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Original article (Orijinal araştırma)

Prodigiosin, a promising biocontrol agent against *Thaumetopoea wilkinsoni* (Tams, 1926) (Lepidoptera: Notodontidae)

Prodigiosin, *Thaumetopoea wilkinsoni* (Tams, 1926) (Lepidoptera: Notodontidae)'ya karşı umut verici bir biyolojik kontrol ajanı

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Abstract

Serratia marcescens Bizio (Enterobacteriaceae: Serratia) is an entomopathogenic bacterium that produces hydrolytic enzymes and toxins. It also produces a pigment with various biological properties called prodigiosin. The study was conducted at Bilecik Seyh Edebali University in 2023. In this study, the effects of medium, incubation temperature and time on the process of prodigiosin production by *S. marcescens* strain Se9 and the extraction efficiency of different solvents were optimized for the first time using the orthogonal Taguchi array design. The optimal yield of pigment was achieved by methanol extraction from bacteria grown in tyriptic soy broth medium at 30ºC for 96 hours. The yield of prodigiosin pigment was 83.4±1.7 mg/L in the validation experiment conducted under the optimum conditions determined. The insecticidal potential of pigment against the larvae of *Thaumetopoea wilkinsoni* (Tams, 1926) (Lepidoptera, Notodontidae) was demonstrated for the first time. While the mortality rate in larvae exposed to 1000 ppm of the pigment was only 40%, it was observed that doubling the applied concentration led to a significant increase in larval mortality, reaching 91%. The LC_{50} value of the pigment for the fourth larval stage of *T. wilkinsoni* was determined to be 1192 ppm. The study showed that the pigment prodigiosin may be a promising biocontrol agent for the control of *T. wilkinsoni.*

Keywords: Insecticidal activity, microbial control, pigment, Serratia, *Thaumetopoea wilkinsoni*

Öz

Serratia marcescens Bizio (Enterobacteriaceae: Serratia) hidrolitik enzimler ve toksinler üreten entomopatojenik bir bakteridir. Ayrıca, prodigiosin adı verilen çeşitli biyolojik özelliklere sahip bir pigment de üretir. Çalışma, 2023 yılında Bilecik Şeyh Edebali Üniversite'sinde gerçekleştirilmiştir. Bu çalışmada, besiyeri, inkübasyon sıcaklığı ve süresinin *S. marcescens* Se9'un prodigiosin üretim sürecine etkileri ve farklı çözücülerin ekstraksiyon verimliliği ilk kez ortogonal Taguchi dizi tasarımı kullanılarak optimize edilmiştir. Optimum pigment verimi, 30ºC'de 96 saat boyunca triptik soya sıvı besiyeri ortamında büyütülen bakterilerden metanol ekstraksiyonuyla elde edilmiştir. Belirlenen optimum koşullar altında yapılan validasyon deneyinde prodigiosin pigment verimi 83,4±1,7 mg/L olarak belirlenmiştir. Prodigiosin pigmentinin *Thaumetopoea wilkinsoni* (Tams, 1926) (Lepidoptera, Notodontidae) larvaları üzerindeki insektisidal potansiyeli ilk kez gösterilmiştir. 1000 ppm pigmente maruz kalan larvalarda ölüm oranı sadece %40 iken, uygulanan konsantrasyonun iki katına çıkarılmasının larva ölümlerinde önemli bir artışa neden olarak %91'e ulaştığı gözlenmiştir. *T. wilkinsoni'*nin dördüncü larva dönemi için pigmentin LC_{50} değeri 1192 ppm olarak belirlenmiştir. Çalışma, prodigiosin pigmentinin *T. wilkinsoni'*nin kontrolü için umut verici bir biyokontrol ajanı olabileceğini göstermiştir.

Anahtar sözcükler: İnsektisidal aktivite, mikrobiyal kontrol, pigment, Serratia, *Thaumetopoea wilkinsoni*

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Introduction

The pine processionary moths (PPM), represented in Europe and North Africa by *Thaumetopoea pityocampa* (Denis & Schiffemüller, 1775) and in Asia and the Middle East by *Thaumetopoea wilkinsoni* (Tams, 1926) (Lepidoptera: Notodontidae), are the most damaging defoliating pests of pine forests in the coastal zone of Mediterranean countries. In most previous studies on *Thaumetopoea* sp. in Turkey, the species was referred to as *T. pityocampa* (Kanat & Alma, 2004; Er et al., 2007; Ince et al., 2008; Sevim et al., 2010). However, recent studies using molecular techniques have shown that the populations in Turkey are *T. wilkinsoni* (İpekdal et al., 2020).

Thaumetopoea wilkinsoni affects the yield of trees by feeding on the needles of various pine species [*Pinus brutia* Ten., *Pinus nigra* Arnold., *Pinus sylvestris* L., *Pinus pinea* L., *Pinus halepensis* Mill. (Pinales: Pinaceae)] during the larval stages in autumn and winter (İpekdal, 2005; Castagneyrol et. al., 2016). The pest causes deformities in seedlings and stunting and even tree mortality in young stands (Kanat et al., 2005). The larval stages cause the pines to shed their leaves and allow the invasion of other opportunistic pine pests, resulting in reduced viability of the pines and a lower yield of edible pine nuts (Faria, 2021). In addition to its serious impact on biodiversity and the environment, the pest also has a dangerous effect due to the stinging hairs it produces from the third larval stage onwards, which cause severe allergic reactions on contact with humans and animals (Olivieri et al., 2023). The stinging hairs are produced in large quantities in special abdominal pouches of the larvae and contain a protein-like toxin, thaumetopoein, which causes allergic reactions to the skin, respiratory tract, mouth and eyes (Bonamonte et al., 2013).

Various methods are used to control PPM, including the use of sex pheromones and mass trapping programs (Salvato et al., 2005). The nests are physically removed and destroyed, or insecticide-soaked tape is placed around the trees. Commercial tree boot barrier traps show high effectiveness in trapping larvae in the last larval stage during their migration and prevent them from reaching the ground to pupate (Colacci et al., 2018).

Chemical insecticides, especially insect growth inhibitors such as diflubenzuron, dimilin and deltamethrin, have been used mainly to control PPM (Demolin & Martin, 1986; Halperin, 1980). However, these agents have potentially undesirable side effects, particularly on predators and parasites of the pest, humans, plants and other non-target animals. Microbial control with entomopathogenic bacteria and bacterial secondary metabolites is an excellent alternative to conventional insecticides because it is cost-effective and high-yielding, does not harm beneficial organisms and releases fewer chemical residues into the fields.

Serratia marcescens Bizio (Enterobacteriaceae: Serratia) is a rod-shaped gram negative bacterium. It infects both invertebrates and vertebrates but is primarily known as an important pathogen of insects, causing rapid insect death (Mohan et al., 2011; Zhang et al., 2021). In addition, *S. marcescens* synthesizes a red pigment known as prodigiosin, which has biological properties including antibacterial (Lapenda et al., 2015), antifungal (John et al., 2021), antimalarial (Arifiyanto et al., 2022), nematicidal (Gomez Valdez et al., 2022), immunosuppressive (D'Alessio et al., 2000) and anticancer (Li et al., 2018). Its toxicity is mainly attributed to its secondary metabolite prodigiosin (Suryawanshi et al., 2015). Just as the culture conditions influence the production of prodigiosin, the extraction of the pigment produced has a considerable influence on the amount of pigment obtained. For these reasons, the production and extraction process of the pigment must be optimized.

Thus, the aim of the present study was to optimize the prodigiosin production of the local *S. marcescens* strain Se9 using Taguchi's orthogonal array method and to demonstrate its insecticidal potential against the field population of *T. wilkinsoni* larvae. This is the first study to show that the pigment prodigiosin is a promising biocontrol agent against *T. wilkinsoni*.

Materials and Methods

Culture of *Serratia marcescens*

Serratia marcescens strain (Se9), a red pigmented bacterium was kindly provided by Laboratory of the Department of Biology at Karadeniz Technical University. 100 µl of the bacteria spread onto tryptic soy agar (TSA) medium and incubated overnight at 30ºC. A single colony was then transferred to fresh TSA medium and used as a seed culture in experiments.

Optimization of prodigiosin production

The Taguchi method was used here to determine the optimal process conditions for the production of prodigiosin from *S. marcescens* strain Se9. The critical fermentation factors such as the medium, incubation temperature and incubation time as well as the solvent to be used for extraction were selected for the optimization process. The factors and their levels are listed in Table 1. A total of 9 experiments were performed based on the orthogonal array method of Taguchi's L9 design using the four target parameters (Table 2). Shaking flask experiments were performed according to the experimental design listed in Table 2. Erlenmeyer containing 90 ml medium was inoculated with 10 ml of overnight culture of *S. marcescens* strain Se9 and incubated on a rotary shaker at 200 rpm for pigment production. Each experiment was repeated three times.

Table 1. Factors and their levels used in optimization process

Factors	Level 1	Level 2	Level 3
Medium	Nutrient broth	Tryptic soy broth	Peptone glycerol broth
Incubation temperature (ºC)	25	30	37
Incubation time (hour)	48	72	96
Solvent	Acetone	Methanol	Petroleum ether

Table 2. Taguchi L9 orthogonal array and experiments to be performed

Experiments	Process parameters			
	Medium	Temperature (ºC)	Incubation time (hour)	Solvent
1	1	1	1	1
2	1	2	2	2
3	1	3	3	3
4	2	1	2	3
5	2	2	3	1
6	2	3	1	2
7	3	1	3	2
8	3	2	1	3
9	3	3	2	1

Extraction of solvent

After incubation, the pigmented culture was centrifuged at 10,000 rpm for 15 minutes at 4ºC and the supernatant discarded. Following the method of Suryawanshi et al (2015) with some modifications, the pellet was resuspended with the solvent indicated for each experiment and vortexed vigorously for at least 10 minutes. The mixture was then centrifuged again at 10,000 rpm for 15 minutes and the supernatant, which contained prodigiosin, was carefully transferred to a sterile Falcon tube. The solvent was removed under vacuum using a rotary evaporator (Bibby Scientific Ltd, Staffordshire, UK) at 50ºC, keeping the cooling temperature below 10ºC, until a dry red pigment was obtained. The crude pigment was collected, quantified on a dry weight basis and stored at 4ºC until use in bioassays.

Validation of the model

The prodigiosin yield was considered as a quality characteristic using the "the larger is the better approach" and the signal-to-noise ratio was determined. In addition, the effect of the selected factors on the pigment production was determined using analysis of variance (ANOVA). All analyses were carried out using Minitab statistical software. A validation experiment was then performed under optimal conditions, which were determined using the Taguchi method. The results were compared with the estimated values obtained with the Minitab software.

Bioassay

The PPM larvae used for the bioassay were collected between October and November 2023 in *Pinus brutia* forests in Bilecik, Türkiye (40.1587ºN, 29.9758ºE and altitude of 850 m above sea level). The silk nests collected with the help of ligature scissors were placed in disinfected containers and brought to the laboratory. Also, it has been confirmed that the pest is *T. wilkinsoni* (K. İpekdal, personal communication). Fourth instar larvae were selected for use in bioassays based on the size of the head capsule and body morphology (EPPO/CABI 1997).

After resuspending ten mg of the dry pigment in 5 ml of methanol, different concentrations (2000, 1000, 500, 250 and 125 ppm) were prepared by 1:2 serial dilution with methanol. The pigment (5 ml) was sprayed onto the needle using a mini hand sprayer. After evaporation of the solvent, the field collected twenty larvae (4th instar) were placed on pine needles and transferred to boxes (30×20×15 cm) with perforated covers to allow air flow. The boxes were incubated in a climate-controlled room at 25±1ºC, 60% relative humidity and a photoperiod of 16:8 hours light: dark. The control group was sprayed with the solvent and twenty larvae were placed on pine needles after evaporation of the solvent. Bioassays were repeated three times for each concentration. Mortality was recorded daily for 14 days.

Statistical analysis

The mortality data were corrected using the Abbott formula (Abbott, 1925). Cumulative mortality was analyzed with survival analysis using Kaplan-Meier estimates of survival probabilities and pairwise comparisons were performed using the log-rank test at 0.05 probability (Mantel-Cox). Concentration-response data were subjected to probit regression analysis and lethal concentrations (LC_{50} and LC_{95}) of the pigment were estimated. Statistical analyses were performed using the IBM SPSS version 25 (IBM, 2017).

Results

Optimization of pigment production

The effectiveness of culture conditions, incubation time and temperature, differences in medium and solvent used in extraction on the production of prodigiosin by *S. marcescens* strain Se9 was tested in 9 experiments using the Taguchi experimental design. The results showed significant variations in the yield of prodigiosin, and it was found that the production was highly dependent on the culture conditions and solvent. The yield of prodigiosin ranged from 0 to 88.7 mg/L (Table 3).

The optimal conditions for each factor were determined by calculating the signal-to-noise ratio of the tested factors. The signal-to-noise ratio should have a maximum value according to the Taguchi method in order to achieve optimal prodigiosin production. It was found that the optimum yield of pigment can be obtained by methanol extraction from bacteria incubated in TSB medium at 30ºC for 96 hours (Figure 1).

In addition, an analysis of variance (ANOVA) was performed to determine the contribution of the individual factors to the variation. The results are shown in Table 6. While the incubation temperature had the greatest positive influence on prodigiosin production among the tested factors with 56.54%, the medium

had the least influence with 1.22% (Table 4). The model obtained from ANOVA showed that the multiple correlation coefficient R2 is 1.0, i.e. the model can explain 100% of the variation in the response.

Table 3. Experiments carried out for optimization and the pigment yield obtained

Experiments	Medium	Incubation temperature (ºC)	Incubation time (hour)	Solvent	Pigment yield (mg/L)±SD
1	Nutrient broth	25	48	Acetone	13.0±0.75
2	Nutrient broth	30	72	Methanol	88.7±0.67
3	Nutrient broth	37	96	Petroleum ether	1.20±0.12
4	Tryptic soy broth	25	72	Petroleum ether	22.7±0.02
5	Tryptic soy broth	30	96	Acetone	52.5±0.70
6	Tryptic soy broth	37	48	Methanol	3.70±0.25
7	Peptone glycerol broth	25	96	Methanol	65.7±0.22
8	Peptone glycerol broth	30	48	Petroleum ether	27.5±0.10
9	Peptone glycerol broth	37	72	Acetone	0.0±0.0

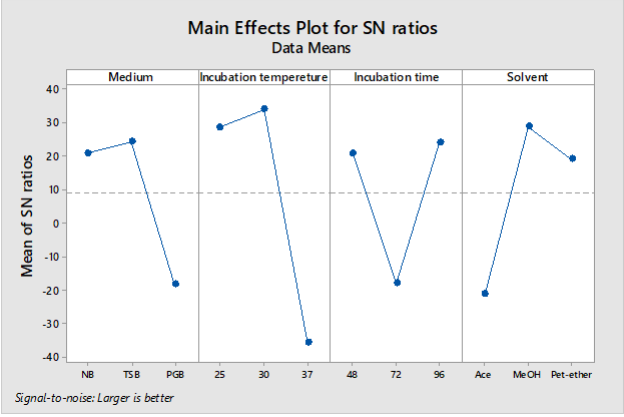

Figure 1. The signal-to-noise ratio plot of tested factors for prodigiosin production. Ace: acetone, MeOH: Methanol, Pet-eter: petroleum ether.

Table 4. Analysis of variance (ANOVA) for the yield of prodigiosin

Factors	DF	Contribution (%)	Adj SS	Adj MS	F-value	P-value
Medium	2	1.22	97.18	48.59	12.86	0.044
Incubation temperature	2	56.54	4519.11	2259.55	46.74	0.021
Incubation time	2	14.23	1137.21	568.6	18.56	0.042
Solvent	2	28.02	2239.83	1119.91	30.06	0.032
Error	0	-	-	-		
Total	8	100				

DF: degree of freedom, Seq SS: sequential sums of squares, Adj SS: adjusted sum of squares, Adj MS: Adjusted mean squares, R2=100%.

The validation experiment was performed with the optimized factors at the recommended levels. The result was a prodigiosin production of 83.93±1.75 mg/L, which is comparable to the predicted value (83.41 mg/L). Therefore, the Taguchi method is validated for the production of prodigiosin pigments.

Insecticidal properties of pigment

The insecticidal potential of the pigment prodigiosin was investigated on the larvae of *T. wilkinsoni.* No mortality was observed at a concentration of 125 ppm, but the mortality rate increased with increasing concentration, reaching 92% at a concentration of 2000 ppm. The log-rank analysis showed that the survival rates of *T. wilkinsoni* larvae treated with the pigment at concentrations of 2000, 1000 and 500 ppm differed from those of the control group (Table 5). However, it was found that the survival rates of larvae treated with the pigment at concentrations of 250 and 125 ppm did not differ from those of the control group ($p>0.05$).

Table 5. Pairwise comparison using Log-Rank (Mantel-Cox) test for *Thaumetopea wilkinsoni* larvae exposed to different concentration of prodigiosin

Concentrations (ppm)	125		250		500		1000		2000		Control	
	X^2	*p*	X^2	*p*	X^2	*p*	X^2	*p*	X^2	*p*	X^2	*p*
125			1.02	0,31	11.11	0.00	47.29	0.00	184.24	0.00	1.00	0.31
250	1.02	0.31			6.71	0.01	40.55	0.00	174.51	0.00	3.03	0.08
500	11.11	0.00	6.71	0.01			19.18	0.00	145.70	0.00	13.84	0.00
1000	47.29	0.00	40.55	0.00	19.18	0.00			75.00	0.00	50.17	0.00
2000	184.24	0.00	174.51	0.00	145.70	0.00	75.00	0.00			185.42	0.00
Control	1.00	0.31	3.03	0.08	13.84	0.00	50.17	0.00	185.42	0.00		

X^2: Chi-square, *p:* significance at 0.05**.**

Figure 2 illustrates the cumulative survival rates of *T. wilkinsoni* larvae exposed to various concentrations of prodigiosin. While larvae exposed to 125 and 250 ppm of the pigment showed relatively high survival rates, those exposed to 2000 ppm had a survival rate of just 8%. The LC_{50} value for prodigiosin, which is the concentration at which 50% of the larvae are expected to die, was determined to be 1192 ppm (Table 6).

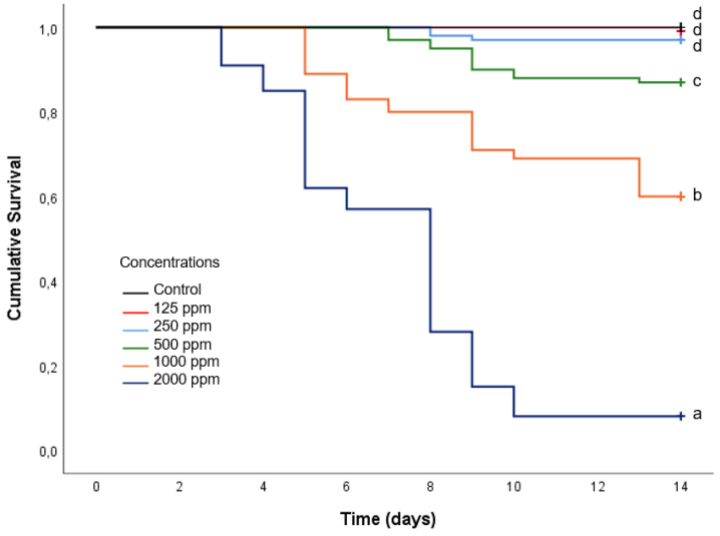

Figure 2. Kaplan-Meier plot showing survival of *Thaumetopea wilkinsoni* larvae after exposure with different concentration of prodigiosin pigment. Curves bearing different letters are significantly different in survival mode (Log-rank test, p<0.05).

Table 6. Lethal concentrations (LC_{50} and LC_{95}) of prodigiosin pigment for the larvae of *Thaumetopea wilkinsoni*

Pigment	LC_{50} (ppm)	95% *FL*		Slope ± SE	LC_{95} (ppm)	95% *FL*		*df*	X^2
		Lower bound	Upper bound			Lower bound	Upper bound		
Prodigiosin	1192	1103	1291	0.02 ±0.00	2064	1901	2275	3	5.06

A total of 360 individuals, 60 for each concentration and controls, were used.

Discussion

The emergence of resistance to conventional insecticides has revived interest in the search for and development of pesticides. Microbial control agents and their products offer alternatives to conventional insecticides as they can be more selective. The insecticidal potential of *S. marcescens*, an entomopathogenic bacterium, has already been reported, but the use of the whole microorganism is limited due to its pathogenicity for animals and humans. Therefore, the active compounds produced by the bacterium could be more useful than the bacterium itself.

The pigment prodigiosin produced by *S. marcescens* is one of the secondary metabolites that confer insecticidal properties to the bacterium. As with many secondary metabolites, the biosynthesis of prodigiosin is dependent on the growth phase and reaches its maximum when the cell enters the stationary phase. Prodigiosin synthesis depends on cultural conditions such as media, incubation time and temperature. This is the first report describing the application of the Taguchi method to prodigiosin production in an entomopathogenic bacterium.

The type of culture medium is an important factor for the production of prodigiosin, with tryptic soy broth proving to be the most effective compared to other media in our experiments The differences in the amount of prodigiosin are directly related to the composition of the media. Since each microorganism has its own nutrient requirements for growth, the tryptic soy broth, which is favorable for the production of prodigiosin in the present study, could contain the necessary nutrients. Peptone from casein, peptone from soymeal, glucose monohydrate, sodium chloride and di-potassium hydrogen phosphate are the main components of the tryptic soy broth. According to Paul et al (2024), peptone is an excellent source of nitrogen as it provides essential fatty acids and amino acids that are important for bacterial growth. However, there is no single nitrogen source that is specifically optimized for the production of prodigiosin in *S. marcescens*, as different nitrogen sources provide different amounts of amino acids and growth-promoting compounds. Furthermore, Giri et al. (2004) point out that the choice of carbon source is crucial for the production of prodigiosin in *Serratia marcescens* strains. In our study, a TSB medium containing 2.5% (w:v) glucose proved to be the most effective for maximizing prodigiosin production. This finding contrasts with previous research by Su et al. (2011), which indicated that glucose and lactose can inhibit prodigiosin production.

In addition to the composition of the medium, the incubation temperature and duration are crucial factors that influence the production of prodigiosin. Paul et al. (2024) reported that the highest prodigiosin yield (15 mg/L) occurred at 30ºC when *S. marcescens* was grown in nutrient broth. Temperatures above 30ºC resulted in a decrease in pigment production, a finding consistent with our results. We found that 30ºC is the optimal temperature for prodigiosin synthesis, with production decreasing significantly at 37ºC. At temperatures above 30ºC, *S. marcescens* ceases pigment production and colonies appear creamy white, which is due to the fact that temperatures below or above the optimal temperature (30ºC) can lead to deactivation of ribosomes and enzymes involved in the prodigiosin synthesis pathway (de Araujo et al., 2010). Incubation time is also an important factor for pigment production. In our study, the optimal incubation time for the production of prodigiosin was determined as 96 hours. Koyun et al (2022) found that the strain *S. marcescens* MB703 showed maximum production of prodigiosin pigments on the 6th day in NB medium. Similarly, maximum production of prodigiosin by *S. marcescens* strain MN5 was observed 6 days after incubation (Elkenawy et al., 2017). Interestingly, Su et al. (2011) achieved high prodigiosin yields from a 1-day culture, although it is generally assumed that microorganisms must reach stationary phase to produce secondary metabolites like prodigiosin.

In addition to the culture conditions for the production of prodigiosin, the extraction of the pigment produced by the bacterium is also an important task. Typically, solvent-assisted extraction is employed, and the choice of organic solvent significantly affects the yield. Park et al. (2012) reported that ethanol is the best solvent in terms of extraction efficiency and that acetone has an extraction efficiency of 98.5% compared to ethanol. However, methanol has high extraction efficiency and unlike Park et al. (2012), acetone showed low extraction efficiency in our study. Overall, highly polar solvents such as ethanol, methanol, acetone, and acetonitrile generally offer high extraction efficiency for prodigiosin (Park et al., 2012).

Prodigiosin is a promising biomolecule with many potential applications. So far, many studies have reported the antibacterial, antifungal, antiviral, anticancer and algicidal effects of prodigiosin extracted from various microorganisms (Islan et al., 2022). In addition, the insecticidal property of prodigiosin was first

determined in three different species of lepidoptera (Asano et al., 1999). They were administered to the larvae of *Spodoptera litura* Fab., 1775 (Lepidoptera: Noctuidae), *Plodia xylostella* L., 1758 (Lepidoptera, Pyralidae) larvae and *Adoxophyes honmai* Yasuda, 1998 (Lepidoptera Tortricidae) by the ingestion method at a dosage of 8 µg/g diet. The mortality rate of the larvae of *P. xylostella* reached up to 96%, while very low mortality rates were observed in the larvae of *S. litura* and *A. honmai*. However, Patil et al. (2013) reported 100% mortality in larvae of *S. litura* treated with high concentrations (30 mg/mL) of prodigiosin. In this study, prodigiosin caused 92% mortality in another lepidopteran, *T. wilkinsoni*, at a concentration of 2000 ppm. This shows that the insecticidal effect of prodigiosin is concentration dependent. Similarly, Eski & Özdemir (2022) reported that at low concentrations (125 and 250 ppm), prodigiosin caused less than 10% mortality in *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae) larvae, while at high concentrations (1000 and 2000 ppm), the mortality rate was over 90%. They also reported an LC_{50} value of prodigiosin for *T. molitor* larvae of 924 ppm. In this study, the LC_{50} value for *T. wilkinsoni* larvae was determined to be 1192 ppm. Compared to previous studies, the LC_{50} value was high. Wang et al. (2012) reported the LC_{50} value of the pigment prodigiosin extracted from *S. marcescens* TKU011 as 230 ppm for the larvae of *Drosophila melanogaster* Meigen, 1830 (Diptera: Drosophilidae). Similarly, the LC_{50} value of prodigiosin extracted from *S. marcescens* NMCC46 was determined to be 103.95 and 105.52 ppm for *Aedes aegypti* L., 1762 (Diptera: Culicidae) and *Anopheles stephensi* Liston, 1901 (Diptera: Culicidae) larvae, respectively. (Patil et al., 2011). The difference in prodigiosin activity in different insects can be explained by the detoxification mechanism through high activity of esterases (Suryawanshi et al., 2015). Therefore, the susceptibility of insects to the pigment prodigiosin can vary. Although there are many studies showing that prodigiosin has an insecticidal effect, Zhou et al. (2016) tested the pigmented strain and its non-pigmented mutant on *Bombyx mori* L., 1758 (Lepidoptera: Bombycidae) and found no difference between the LC_{50} values. Therefore, it was reported that prodigiosin is not a necessary virulence factor. However, in a study conducted by Suryawanshi et al. (2015) to understand the mode of action of the prodigiosin, it was found that immune system enzymes such as protease, acetylcholinesterase, esterase and phosphatase change significantly when mosquito larvae are exposed to the pigment. In addition, nutrient absorption decreases, and the insect dies due to the decreasing pH value in the insect's midgut.

In conclusion, it has been demonstrated that the pigment prodigiosin has the potential to be used in the control of *T. wilkinsoni*, an important pest in pine forests. The efficacy of the pigment should be tested under field conditions. Furthermore, the usability of the pigment together with other control agents should be investigated as part of integrated pest management strategies for this pest.

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