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Nanoemulsion from *Piper aduncum*, *Cymbopogon nardus*, and *Bacillus thuringiensis* to Control *Xanthomonas axonopodis* pv. *allii*

Ly Lan Phuong*[a]*, Eka Candra Lina*[b], *Yulmira Yanti*[b]

[a] *Student of Plant Protection, Post Graduated Program, Andalas University, Kampus Unand Limau Manis, Padang 25163, Indonesia*
[b] *Plant Protection Department, Faculty of Agriculture, Andalas University, Kampus Unand Limau Manis, Padang 25163, Indonesia*

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Correspondence:
E-mail: lylanphuong.agu@gmail.com

A B S T R A C T

The bacterial species *Xanthomonas axonopodis* pv *allii* (Xaa) is an important pathogen causing leaf blight in shallots. The use of botanical pesticides with nanoemulsion formulations has become a common alternative. This study aims to determine the characteristics and optimum concentration of the mixture of essential oil of *Piper aduncum* and fragrant *Cymbopogon nardus* waste. Nanoemulsion formulations are made using spontaneous emulsification methods. Besides, testing *Bacillus thuringiensis* strain MRSNR3.1 and its secondary metabolites toxicity against Xaa was carried out by the diffusion method using paper discs to determine the diameter of the inhibition zone. The results demonstrate that all four concentrations, 1%, 2.5%, 5%, and 7.5%, could control Xaa bacteria. A concentration of 1% is considered more optimal than the other three concentrations in bactericidal effects against Xaa, as manifested in the formed clear zone (diameter of 3.17 cm). Besides, *Bacillus thuringiensis* strain MRSNR3.1 and its secondary metabolites were also effective against Xaa after four days of incubation with inhibition zones of 3.04 ± 0.44 and 2.21 ± 0.28, respectively. Hence, it is concluded that nanoemulsion at 1% concentration and *Bacillus thuringiensis* strain MRSNR3.1 have bactericidal properties that can be used to control Xaa.

INTRODUCTION

Shallot (*Allium cepa* L.) is an important crop distributed widely in tropical regions. During the growing process, various diseases appear to reduce the yield and quality of shallot (Gent & Schwartz, 2005). It is one of the most dangerous diseases affecting many other countries and the areas of the country that produce shallots (Alvarez, Buddenhagen, Buddenhagen, & Domen, 1978). The disease progresses rapidly, leading to significant yield loss due to the destruction of leaves in an environment with high relative humidity, persistent rain, and warm temperatures (Conn, Lutton, & Rosenberger, 2012). If no control methods are used, yield losses (including tuber size and quality) might reach 100%, especially in the rainy season (Picard et al., 2008). Besides the use of chemical pesticides, people also use antibiotics to prevent disease-causing bacteria, while this is limited use in agriculture due to the antibiotic content that can remain in the product (Schlegel & Zaborosch, 1993).

Pesticides should not be used continuously or improperly since they might harm the environment. Instead, biological control is used using microorganisms from native plants that encourage disease-resistant growth, where the *Bacillus thuringiensis* group is used to make plants more resistant to pathogens (Rosliani, Palupi, & Hilman, 2013). Through secreting extracellular

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compounds such as antibiotics, cell wall hydrolases, and accessory cells, the *Bacillus thuringiensis* engaged in antagonistic activities (Sansinenea, 2012). These bacteria have the capacity to create a large variety of secondary metabolites, each having a very distinct nature, structure, and range of activity (Alvarez et al., 1978).

These metabolites were first created to help the bacteria survive in their natural environment and include antibiotics, pigments, poisons, growth promoters (for both animals and plants), ecological competition effectors, pheromones, enzyme inhibitors, and other bioactive substances (Stein, 2005). The majority of *Bacillus* species and the products they produce are thought to be environmentally friendly. *Bacillus thuringiensis* induced inhibition of bacterial growth through a variety of processes, including competition for nutrients and space, generation of antibiotics and hydrolytic enzymes, formation of cellular appendages, and/or generation of systemic resistance (Wraight, Zangerl, Carroll, & Berenbaum, 2000). Additionally, *Bacillus thuringiensis* can function as biofertilizers or biostimulants by promoting the uptake of certain nutrients from the environment (nitrogen fixation, phosphate solubilization) or by giving plants substances (plant hormone production) (Xu, Yu, Wu, & microbiology, 2005). *Bacillus thuringiensis* is a biocontrol agent that can control many pathogens such as *Pseudomas syringae* pv Arabidopsis, *Xanthomonas campestris* pv on cabbage (Dimkić et al., 2022), *Xanthomonas axanopodis* pv glycine on soybean, *Xanthomonas vesicatoria* on tomato (Khosro, Sara, Saeed, & Mohammad, 2012), *Ralstonia solanacearum* on medicinal plants leaves and mulberry plants (Hyakumachi et al., 2013), and *Ralstonia syzygii* sub sp. (Zwahlen, Hilbeck, Gugerli, & Nentwig, 2003). Hence, disease control strategies with antagonistic bacteria or botanical pesticides with relatively lower negative effects than synthetic pesticides have received attention.

Botanical pesticides contain natural active ingredients derived from plants that are readily degraded in nature and are selective so that they are safe for non-target organisms and the environment (Dubey, Shukla, Kumar, Singh, & Prakash, 2010). Other advantages include the fact that it may be combined with other pest management methods, is not likely to quickly lead to resistance, and that easy preparation can lessen dependency on chemicals and synthetic pesticides (Dubey et al., 2010).

According to Brazao et al. (2014), the bactericidal activity of essential oil from *Piper aduncum* was used against multi-resistant strains of *Staphylococcus* spp. (Brazao, Brazao, Guilherme, & Monteiro, 2014). Dilapiol, which had a peak area of 68.8% on chromatograms based on a gas chromatographic examination, was the primary component in the active fraction of the n-hexane extract of *Piper aduncum* fruit (Wibawa et al., 2019). In addition to its insecticidal activity, dilapiol was isolated from the essential oil of *Piper aduncum* leaves and also exhibits antifungal and antibacterial activities in addition to its insecticidal effect. By preserving the chemicals and improving their release and coverage of the leafy produce, the formulation of essential oils in nanoemulsions offers a new way to increase their effectiveness in food systems (Hasyim, 2011).

The antibiotic capabilities of *P. aduncum* were used against bacteria (*Streptococcus mutans*) and health-related bacteria in addition to its capacity to combat disease-causing insects (*Streptococcus sanguinis*) (Ferreira et al., 2022). *P. aduncum* extracts may inhibit sucrose-dependent adhesion and decrease the acid production of *S. mutans*. The extracts are more effective against *S. mutans* than *S. sanguinis*, so there is a chance that this plant species can stop harmful bacteria from growing (*Streptococcus mutans*) (Magalhães et al., 2016).

Cymbopogon nardus contains citronellal (monoterpene aldehyde) as its main constituent and the other active compounds, citronellol, and geraniol, respectively (Mahalwal & Sanjrani, 2003). The antibacterial activity of the methanol extract of Citronella at concentrations of 20, 30, 50, and 400 mg/ml was shown that inhibit the growth of *Staphylococcus aureus*, *Bacillus cereus*, and *Escherichia coli*. Its antibacterial action is enhanced by higher doses (Jafari et al., 2012). The antibacterial activity of *Cymbopogon nardus* essential oils may be due to terpenoids and phenolic compounds (Kamal et al., 2020). The mixture from *Piper aduncum* and *Cymbopogon nardus* has a higher value in bactericidal activity than the single extract and was strongly synergistic. This will certainly positively impact agricultural production because there were many previous cases where there was an outbreak of certain pests due to the inappropriate use of pesticides. Biological control is thus considered an alternative or supplemental way of reducing the use of chemicals in agriculture (Lengai, Muthomi, & Mbega, 2020).

Therefore, this study aimed to find the concentration of nanoemulsion of *Piper aduncum* essential oil and mix fragrant *Cymbopogon nardus* distilled waste, *Bacillus thuringiensis* strain MRSNR3.1, and its secondary metabolites toxicity against *Xanthomonas axonopodis* pv. *allii*.

METHOD

The research was conducted at the Laboratory of Microbiology, Insect Bioecology, and Greenhouse of Faculty Agriculture at Andalas University, Padang. The research begins in June to October 2022.

Materials used are the fruit of *Pipper aduncum* and hydrosol from *Cymbopogon nardus* obtained from Limau Manis and *Xanthomonas axonopodis* pv. allii, *Bacillus thuringiensis* strain MRSNR3.1, Secondary metabolites of

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Bacillus thuringiensis strain MRSNR3.1, MgSO4, tween 80, distilled water.

Making nanoemulsion from *Piper aduncum* and *Cymbopogon nardus*

Cymbopogon nardus was taken from the city of Solok and brought to the Insect Bioecology Laboratory of Andalas University, which will be used as a solvent for nanoemulsions.

Piper aduncum, which was obtained from Bukit Lampu, was cleaned with tissue paper from the attached dirt. Five hundred grams of plant material were cut into pieces that weighed as much as 100 grams. Then put it into a boiling flask with a volume of 3 liters. 2,5 liters of distilled water was added to a flask that already contained plant material. The distillation process is carried out for four hours since the water in the flask boils. The oil that drips on the collecting column is then transferred slowly into a glass bottle. Magnesium sulfate is used to remove the remaining water in the obtained volatile oil liquid (Erlina, Lina, & Djamaan, 2020).

Preparation of bacterial cultures

Colonies of each *X. axonopodis* pv. *allii* grew for 48 h on YPGA medium. A bacterial concentration of about 10^7 colony-forming units (CFU)/ml was used. 100-microlitre suspensions were diluted continuously 10-fold, then coated on the surface of each Petri dish in replicates. Bacterial density after culture in YPGA medium was determined by *MacFarland* 10^7 (Figure 1)

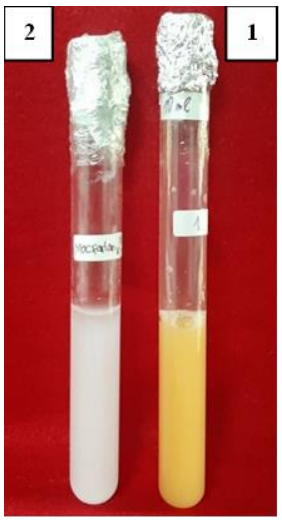

Figure 1. Propagation of *X. axonopodis* pv. *allii* (1) Suspension *X. axonopodis* pv. *allii*, (2) *McFarland*'s solution scale 7 with a population density of 10^7

The secondary metabolite of *Bacillus thuringiensis* strain MRSNR3.1 was carried out by adding 9 ml of sterile distilled water to a petri dish. It was then suspended with the help of a needle, put into a test tube, and homogenized with a vortex. A total of 2 ml was inoculated into 200 ml of sterile NB medium and incubated on a shaker for 24 hours. The cells were separated from the solution by centrifugation to obtain the *Bacillus thuringiensis* strain MRSNR3.1 supernatant (4100 rpm for 15 minutes) (Okulate, 2009).

Test of nanoemulsion and *Bacillus thuringiensis* strain MRSNR3.1 and secondary metabolites of *Bacillus thuringiensis* strain MRSNR3.1 to attack *Xanthomonas axonopodis* pv. *allii*

Treatments were arranged factorially in the Randomized completely block design (RCBD) with five treatments (Nanoemulsion, *Bacillus thuringiensis* strain MRSNR3.1, Secondary metabolites of *Bacillus thuringiensis* strain MRSNR3.1, Nanoemulsion + *Bacillus thuringiensis* strain MRSNR3.1, Nanoemulsion + Secondary metabolites of *Bacillus thuringiensis* strain MRSNR3.1) 4 replications.

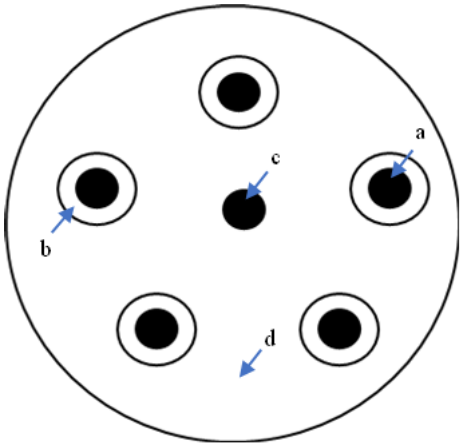

Figure 2. Test sketch of inhibit *Xanthomonas axonopodis* pv *allii*, (a) treatment, (b) Inhibition zone, (c) Control, (d) Xaa.

The nanoemulsion formulation was tested by preparing concentrations 1, 2.5, 5, and 7.5, respectively. Filter paper discs were prepared and dipped one by one in the nanoemulsion formula with a certain concentration, including the control solution and the other treatments.

The experiment is followed by using a 100 µl pipette of bacteria of each type (cell density 10^6 CFU/ml), then mixing evenly on the stable dried nutrient agar (NA) medium and waiting for the surface to dry. Disc paper 6mm sterile absorbent saturated with each treatment and wait to dry, then put on the surface of the agar that has contained bacteria, gently press the paper disc to fix on the agar surface (Figure 2).

The disc paper in the middle is impregnated with the control solution (distilled water), and the surrounding paper discs are soaked with successive treatments. Transfer the petri dishes to the refrigerator (10ºC) for about 4 - 8 hours for the essential oils to diffuse into the agar, then incubate at 37ºC for 24 – 48 hours. After incubation, the diameter of the inhibition ring (Dd) was

determined by the difference between the diameter of the outer ring (D, mm) and the diameter of the paper plate (d = 6 mm) when Dd > 0 mm, the essential oil was extracted considered antibacterial. The recorded result D is the average measurements on the same experimental unit. Data will be recorded daily up to 96 hours after incubation.

RESULTS AND DISCUSSION

Nanoemulsion made from *Piper aduncum* essential oil and fragrant *Cymbopogon nardus* distilled waste.

The process of steam distillation uses saturated or superheated steam as a separation agent and energy to extract volatile components with high boiling points from inert and difficult substrates, both in solid and liquid form. The procedure of obtaining essential oils from plants has been widely employed (Cerpa et al., 2009). After cooling and condensing the resultant vapor phase, the water and organic phase were separated depending on their immiscibility. The compounds are heated by the steam, which causes them to volatilize before being transported to the steam through diffusion. The distillation chamber receives heat from the burner flame, which causes the water to boil and evaporate compounds. The condenser cools the water vapour and drip-by-drip transfers it to the Erlenmeyer flask. Condensation of the water vapour is aided by the flowing water in the outer cooling tube enclosing the inner condensing tube. After cooling and condensing the resultant vapor phase, the water and organic phase are separated depending on their immiscibility. Essential oil and hydrosol are the two products that are produced as a result of the procedure (Figure 3). Nanoemulsion is generated in two stages. First, for the aqueous phase, the hydrosol was mixed with the surfactant (Tween 80) in the respective ratio of 87:3, then homogenized the mixture by placing them on Stirrer for 30 minutes (Stir bar was added in an elernmeyer flask containing the aqueous phase). Organic phases consist of essential oil from *P.aduncum* and ethanol (1:1 ratio). In the next stage, nanoemulsion was prepared by dropping the organic phase containing oil and ethanol. The organic phase is dripped into the aqueous phases until the end using a magnetic stirrer for 40 min at 4000 rpm. At the end of the process, nanoemulsion will be obtained with the ratio of water phase and organic phase of 9:1, respectively. The nanoemulsions should have maximum stability, which is not a phase separation (creaming and cracking) (Drais & Hussein, 2015).

Figure 3. Nanoemulsion mixture of fragrant *Cymbopogon nardus* waste (hydrosol) and *Piper aduncum* essential oil

Nanoemulsion and *Bacillus thuringiensis* strain MRSNR3.1 to attack *Xanthomonas axonopodis* pv. *allii* invitro.

The results of the antibacterial activity test showed that nanoemulsion from *Piper aduncum* and *Cymbopogon nardus* could inhibit the growth of *Xanthomonas axonopodis* pv. *allii* bacteria, which was shown by the presence of a clear zone around the paper discs. The results of the antibacterial activity test of nanoemulsion at different concentrations can be seen in Table 1 and Figure 4.

Table 1. The average diameter of the inhibition zone of the nanoemulsion from a mixture of fragrant *Cymbopogon nardus* waste and *Piper aduncum* essential oil against *Xanthomonas axonopodis* pv. *allii* bacteria.

Concentrations	Average resistance area diameter (cm) (day) (X ± SD)			
%	1	2	3	4
1	0.87 ± 0.15 a	1,50 ± 0,10 a	2.70 ± 0.30 a	3.17 ± 0.80 a
2.5	0.73 ± 0.06 a	1.33 ± 0.55 a	1.67 ± 0.58 b	2.30 ± 0.26 b
5	0.93 ± 0.21 a	1.33 ± 0.29 a	1.53 ± 0,50 b	1.53 ± 0.23 b
7.5	0.90 ± 0.10 a	1.40 ± 0.47 a	1.43 ± 0.31 b	1.47 ± 0,31 b
0 (control)	0.00 ± 0.00 b	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 c

Numbers followed by the same letter are no different from Duncan's results X = Average, SD = Standard deviation

Based on the result of research on nanoemulsion made from *Piper aduncum* and *Cymbopogon nardus*, it can be seen that all four concentrations of nanoemulsion, 1%, 2.5%, 5%, and 7.5%, were able to attack Xaa bacteria. In

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general, results after 1 and 2 days of incubation for all four concentrations were not significantly different from 0.00% concentration (control). The observation results can be seen in Table 1. The concentration considered less controllable Xaa is 7.5%, with a diameter (cm) after four days are 1,47 ± 0.31. However, after 3 and 4 days, the concentration of 1% was considered to be the most optimal compared with the remaining concentrations with an average diameter of the inhibition of 2,70 ± 0.30 (cm); 3,17 ± 0,80 (cm), respectively.

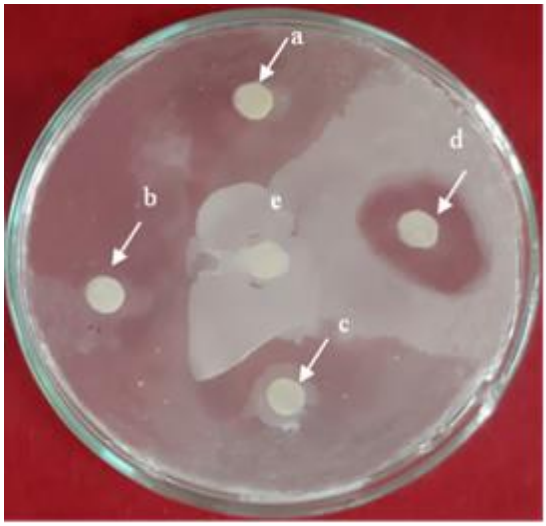

Figure 4. Nanoemulsion concentration inhibition zone from *P. aduncum* essential oil and hydrosol of *C. nardus* (a) 1%, (b) 2.5%, (c) 5%, (d) 7.5 %, (e) Control (distilled water)

Analysis of the *P. aduncum* essential oil from fresh leaves revealed that the main constituent found in the leaf of essential oil of *P. aduncum* is dillapiole, a phenylpropanoid derivative that ranges from 31% to 97% bioactivity that is antimicrobial due to these substances (Arze, Collin, Garneau, Jean, & Gagnon, 2008). The majority of *P. aduncum's* biological activities are governed by dillapiole (Navickiene et al., 2006). Additionally, dillapiole was reported to operate in synergy with a number of organic insecticides, such as carbamates, organochlorides, pyrethrum, tenulin, and azadirachtin. Dillapiole interacts synergistically with other volatiles in *P. aduncum* oil to increase stability, insecticide activity, and antibacterial activity. The essential oil of *P. aduncum* was active against protozoa parasites and diverse microorganisms, including pathogenic bacteria and fungi, showing properties as antimicrobial, molluscicidal, and cytotoxic (de Morais et al., 2007).

Besides, waste distilling of fragrant *Cymbopogon nardus* has been utilized as a mixed material for botanical insecticides, where the potential of hydrosol as a pest controller or botanical insecticide can be further developed to have insecticide activity (Abena et al., 2007). Alkaloids, terpenoids, polypeptides, phenolics, polyphenols, and other compounds found in *Cymbopogon nardus* plant extracts are natural and potent substitutes for antibiotics, agrochemicals, and other synthetic substances (Nakahara, Alzoreky, Yoshihashi, Nguyen, & 2003). The main active ingredients in fragrant lemongrass oil are aldehydes (citronellol-C10H18O) by 30 - 45%, alcohol compounds (cyronelolC10H20O and geraniol-C10H18O) by 55- 65%, and other compounds such as geraniol, cyral, nerol, metal, heptonon, and dipentene (Carmo et al., 2012). These compounds function as immune system stimulants and have growth-promoting, antibacterial, and substitutive qualities (Mekonnen et al., 2015). The concentration of *Cymbopogon nardus* has an inhibitory effect on the growth of bacteria, especially Gram-positive bacteria (Chisowa, Hall, Farman, & Journal, 1998).

Table 2. The average diameter of the inhibition zone area of *Bacillus thuringiensis* strain MRSNR3.1 and secondary metabolites of *Bacillus thuringiensis* strain MRSNR3.1 against *Xanthomonas axonopodis* pv. *allii* bacteria

Treatment	Average resistance area diameter (cm) (day) (X ± SD)			
	1	2	3	4
Bacillus thuringiensis strain MRSNR3.1	1.03 ± 0,09 a	1.57 ± 0,58 a	1.91 ± 0,61 a	3.04 ± 0,44 a
Secondary metabolites of *Bacillus thuringiensis* strain MRSNR3.1	0.55 ± 0,51 a	1.07 ± 0,23 a	1.19 ± 0,21 a	2.21 ± 0,28 a
Control	0,00 ± 0,00 b	0,00 ± 0,00 b	0,00 ± 0,00 b	0,00 ± 0,00 b

Numbers followed by the same letter are no different from Duncan's results X = Average, SD = Standard deviation.

According to Table 2, the strain Bacillus thuringiensis MRSNR3.1 and its second metabolites (supernatant) could attack Xaa (Figure 5). It can be seen that after four days of incubation, the diameter of *strain Bacillus thuringiensis* MRSNR3.1 is larger than that of its secondary metabolites with an inhibition zone of 3.04 ± 0.44; 2.21 ± 0.28, respectively.

Bacillus thuringiensis can produce spores under unfavorable circumstances and is more resilient to harsh circumstances. Due to its long-term storage capacity and ability to trigger an immune reaction in the host plants leads to systemic resistance (Induced SR), rendering the plant less susceptible to pathogen infection (Jung et al., 2008). *Bacillus thuringiensis* can exist in soil under

various environmental circumstances, can move freely, competes in the rhizosphere and plant tissue, and is a facultative anaerobe (Yanti, Habazar, Reflinaldon, Nasution, & Felia, 2017).

Bacillus thuringiensis, as a biocontrol, be able to test for pathogens such as *Pseudomas syringae* pv Arabidopsis (Niazi et al., 2014), *Xanthomonas campestris* pv. in cabbage (Wulff et al., 2002), *Xanthomonas axanopodis* pv glycine in soybean (Choudhary & Johri, 2009), (*Xanthomonas vesicatoria* in tomato (Cook & Stall, 1969). Exotoxinthermostable exotoxin generated by *B. thuringiensis* is a broad-spectrum vegetative factor released in the supernatant of *B. thuringiensis* during vegetative development (Sharma, Prasad, Pai, & Sharma, 2000).

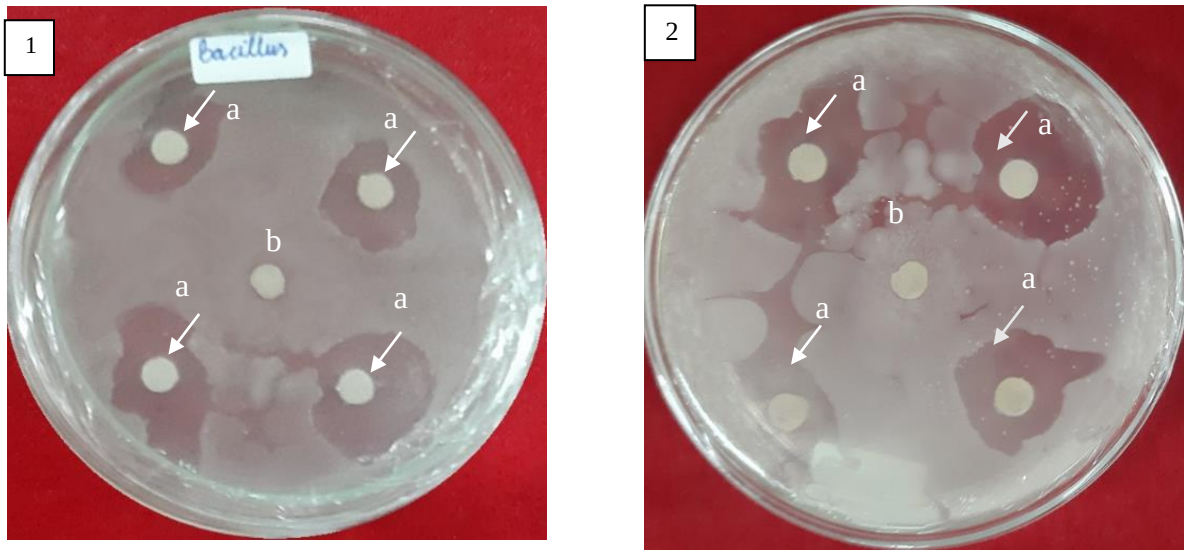

Figure 5. Zone of inhibition of (1) *Bacillus thuringiensis* strain MRSNR3.1 (1a) *Bacillus thuringiensis* strain MRSNR3.1 (1b) Control (2) Secondary metabolites of *Bacillus thuringiensis* strain MRSNR3.1 (2a) Secondary metabolites of *Bacillus thuringiensis* strain MRSNR3.1 (2b) Control.

Due to secondary metabolites, *Bacillus thuringiensis* is more competitive with other microbes (Liu et al., 2014). Specifically, Nonribosomal peptides (NRPs) have a considerable antibacterial potential through bacterial protein synthesis suppression, fungal membrane perforation, enzyme inhibition, and cell lysis (Schwartz & Laprade, 2000). Secondary metabolites provide *Bacillus thuringiensis* with increased competitiveness towards other microorganisms (Kamoun, Zouari, Saadaoui, Jaoua, & biotechnology, 2009). They have a tremendous antibacterial potential by disrupting bacterial protein production, perforating fungal membranes, and inhibiting enzymes (Sansinenea & Ortiz, 2011).

Table 3. The average diameter of the inhibition zone of the nanoemulsion from *Piper aduncum* and *Cymbopogon nardus*, *Bacillus thuringiensis* strain MRSNR3.1, and secondary metabolites of *Bacillus thuringiensis* strain MRSNR3.1 against *Xanthomonas axonopodis pv. allii* bacteria

Treatment	Average resistance area diameter (cm) (day) (X ± SD)			
	1	2	3	4
1 %	1.07 ± 0.47 a	1.80 ± 0.26 a	3.10 ± 0.69 a	3.33 ± 0.29 a
2.5 %	0.77 ± 0.12 a	1.70 ± 0.52 a	2.33 ± 1.04 ab	2.77 ± 0.25 ab
5 %	1.10 ± 0.17 a	1.53 ± 0.64 a	2.20 ± 0.17 ab	2.63 ± 0.64 b
7.5 %	0,00 ± 0.00 b	1.50 ± 0.36 a	2.00 ± 0.46 b	2.53 ± 0.72 b
Bacillus thuringiensis strain MRSNR3.1	1.00 ± 0.10 a	1.93 ± 0.25 a	2.60 ± 0.36 ab	2.83 ± 0.29 ab
Secondary metabolites of *Bacillus thuringiensis* strain MRSNR3.1	0.60 ± 0.53 a	1.27 ± 0.06 a	2.70 ± 0.17 ab	2.83 ± 0.38 ab
Control	0,00 ± 0,00 b	0,00 ± 0,00 b	0,00 ± 0,00 c	0,00 ± 0,00 c

Numbers followed by the same letter are no different from Duncan's results X = Average, SD = Standard deviation.

Based on the results of measuring the average diameter of the inhibition zone on nanoemulsions made from *Piper aduncum* and *Cymbopogon nardus*, it can be seen that the concentration of nanoemulsions (1%, 2.5%, 5%, 7.5%)) strain of *Bacillus thuringiensis* MRSNR3.1 and supernatant of the strain *Bacillus thuringiensis*

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MRSNR3.1 were able to inhibit Xaa at four days after incubation (Table 3 and Figure 6). According to the results from Table 3, in the nanoemulsion treatment, a concentration of 1% with an inhibition zone of 3.33 ± 0.29 (cm) was considered to be more optimal than the rest of the treatments for effectively inhibiting Xaa at four days after incubation. *P. aduncum* oil is an essential oil that acts as an antibacterial by disrupting the process of forming membranes or cell walls so that they are not formed or formed imperfectly (Brazao et al., 2014). The hydrophobicity of essential oils can break down lipids in bacterial cell membranes, damage the membrane structure, causing cell membrane leakage and ultimately causing bacterial cells to die (Benchimol, Sutton, Bastos, & Dias-Filho, 2001).

Bacillus thuringiensis shows antimicrobial activity in the nanomolar range against a broad spectrum of Gram-positive bacteria (Kamoun et al., 2011). Its antibacterial efficacy is caused by cytoplasmic membrane penetration of susceptible bacteria (Wirth, Walton, & Federici, 2010). These bacteria can produce a wide range of secondary metabolites with very different natures and structures and display broad-spectrum activities (de la Vega, Barboza-Corona, Aguilar-Uscanga, & Ramírez-Lepe, 2006). In especially, nonribosomal peptides (NRPs) have an enormous antimicrobial potential by causing cell lysis, perforation of fungal membranes, enzyme inhibition, or disruption of bacterial protein synthesis. Antibiotics and other secondary metabolites found in bacterial isolates have been suggested to have a role in the prevention of pathogen development, for which antibiosis is the primary mechanism of action for disease control (Ortiz-Rodríguez, De La Fuente-Salcido, Bideshi, Salcedo-Hernández, & Barboza-Corona, 2010).

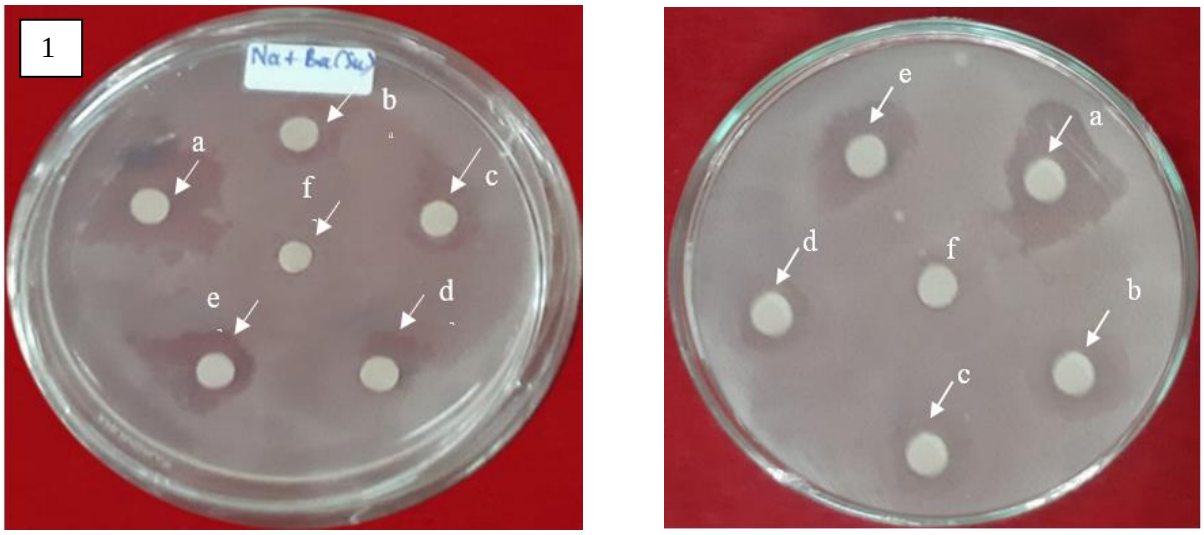

Figure 6. Zone of inhibition of (1) Concentrations of Nanoemulsion + *Bacillus thuringiensis* strain MRSNR3.1, (1a) 1%, (1b) 2.5%, (1c) 5%, (1d) 7.5%, (1e) *Bacillus thuringiensis* strain MRSNR3.1 (1f) Control; (2) Concentrations of Nanoemulsion + Secondary metabolites of *Bacillus thuringiensis* strain MRSNR3.1 (2a) 1%, (2b) 2.5%, (2c) 5%, (2d) 7.5%, (2e) Secondary metabolites of *Bacillus thuringiensis* strain MRSNR3.1 (2f) Control.

CONCLUSIONS

Based on the research that has been done, it can be concluded that the nanoemulsion mixture of *Piper aduncum* essential oil and *Cymbopogon nardus* waste is effective to be used as an alternative in control *Xanthomonas axonopodis* pv. *allii*. The study promoted the possibility that the essential oil of *P. aduncum* and *C. nardus* waste could be developed into the industrial production of bactericides, fungicides, and insecticides. In order to obtain greater benefits, the mixed nanoemulsion should be tested for its effectiveness against other pathogens and its effectiveness in controlling pathogens in the field. Besides, *Bacillus thuringiensis* species represent a rich source of secondary metabolites that exhibit strong antifungal and antibacterial activities and enable the bacterium to survive in its natural environment. In this perspective, the development of *Bacillus thuringiensis* was able to control Xaa for plant growth.

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REFERENCES

Abena, A. A., Gbenou, J. D., Yayi, E., Moudachirou, M., Ongoka, R., Ouamba, J. M., & Silou, T. (2007). Comparative chemical and analgesic properties of essential oils of Cymbopogon nardus (L) Rendle of Benin and Congo. *African Journal of Traditional, Complementary and Alternative Medicines*, *4*(3), 267-272.

Alvarez, A. M., Buddenhagen, I. W., Buddenhagen, E. S., & Domen, H. Y. (1978). Bacterial blight of onion, a new disease caused by Xanthomonas sp. *Phytopathology*, *68*(8), 1132-1136.

Arze, J. B. L., Collin, G., Garneau, F. X., Jean, F. I., & Gagnon, H. (2008). Essential oils from Bolivia. VIII. Piperaceae: Piper heterophyllum Ruiz et Pavón, P. aduncum L. *Journal of Essential Oil Bearing Plants*, *11*(1), 53-57.

Benchimol, R. L., Sutton, J. C., Bastos, C. N., & Dias-Filho, M. (2001). Survival of black pepper plants in soil infected with Fusarium solani f. sp. piperis and amended with extracts or residues of Piper aduncum. *Can J Plant Pathol*, 23, 194.

Brazao, M. A. B., Brazao, F. V., Guilherme, J., & Monteiro, M. C. (2014). Antibacterial activity of the Piper aduncum oil and dillapiole, its main constituent, against multidrug-resistant strains. *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas*, *13*(6), 517-526.

Carmo, E. S., Pereira, F. D. O., Moreira, A. C. P., Brito, L. L., Gayoso, C. W., Costa, J. D., & Lima, E. D. O. (2012). Essential oil from Cymbopogon citratus (DC) Stapf: a promising natural product against Malassezia spp. *Rev Inst Adolfo Lutz, Rio de Janeiro*, *2*(71), 386-391.

Cerpa, M. G., Mato, R. B., Cocero, M. J., Ceriani, R., Meirelles, A. J. A., Prado, J. M., ... & Meireles, M. A. A. (2009). *Steam distillation applied to the food industry*. CRC Press: Boca Raton, FL.

Magalhães, C.F., de Siqueira E.P., de Oliveira, E.A., Zani C.L., Peres R.L., dos Santos K.V. & Andrade A. A. (2016). Antimicrobial activity of Piper aduncum leaf extracts against the dental plaque bacteria Streptococcus mutans and Streptococcus sanguinis. *JMPR 10*(23), 331-337.

Chisowa, E. H., Hall, D. R., & Farman, D. I. (1998). Volatile constituents of the essential oil of Cymbopogon citratus Stapf grown in Zambia. *Flavour and Fragrance Journal*, *13*(1), 29-30.

Choudhary, D. K., & Johri, B. N. (2009). Interactions of Bacillus spp. and plants–with special reference to induced systemic resistance (ISR). *Microbiological Research*, *164*(5), 493-513.

Conn, K. E., Lutton, J. S., & Rosenberger, S. A. (2012). Onion disease guide: a practical guide for seedsmen, growers and agricultural advisors. *Senminis Vegetable Seeds. Woodland.*

Cook, A. A., & Stall, R. E. (1969). Differentiation of pathotypes among isolates of Xanthomonas vesicatoria. *Plant disease reporter.*

de la Vega, L. M., Barboza-Corona, J. E., Aguilar-Uscanga, M. G., & Ramírez-Lepe, M. (2006). Purification and characterization of an exochitinase from Bacillus thuringiensis subsp. aizawai and its action against phytopathogenic fungi. *Canadian Journal of Microbiology*, *52*(7).

de Morais, S. M., Facundo, V. A., Bertini, L. M., Cavalcanti, E. S. B., dos Anjos Júnior, J. F., Ferreira, S. A., ... & de Souza Neto, M. A. (2007). Chemical composition and larvicidal activity of essential oils from Piper species. *Biochemical Systematics and Ecology*, *35*(10), 670-675.

Dimkić, I., Janakiev, T., Petrović, M., Degrassi, G., & Fira, D. (2022). Plant-associated Bacillus and Pseudomonas antimicrobial activities in plant disease suppression via biological control mechanisms-A review. *Physiological and Molecular Plant Pathology*, 117, 101754.

Drais, H. K., & Hussein, A. A. (2015). Formulation and characterization of carvedilol nanoemulsion oral liquid dosage form. *Int J Pharm Pharm Sci*, *7*(12), 209-216.

Dubey, N. K., Shukla, R., Kumar, A., Singh, P., & Prakash, B. (2010). Prospects of botanical pesticides in sustainable agriculture. *Current Science*, *98*(4), 479-480.

Erlina, L. H., Lina, E. C., & Djamaan, A. (2020, March). Insecticidal activity of nanoemulsion of Piper aduncum extracts against cabbage head caterpillar Crocidolomia pavonana F.(Lepidoptera: Crambidae). In *IOP Conference Series*: *Earth and Environmental Science* (Vol. 468, No. 1, p. 012001). IOP Publishing.

Ferreira, O. O., Cruz, J. N., de Moraes, Â. A. B., de Jesus Pereira Franco, C., Lima, R. R., Anjos, T. O. D., ... & Andrade, E. H. D. A. (2022). Essential oil of the plants growing in the Brazilian Amazon: Chemical composition, antioxidants, and biological applications. *Molecules*, *27*(14), 4373.

FroÃ, C., de Siqueira, E. P., de Oliveira, E. A., Zani, C. L., Peres, R. L., ValÃ, K., & AssunÃ, A. (2016). Antimicrobial activity of Piper aduncum leaf extracts against the dental plaque bacteria Streptococcus mutans and Streptococcus sanguinis. Journal of Medicinal Plants Research, 10(23), 331-337.

Gent, D. H., & Schwartz, H. F. (2005). Management of Xanthomonas leaf blight of onion with a plant activator, biological control agents, and copper bactericides. *Plant disease*, *89*(6), 631-639.

Hasyim, D. M. (2011). *Potensi buah sirih hutan (Piper aduncum) sebagai insektisida botani terhadap larva Crocidolomia pavonana* (Doctoral dissertation, Tesis MSi. Bogor: Institut Pertanian Bogor).

Hyakumachi, M., Nishimura, M., Arakawa, T., Asano, S., Yoshida, S., Tsushima, S., & Takahashi, H. (2013). Bacillus thuringiensis suppresses bacterial wilt disease caused by Ralstonia solanacearum with systemic induction of defense-related gene expression in tomato. *Microbes and Environments*, *28*(1), 128-134.

Jafari, B., Ebadi, A., Aghdam, B. M., & Hassanzade, Z. (2012). Antibacterial activities of lemon grass methanol extract and essence on pathogenic bacteria. *American-Eurasian J Agric and Environ Sci*, 2, 1042-6.

Jung, W. J., Mabood, F., Souleimanov, A., Zhou, X., Jaoua, S., Kamoun, F., & Smith, D. L. (2008). Stability and antibacterial activity of bacteriocins produced by Bacillus thuringiensis and Bacillus thuringiensis ssp. kurstaki. *Journal of Microbiology and Biotechnology*, *18*(11), 1836-1840.

Kamal, H. Z. A., Ismail, T. N. N. T., Arief, E. M., & Ponnuraj, K. T. (2020). Antimicrobial activities of citronella (Cymbopogon nardus) essential oil against several oral pathogens and its volatile compounds. *Padjadjaran Journal of Dentistry*, *32*(1), 1-7.

Kamoun, F., Fguira, I. B., Hassen, N. B. B., Mejdoub, H., Lereclus, D., & Jaoua, S. (2011). Purification and characterization of a new Bacillus thuringiensis bacteriocin active against Listeria monocytogenes, Bacillus cereus and Agrobacterium tumefaciens. *Applied biochemistry and biotechnology*, 165, 300-314.

Kamoun, F., Zouari, N., Saadaoui, I., & Jaoua, S. (2009). Improvement of Bacillus thuringiensis bacteriocin

 DOI: 10.25077/ijasc.6.2.95-103.2022

production through culture conditions optimization. *Preparative biochemistry & biotechnology*, *39*(4), 400-412.

Issazadeh, K., Rad, S. K., Zarrabi, S., & Rahimibashar, M. R. (2012). Antagonism of Bacillus species against Xanthomonas campestris pv. campestris and Pectobacterium carotovorum subsp. carotovorum. *African Journal of Microbiology Research*, *6*(7), 1615-1620.

Lengai, G. M., Muthomi, J. W., & Mbega, E. R. (2020). Phytochemical activity and role of botanical pesticides in pest management for sustainable agricultural crop production. *Scientific African*, *7*, e00239.

Liu, X., Ruan, L., Peng, D., Li, L., Sun, M., & Yu, Z. (2014). Thuringiensin: a thermostable secondary metabolite from Bacillus thuringiensis with insecticidal activity against a wide range of insects. *Toxins*, *6*(8), 2229-2238.

Mahalwal, V. S., & Ali, M. (2003). Volatile constituents of Cymbopogon nardus (Linn.) Rendle. *Flavour and fragrance journal*, *18*(1), 73-76.

Mikonnen, M., Abate, S., & Manhile, B. (2015). Adaptation of citronella grass oil (Cymbopogon winterianus Jowitt) technologies as an alternative method for cockroaches (Blatella germanica L.). *Int. J. Innov. Agric. & Biol. Res*, *3*(1), 29-33.

Nakahara, K., Alzoreky, N. S., Yoshihashi, T., Nguyen, H. T., & Trakoontivakorn, G. (2013). Chemical composition and antifungal activity of essential oil from Cymbopogon nardus (citronella grass). *Japan Agricultural Research Quarterly*: JARQ, *37*(4), 249-252.

Navickiene, H. M. D., Morandim, A. D. A., Alécio, A. C., Regasini, L. O., Bergamo, D. C. B., Telascrea, M., ... & Kato, M. J. (2006). Composition and antifungal activity of essential oils from Piper aduncum, Piper arboreum, and Piper tuberculatum. *Química Nova*, *29*, 467-470.

Niazi, A., Manzoor, S., Asari, S., Bejai, S., Meijer, J., & Bongcam-Rudloff, E. (2014). Genome analysis of Bacillus amyloliquefaciens subsp. plantarum UCMB5113: a rhizobacterium that improves plant growth and stress management. *PLoS One*, *9*(8), e104651.

Okulate, M. A. (2009). Antimicrobial activity of bioactive compounds produced by Bacillus species. *A final report of microbial diversity course*.

Ortiz-Rodríguez, T., De La Fuente-Salcido, N., Bideshi, D. K., Salcedo-Hernández, R., & Barboza-Corona, J. E. (2010). Generation of chitin-derived oligosaccharides toxic to pathogenic bacteria using ChiA74, an endochitinase native to Bacillus thuringiensis. *Letters in applied microbiology*, *51*(2), 184-190.

Picard, Y., Roumagnac, P., Legrand, D., Humeau, L., Robène-Soustrade, I., Chiroleu, F., ... & Pruvost, O. (2008). Polyphasic characterization of Xanthomonas axonopodis pv. allii associated with outbreaks of bacterial blight on three Allium species in the Mascarene archipelago. *Phytopathology*, *98*(8), 919-925.

Rosliani, R., Palupi, E. R., & Hilman, Y. (2016). Pengaruh benzilaminopurin dan boron terhadap pembungaan, viabilitas serbuk sari, produksi, dan mutu benih bawang merah di dataran rendah.

Sansinenea, E., & Ortiz, A. (2011). Secondary metabolites of soil Bacillus spp. *Biotechnology letters*, *33*, 1523-1538.

Sansinenea, E. (2012). *Bacillus thuringiensis biotechnology* (Vol. 528). Netherlands: Springer.

Schlegel, H. G., & Zaborosch, C. (1993). *General microbiology*: Cambridge university press.

Schwartz, J. L., & Laprade, R. (2000). Membrane permeabilisation by Bacillus thuringiensis toxins: protein insertion and pore formation. *Entomopathogenic Bacteria: from laboratory to field application*, 199-217.

Sharma, C. B. S. R., Prasad, S. S. V., Pai, S. B., & Sharma, S. (1976). The exotoxin of Bacillus thuringiensis: A new C-mitotic agent. *Experientia*, *32*(11), 1465-1466.

Stein, T. (2005). Bacillus subtilis antibiotics: structures, syntheses, and specific functions. *Molecular microbiology*, *56*(4), 845-857.

Wibawa, I. P. A. H., Saraswaty, V., Kuswantoro, F., Andila, P. S., Wardhani, P. K., Tirta, I. G., & Sujarwo, W. (2019). A study of essential oil from an invasive Piper aduncum L. *Jurnal Biologi Udayana*, *23*(2), 50-58.

Wirth, M. C., Walton, W. E., & Federici, B. A. (2010). Inheritance patterns, dominance, stability, and allelism of insecticide resistance and cross-resistance in two colonies of Culex quinquefasciatus (Diptera: Culicidae) selected with Cry toxins from Bacillus thuringiensis subsp. israelensis. *Journal of medical entomology*, *47*(5), 814-822.

Wraight, C. L., Zangerl, A. R., Carroll, M. J., & Berenbaum, M. R. (2000). Absence of toxicity of Bacillus thuringiensis pollen to black swallowtails under field conditions. *Proceedings of the National Academy of Sciences*, *97*(14), 7700-7703.

Wulff, E. G., Mguni, C. M., Mansfeld-Giese, K., Fels, J., Lübeck, M., & Hockenhull, J. (2002). Biochemical and molecular characterization of Bacillus amyloliquefaciens, B. subtilis, and B. pumilus isolates with distinct antagonistic potential against Xanthomonas campestris pv. campestris. *Plant pathology*, *51*(5), 574-584.

Xu, X., Yu, L., & Wu, Y. (2005). Disruption of a cadherin gene associated with resistance to Cry1Ac δ-endotoxin of Bacillus thuringiensis in Helicoverpa armigera. *Applied and environmental microbiology*, *71*(2), 948-954.

Yanti, Y., Habazar, T., Reflinaldon, R., Nasution, C. R., & Felia, S. (2017). Indigenous Bacillus spp. ability to growth promoting activities and control bacterial wilt disease (Ralstonia solanacearum). *Biodiversitas Journal of Biological Diversity*, *18*(4), 1562-1567.

Zwahlen, C., Hilbeck, A., Gugerli, P., & Nentwig, W. (2003). Degradation of the Cry1Ab protein within transgenic Bacillus thuringiensis corn tissue in the field. *Molecular Ecology*, *12*(3), 765-775.