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Research Article

Determination of the Antifungal Effect of Bacterial Metabolites of *Xenorhabdus szentirmaii* Against Some Phytopathogenic Fungi

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ABSTRACT

This study was carried out to determine the antifungal effects of the supernatant produced by the bacterium *Xenorhabdus szentirmaii*, which is associated with soil-inhabiting entomopathogenic nematodes, on important plant pathogenic fungi, *Fusarium verticilliodes*, *Fusarium oxysporum* f.sp *lycopersici*, *Fusarium oxysporum* f.sp *radicis lycopersici*, *Botrytis cinerea*, *Sclerotinia sclerotiorum* and *Phytophthora nicotianae*. The effects of 1, 3, 5 and 7% concentrations of the supernatant produced by *X. szentirmaii* bacteria on mycelium growth of the fungal pathogens was determined at 3, 7 and 14 days after application (dap). The effects of the supernatant on the formation of sporangia and the emergence of zoospores from the sporangium of *Phytophthora nicotianae* was also determined. According to the results of the study, the highest dose of the supernatant (7% concentration) prevented mycelium development of all tested pathogens. The effect of the supernatant decreased at 14 dap against *F. verticilliodes*, *F. oxysporum* f.sp *lycopersici*, *F. oxysporum* f.sp *radicis lycopersici*. The antifungal effect on *S. sclerotiorum* completely disappeared in 14 days whereas the effect continued even in 14 days against *B. cinerea* and *P. nicotianae*. *Xenorhabdus szentirmaii* supernatant at a concentration of 7% was highly efficacious on the formation of sporangia and the emergence of zoospores within the sporangium of *P. nicotianae* at a concentration of 7%.

Keywords: *Botrytis, Fusarium, Phytophthora, Sclerotinia, Sporangium, Xenorhabdus*.

Xenorhabdus szentirmaii Bakteri Metabolitlerinin Bazı Önemli Bitki Patojeni Funguslara Karşı Antifungal Etkilerinin Belirlenmesi

ÖZ

Bu çalışmada entomopatojenik nematodların simbiyotik bakterilerinden birisi olan *Xenorhabdus szentirmaii* tarafından üretilen metabolitlerin önemli bitki patojenlerinden olan *Fusarium verticilliodes*, *Fusarium oxysporum* f.sp *lycopersici*, *Fusarium oxysporum* f.sp *radicis lycopersici*, *Botrytis cinerea*, *Sclerotinia sclerotiorum* ve *Phytophthora nicotianae*'ya karşı antifungal etkileri araştırılmıştır. Buna göre farklı oranlarda (%1, 3, 5 ve 7'lik) *X. szentirmaii* bakteri süpernatantı içeren besiyerlerinde fungusların misel gelişimi 3, 7 ve 14 gün sonra değerlendirilmiştir. Ayrıca supernatantın *P. nicotianae*'nın sporangia oluşumuna ve zoospor çıkışı üzerindeki etkisi de değerlendirilmiştir. Elde edilen sonuçlara göre test edilen tüm patojenlerde en yüksek süpernatant konsantrasyonu (%7) misel gelişimini önemli oranda engellemiştir. Süpernatantın etkisi *F. verticilliodes*, *F. oxysporum* f.sp *lycopersici*, *F. oxysporum* f.sp *radicis lycopersici*'ye karşı 14 gün sonunda azalmıştır. *S. sclerotiorum* için antifungal etkinin 14 gün sonunda tamamen kaybolduğu, fakat *B. cinerea* ve *P. nicotianae* için devam ettiği gözlenmiştir. Ayrıca *X. szentirmaii* süpernatantının %7'lik konsantrasyonunun *P. nicotianae*'nın sporangia oluşumu ve zoospor çıkışını engellediği bulunmuştur.

Anahtar Kelimeler: *Botrytis, Fusarium, Phytophthora, Sclerotinia, Sporangium, Xenorhabdus*

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I. INTRODUCTION

Fungal plant pathogens cause serious diseases and considerable yield losses in cultivated plants. Species such as *Fusarium verticilliodes*, *Fusarium oxysporum* f.sp *lycopersici*, *Fusarium oxysporum* f.sp *radicis lycopersici*, *Botrytis cinerea*, *Sclerotinia sclerotiorum* and *Phytophthora nicotianae* attack the leaves, shoots, roots, stems, fruits, flowers or woody tissues, and produce enzymes and toxins that break down and kill these plant tissues causing enormous losses in yield and quality of plant products [1]. Along with the use of resistant varieties and disposal of infected plants, fungicides, which are an expensive and short fix, are generally used to combat these pathogens. For example, it is estimated that the average cost for chemical control of *Botrytis* spp., for example, in all affected crops worldwide is about €40/ha [2]. However, due to various problems associated with fungicide use, alternative methods of control, including biological control, have been investigated and developed and it is widely known that natural products from microbes in nature are a possible source of novel antimicrobial compounds.

Xenorhabdus spp. bacteria are obligate insect pathogens mutually associated with *Steinernema* nematodes found in soil. These bacteria are virulent in insect hemolymph where they produce antimicrobial secondary metabolites to protect insect cadavers infected by their host nematodes from saprophyte and predatory microorganisms. This enables the nematode/bacteria complex to benefit from insect cadavers for a longer time untroubled [3]. Several studies have been conducted to investigate the antifungal effects of secondary metabolites obtained from different species of *Xenorhabdus* against important phytopathogens like *Phytophthora nicotianae*, *Phytophthora infestans*, *Phytophthora cactorum*, *Armillaria tabescens*, and *Fusicladium tabesum* and their potential as novel sources of fungicidal compounds [4]. So far, some studies have determined that species such as *X. nematophila*, *X. bovienii*, *X. cabanillasii* and *X. szentirmaii* produce secondary metabolites like indole, xenocoumacin, xenofuranones, xenorhabdin and cabanillasin derivatives that have antibacterial and antifungal properties [5]. The antifungal agents such as trans-cinnamic acid (TCA) have been identified from *Photorhabdus luminescens* which is mutually associated with *Heterorhabditis* spp. nematodes [6]. This compound has been reported as a highly efficacious antifungal compound against plant pathogens [7-8]. TCA even synergizes the antifungal effectiveness of some commercial fungicides like Elast® (dodine), Prophyt® (potassium phosphite), PropiMax® (propiconazole) Serenade®, and Regalia® [9]. It reported that xenocoumacin 1 from *Xenorhabdus* bacteria and methanol extract from secondary metabolites of *X. nematophila* species showed antifungal effect [10].
The potential roles of *Xenorhabdus* and *Photorhabdus* metabolites have been exhibited in the various studies. In this study, the effect of the secondary metabolite produced by *X. szentirmaii* bacteria on important plant pathogens *Fusarium verticilliodes*, *Fusarium oxysporum* f.sp *lycopersici*, *Fusarium oxysporum* f.sp *radicis lycopersici*, *Botrytis cinerea*, *Sclerotinia sclerotiorum* and *Phytophthora nicotianae* were assessed. Additionally, the effect of the bacterial supernatant on the sporangium formation and zoospore discharge in *Phytophthora nicotianae* was evaluated.

II. MATERIALS AND METHODS

A. 1. Fungal Isolates

Fungal pathogens *Fusarium verticilliodes*, *Fusarium oxysporum* f.sp *lycopersici*, *Fusarium oxysporum* f.sp *radicis lycopersici*, *Botrytis cinerea* and *Sclerotinia sclerotiorum* used in the experiment were obtained from the Mycology Laboratory of Department of Plant Protection, Faculty of Agriculture, Düzce University. *Phytophthora nicotianae* isolate was obtained from Western Mediterranean Agricultural Research Institute of Antalya- Turkey.

A. 2. Preparation Of Bacterial Supernatant

Xenorhabdus szentirmaii bacteria (DSMZ 16338) was used in the experiments. It was provided by Dr. Selcuk Hazir from the Department of Biology, Adnan Menderes University. After the stock cultures were produced in liquid medium, they were stored in mixture of 50% medium, and 50% glycerol at -80°C. For the experiments, the bacterium was streaked on NBTA (2.3% nutrient agar, Difco; 0.0025% bromothymol blue, Merck; 0.004%, 2, 3, 5- triphenyltetrazolium) from the stock culture with the help of a loop. *Xenorhabdus* bacteria have two different colony variants as Phase-I and Phase-II and it is known that Phase I form positively affects nematode growth when compared to those in Phase-II and produces secondary metabolites with antimicrobial properties [3]. NBTA medium was used to ensure that bacteria used in the experiments were of phase 1 according to cell and colony morphologies.
To obtain bacterial supernatant, firstly a loop full of bacteria from the selected colonies was first transferred to 100 ml TSBY (Tryptic soy broth, Difco + 0.5% yeast extract, Sigma) medium and incubated at 25°C, 130 rpm for 24 hours in complete darkness to prepare an overnight culture [6-7]. From the grown bacterial culture, 2% of the medium volume was transferred to a larger volume (500 ml) of TSBY medium. It was incubated for 120 hours under the same conditions. Afterwards the growth culture was centrifuged at 4°C, 10,000 rpm for 20 minutes; and the separated supernatant was filtered through a 0.22 µm diameter filter (Thermo Scientific, NY) to get rid of bacteria in the culture medium. The supernatants were transferred to 50 ml falcon tubes and stored at 4 °C for use within a maximum of 2 weeks [8].

A. 3. The Effect Of Bacterial Supernatant On Mycelium Growth

This study investigated the effects of *X. szentirmaii* bacterial supernatant at 1, 3, 5 and 7% concentrations on mycelium growth of the fungal pathogens mentioned above. Since [9] established that high temperature had no adverse effects on the antifungal activities of the bioactive compound/s in the supernatant, the required amount of supernatant for the determined concentrations (1, 3, 5, 7%) was added into Potato Dextrose Agar (PDA) (Merck, Germany) before autoclaving. PDAs containing the different concentrations of the bacterial supernatant were autoclaved (121°C, 15 min.) and then poured into 90 mm plastic petri dishes.

Agar discs of 5 mm diameter were taken from the edges of one-week-old cultures of the pathogens with the help of cork-borer and placed in the center of the petri dishes with PDA supplemented with bacterial supernatant. Control group only had PDA medium. Inoculated petri dishes were left to incubate at 24 ± 1°C for two weeks. Measurements were made at 3, 7 and 14 days after the application (dap). Diameters of the mycelial colony were measured at two different points perpendicular to each other and the obtained mean colony diameters was used to calculate the inhibition rates of the different concentrations of secondary metabolite on mycelium growth using the formula:

Prevention of mycelium growth

$$(\%) = [(dc - dt) / dc] \times 100\ (\%) \quad (1)$$

dc: average of colony diameter in control, dt: average of treated colony diameter [11].

A. 4. The Effect Of The Bacterial Supernatant On The Formation Of *Phytophthora Nicotianae* Sporangium

This experiment was conducted using sterile soil suspension (SSS). Ten grams of garden soil was placed in 1 liter of pure water and mixed in a magnetic stirrer for 1 hour. The soil particles in the suspension were allowed to settle for 24 hours. At the end of this period, the suspension was filtered through 4-layer cheese cloth and sterilized in an autoclave at 120°C at 1 atm [12]. Different volumes of bacterial supernatant and SSS were added to 90 mm petri dishes in order to prepare environments with 1, 3, 5 and 7% supernatant concentrations and a final volume of 25 ml. Only SSS was used in the

control group. Five mm diameter agar discs were taken from a new *Phytophthora nicotianae* culture in PDA medium with the help of cork-borer and placed on petri dishes containing SSS-supernatant combinations. The Petri dishes were incubated at 24 ± 1℃ for 48 hours and at the end of this period, photographs were taken from three different regions of each mycelial disc using a computer-connected camera (Olympus SZ61 model microscope, DP25 model camera) and measurement program (cellSens v.1.11). The average number of sporangium in each image obtained at 10x magnification was determined [13] and the effects of different concentrations of the bacterial supernatant on the formation of sporangium was determined using the formula:

The effect of sporangium formation

$$(\%) = [(dc - dt) / dc] \times 100\ (\%) \quad (2)$$

dc: sporangium number in control, dt: sporangium number in the treated [11].

A. 5. Effect Of Bacterial Supernatant *Phytophthora Nicotianae* On Zoospore Discharge

Five agar discs (5 mm in diameter) were taken from the edges of a freshly grown *P. nicotianae* culture in PDA medium with the help of cork-borer and placed on SSS. These petri dishes were incubated at 24 ± 1℃ for 48 hours to allow sporangium formation. At the end of this period, different concentrations of supernatant were added to the petri dishes. Only SSS was used in control plates. Petri dishes were initially kept at 4 ± 1℃ for 30 minutes and then incubated at 24 ± 1℃ for 30 minutes in order to encourage the discharge of zoospore. At the end of the incubation, 3 different images were obtained from each mycelium disc and the ratio of empty sporangium number to total sporangium number was determined [13]. The experiment was conducted in 5 replications. The effects of the different concentrations of the supernatant on the zoospore discharge:

The effect of the zoospore discharge

$$(\%) = [(dc - dt) / dc] \times 100\ (\%) \quad (3)$$

dc: the empty sporangium ratio in the control, dt: the empty sporangium ratio in the treated cases) was determined using the formula [11].

A. 6. Statistical Analysis

One-way analysis of variance was performed using the SPSS 16.0 statistical program for the obtained mean values from each concentration of the secondary metabolite. The Duncan test was applied to the groups for significant differences

III. RESULTS AND DISCUSSION

Different concentrations of bacterial secondary metabolite were used to determine their effect on the mycelium development of tested plant pathogens. The effect of supernatant on mycelium development at 3, 7 and 14 dap are given in Table 1.

Table 1 *Effect of different concentrations of bacterial supernatant on mycelial growth of fungal pathogens*

Fungal pathogens	Assessment period	Percentage effect			
		Concentration			
		1%	3%	5%	7%
Fusarium oxysporum f.sp. *lycopersici*	3 dap	32.25Ab*	49.40Aa	57.48Aa	58.35Aa
	7 dap	36.18Ab	52.20Aa	61.11Aa	65.15Aa
	14 dap	0Bd	26Bc	35.50Bb	45Ba
Fusarium oxysporum f.sp.*radicis lycopersici*	3 dap	39.89Ac	56Bb	66.87Ba	69.43Aa
	7 dap	43.66Ac	62.10Ab	70.75Aa	71.47Aa
	14 dap	0Bd	21.88Cc	35.31Cb	43.44Ba
Fusarium verticillioides	3 dap	31.54Ac	46.32Ab	67.75Aa	69.79Aa
	7 dap	27.96Bd	47.91Ac	58.63Bb	64.58Ba
	14 dap	0Cd	6Bc	14.25Cb	33Ca
Sclerotinia sclerotiorum	3 dap	24.25Ac	66.75Ab	98.00Aa	98.75Aa
	7 dap	0Bb	0Bb	65.50Ba	65.75Ba
	14 dap	0B	0B	0C	0C
Botrytis cinerea	3 dap	71.86Ab	100Aa	100Aa	100Aa
	7 dap	57.50Bc	81.25Bb	90.00Bab	94.25Ba
	14 dap	0Cd	68.50Cc	77.50Cb	87.50Ba
Phytophthora nicotianae	3 dap	32.22Ac	56.67Ab	100Aa	100Aa
	7 dap	1.18Bd	56.51Ac	78.20Bb	85.53Ba
	14 dap	9.02Bd	44.75Ac	70.73Cb	83.25Ba

**= different small letters in the rows show a statistically significant difference between the means for the each pathogen and application period. Different capital letters in the columns show a statistically significant difference between the means for the each pathogen and concentration. (P<0.05).*

Our data showed that the effectiveness of the supernatant against each fungus were different. The effect of 1% concentration of supernatant against *F. oxysporum* f.sp *lycopersici* and *F. oxysporum* f.sp *radicis lycopersici* ranged between 32% and 39.8% at 3 dap, butthis effect increased with increase in concentration. Seven percent supernatant concentration inhibited fungal growth by 58 and 69% at 3 dap and these effects recorded as 65.15% and 71.47% at 7 dap. The inhibition effects of supernatant concentrations against *F. verticilliodes* were determined as 31.54, 46.32, 67.75 and 69.79% for 1, 3, 5 and 7% concentrations, respectively.

However, it was determined that the efficacy of the bacterial supernatant on *F. oxysporum* f.sp *lycopersici*, *F. oxysporum* f.sp *radicis lycopersici* and *F. verticilliodes* decreased with expanding time (14 dap) and the effects were decreased to 45%, 43% and 33% respectively.

In *S. sclerotiorum*, *Xenorhabdus szentirmaii* supernatants were highly effective at tested higher doses at 3 dap; 1% dose exhibited 24% antifungal effects against the pathogen whereas the effects of the highest tested dose (7% concentration) was 98. 75%. The effect decreased rapidly after 7 and 14 days; 1% and 3 % concentrations had no effects on the pathogen whereas 5% and 7% were 65% effective. By 14th day, it was determined that the effect completely disappeared at all doses.

For *B. cinerea*, the supernatant at 1% was 71% effective whereas 3, 5 and 7% concentrations were 100% effective at 3dap. At 7 dap, the effects of the supernatants were decreased slightly and ranged between 57.5 and 94%. While 1% concentration lost it antifungal effect, the other tested concentrations were still efficacious and continued even 14 dap, especially for 7% concentration.

For *Phytophthora nicotianae*, 1, 3, 5% bacterial supernatants were 32, 56, and 100% effective at 3dap, but at 7 dap, they were 1, 56 and 78% effective, respectively. The 7% concentration presented the highest antifungal effect against *P. nicotiana*. It totally inhibited (100%) fungal growth at 3 dap, but antifungal effects were 85.33 and 83.25% at 7 and 14 dap, respectively (Table 1).

In our study, we also tested the effects of different concentrations of bacterial supernatant on *phytophthora nicotianae* sporangium formation and zoospore discharge (Table 2).

The effects of different supernatant concentrations on *P. nicotiana* sporangium formation were pronounced at the 14th days.

Table 2. *Effects of different concentrations of bacterial supernatant on Phytophthora nicotianae sporangium formation and zoospore discharge.*

Concentrations of bacterial supernatant	Number of sporangia	Effect *(%)*	Percent of zoospore discharge *(%)*	Effect *(%)*
0% (Control)	81.31	0,00	57.19	0,00
1%	36.41	54.40c*	38.85	31.41c*
3%	7.47	90.90b	34.26	40.33bc
5%	0	100a	25.83	54.88b
7%	0	100a	2.04	93.97a
		F=105.229		F=16.983

**= Different letters in the columns show a statistically significant difference between the means. (P<0.05).*

The 5 and 7% supernatant concentrations completely inhibited *P. nicotianae* sporangium formation. The 1% concentration was 54.40% effective against sporangium formation whereas the 3% concentration was 90.90% effective. In the study conducted to determine the effect of bacterial supernatant on zoospore discharge from the sporangium, 7% concentration had the highest effect with 93.97%. This was followed by 5% concentration with an effect of 54.88%. The other concentrations were less effective than 50% (Table 2).

Our study revealed that the supernatant obtained from *X. szentirmaii* bacteria was effective against important plant pathogenic fungi. However, the duration of this effect varied according to the fungal species. It is thought that this antifungal effect may be due to the compounds in the supernatant. Bacteria in the *Xenorhabdus* genera, which are in a mutualistic relationship with *Steinernema* spp. nematodes, produce metabolites that exhibit antifungal and antibacterial activity. Several studies have investigated the effects of cell cultures or cell-free supernatants (CFS) of *Xenorhabdus* and a

close relative, *Photorhabdus*, on different fungal phytopathogens such as *Botrytis* spp., *Fusicladium* spp, *Fusarium* spp., *Monilinia* spp., *Moniliophthora* spp., *Rhizoctonia* spp. etc. [6-7-8]. These studies have reported that there are differences in the antifungal effects of different bacteria in the genera *Xenorhabdus* and *Photorhabdus*. These differences are due to the differences in the supernatants they produce [14].

Xenorhabdus szentirmaii bacteria is known to produce antimicrobial compounds such as xenofuranone A and B, poly-iodinin crystal, szentiamide, xenematide, anthrarufn (anthraquinone) and fabclavines [5-15]. In addition, it also synthesizes exoenzymes such as lipases, chitinases, proteases and phospholipases [16] which might be effective on fungal cell wall generally composed of polysaccharides like chitin, glucan, although this varies according to fungal species [17]. The effects of antibiotics and enzymes inside the supernatant on the compounds forming the fungal cell wall should be investigated.

We observed that the bacteria supernatant were highly effective on mycelium development of *S. sclerotiorum* at 3 days after the application and against *F. verticilliodes*, *F. oxysporum* f.sp *lycopersici*, *F. oxysporum* f.sp *radicis lycopersici* fungi at 7 days after the application, however by the 14 dap, the effect against these factors decreased or completely disappeared. Likewise, Fang et al [10]'s study conducted with 10% concentration of the supernatant from *X. nematophila* bacteria, reported a 37.14% effect against *Fusarium graminearum* and 62.17% against *Fusarium oxysporum* in the evaluation performed at 7 dap. We observed that at 7 dap, *X. szentirmaii* was 65% effective against *S. scleroiorum* at the highest tested concentration compared to Fang et al [9] who reported that the supernatant of *X. nematophila* bacteria at 10% concentration exhibited 91.23% effect on *S. sclerotiorum* at 7 dap. Most recently, in another study, *X. szentirmaii* bacteria supernatants were obtained after 3, 6 and 9 days of incubation and 3%, 10% and 33% supernatant concentrations were tested against S. *sclerotiorum* [18]. The highest effect was observed when used the 33% concentration of supernatant obtained after 6 days of incubation [18].

The supernatant obtained from the *X. szentirmaii* bacterium, in our study, had a high effect against *B. cinerea*. Sufficient effect was obtained even in 14 dap of the supernatant. Unlike, the antifungal effects of *X. szentirmaii* bacterial supernatant against the other tested fungal pathogens were long lasting. Similarly, 10% concentration of *X. nematophila* supernatant was 98.51% effective against *B. cinerea* at 7 dap [11]. In the study conducted by Adlığ and Gulcu [19], the efficacy of *X. szentirmaii* bacterial supernatant and trans cinnamic acid synthesized from *P. luminescens* bacteria were tested against *B. cinerea* fungi. In their evaluation, at 5 dap, the highest effect was obtained at 10% concentration of the supernatant of *X. szentirmaii* bacteria and 2% dose of TCA. These results show that metabolites obtained from *Photorhabdus* spp. and *Xenorhabdus* spp. show a strong antifungal effect against *B. cinerea*.

Phytophthora nicotianae has a wide host array and causes significant damage on cultivated plants. This fungus infects its hosts either by direct germination of the sporangium or by the two flagellated zoospores formed within the sporangium. Since zoospores can move easily in soil water, irrigation water and hydroponic solutions with their flagella, they are very important in terms of spreading this pathogen in agricultural areas. Few studies carried out so far have investigated the effects of supernatants obtained from *Xenorhabdus* and *Photorhabdus* on the mycelium growth and zoospore viability of oomycotes. For instance, 0.1 and 1 ppm doses of the metabolite obtained from *Xenorhabdus bovienii* strain A2 completely prevented the mycelium growth of *P. infestans*, which is an important pathogen [19]. In our study, 5 and 7% supernatant of doses *X. szentirmaii* inhibited the mycelium growth of *Phytophthora nicotianae*. Boszormenyi et al [4] stated that the filtrates obtained from *X. szentirmaii* and *Xenorhabdus budapestens* inhibited the colony development of *P. nicotianae* by 56.2%, 77.1% and 84% at 6.25, 25, 50 ppm, respectively. In the same study, it was reported that zoospore activity was inhibited within 1 minute of contact with 6.25 ppm dose and approximately 90% of the zoospores were degraded. It has also been determined that the methanol extract synthesized from the culture filtrate of *X. nematophila* bacteria is effective on the mycelial growth and zoospore germination of *P. capsici* [10].

There is no study on the effects of the supernatant of *X. szentirmaii* on the production of sporangium, which is the asexual reproductive structure of *Phytophthora* spp. fungi, and on the discharge of zoospores formed in them. In our study, 7% concentration of *X. szentirmaii* supernatant prevented both the formation of sporangia and the discharge of zoospores from the sporangia. There are studies on the effects of other species in the genus *Xenorhabdus* on the formation of sporangium. It has been determined that Xenocoumacin 1 (Xcn1) obtained from the bacteria *X. nematophila* inhibited the formation of sporangium of *P. infestans* and *P. capsici* [21]. In general, it is predicted that the effect of Xenocoumacin 1 is on protein synthesis. The emergence of zoospore from sporangium increase the rate of spread of *P. nicotianae* and the severity of the disease. Our study reveals that the supernatant of the bacteria *X. szentirmaii* prevented the discharge of zoospores from the sporangium. Miao et al [13] reported that oxathiapiprolin, a fungicide developed for the control of plant pathogenic oomycete species prevents the emergence of zoospores from *Phytophthora capsici* sporangium.

IV. CONCLUSION

As a result, it has been demonstrated that the supernatant of *X. szentirmaii* bacteria inhibits the growth of important plant pathogenic fungi. However, as seen in our study, there were differences in the effectiveness of this supernatant against fungal pathogens. Studies should be conducted to determine the factors affecting this in the future. The supernatant also influenced the mycelial development, the formation of sporangia and the release of zoospores from sporangia of *P. nicotianae*. Secondary infections caused by *P. nicotianae* in nature occur through direct germination of the sporangium or by zoospores formed within the sporangia. It is thought that the supernatant of the bacterium *X. szentirmaii* was effective on both fungal formation. These metabolites can be used successfully in Integrated Pest Management (IPM).

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