acid is also widely used in the tanning and textile industries [18]. The study aims to test the efficiency of lactic acid in the laboratory as an environmentally friendly organic pesticide instead of synthetic chemical pesticides by testing different infestations on the nymphs of A. gossypii on okra plants.

Materials and methods

The place where the experiment was conducted :

The experiment was conducted in the General Insects Laboratory for Postgraduate Studies of the Department of biological control technologies at Al-Mussaib Technical College in the year (2024 .(

Collecting and breeding aphids:

A. gossypii insects were collected from fields planted with okra plants in Babil Governorate, by observing their presence on the plants. Then, the leaves infected with the insect were taken and placed in plastic bags perforated with small holes for ventilation. Then they were growed and multiplied on okra plants grown in plastic pots inside a house. A small plastic house was built for the purpose of raising aphids with a size of $(3 \times 1.5 \times 1.5)$ m. They were left to grow and reproduce for the purpose of use in subsequent laboratory experiments. Also, to avoid changes in climatic conditions and to preserve the presence of the insect in order to conduct laboratory experiments, a number of okra plants were kept in the laboratory after being infected with A. gossypii inside an insect breeding box .

Preparation of lactic acid dilutions:

Lactic acid (PanReac AppliChem) was obtained from one of the chemical sales centers in Babylon Governorate. A series of dilutions were prepared using a serial dilution method (10-1 - 10-5) by taking 1 ml of the basic solution using a Micropipette and adding it to 9 ml of distilled water to obtain the dilution 10-1, then 1 ml of this dilution was taken to a second tube containing 9 ml of distilled water, to prepare 10-2 dilution, and so on to obtain the required dilutions. Then the dilutions (10-1, 10-3, 10-5) were selected and transferred to 10 ml hand sprayers to conduct the experiments.

Laboratory assaying the effect of lactic acid on the mortality of cotton nymphs A. gossypii : Okra plants free of any infection were selected and planted in plastic pots with a diameter of (12) cm (1 kg). It was carefully confirmed that they were free of aphid infection and cleaned well with a small, soft brush. Then, paper in the form of a funnel was placed around the stem of the plant to count the number of mortal and falling insects from the leaves. 25 nymphs were placed on each plant with three replicates of each concentration in addition to the control treatment. The pots were then treated with dilutions of lactic acid (10-1, 10-3, 10-5) at a rate of 2 sprays on the location of the nymphs and from a distance of 15 cm. For the control treatment, they were sprayed with distilled water only, after which the pots treated with the specified dilutions were transferred to (Isolation box for experiments) Each dilution treatment was in a separate strip, while the control treatment was separated in another place away from the box in order to ensure that insects did not move from the control to the replicates that were treated with lactic acid dilutions.

Statistical analysis:

The study results were statistically analyzed using GenStat Release 2009 V12.1 software according to a completely randomized design (C.R.D) factorial experiments with completely randomized design for laboratory experiments. The least significant difference (L.S.D) test was used under the 5% probability level (P \geq 0.05) to test the significance of the results. The percentage of loss was corrected according to the Abbott equation [19.] Results and discussion

The effect of lactic acid on the mortality of cotton nymphs A. gossypii laboratory:

The results listed in Table No. (1) showed the effect of lactic acid on the mortality of A. gossypii nymphs, where the mortality rate increased with an increase in the time period and a decrease in dilution (increasing the acid concentration). The highest mortality rate was at the lowest dilution (10-1) (highest acid concentration) which reached (82.68, 100, 100) % after (24, 48, 72) hours, respectively, compared to (0.00) % in the comparison treatment, while the lowest percentage of mortality was at the highest dilution (10-5), where It reached (5.32, 22.68, 54.68) % after the same period of time respectively, compared to (0.00) % in the comparison treatment. Figure (1): showed the effect of lactic acid dilution (10-1) on A. gossypii nymphs.

Dilutions	Percentage of mortal insects/ hour			Dilution note
	24	48	72	- Dilution rate
Control	0.00	0.00	0.00	0.00
10 ⁻⁵	5.32	22.68	54.68	27.56
10⁻³	14.68	46.68	85.32	48.89
10⁻¹	82.68	100	100	94.22
Average period	25.67	42.34	60	

Table No. (1) shows	the effect	of lacti	c acid	l on the	e mortality	of nymphs of cotton A. gossypii
	D		•			

L.S.D ($P \le 0.05$) :- For dilutions = 2.070 For periods = 1.793 For interference = 3.586



Figure (1): showed the effect of lactic acid on A. *gossypii* nymphs.

The general trend in the field of plant protection is to reduce the use of pesticides with environmentally and replace them friendly biological products. The most promising strategy for plant protection is the use of microbial metabolites [20]. Lactic acid is an organic compound and is one of the most important acids produced by lactic acid bacteria (LAB). [21] showed the effect of lactic acid on the third instar larvae of the insect Aedes aegypti, where it gave a percentage of mortality at concentrations (0.8, 0.6) of (100) % after (16, 24) hours of exposure, respectively. While [22] found that lactic acid (10%) was toxic to silkworm caterpillars (Bombyx mori) and led to a noticeable decrease in their growth, which led to the mortality of the caterpillars. The results are agreeing with a study [23] that showed the effect of synthetic kojic acid on the mortality of A. gossypii, as the mortality rate on the first day when treated at a concentration of (100 parts per million) reached (5.0) %. On the second day of treatment, the mortality rate increased (20.0) %. In addition, after three days of exposure, the effect appeared to double in the mortality rate, reaching (40.0)%. The mortality rate of A. gossypii varies

greatly depending on the concentration and time duration (days). The mortality rate of A. increased gossypii with increasing concentrations. In addition. mortality increased when the time period was prolonged, and this was indicated in a study [23]. While the results of a study [24] showed that citric acid was effective against all tested stages of Aphis craccivora at all exposure times. It was noted that this activity had a minimal or minor effect after two hours, and its effectiveness gradually increased until it became very effective after 12 hours. And in another study of the effect of some compounds produced from the fungal filtrate Trichoderma harzianum on some aspects of the life of the insect Myzus persicae, where the percentage of mortality of nymphs and adults increased when concentrations increased to (39.15, 36.22) respectively, compared to (12.29, 6.1). % in the control treatment three days after the treatment, this study was aligning with our results, the mortality of A. gossypii was increase with the increasing concentration (low dilution) of lactic acid [25.] Conclusion

The results of the current study showed an effective effect of lactic acid on the mortality

of A. gossypii nymphs in pots planted with okra plants at different dilutions and time periods. There was a direct relationship between the time period and the rate of mortality of nymphs and an inverse relationship between dilution of lactic acid and the rate of mortality of the nymphs. A. gossypii.

Acknowledgment: I would like to thank the staff of the postgraduate studies laboratory at Al-Mussaib Technical College for giving me the opportunity to work in the laboratory .

References

[1] Daliu, P., Annunziata, G., Tenore, G. C., & Santini, A. 2020. Abscisic acid identification in Okra, Abelmoschus esculentus L.(Moench): Perspective nutraceutical use for the treatment of diabetes. Natural Product Research, 34(1), 3–9.

[2] Singh, J., & Nigam, R. 2023. Importance of Okra (Abelmoschus esculentus L.) and It's Proportion in the World as a Nutritional Vegetable. International Journal of Environment and Climate Change, 13(10), 1694–1699.

[3] Dantas, T. L., Alonso Buriti, F. C., & Florentino, E. R. 2021. Okra (Abelmoschus esculentus L.) as a potential functional food source of mucilage and bioactive compounds with technological applications and health benefits. Plants, 10(8), 1683.

[4] Nasab, F. K., Zare, M., MEHRABIAN, A., & Ghotbi-Ravandi, A. A. 2022. Ethnopharmacological survey of medicinal plants used to treat skin diseases among herbal shops in Jahrom, Iran. Collectanea Botanica, 41, e001.

[5] Lal, B., Singh, U. C., Bhaduaria, N. S., Tomar, S. P. S., & Singh, P. 2020. Seasonal incidence of major insect pests of okra, Abelmoschus esculentus (L.) and their natural enemies. Journal of Entomology and Zoology Studies, 8(3), 736–740.

[6] Chandio, M. A., Kubar, M. I., Butt, N. A., Magsi, F. H., Mangi, S., Lashari, K. H., Channa, N. A., & Roonjha, M. A. 2017. Varietal resistance of okra against cotton jassid, Amrasca biguttula biguttula (Ishida). Journal of Entomology and Zoology Studies, 5(3), 1647–1650.

[7] Im, Y., Park, S.-E., Lee, S. Y., Kim, J.-C., & Kim, J. S. 2022. Early-stage defense mechanism of the cotton aphid Aphis gossypii against infection with the insect-killing fungus Beauveria bassiana JEF-544. Frontiers in Immunology, 13, 907088.

[8] Singh, R., & Singh, G. 2021. Aphids. Polyphagous Pests of Crops, 105–182.

[9] Alengebawy, A., Abdelkhalek, S. T., Qureshi, S. R., & Wang, M.-Q. 2021. Heavy metals and pesticides toxicity in agricultural soil and plants: Ecological risks and human health implications. Toxics, 9(3), 42.

[10] Subbanna, A., Stanley, J., Rajasekhara, H., Mishra, K. K., Pattanayak, A., & Bhowmick, R. 2020. Perspectives of microbial metabolites as pesticides in agricultural pest management. Co-Evolution of Secondary Metabolites, 925–952.

[11] Maharana, C., Padala, V. K., Hubballi, A. B., Nikhil Raj, M., Paschapur, A., Bhat, C., Singh, A. K., & Subbanna, A. 2022. Secondary metabolites of microbials as potential pesticides. In Sustainable Management of Potato Pests and Diseases (pp. 111–142). Springer.

[12] Cai, H., Tao, L., Zhou, X., Liu, Y., Sun, D., Ma, Q., Yu, Z., & Jiang, W. 2024. Lactic Acid Bacteria in Fermented Fish: Enhancing Flavor and Ensuring Safety. Journal of Agriculture and Food Research, 101206. [13] Abedi, E., & Hashemi, S. M. B. 2020. Lactic acid production–producing microorganisms and substrates sources-state of art. Heliyon, 6(10.(

[14] Ferguson, B. S., Rogatzki, M. J., Goodwin, M. L., Kane, D. A., Rightmire, Z., & Gladden, L. B. 2018. Lactate metabolism: historical context, prior misinterpretations, and current understanding. European Journal of Applied Physiology, 118, 691–728.

[15] Kim, J., Kim, Y.-M., Lebaka, V. R., & Wee, Y.-J. 2022. Lactic acid for green chemical industry: recent advances in and future prospects for production technology, recovery, and applications. Fermentation, 8(11), 609.

[16] Cubas- Cano, E., González-Fernández, C., Ballesteros, M., & Tomás-Pejó, E. 2018. Biotechnological advances in lactic acid production by lactic acid bacteria: lignocellulose as novel substrate. Biofuels, Bioproducts and Biorefining, 12(2), 290–303.

[17] P Pawar, R., U Tekale, S., U Shisodia,
S., T Totre, J., & J Domb, A. 2014.
Biomedical applications of poly (lactic acid).
Recent Patents on Regenerative Medicine,
4(1), 40–51.

[18] Yadav, P., Chauhan, A. K., Singh, R. B., Khan, S., & Halabi, G. 2022. Organic acids: microbial sources, production, and applications. In Functional foods and nutraceuticals metabolic in and noncommunicable diseases 325-337). (pp. Elsevier.

[19] Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide.J. Econ. Entomol, 18(2), 265–267.

[20] I. Morgunov, I. G., Kamzolova, S. V, Dedyukhina, E. G., Chistyakova, T. I., Lunina, J. N., Mironov, A. A., Stepanova, N. N., Shemshura, O. N., & Vainshtein, M. B. 2017. Application of organic acids for plant protection against phytopathogens. Applied Microbiology and Biotechnology, 101, 921– 932.

[21] Chakraborty, S., Singha, S., & Chandra, G. 2010. Mosquito larvicidal effect of orthophosporic acid and lactic acid individually or their combined form on Aedes aegypti. Asian Pacific Journal of Tropical Medicine, 3(12), 954–956.

[22] He, Z., Fang, Y., Li, D.-C., Chen, D.-S., & Wu, F. 2021. Effect of lactic acid supplementation on the growth and reproduction of Bombyx mori (Lepidopteria: Bombycidae). Journal of Insect Science, 21(2), 7.

[23] Ahmed, F. S., Mahmoud, A.-B. S. E.-D., EL-Swaify, Z. A., & Salah El-Din, R. A. 2023. A comparative Evaluation of Phytochemical and Antimicrobial Properties of Selected Aquatic and Terrestrial Halophyte Plants Growing in Egypt. International Journal of Theoretical and Applied Research, 16–29.

[24] El-kady, A. M., Mohamed, A. I., & Mohamady, A. H. 2010. Insecticidal activity of citric acid and their soluble powder formulations against Aphis craccivora under laboratory conditions. Egyptian Academic Journal of Biological Sciences, F. Toxicology & Pest Control, 2(1), 7–12.

[25] Hatem, R. B., Al-jedawi, Y. D. R., & Mohmed, A. S. 2020. Lethal Effects of Crude Secondary Metabolites of Trichoderma Harzianum Against Myzus Persicae (Homoptera : Aphididae). 20(2), 4997–5000.