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THEME

Assessment of the Biocontrol Potential of *Pseudomonas chlororaphis* subsp. *aureofaciens* and *Virgibacillus halodenitrificans* Against Stored-Product Beetles

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Before the jury:

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الملخص

تمثل حشرات المواد المخزنة تحدياً رئيسياً لحفظ الحبوب في جميع أنحاء العالم، حيث تتسبب في خسائر كمية ونوعية كبيرة أثناء التخزين. وقد أدى الاستخدام المكثف للمبيدات الحشرية الكيميائية إلى مشكلات بيئية وآثار سلبية على الكائنات غير المستهدفة، بالإضافة إلى ظهور مقاومة لدى الحشرات، مما يبرز الحاجة إلى بدائل مستدامة وصديقة للبيئة. هدفت هذه الدراسة إلى تقييم الفعالية الضد حشرية لسلالتين بكتيريتين من منطقة الجذور هما *Pseudomonas chlororaphis* subsp. *aureofaciens* و Virgibacillus *halodenitrificans* ضد نوعين مهمين من آفات الحبوب المخزنة هما *Oryzaephilus surinamensis* و *Tribolium confusum*. أُجريت الاختبارات الحيوية باستخدام خلايا بكتيرية مغسولة و بكتيريا نامية في وسطي Nutrient Broth Yeast Extract (NBY) و King B اللذين اختيرا لتحفيز إنتاج المركبات الحيوية الفعالة. عُرّضت الحشرات البالغة لحبوب معالجة بالبكتيريا لمدة 28 يوماً، وسُجلت معدلات الوفيات. أظهرت النتائج أن *P. chlororaphis* تمتلك أعلى نشاط ضد حشري، حيث بلغت نسبة الوفيات 60٪ ضد *O. surinamensis* عند استخدام وسط King B ، و40٪ ضد كل من *O. surinamensis* و *T. confusum* عند استخدام وسط NBY. في المقابل، أظهرت *V. halodenitrificans* نشاطاً محدوداً، إذ بلغت أعلى نسبة وفيات 25٪ ضد *O. surinamensis* في وسط NBY ، ولم يظهر أي تأثير ملحوظ ضد *T. confusum*. كما لم تُسجل أي وفيات في المعاملات الشاهدة. يُحتمل أن تعود الفعالية العالية لـ *P. chlororaphis* إلى إنتاجها لسيانيد الهيدروجين والفينازينات ومركبات أخرى ذات نشاط حشري، في حين قد ترتبط الفعالية المحدودة لـ *V. halodenitrificans* بمستقلبات ثانوية لا يزال دورها في إحداث وفيات الحشرات غير مفهوم بشكل كامل. تؤكد هذه النتائج الإمكانات الواعدة لـ *P. chlororaphis* كعامل للمكافحة الحيوية ضد آفات الحبوب المخزنة. إضافة إلى ذلك، فإن خصائصها المعروفة في تعزيز نمو النبات ومكافحة الفطريات تجعلها كائناً دقيقاً متعدد الوظائف ذا أهمية كبيرة في الزراعة المستدامة. وتوصى الدراسات المستقبلية بإجراء تحليلات أيضية وجزيئية لتحديد المركبات المسؤولة عن النشاط الضد حشري وفهم آليات عملها بشكل أفضل.

الكلمات المفتاحية *Pseudomonas chlororaphis* ، *Virgibacillus halodenitrificans* ، المكافحة الحيوية، حشرات المواد المخزنة، *Oryzaephilus surinamensis* ، *Tribolium confusum* ، سيانيد الهيدروجين، الفينازينات.

Abstract

Stored-product insect pests represent a major challenge to cereal preservation worldwide, causing substantial quantitative and qualitative losses during storage. The extensive use of synthetic insecticides has led to environmental concerns, adverse effects on non-target organisms, and the development of insect resistance, highlighting the need for sustainable alternatives. The present study aimed to evaluate the insecticidal potential of two rhizosphere bacterial strains, *Pseudomonas chlororaphis* subsp. *aureofaciens* and *Virgibacillus halodenitrificans*, against two important stored-product pests, *Oryzaephilus surinamensis* and *Tribolium confusum*. Bioassays were conducted using bacterial washed cells and cultures grown in Nutrient Broth Yeast Extract (NBY) and King B media, selected for their ability to stimulate the production of bioactive metabolites. Adult insects were exposed to treated cereal grains for 28 days, and mortality rates were recorded. The results demonstrated that *P. chlororaphis* exhibited the highest insecticidal activity, achieving mortality rates of 60% against *O. surinamensis* in King B medium and 40% against both *O. surinamensis* and *T. confusum* in NBY medium. In contrast, *V. halodenitrificans* showed limited activity, with a maximum mortality rate of 25% against *O. surinamensis* in NBY medium and no significant effect against *T. confusum*. No mortality was observed in the negative controls during the 28-day exposure period. The enhanced efficacy of *P. chlororaphis* is likely associated with the production of hydrogen cyanide, phenazines, and other insecticidal metabolites, whereas the weak activity of *V. halodenitrificans* may be related to secondary metabolites whose role in insect mortality remains poorly understood. These findings confirm the potential of *P. chlororaphis* as a promising biological control agent for stored-product insect management. Furthermore, its known plant growth-promoting and antifungal properties make it a particularly valuable multifunctional microorganism for sustainable agriculture. Future studies should focus on metabolomic and molecular analyses to identify the compounds responsible for insect mortality and to clarify their mechanisms of action.

Keywords: *Pseudomonas chlororaphis*, *Virgibacillus halodenitrificans*, biological control, stored-product insects, *Oryzaephilus surinamensis*, *Tribolium confusum*, hydrogen cyanide, phenazines.

Résumé

Les insectes ravageurs des produits stockés représentent une menace majeure pour la conservation des céréales dans le monde, provoquant d'importantes pertes quantitatives et qualitatives durant le stockage. L'utilisation intensive des insecticides chimiques a entraîné des problèmes environnementaux, des effets néfastes sur les organismes non ciblés ainsi que l'apparition de phénomènes de résistance, ce qui souligne la nécessité de développer des alternatives durables. La présente étude avait pour objectif d'évaluer le potentiel insecticide de deux souches bactériennes rhizosphériques, *Pseudomonas chlororaphis* subsp. *aureofaciens* et *Virgibacillus halodenitrificans*, contre deux ravageurs importants des denrées stockées, *Oryzaephilus surinamensis* et *Tribolium confusum*. Les bioessais ont été réalisés à l'aide de cellules bactériennes lavées et de cultures développées sur les milieux Bouillon nutritif à l'extrait de levure (NBY) et King B, choisis pour leur capacité à stimuler la production de métabolites bioactifs. Les insectes adultes ont été exposés à des grains de céréales traités pendant 28 jours et les taux de mortalité ont été enregistrés. Les résultats ont montré que *P. chlororaphis* présentait l'activité insecticide la plus élevée, avec des taux de mortalité atteignant 60 % contre *O. surinamensis* sur milieu King B et 40 % contre *O. surinamensis* et *T. confusum* sur milieu NBY. En revanche, *V. halodenitrificans* a montré une activité limitée, avec un taux maximal de mortalité de 25 % contre *O. surinamensis* sur milieu NBY et aucun effet significatif contre *T. confusum*. Aucune mortalité n'a été observée dans les témoins négatifs pendant les 28 jours de l'expérimentation. L'efficacité supérieure de *P. chlororaphis* pourrait être liée à la production de cyanure d'hydrogène, de phénazines et d'autres métabolites à activité insecticide, tandis que la faible activité de *V. halodenitrificans* pourrait être attribuée à des métabolites secondaires dont le rôle exact dans la mortalité des insectes reste à élucider. Ces résultats confirment le potentiel de *P. chlororaphis* comme agent prometteur de lutte biologique contre les insectes des produits stockés. De plus, ses propriétés reconnues de promotion de la croissance végétale et de lutte antifongique en font un microorganisme multifonctionnel particulièrement intéressant pour une agriculture durable. Des études complémentaires fondées sur des analyses métabolomiques et moléculaires sont nécessaires afin d'identifier les composés responsables de l'activité insecticide et de mieux comprendre leurs mécanismes d'action.

Mots-clés : *Pseudomonas chlororaphis*, *Virgibacillus halodenitrificans*, lutte biologique, insectes des produits stockés, *Oryzaephilus surinamensis*, *Tribolium confusum*, cyanure d'hydrogène, phénazines.

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Introduction

Introduction

For more than 10,000 years, wheat (*Triticum* spp.), belonging to the *Poaceae* family, has represented a fundamental element of human civilization. Showing remarkable adaptability and tolerance, this crop flourishes across diverse agroclimatic zones, including temperate, subtropical, and semi-arid areas **(Sharma et al., 2025).** As it is the most important primary crop in these regions, its demand continues to escalate in countries experiencing urbanization and industrialization. Wheat is known for its high content of carbohydrates as it's regarded as a major source of energy. In addition, it also provides notable amounts of proteins, fiber, as well as micronutrients components, including lipids, vitamins, minerals, and phytochemicals, all of which are essential for a healthy diet **(Shewry and Hey, 2015).**

In terms of global importance, wheat ranks as the second most cultivated and produced crop worldwide based on cultivated area and total production volume. During the marketing year of 2024/25, the global production of wheat reached nearly 793 million metric tons **(Anonymous, 2025a)**. It is extensively cultivated in temperate regions worldwide **(Encyclopaedia Britannica, 2024)**. In Algeria, wheat production and consumption are projected to remain constant in 2025-26, with the harvest forecast at 3 million tons and consumption at 11.9 million tons **(Anonymous, 2025b)**.

Each year, nearly 20% of wheat is lost due to diseases, among 200 diseases, 50 cause economic losses and are widely spread, including rusts, smut, tan spot, spot blotch, Fusarium head blight, common root rot, Septoria blotch, powdery mildew, blast, various viral, nematode, and bacterial diseases cause mortality of the plants **(Singh et al., 2023)**. Throughout the plant's life stages, several arthropods cause significant harm; most of these pests are either polyphagous or oligophagous and are rarely monophagous **(Bin Farook et al., 2019)**.

Among the biotic elements that affect the stored cereal grains, storage pests cause serious damage **(Guru et al., 2022)**. Globally, around one thousand species of insects have been identified as storage infesting pests, where they cause grain damage and contamination with undesirable odours and flavours **(Srivastava and Subramanian, 2016)**.

Wheat-related stored pests can invade throughout various stages, starting from the field, in storage sacks, agricultural machinery, during transportation, handling, processing or storage channels **(Negbenebor et al., 2024)**.

Many factors facilitate the distribution of stored product pests, including grain movement between regions either passively or by the active flight of adult insects **(Srivastava and Subramanian, 2016)**.

Several strategies have been developed to control the threat of insect pests in stored wheat grains, including both non-chemical and synthetic pesticides. Nevertheless, synthetic pesticides remain the most widely used despite their disadvantages, such as risks to the environment and non-target organisms, including humans, and the development of pest resistance. These problems lead to a search for safer and more eco-friendly pest control methods **(Negbenebor et al., 2024)**.

The objective of the present work is to experimentally evaluate the effectiveness of two bacterial strains already described as antifungal agents, against insect pests affecting cereals.