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EVALUATING THE PATHOGENICITY FROM THE STRAIN CAUSING LEAF BLIGHT ON *Allium chinense* AND THE INHIBITORY EFFECTIVENESS OF SOME ANTIBIOTIC AND CHEMICAL COMPOUNDS

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Abstract

The leaf blight disease from Allium chinense caused by Xanthomonas spp. has led to significant economic losses for farmers of this crop in Dong Thap province. This study aims to evaluate the pathogenicity of Xanthomonas spp. on Allium chinense and Allium sativum (garlic) and assess the effectiveness of some antibiotics and chemical compounds in controlling this pathogen. The results indicate that the bacteria responsible for leaf blight on Allium chinense can also infect garlic, causing characteristic symptoms such as small, pale yellow lesions at the leaf tips or along the veins. These lesions subsequently expand, leading to leaf yellowing, browning, and necrosis. Additionally, in vitro experiments revealed that oxolinic acid 20% exhibited the highest antibacterial efficacy, with an inhibition zone diameter of 36.3 mm after a 72 hour test. These findings suggest that oxolinic acid has strong potential for controlling the leaf blight pathogen in Allium chinense and Allium sativum (garlic).

Keywords: *Allium chinense, Antibiotic and chemical Compounds, Leaf blight, Pathogenicity, Oxolinic acid.*

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KHẢ NĂNG GÂY HẠI CỦA DÒNG VI KHUẨN GÂY BỆNH CHÁY LÁ TRÊN CÂY KIỆU (*Allium chinense*) VÀ HIỆU QUẢ ỨC CHẾ CỦA MỘT SỐ HOẠT CHẤT THUỐC HÓA HỌC

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Tóm tắt

Bệnh cháy lá trên cây kiệu (Allium chinense) do Xanthomonas spp. gây ra đã gây thiệt hại kinh tế đáng kể cho nông dân trồng kiệu tại tỉnh Đồng Tháp. Nghiên cứu này nhằm đánh giá khả năng gây bệnh của dòng vi khuẩn này trên cây kiệu và cây tỏi (Allium sativum), đồng thời khảo sát hiệu quả kiểm soát tác nhân gây bệnh của một số hoạt chất thuốc hóa học phổ biến. Kết quả ghi nhận vi khuẩn gây bệnh cháy lá trên cây kiệu cũng có thể lây nhiễm sang cây tỏi, gây ra các triệu chứng đặc trưng như xuất hiện vết bệnh nhỏ màu vàng nhạt trên chóp lá hoặc dọc theo gân lá. Các vết bệnh sau đó lan rộng, khiến lá chuyển vàng, vàng nâu và có dấu hiệu cháy khô. Bên cạnh đó, từ thử nghiệm in vitro, Oxolinic acid 20% đã thể hiện khả năng ức chế vi khuẩn gây bệnh cao nhất với đường kính vòng đối kháng đạt 36,3 mm sau 72 giờ thử nghiệm. Kết quả này ghi nhận tiềm năng kiểm soát tác nhân gây bệnh cháy lá trên cây kiệu và cây tỏi bằng oxolinic acid.

Từ khóa: *Allium chinense, Bệnh cháy lá, Hợp chất kháng sinh và hóa học, Khả năng gây bệnh, Oxolinic acid.*

1. Introduction

Allium chinense, belonging to the Alliaceae family, is a commercially valuable plant species worldwide (Bah et al., 2012). Recently, bacterial leaf blight has severely affected this crop production in the Mekong Delta, particularly in Tam Nong district, Dong Thap province (Thuy, 2022). The pathogen has a wide host range and uses various virulence factors to invade crops (Timilsina et al., 2020).

Currently, disease control in the area primarily relies on chemical pesticides. However, no studies have reported the type of chemical compound capable of inhibiting the pathogen in *in vitro* conditions. Furthermore, the overuse of chemicals negatively impacts human health and the environment and increases the risk of pathogen resistance. Applying chemical pesticides in controlling harmful bacteria is a feasible solution (Longhi et al., 2022). However, it is important to use the correct pathogen, as well as the proper ingredients and dosages of chemical pesticides. Therefore, this study evaluates *Xanthomonas* spp. pathogenicity on allium chinense, galic and identifies effective compounds to control the pathogen *in vitro*. The research results contribute to reducing pesticide overuse, decreasing the risk of resistance, optimizing production costs, and protecting public health as well as the agricultural environment.

2. Materials and research methods

2.1. Materials, Time, and Location of the Study

- Pathogenic bacterial strain with the *Xanthomonas* spp. XA32 was isolated from leaf blight samples from *Allium chinense* and is stored in the Biotechnology Laboratory, Dong Thap University.

- Experimental plants include: *Allium chinense*, a variety commonly grown in Tam Nong district, Dong Thap province; Garlic (*Allium sativum*), a variety commonly grown in Vinh Chau district, Soc Trang province.

- Culture medium used with King's B (Pepton 20 g; $MgSO_4$ 1.5 g; K_2HPO_4 1.5 g; Glycerol 15 mL; Agar 15 g; 1000 mL water).

- The experiment was conducted from November 2024 to February 2025 at the Biotechnology Laboratory and experimental area of Dong Thap University.

2.2. Research methods

2.2.1. Investigation of the pathogenicity of Xanthomonas spp. *strain on Allium chinense and Allium sativum (garlic)*

This study aims to investigate the pathogenicity of *Xanthomonas* spp. XA32 on *allium chinense* and garlic. The experiment was conducted on two types of plants, *Allium chinense* and garlic, with three replications. Each replication consisted of 10 plants per pot. There are three treatments, including XA1, which was the treatment for artificial infection with *Xanthomonas* spp. XA32 on garlic, and Control as the control treatment without artificial infection.

Experimental plants were prepared in the net-house, with 10 disease sympton-free seedlings per pot. After 60 days of growth, the experiment began. Meanwhile, the *Xanthomonas* spp. XA32 was cultured on King's B medium for 48 hours to allow growth. The bacteria colonies were then harvested and adjusted in sterile water to create a suspension with a concentration of 10^8 CFU/mL.

Artificial infection was performed by spraying the disease suspension onto the leaves. The control treatment was sprayed only with sterile water. The pots were then placed under humid, dark conditions (25°C) for 24 hours to create favorable conditions for infection. Afterward, the plants were transferred back to the net-house, where the leaves were sprayed with fresh water four times a day. Symptoms of the disease on the plants were observed and described. The bacteria were re-isolated from the diseased samples and compared with the strain used for infection to confirm the pathogen.

2.2.2. Investigation of in vitro inhibition against Xanthomonas spp. *causing leaf blight on Allium chinense by some antibiotic and chemical compounds*

The experiment was designed as a completely randomized design with three replications. The test was conducted with 11 groups of chemical compounds, including:

Table 1. List of antibiotic and chemical agents used in the experiment

Antibiotic and chemical Compounds	Usage Concentration
Oxolinic acid	20%
Streptomycin sulfate	10%
Bronopol	40%
Bronopol	20%
Bismerthiazol+ Streptromycin sulfate	Bismerthiazol (20%)
	Streptromycin sulfate (5%)
Thiodiazole-Zn	5%
Ningnamycin	3%
Bismerthiazol + Kasugamycin	Bismerthiazol (19%)
	Kasugamycin (1%)
Streptomycin + Ningnanmycin + Polyoxin B	Streptomycin (0,5%)
	Ningnanmycin (0,5%)
	Polyoxin B (1,1%)
Kasugamycin	6%
Bismerthiazol	30%

The *Xanthomonas* spp. XA32 was cultured on King's B medium for 48 hours. Then, 100 µL of the bacterial suspension (OD_{600} = 0.3) was mixed to a petri containing 15 mL of King's B medium at 40°C, gently shaken, and incubated in a sterile incubator for 5 minutes as a pathogenic bacteria petri. Besides, eleven types of antibiotic and chemical compounds were prepared depending on the recommended concentrations in sterile distilled water.

Testing the antibacterial inhibition was practiced by soaking each of the sterile filter paper discs (5 mm in diameter) in each compound solution for 15 minutes and then placed in a specific position on the petri dish containing the pathogen. The control sample was a filter paper disc soaked in sterile distilled water (Tam et al., 2023). The inhibition zone diameter of each compound was recorded after 24, 72, and 96 hours of testing. The effectiveness of each compound in inhibiting the bacteria was assessed.

2.2.3. Data analysis

Data were processed using Microsoft Excel software. Statistical analysis was performed using Minitab 16.1 software with Tukey's test at a 5% significance level.

3. Results and discussion

3.1. The pathogenicity of *Xanthomonas* spp. XA32 on *Allium chinense* and *Allium sativum* (garlic)

The experiment was conducted to assess the infectivity of the bacterial strain causing leaf blight on *A. chinense* and *A. sativum* (garlic) using artificial infection methods. The results showed that *Xanthomonas* spp. XA32 could cause disease in both tested plants. The symptoms observed on garlic were similar to those of leaf blight previously reported from *A. chinense* shallot.

On *A. chinense*, two days after inoculation (DAI), lesions formed at the leaf tips, becoming water-soaked and turning pale yellow, then gradually spreading to the leaf blade. The lesions turned orange-brown and dried out after five days (Figure 1-B). The plants showed poor growth, possibly due to reduced photosynthetic capacity. The control plants, which were not infected, developed typically.

On garlic, two DAI, the disease appeared as water-soaked lesions, pale yellow in color, at the leaf tips or along the leaf edges. The disease gradually spread down the leaf blade. Four DAI, the lesions turned yellow-brown and eventually black, causing tissue necrosis (Figure 1-E).

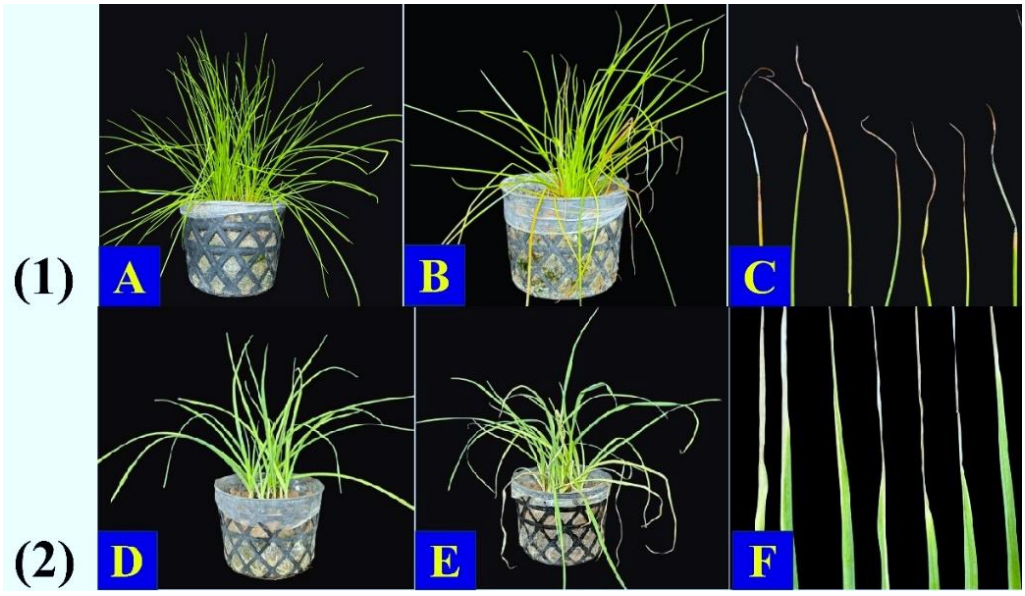

Figure 1. Pathogenicity of *Xanthomonas* spp. XA32 on *Allium chinense* and *Allium sativum* at 5 days after infection.

(1): Pathogenicity on *A. chinense*; (2): Pathogenicity on *A. sativum*;
A, D: Control treatment with no infection; B: Disease symptoms on *A. chinense* after 5 DAI; C: Infected leaves of *A. chinense* showing orange-brown lesions; E: Disease symptoms on *A. sativum* after 5 DAI; F: Infected leaves of *A. sativum* showing yellow-brown lesions.

Both symptoms on *A. sativum* and *A. chinense* were consistent with previous studies by Roumagnac *et al.* (2004), who recorded leaf blight on garlic, chives, and leeks with initial

water-soaked spots, which later turned into dry necrotic areas with a yellow color. The results were also in line with the study by Black et al. (2012) on leaf blight in onions, where plants infected early exhibited necrotic leaves, stunted growth, and smaller bulbs at harvest, as well as the research by Heiden et al. (2023) on monocot plants infected with *Xanthomonas*, which showed water-soaked leaves turning yellow along the veins.

Re-isolation from disease lesions obtained from infected plants on King's B medium showed a colony with characteristics similar to the original bacterial strain used for artificial inoculation. Overall, the research results demonstrate that the *Xanthomonas* spp. XA32 not only causes disease in *Allium chinense* but also can infect and produce similar symptoms in garlic.

3.2. The inhibition *in vitro* against *Xanthomonas* spp. causing leaf blight on *Allium chinense* by some antibiotics and chemical compounds

To select the chemical compounds with the highest effectiveness in inhibiting *Xanthomonas* spp. that causes leaf blight on *Allium chinense*, an in vitro experiment was conducted using 11 antibiotic and chemical compounds on petri containing *Xanthomonas* spp. XA32, which is responsible for causing leaf blight on *A. chinense* (Table 2).

Table 2. Inhibition zone diameters of antibiotic and chemical compounds against *Xanthomonas* spp. causing leaf blight on *Allium chinense*

Treatments	Inhibition zone diameter (mm)		
	24h	72h	96h
Oxolinic acid 20%	37.7^{a}	36.3^{a}	35.7^{a}
Bronopol 20%	8.0^{bc}	6.0^{bc}	6.0^{b}
Bronopol 40%	9.3^{b}	8.7^{b}	8.7^{b}
Average	18,3^{A}	17,0^{A}	16,8^{A}
Significance level	*	*	*

*The average numbers in each column followed by one or more identical letters (in lowercase or uppercase) indicate no statistically significant difference at the 5% significance level according to Tukey's test. * denotes a significant difference at the 5% level.*

Three chemical compounds (Oxolinic acid 20%, Bronopol 20%, Bronopol 40%) were showed effectively inhibited the pathogen after 72 hours of testing (Table 1). Among them, oxolinic acid 20% exhibited the highest inhibition efficiency, with an halo zone diameter of 37.7 mm from 24 hours of testing. Additionally, the two bronopol compounds (bronopol 20% and bronopol 40%) showed inhibitory effects, but were much lower, with halo zone diameters of 8 mm and 9.3 mm, respectively (Figure 2). The average inhibition zone diameters for these three compounds at three time points (24h, 72h, 96h) were similar, indicating that the inhibition capacity did not change over time.

Overall, oxolinic acid 20% demonstrated high efficacy in controlling the bacterial pathogen under *in vitro* conditions. These findings align with the study by Arauz et al. (2023), which examined the sensitivity of bactericides against bacterial pathogens in coffee plants, using bactericides such as oxytetracycline hydrochloride 5 WP, streptomycin plus oxytetracycline 16.5 WP, oxolinic acid 20 WP, and copper sulfate 24 SC. Among these,

oxolinic acid exhibited the most significant effect, as evidenced by larger inhibition zone diameters. Additionally, these results are consistent with studies evaluating the effectiveness of antibiotic and chemical compounds in controlling *Xanthomonas hortorum* pv. *carotae* (*Xhc*), the pathogen responsible for leaf blight on carrots (Kang et al., 2023). In their study, various compounds, including streptomycin, oxolinic acid, and kasugamycin, along with two copper compounds and three rhizobacteria strains (*Burkholderia gladioli* MRL408-3, *Pseudomonas fluorescens* TRH415-2, and *Bacillus cereus* KRY505-3), were tested.The results indicated that streptomycin, oxolinic acid, and the antagonistic bacterium MRL408-3 demonstrated superior control efficacy compared to the other agents.

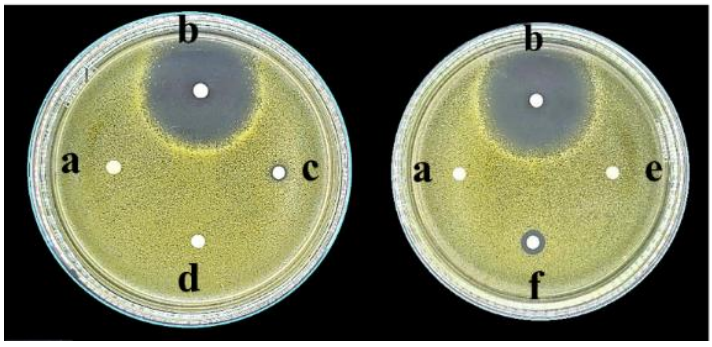

Figure 2. Inhibition of *Xanthomonas* spp. XA32 causing leaf blight on *Allium chinense* by some antibiotics and chemical compounds

a: Control; b: Oxolinic acid 20%; c: Bronopol 20%; d: Streptomycin sulfate 10%; e: Bismerthiazol 20% + Streptomycin sulfate 5%; f: Bronopol 40%.

4. Conclusion

The study demonstrated that *Xanthomonas* spp. XA32 is capable of causing leaf blight on *Allium chinense* and *Allium sativum* (garlic). Disease symptoms appeared 2 to 5 days after inoculation, characterized by water-soaked lesions, yellow-brown discoloration, and necrosis of the leaf tissue, all of which negatively impacted plant growth.

Additionally, the study evaluated 11 groups of chemical compounds to find effective solution for controlling leaf blight caused by *Xanthomonas* spp. The results showed that Oxolinic acid 20% was the most effective compound in inhibiting the bacteria under *in vitro* conditions, exhibiting a significant inhibition zone diameter of 35.7 mm after 96 hours. In contrast, Bronopol 20% and Bronopol 40% showed inhibitory effects, but at much lower levels.

Overall, the study provides valuable insights into the pathogenicity of *Xanthomonas* spp. on *Allium chinense* and *Allium sativum*. In the same time, Oxolinic acid 20% could be a viable option for pathogen control. This approach helps minimize the overuse of chemical pesticides, particularly those with limited efficacy, reduces the risk of antibiotic resistance, optimizes production costs, and contributes to the protection of the agricultural production environment.

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