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Efficacy of Copper Compounds on Transmitted by Seed of Different Dry Bean Cultivars against *Xanthomonas axonopodis* pv. *phaseoli*

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Abstract

Dry beans, which have an important place in human nutrition, are mostly produced in the Central Anatolia Region with a rate of 56.8% in our country. Common bean bacterial blight disease caused by *Xanthomonas axonopodis* pv. *phaseoli* (Xap), which is carried by bean seeds, causes significant economic losses under favorable climatic conditions. The disease has started to be seen at high rates in our cultivation areas, especially in the areas where sprinkler irrigation is applied and in the increasing temperature conditions due to the climate crisis. In this study, efficacy of several copper compounds in seeds of 3 different bean cultivars were determined in comparison with antibiotic in order to prevent the disease / reduce economic losses. According to the findings, copper sulfate was found to be the most successful compound in seeds of all bean varieties, both in reducing bacterial populations *in vitro* and in preventing the disease *in vivo*. Considering the negative effects of copper use in terms of human and environmental health and the risks of copper resistance in the pathogen, it is thought that the success in combating the increasingly common bean blight disease will increase with the use of the most effective copper compound in eco-friendly approaches.

Keywords: *dry bean, copper, seed, Xanthomonas, control*

Farklı Kuru Fasulye Çeşitlerinin Tohumlarıyla Taşınan *Xanthomonas axonopodis* pv. *phaseoli*'ye Karşı Bakır Bileşiklerinin Etkinliği

Özet

İnsan beslenmesinde önemli bir yere sahip olan kuru fasulye, ülkemizde en çok %56,8 oranıyla İç Anadolu Bölgesi'nde üretilmektedir. Fasulye tohumlarıyla taşınan *Xanthomonas axonopodis* pv. *phaseoli*'nin (Xap) neden olduğu fasulye adi bakteriyel yanıklık hastalığı, uygun iklim koşullarında önemli ekonomik kayıplara neden olmaktadır. Hastalık ekim alanlarımızda, özellikle yağmurlama sulama uygulanan alanlarda ve iklim krizi nedeniyle artan sıcaklık koşullarında yüksek oranlarda görülmeye başlanmıştır. Bu çalışmada, hastalığın önlenmesi/ekonomik kayıpların azaltılması amacıyla 3 farklı fasulye çeşidinin tohumlarında çeşitli bakır bileşiklerinin etkinliği antibiyotikle karşılaştırmalı olarak belirlenmiştir. Bulgulara göre, bakır sülfatın tüm fasulye çeşitlerinin tohumlarında hem *in vitro*'da bakteri popülasyonunu azaltmada hem de *in vivo* koşullarda hastalığı önlemede en başarılı bileşik olduğu belirlenmiştir. Bakır kullanımının insan ve çevre sağlığı açısından olumsuz etkileri ve patojendeki bakır direnci riskleri göz önüne alındığında, giderek yaygınlaşan fasulye bakteriyel adi yanıklığı hastalığıyla mücadeledeki başarının, çevre dostu yaklaşımlar içerisinde, en etkili bakır bileşiğinin kullanılmasıyla artacağı düşünülmektedir.

Anahtar kelimeler: *kuru fasülye, bakır, tohum, Xanthomonas, mücadele*

Introduction

Beans (*Phaseolus vulgaris* L.) are an economically significant agricultural product, with a cultivation area of 38,225,000 hectares worldwide, grown in 105 countries. Among food legumes worldwide, beans rank first in economic importance. In Turkey, as of 2021, in terms of production quantity, beans are the second most important crop (29.03%) after chickpeas (45.22%). Approximately 51% of the cultivation area for dry beans is located in the Inner Anatolia Region, followed by the Eastern Anatolia Region at 7.4% (Balçık and Baştaş, 2021).

There are numerous disease agents that affect yield and quality in beans (Kadakoğlu and Karlı, 2022). The most common bacterial agents worldwide are *Xanthomonas axonopodis* pv. *phaseoli*, *Pseudomonas savastanoi* pv. *phaseolicola*, *P. syringae* pv. *syringae*, and *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (Hall, 1994; Howard et al., 1994; Agrios, 1997). It has been reported that these agents can lead to yield losses of over 40% when suitable conditions are present (Seattler, 1989).

In the Central Anatolia Region, especially in bean cultivation areas with overhead irrigation, the bacterial blight agent *Xanthomonas axonopodis* pv. *phaseoli* (Xap) is commonly observed and is often confused with bean anthracnose disease.

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The optimum temperature range required for the development of the disease is 23-32 °C, and disease development stops at temperatures above 35.5 °C (Sönmezalp, 1966; Saettler, 1989). Xap causes more severe infections in plants at 28 °C compared to lower temperatures (Goss, 1940). The symptoms caused by the pathogen on leaves initially appear as wetness, and then these wet areas expand, covering the surface. The areas with wetness turn necrotic, surrounded by a narrow, fine lemon-yellow halo. Lesions are typically observed along the edges of leaves and between veins. Lesions expand and merge, eventually leading to symptoms of leaf blight (Saygılı et al., 2008). In the general management of the disease, several methods are recommended for disease control. These methods include the use of disease-free and certified seeds, disinfection of tools and equipment used during harvest and other operations with chemicals such as chlorine dioxide and sodium hypochlorite, collection and disposal of infected plant residues after harvest, the use of resistant varieties, foliar spraying with copper compounds, and antibiotic application to seeds (Schwartz et al., 2007). Worldwide, different types of copper preparations are used as part of chemical control programs for bacterial diseases. However, it is important to use only a limited number of effective copper compounds against the disease due to issues such as the rapid development of resistance to copper preparations, resulting in a loss of effectiveness, harm to beneficial bacteria, and phytotoxicity.
This study aims to determine the effectiveness levels of various copper compounds used in the control of bean bacterial blight, a disease that has become widespread in recent years in bean production areas, leading to significant economic losses. The goal is to reduce the use of copper while ensuring the most effective chemical for the sake of human and environmental health.

Materials and Methods

Materials

In this research, commercially available seeds of three dry bean varieties, namely Tezgeldi (Sarıkız), Saltan (Magnum) and Göynük-98, were used.
The highly virulent (88%) isolate of the bean bacterial blight pathogen, *Xanthomonas axonopodis* pv. *phaseoli*, with the Xap1 code, was obtained from the culture collection of the Molecular Bacteriology Laboratory at the Department of Plant Protection, Selçuk University. The copper compounds and antibiotics used in the experiment were procured from the companies listed in Table 1 in the commercial chart.

Table 1. Chemicals Used in the Experiment and their Application Dosages

Applications	Commercial Names	Formulation	Usage Dosage
Control (Xap 1)	-	Suspension	10^8 cells ml-1
Copper sulfate	Mastergold	SC	150 ml / 100 liters of water
Copper oxychloride	Cuprocol	SC	200 ml / 100 liters of water
Copper hydroxide	Kocide 2000	WG	200 g / 100 liters of water
Streptomycin sulfate	-	WG	250 ppm (mg/lt)

Method

Before bacterial inoculation, seeds of the Tezgeldi (Sarıkız), Saltan (Magnum), and Göynük-98 dry bean varieties were sterilized with a 3% sodium hypochlorite solution and then dried in a laminar cabinet. Freshly cultures of Xap, grown on Nutrient Agar (NA) for 48 hours, were prepared at a concentration of 10^8 cells ml^{-1}. Bean seeds (1000 seeds) were added to this suspension and shaken at 120 rpm for 30 minutes. Subsequently, they were dried in the laminar cabinet overnight (Mirik and Aysan, 2005; Kendi and Baştaş, 2011).
Copper sulfate, copper oxychloride, copper hydroxide, and streptomycin sulfate were added to the seeds in sterile distilled water at commercial dosages and shaken at 120 rpm for 30 minutes (Mirik and Aysan, 2005).

In vitro Experiments

Following the applications of copper compounds, the population densities in the seeds were determined using the formula:

Bacterial cell count (cells per ml) = CFU x DSD x 10
(CFU; colony-forming units, DSD; dilution series factor)

This formula was used to calculate the bacterial cell count in the seeds after the application of copper compounds (Klement et al., 1990).

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In vivo Experiments
To determine the germination rates, 30 vials were planted with bean seeds that had been treated with copper compounds, antibiotics, and *Xanthomonas axonopodis* pv. *phaseoli*.

Re-isolation of the Pathogen from Bean Seeds and Diagnosis of the Pathogen
To avoid any influence of environmental conditions on bacterial populations, re-isolations were carried out from seeds that had been dried in an environment with 70% relative humidity for one day. Suspensions diluted at a ratio of 10^{-3} were spread onto Petri dishes (Koch, 1884). Petri dishes were incubated at 27°C for 24-48 hours, and the bacterial population densities that developed were determined according to Klement et al. (1990). The experiment was conducted with three replications.
The diagnosis of the pathogen was performed based on biochemical, morphological, physiological, and molecular criteria according to Schaad et al. (2001) (Table 3). For the molecular diagnosis of the pathogen, X4c/X4e primers (5'-GGCAACACCCGATCCCTAAACAGG-3' and 5'-CGCCCGGAAGCACGATCCTCGAAG-3') were used, and the following PCR protocol was employed: 94°C for 3 minutes (1 cycle), followed by 94°C for 1 minute, 65°C for 1 minute, 72°C for 2 minutes (30 cycles), and a final extension at 72°C for 5 minutes (1 cycle) (Audy et al., 1994). The obtained PCR products were evaluated using agarose gel electrophoresis (Sigma) and a Prizma imaging device (Russell and Sambrook, 2001).

Statistical Analysis
The data obtained from the study were subjected to variance analysis using MINITAB version 14 software, and statistical evaluations were performed using the MSTAT program with the Tukey multiple comparison test to determine the interactions between chemicals and the pathogen (Düzgüneş et al., 1987).

Results and Discussion

Bean bacterial blight disease has started to become increasingly prevalent in our dry bean cultivation areas in the Central Anatolia Region, especially in areas where overhead irrigation is practiced and due to rising temperature conditions caused by the climate crisis.
In this study, the effectiveness of different copper compounds was compared to antibiotics in preventing bean bacterial blight disease and reducing economic losses, using the seeds of Tezgeldi (Sarıkız), Saltan (Magnum), and Göynük-98 dry bean varieties.
According to the findings obtained from experiments conducted under *in vitro* and *in vivo* conditions, statistically, the highest efficacy was achieved with copper sulfate, while the lowest efficacy was observed with copper oxychloride ($p < 0.05$) (Figure 1, Table 2).
At the variety level, after the use of copper compounds, the highest bacterial growth was observed in the Saltan (Magnum) and Göynük-98 bean varieties. The Tezgeldi (Sarıkız) variety was identified as the most successful combination in preventing the disease in bacterial x variety x copper interactions (Table 2).

Table 2. Effects of Different Copper Compounds on Bacterial Populations in Seed Trials

Applications	Saltan (Magnum)	Tezgeldi (Sarıkız)	Göynük-98
Control (Xap 1)	$0,469 \times 10^{6}$A	$0,88 \times 10^{5}$ A	$0,41 \times 10^{6}$ A
Copper sulfate	$0,02 \times 10^{5}$ B	$0,023 \times 10^{5}$ C	$0,20 \times 10^{5}$ B
Copper oxychloride	$0,12 \times 10^{5}$ B	0,47 $0,02 \times 10^{5}$ B	$0,72 \times 10^{5}$ B
Copper hydroxide	$0,04 \times 10^{5}$ B	$0,053 \times 10^{5}$ C	$0,73 \times 10^{5}$ B
Streptomycin sulfate	0.033×10^{4} B	$0,01 \times 10^{5}$ C	$0,09 \times 10^{5}$ B

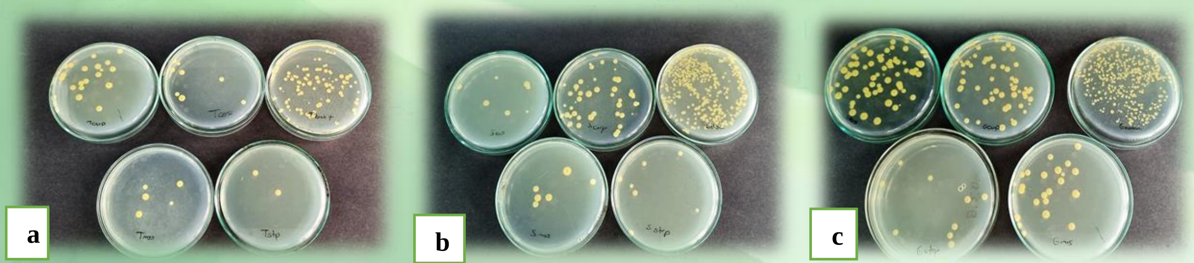

Figure 1. Results from Seed Trials with Different Copper Compounds and Streptomycin through Re-isolation

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Table 3. Germination Rates (%) of Bean Seeds Treated with Copper Compounds and *Xanthomonas axonopodis* pv. *phaseoli*

Applications	Saltan (Magnum) (%)	Tezgeldi (Sarıkız) (%)	Göynük-98 (%)
Control (Xap 1)	77	92	61,9
Copper sulfate	94,4	66,66	85
Copper oxychloride	72	26,66	90
Copper hydroxide	88,8	60	28,57
Streptomycin sulfate	85,5	85,71	85

Table 4. Results of Biochemical, Physiological and Morphological Tests for the Re-isolated Pathogen

Tests	Isolate used in experiments (Xap1)	Re-isolation
Gram reaction	-	-
Growth at 35°C	+	+
Formation of fluorescent pigment on KB agar	-	-
Production of H2S from cysteine	+	+
Levan formation	+	+
Esculin test	+	+
Catalase test	+	+
Oxidase test	-	-
Tobacco hypersensitivity reaction (HR) test	+	+
Production of H_2S from cysteine	+	+

(+)Isolate yielding a positive test result"; (-) Isolate yielding a negative test result

In Table 3, the effects of different copper compounds used in trials on the prevention of bacterial blight disease in beans and their effects on the emergence rates of bean varieties are given. Accordingly, at the variety level, streptomycin sulfate application resulted in the lowest damage to seed emergence rates for all varieties, while other applications caused significant differences at the variety level. It has been observed that copper sulfate applications against the disease have the least impact on seed emergence rates.

Re-isolations were conducted to confirm the presence of the pathogen in seeds and plants after seed treatments and to fulfill Koch's postulates. The pathogen re-isolated through X4c/X4e specific primers and confirmed through biochemical, physiological, morphological tests, and molecular diagnostics was identified as *Xanthomonas axonopodis* pv. *phaseoli* (Table 4).

The chemical control of the disease is generally carried out through antibiotic application to seeds, foliar spraying with antibiotics and other chemicals, and, especially in recent years, through the application of certain chemicals that stimulate systemic acquired resistance in plants (Bozkurt, 2009; Öztürk, 2014).

Liang et al. (1992) applied three different antibiotics (streptomycin, tetracycline, and chlorotetracycline) to bean seeds infected with *X. a.* pv. *phaseoli* and reported that it significantly inhibited the pathogen but also caused phytotoxic effects. In countries where the use of antibiotics is banned or restricted, copper-based preparations are commonly used for the control of bean bacterial diseases.

Balcık and Baştaş (2021) investigated the effectiveness of different copper compounds under field conditions against common bacterial blight disease on the Alberto variety of dry beans grown in Konya and Afyonkarahisar provinces with different climatic characteristics. The most effective copper compound was determined to be Copper oxychloride. However, in our study, it was found that Copper sulfate was the most effective compound for seed treatments in preventing the disease.

The development of resistance to antibiotics and copper preparations has been reported. Therefore, the selection of chemicals with the lowest harm rate to human and environmental health, along with choosing the most successful variety against plant bacterial diseases, would be the most appropriate approach (Kotan, 2002).

Furthermore, minimizing the use of the most effective copper preparation in disease control should also be considered as an essential strategy to reduce the issue of pathogen resistance.

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