Antibacterial Activity of *Crocus sativus* Essential Oil against Selected Phytopathogenic Bacteria and its Insecticidal Potential

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Abstract
Crocus sativus, commonly known as saffron crocus, is a plant that looks delicately fragrant and produces stigmas. When harvested and dried, these stigmas become saffron. Saffron is a spice that has been used by humans since ancient times. It is one of the more expensive spices in the world, so its cultivation is of great economic significance wherever it is grown. The aim of our study was the investigation of the antibacterial potential of *Crocus sativus* essential oil against plant pathogens and its insecticidal activity. The bacterial species *Agrobacterium radiobacter, Pectobacterium carotovorum, Priestia megaterium, Pseudomonas syringae* and *Xanthomonas arboricola* were used to test the antimicrobial activity and insect *Megabruchidius dorsalis* was used for insecticidal effect. The antimicrobial activity was monitored by disk diffusion method under *in vitro* and gas phase conditions on fruit (strawberry) and vegetable (carrot) models. Under *in vitro* conditions, the best antimicrobial activity was found against *X. arboricola*, and under *in situ* condition, the best antimicrobial activity were found against *A. radiobacter* on strawberry model and against *P. megaterium* on carrot model at the lowest concentration of *C. sativus* essential oil. The best insecticidal activity was found at the highest concentrations 100 % of *C. sativus* essential oil used. Our study demonstrated the antibacterial and insecticidal activity of *C. sativus* essential oil.

Keywords: *Crocus sativus*, antibacterial and insecticidal activity, *in vitro, in situ*

1. Introduction

Crocus sativus, commonly known as saffron crocus, is a delicate-looking lavender plant that produces stigmas. When harvested and dried, these stigmas create saffron, a spice that has been used by humans since ancient times. It is one of the costliest spices in the world, and as such, its cultivation has great economic significance wherever it takes place. The plant is indigenous to south-west Asia. The precise geographical origin of *C. sativus* is not fully established; however, it is currently cultivated in Western Asia, Turkey, Iran, Greece, India and Spain [1]. Currently, Iran is responsible for approximately 90 % of global saffron production [2].

Saffron oil, obtained through the distillation of saffron flowers, is considered a valuable commercial product due to its pharmacological properties and its olfactory characteristics. A substantial corpus of reviews has emerged addressing the multifaceted facets of saffron, encompassing domains such as cultivation, extraction methodologies, historical applications, and pharmacological properties [3-11]. As early as the earliest of days, the utilization of saffron oil has been recorded in a variety of regions

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throughout the world in the sphere of conventional medicine for the prevention and treatment of a wide range of diseases and health conditions [8].
As evidenced by Bakkali et al. [12], essential oils have various biological activities, which include but are not limited to antimicrobial, antiviral, antioxidant, and anticancer properties. Saffron oil, in particular, has significant applications in the food industry. It is used to color and flavor a variety of food products, drinks, and beverages. In addition to its coloring and flavoring properties, saffron oil has antimicrobial and antioxidant properties [13]. This means that it can be used to improve the quality of food products. The food industry is currently experiencing a period of rapid change, with the increasing production of preserved foods for consumers. It is evident that consumers have a clear preference for foodstuffs that are preserved using safe methods [14]. Microbial activity is one of the most prevalent causes of spoilage, which is a major concern in the food industry. This is due to the significant economic losses that it causes, as well as its potential to have serious public health consequences. Microorganisms produce saccharolytic, proteolytic, pectinolytic, and lipolytic enzymes, which are associated with metabolic end products that are characteristic of spoilage [15]. Therefore, it can be concluded that microbial activity is of great importance in the manifestation of spoilage. The manifestation of microbial food spoilage is characterized by the occurrence of visible growth and changes.
The objective of the present study was twofold. Firstly, it set out to investigate the antibacterial potential of Crocus sativus essential oil against plant pathogens. Secondly, it investigated its insecticidal activity.

2. Materials and methods

2.1. Essential oil
Crocus sativum essential oil was purchased from Inevita SK (Bratislava, Slovakia). The essential oil was stored at 4 °C before use. The essential oil was produced by CO_2 extraction.

2.2. Bacterial strains
The antibacterial activity of *Crocus sativum* essential oil (CSEO) was evaluated against a range of Gram-negative (G^-) bacterial strains including *Agrobacterium radiobacter* CCM 2926, *Pectobacterium carotovorum* CCM 1008^T, and *Pseudomonas syringae* CCM 2868 and *Xanthomonas arboricola* CCM 1441 and Gram-positive (G^+) bacteria *Priestia megaterium* CCM 2007**.** All bacterial strains were obtained from the Czech Collection of Microorganisms in Brno, Czech Republic. Bacterial inocula were cultured in Mueller-Hinton broth (MHB, Oxoid, Basingstoke, UK) for 24 h at 37 °C before analysis. The optical density of the inocula was adjusted to 0.5 McFarland standard on the day of the experiment.

2.3. Disc diffusion method
In order to evaluate the antibacterial activity, the disc diffusion method was employed, as previously described. Small discs (6 mm in diameter) saturated with CSEO were placed on Mueller-Hinton agar (MHA) for bacterial strains. The bacterial strains were incubated at 37°C for 24 hours. The measurement of the inhibition zones was conducted in mm. The blank discs functioned as negative controls, while the antibiotic discs (cefoxitin for Gram-positive bacteria, gentamicin for Gram-negative bacteria, from Oxoid, Basingstoke, UK) served as positive controls [16].

2.3. In situ antimicrobial activity
In order to assess the *in situ* antimicrobial activity of CSEO, a range of substrates were tested, including commercial strawberry and carrot, as well as specific both Gram-positive and Gram-negative bacteria. The substrates were sliced into pieces measuring 0.5 mm, cleaned, and placed in 60 mm Petri dishes which had been inoculated with bacteria. CSEO was dispersed in ethyl acetate at concentrations of 500, 250, 125, and 62.5 μg/mL. Ethyl acetate filter sheets served as controls. The plates were sealed and incubated at 37 °C for a period of seven days. Microbial colony growth was assessed using ImageJ to calculate bacterial volume densities, along with standard methods for measuring in situ colony development [16].

2.4. Insecticidal activity
The insecticidal activity of CSEO was assessed using *Megabruchidius dorsalis* Fahreus, 1839 as the model organism. Groups of fifty *M. dorsalis* insects were placed in Petri dishes, each lined with sterile filter paper. Various concentrations of

CSEO (100 %, 50 %, 25 %, 12.5 %, 6.25 %, and 3.125 %) were prepared by diluting the CSEO with a 0.1% polysorbate solution. After saturating sterile filter paper discs with 100 µL of each CSEO concentration, the plates were sealed with parafilm and left at room temperature for 24 hours. The control group received 100 µL of the 0.1 % polysorbate solution. After one full day, the number of living and dead insects was counted. This experimental procedure has been replicated successfully in three separate studies.

3. Results and discussion

The aim of our work was the antibacterial activity of CSEO under *in vitro* and *in situ* conditions against 5 plant pathogens. The best antimicrobial activity was found against bacteria *X. arboricola* (Figure 1). The lowest antibacterial activity was found against *A. radiobacter* 7.33 mm). Next, we investigated the antibacterial activity of CSEO in vapor phase on strawberry and carrot model. The best inhibited bacteria was *A. radiobacter* (89.76 %) at the lowest concentration (Figure 2), while on the carrot model the best inhibited bacteria was *P. megaterium* (88.67 %) at the lowest concentration tested (Figure 3). It was determined that the active constituents of saffron, namely safranal (8–16 mg/mL) and crocin (64–128 mg/mL), exhibited antibacterial activity, particularly against *Salmonella* [17]. This is a primary reason why bacteria do not proliferate in saffron spices. A further study demonstrated the efficacy of an ethyl acetate extract of saffron stigma, employing the cup plate diffusion method, against a range of bacterial (*Micrococcus luteus, Staphylococcus epidermitis, Staphylococcus aureus*, and *Escherichia coli*) and fungal (*Candida albicans, Aspergillus niger*, and *Cladosporium* sp.) strains. The ethanolic and methanolic extracts of saffron also demonstrated moderate anti-*Brucella* activity using the disc diffusion method [18, 19].

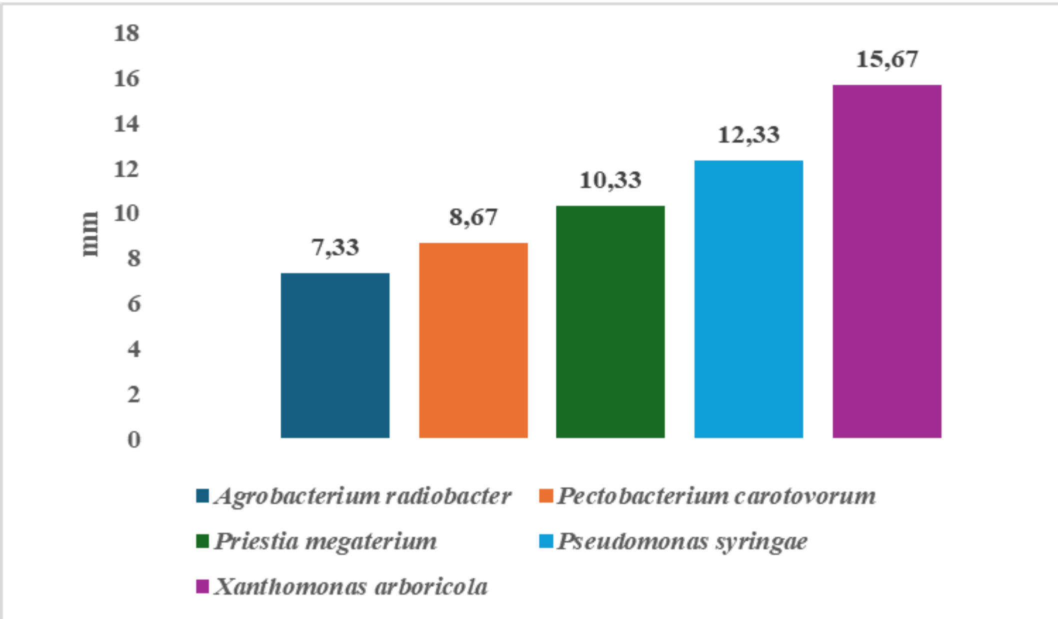

Figure 1. Antimicrobial activity of CSEO with disc diffusion method in mm

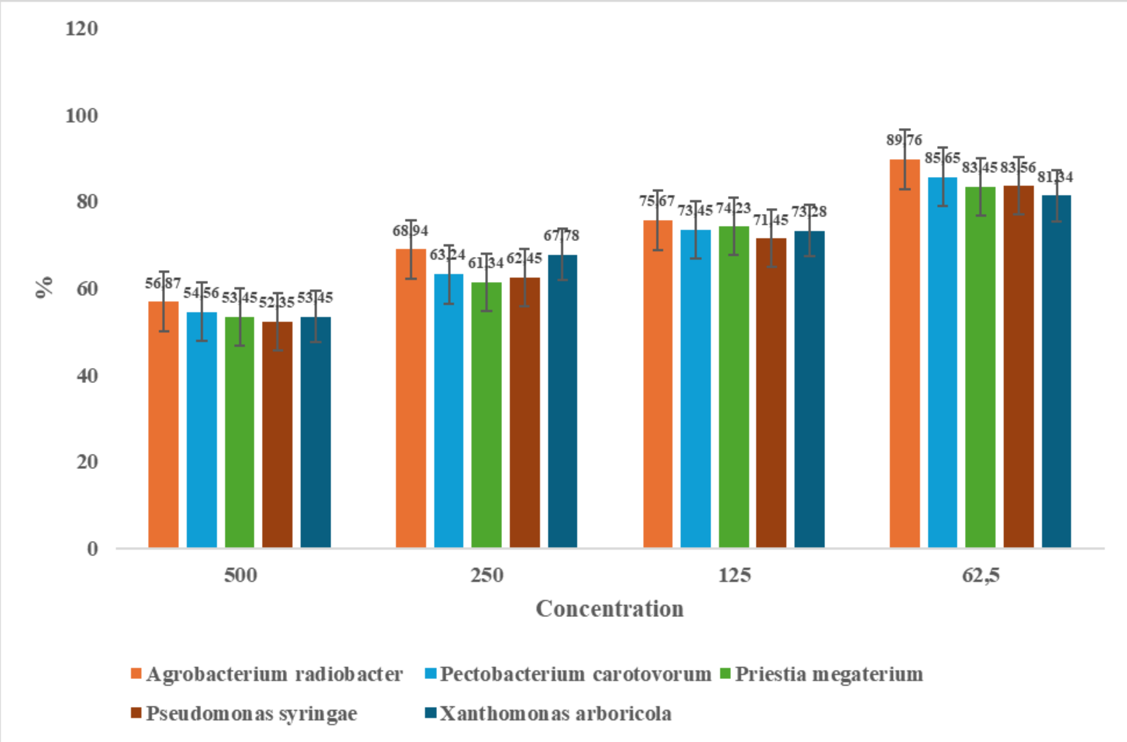

Figure 2. Antimicrobial activity *in situ* on strawberry in %

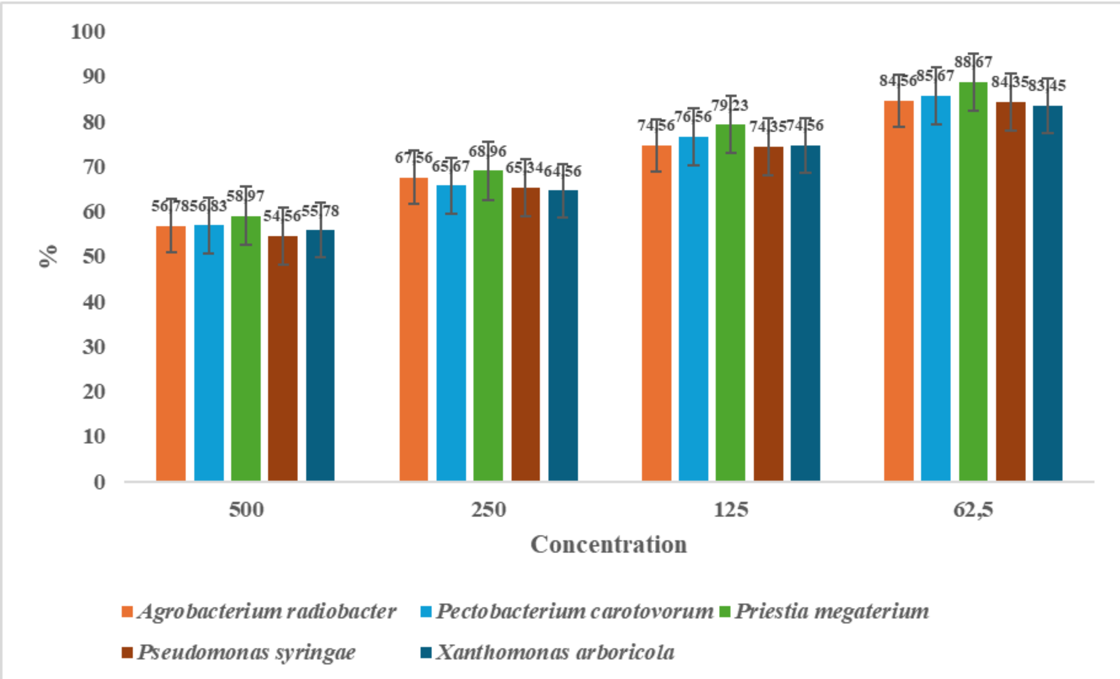

Figure 3. Antimicrobial activity *in situ* on carrot in %

As illustrated in Table 1, the study examined the insecticidal efficacy of CSEO against *M. dorsalis*. The findings indicated that the highest levels of insecticidal activity were achieved when 50 % and 100 % of the CSEO solution was applied. However, concentrations of 6.25 % and 3.125 % of CSEO did not demonstrate a significant repellent effect against *M. dorsalis*. It is worthy of note that the *M. dorsalis* population (50 %) was impacted by a concentration of 12.5 %. Conversely, 25 % of the insects were effectively killed by that concentration. The study set out to determine the effects of saffron petal extract on *T. confusum* adults. The findings revealed that the extract caused fumigant toxicity, and that mortality increased with both increasing concentration and duration of exposure [19]. Since repellents are regarded as one of the new methods of controlling stored-products insect pests, the repellency of saffron petal extract was evaluated in *T. confusum* adults. The study found that the repellency of saffron petal extract on *T. confusum* adults increased with increased time of exposure. The chemical compounds of saffron petals have been shown to confirm the presence of terpenoids, ethyl acetate, methanol, and an aqueous extract of saffron petals. The presence of these chemical compounds has been proven to demonstrate the antibacterial properties of saffron petal extract [20]. Essential oils (EOs) have been shown to possess significant pesticidal properties, including insecticidal effects. Consequently, they have become the focus of research in recent decades, with the aim of identifying EOs that could be used as active ingredients in botanical insecticides. While the insecticidal efficacy of numerous EOs is well documented, there is a paucity of information regarding the efficacy of their individual major compounds against different insects. This is despite the fact that several insects are among the most common pests of many cultivated crops, and they are becoming resistant to traditional active substances such as pyrethroids, neonicotinoids, and carbamates [21].

Table 1. Insecticidal activity of CSEO against *Megabruchidius dorsalis* (n=50)

Concentration (%)	Number of Living Individuals	Number of Dead Individuals	Insecticidal Activity (%)
100	0	100	100.00 ± 0.00
50	10	90	90.00 ± 0.00
25	30	80	80.00 ± 0.00
12.5	70	30	30.00 ± 0.00
6.25	100	0	0.00 ± 0.00
3.125	100	0	0.00 ± 0.00
Control group	100	0	0.00 ± 0.00

4. Conclusions

The results of our experiments revealed good antimicrobial activity of saffron essential oil against various species of bacteria belonging to plant pathogens under in vitro and in situ conditions. The insecticidal effect against *M. dorsalis* was also demonstrated. There is considerable interest in the screening and development of safer alternatives, with botanicals being the focus of a significant degree of attention. It is evident that botanicals pose minimal threat to both human health and the environment. This can be ascribed to their low mammalian toxicity and minimal environmental persistence. Essential oils can be extracted from various plant parts, including barks, flowers, buds, leaves, peels and resins. The extraction process principally involves the utilization of steam distillation, a method that has been employed for centuries. These oils are recognized for their composition of a diverse array of monoterpenes, sesquiterpenes, and their derivatives, numbering in the hundreds. The biological activities of essential oils have been demonstrated to extend to a variety of effects, including alterations to the shelf life of products, food safety and insecticidal properties.

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References

1. Hill, T., The Contemporary Encyclopedia of Herbs and Spices: Seasonings for the Global Kitchen (first ed.), Wiley 2004.

2. Ghorbani, M., The efficiency of saffron's marketing channel in Iran, World Applied Science Journal, 2008, 4, 523-527.

3. Liakopoulou-kyriakides, M., Kyriakidis, D.A., Crocus sativus-biological active constituents Studies in Natural Products Chemistry, 2002, pp. 293-312.

4. Ahmed, H., Dharamveer, R. Dixit, S. Saraf, Srivastava, R., *Crocus sativus* L., A comprehensive review, Pharmacognosy Reviews, 2010, p. 200.

5. Bhargava, V.K., Medicinal uses and pharmacological properties of *Crocus sativus* Linn (saffron), International Journal of Pharmacology and Pharmaceutical Sciences, 2011, pp. 22-26.

6. Katariya, D.C., Nerkar, N., Gadiya, R.V., Abhyankar, M.M., Detailed profile of *Crocus sativus* International Journal of Pharmacology and Biological Science, 2011, pp. 530-540.

7. Ulbricht, C., Conquer, J., Costa, D., Hollands, W., Iannuzzi, C., Isaac, R., Jordan, J.K., Ledesma, N., Ostroff, C., Serrano, J.M.G., Shaffer, M.D., Varghese, M., An evidence-based systematic review of saffron (*Crocus sativus*) by the Natural Standard Research Collaboration, Journal of Dietary Supplements, 8 (2011), pp. 58-114

8. Hosseinzadeh, H., Nassiri-Asl, M., Avicenna's (Ibn Sina) the Canon of Medicine and saffron (*Crocus sativus*): a review, Phytotherapy Research, 2013, pp. 475-483.

9. Wright, K., *Crocus sativus* (Saffron): a monograph Australian Journal of Herbal and Naturopathic Medicine, 2014, pp. 18-21

10. Amin, B. Hosseinzadeh, H., Bioactive nutraceuticals and dietary supplements in neurological and brain disease, R.R. Watson, V.R. Preedy (Eds.), Chapter 33: Analgesic and Anti-inflammatory Effects of Crocus Sativus L. (Saffron), Academic Press 2015, pp. 319-324

11. Nassiri-Asl, M. Hosseinzadeh, H., Bioactive nutraceuticals and dietary supplements in neurological and brain disease, R.R. Watson, V.R. Preedy (Eds.), Chapter 3: Neuropharmacology Effects of Saffron (*Crocus sativus*) and Its Active Constituents, Academic Press 2015, pp. 29-39

12. Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M., Biological effects of essential oils—a review, Food Chemical Toxicology, 2008, pp. 446-475

13. Armellini, R., Peinado, I., Pittia, P., Scampicchio, M., Heredia, A., Andres, A., Effect of saffron (*Crocus sativus* L.) enrichment on antioxidant and sensorial properties of wheat flour pasta, Food Chemistry, 2018, pp. 55–63.

14. Hassoun, A., Jagtap, S., Trollman, H., Garcia-Garcia, G., Abdullah, N. A., Goksen, G., Bader, F., Ozogul, F., Barba, F. J., Cropotova, J., Munekata, P. E. S., Lorenzo, J. M., Food processing 4.0: Current and future developments spurred by the fourth industrial revolution, Food Control, 2023, 109507.

15. Lorenzo, J. M., Munekata, P. E., Dominguez, R., Pateiro, M., Saraiva, J.A., Franco, D., Main Groups of Microorganisms of Relevance for Food Safety and Stability: General Aspects and Overall Description, Innovative Technologies for Food Preservation. 2018, pp. 53–107.

16. Kačániová, M., Čmiková, N., Vukovic, N. L., Verešová, A., Bianchi, A., Garzoli, S., Ben Saad, R., Ben Hsouna, A., Ban, Z., Vukic, M. D., *Citrus limon* Essential Oil: Chemical Composition and Selected Biological Properties Focusing on the Antimicrobial (In Vitro, In Situ), Antibiofilm, Insecticidal Activity and Preservative Effect against *Salmonella enterica* Inoculated in Carrot, Plants, 2004, 524.

17. Mafe, A. N., Edo, G. I., Makia, R. S., Joshua, O. A., Akpoghelie, P. O., Gaaz, T. S., Jikah, A. N., Yousif, E., Isoje, E. F., Igbuku, U. A., Ahmed, D. S., Essaghah, A. E. A., Umar, H., A review on food spoilage mechanisms, food borne diseases and commercial aspects of food preservation and processing, Food Chemistry Advances, 2024, 100852.

18. Pintado, C., Miguel, A.D., Acevedo, O., Nozal, L., Novella, J.L., Rotger R., Bactericidal effect of saffron (*Crocus sativus* L.) on *Salmonella enterica* during storage, Food Control, 2011, pp. 638-642.

19. Motamedi, H., Darabpour, E., Gholipour, M., Seyyed, N.S.M., *In vitro* assay for the anti-Brucella activity of medicinal plants against tetracycline-resistant *Brucella melitensis*, Journal of Zhejiang University Science B, 2010, pp. 506-511

20. Ibrahim, S. S., Salem, N. Y., Insecticidal efficacy of nano zeolite against *Tribolium confusum* (Col., Tenebrionidae) and *Callosobruchus maculatus* (Col., Bruchidae). Bulletin of the National Research Centre, 2019.

21. Gong, P., Li, X., Wang, C., Zhu, S., Li, Q., Zhang, Y., Li, X., Li, G., Liu, E., Gao, H., et al. The Sensitivity of Field Populations of *Metopolophium dirhodum* (Walker) (Hemiptera: Aphididae) to Seven insecticides in Northern China, Agronomy, 2021, pp. 1556.