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Serratia marcescens strain NPKC3_2_21 as endophytic phosphate solubilizing bacteria and entomopathogen: Promising combination approach as rice biofertilizer and biopesticide

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Abstract. *Sutio G, Afifah AN, Maharani R, Basri M. 2023*. Serratia marcescens *strain NPKC3_2_21 as endophytic phosphate solubilizing bacteria and entomopathogen: promising combination approach as rice biofertilizer and biopesticide. Biodiversitas 24: 901-909*. Accumulation insoluble Phosphorus (P) and rice stem borer pest (*Scirpophaga innotata*) are two of the primary constraints in rice (*Oryza sativa*) production systems. The availability of soluble forms of P for plants in the soils is limited because it is fixed as insoluble P by iron (Fe) and aluminum (Al) in acidic soil as well as calcium (Ca) and magnesium (Mg) in alkaline soil causing P accumulation in the soil. Another problem is the rice pest which caused the most by rice stem borer (*Scirpophaga innotata*), should take in the first place because causing annual losses of the rice crop. Besides, soil acidity can affect the growth of bacteria in soil and pest management. The study highlighted the contribution of *Serratia marcescens* strain NPKC3_2_21 as endophytic root-associated microorganisms in solubilizing P to enhance the availability of P in soil for the plant. Besides, we investigated the effect of entomopathogenic bacteria *Serratia marcescens* strain NPKC3_2_21 on pests *Spodoptera litura* as a contribution to the knowledge of the efficacy of *Serratia marcescens* strain NPKC3_2_21 as an entomopathogenic bacteria for pest controlling management in rice plant. In addition, we assessed the growth ability of *Serratia marcescens* strain NPKC3_2_21 in alkaline, neutral and acid pH conditions as an indicator that these bacteria are able to be grown at various pH conditions. These analyses revealed that *Serratia marcescens* strain NPKC3_2_21 has potential as 1) endophyte that can enter with no visible harmful effects for plants, 2) P-solubilizing bacteria that enhance the availability of P in soil by producing organic acids, and 3) entomopathogenic bacteria to insects. Besides, *Serratia marcescens* strain NPKC3_2_21 can grow in the soil at various pH (acid, neutral and alkaline) conditions. Therefore, we suggested the bacterium *Serratia marcescens* strain NPKC3_2_21 might be an alternative strategy to enhance available P taken up by the roots, besides be a promising role as bio-insecticide applied in rice crops.

Keywords: Bacterial endophyte, biofertilizer, biofungicide, entomopathogenic bacteria, P-solubilization

INTRODUCTION

Phosphate is one of the major growth-limiting macronutrients needed by plants because it plays a vital role in overall plant growth and development. It accounts for between 0.2 to 0.8% of the dry weight of plants (Sharma 2013; Kalayu 2019). The deficiency of P could reduce plant growth as weak stem, brown and small leaves and slow plant development. Most soils possess considerable amounts of P but in insoluble form. Yet, the availability of soluble forms of P for plants in the soils is limited because it is fixed as insoluble phosphates by Fe, Al, Ca and Mg, causing P accumulation in the soil. The application of P fertilizer used by farmer to P-deficient soils can result in P accumulation as well. Therefore, the alternative techniques to provide P in soil are needed.

One of the most well-known mechanisms of P solubilization is the release of weak organic acids by P-solubilizing microorganisms (Kaur 2021). Phosphate solubilizing microbes produce organic acids by dissolving the insoluble soil phosphates by chelation of cations. The insoluble forms of P, such as tricalcium phosphate ($Ca_3PO_4)_2$, aluminium phosphate (Al_3PO_4), iron phosphate (Fe_3PO_4), etc. may be converted to soluble P by P-solubilizing organisms inhabiting different soil ecosystems (Gupta et al. 2007; Song et al. 2008; Khan et al. 2013; Sharma et al. 2013; Khan et al. 2014) by producing of organic acids (Kalayu 2019). Most often different divalent and trivalent organic anions such as malate, citrate and oxalate are produced by the microbes and are implicated to play an important role in the solubilization of P (Pohlman 1986; Kaur 2021). Till now, several kinds of bacteria have been proved efficient in promoting P-solubilizing, such as *Bacillus* sp. (Hanif 2015), *Serratia* sp. (Gong 2022), *Bacillus subtilis* PH, *Serratia marcescens* PH1, and *Serratia marcescens* PH2 (Mohamed 2018).

Besides, the role of endophytic property of microbe must be considered due to a symbiotic relationship established as part of a stable ecosystem. Over the past few years, there has been a sharp increase in the use of endophytic microorganisms as agricultural inoculants. They promote plant growth; antagonize phyto pathogens along with production of many industrial metabolites (Azevedo et al. 2000; Chauhan et al. 2016: Mehta et al. 2019). Unlike non root-associated microbe, which can limited by abiotic ecosystem factors. The advantages of

endophytic bacteria as P-solubilizing, which are defined by Yandila et al. (2018) that endophytic bacteria are bacteria that live in plant tissues and have symbiotic mutualism with their hosts by producing secondary metabolites in the form of bioactive substances. Based on the mechanism of endophytic bacteria as P-solubilizing, so the bacteria could enter the plant tissues, have a positive symbiotic relationship with plants, not influenced by environmental stress factors, and could optimize the P absorption by plants.

In line with this point, pest management also needs to be a concern. Insect pests with high population level cause enormous damage to agricultural crops and economy, one of them are rice stem borer (*Scirpophaga innotata*) which are able to enter inside of rice stem base. For controlling insect pests, several microbial agents have been developed to manage insect pests. Entomopathogenic microbe in biological plant protection plays a key role in the program of sustainable pest management. Entomopathogenic microbe producing several hydrolytic enzymes such as protease, chitinase, nuclease and lipase, which are toxins (Flyg et al. 1983; Dalahi 2014). Niu (2022) explained that one of bacterial entomopathogens, such as *Serratia marcescens* promotes plant growth and improves resistance against *Nilaparvata lugens* in rice.

In addition, the ability of bacteria to survive at a certain pH range also must also be considered. This is because most bacteria could grow in the range of neutral and alkaline pH values, but most bacteria are hard to overcome in order to survive and growth at low pH. The study highlights the contribution of *Serratia marcescens* strain NPKC3_2_21 as endophytic root-associated microorganisms in solubilizing P to enhance the availability of P in soil for the plant. Besides, we investigated the effect of entomopathogenic bacteria *Serratia marcescens* strain NPKC3_2_21 on pests *Spodoptera litura* as a contribution to the knowledge of the efficacy of *Serratia marcescens* strain NPKC3_2_21 as an entomopathogenic bacteria for pest controlling management in rice plant. In addition, we assessed the growth ability of *Serratia marcescens* strain NPKC3_2_21 in alkaline, neutral and acid pH conditions as an indicator that these bacteria are able to be grown at various pH conditions.

MATERIALS AND METHODS

Assessment of P-solubilizing ability in different potential bacteria

This study aimed to asses the ability of phosphate solubilizing potential bacteria as a promising biological agent for managing soil phosphorus deficiency because it enhances the available P taken up by organic acids production. The microbes like *Serratia marcescens* strain NPKC3_2_21, *Bacillus amyloliquefaciens* strain 50, *Pseudomonas fluorescens* strain YLSS3, *Trichoderma harzianum* strain MGQ2 and *Streptomyces thermovulgaris* strain 09924-c8Ka-50-3 as well as pikovskaya liquid medium, molybdenum blue reagent and aquades were used in this study. While, the tools used were well dish, centrifuge, and spectrophotometer. To assess the ability of several microbes to solubilize P, isolates were cultured in liquid Pikovskaya medium and the turbidity was measured at a wavelength of 693 nm using a spectrophotometer.

Assessment of P-solubilizing ability, organic acids availability and growth ability under pH stress conditions

This study aimed to asses the ability of phosphate solubilizing bacteria *S. marcescens* strain NPKC3_2_21, their ability to produce organic acids and their growth ability at various pH conditions (was set at 4,0, 4,5, 6,75, and 8,0). Apart from bacterial strain *S. marcescens* NPKC3_2_21, LB medium, aquades, pikovskaya liquid medium, molybdenum blue reagent, aquades, $Ca_3(PO_4)_2$, $AlPO_4$, $FePO_4$ and organic acids were used in this step. While, the tools used were pH meter, well dish, petri dish, tubes, vortex, bunsen, centrifuge and spectrophotometer. To assess the ability to solubilize P and producing of organic acids, isolates were cultured in liquid Pikovskaya medium and the turbidity was measured. To assess the growth ability of *S. marcescens* strain NPKC3_2_21 at alkaline and acidic pH conditions, isolates were cultured in LB medium with pH set at 4.0, 4.5, 6.75, and 8.0. After that the population of *S. marcescens* strain NPKC3_2_21 was measured using Total Plate Count (TPC) method.

Assessment of the presence of endophytic isolates in plant tissues

The purpose of this study was to asses the presence of bacteria *S. marcescens* strain NPKC3_2_21 in rice plant as evidence of endophytic properties. The materials used were *S. marcescens* strain NPKC3_2_21, IPB 3S rice seed, Urea, SP36, NPK-16-16-16, 70% alcohol, and chlorox. While, the tools used were Petri dish, vortex, bunsen, rockwoll and spray. To assess the presence of endophytic isolates in plant tissues, isolates were applied to the surface of rockwoll to wet on rice aged 10 / >10 DAC. Samples were taken at the base of the stem, base of the leaves and roots, and then isolated on solid LB media with washing in 70% alcohol and 4% chlorox for 30 seconds.

Assessment of entomopathogenic role

Preliminary test

This study aimed to asses the ability of *S. marcescens* strain NPKC3_2_21 as entomopathogenic bacteria. The bacteria viz. *S. marcescens* strain NPKC3_2_21 and *Spodoptera litura* were here in this step. To assess the ability of *S. marcescens* as an entomopathogenic bacteria, *S. litura* was inoculated with *S. marcescens* by spraying and observing total death of larvae.

Field test

This study aimed to control the pest of rice stem borer worm by utilizing biological agent *S. marcescens* strain NPKC3_2_21 bacteria. The materials used were *S. marcescens* strain NPKC3_2_2 consist in product of BT-MAX, IPB 3S rice seeds, Urea, SP36, and chemical insecticide of Virtako (Chlorantraniliprole, Thiamethoxam).

The application was carried out at the same application interval as chemical fertilizer. Every 50 grams of BT-MAX was mixed with 25 kg of chemical fertilizer then sprinkled evenly over the area in the age of 10 DAC and 30 DAC.

Assessment of toxicological effects on mammals through pre-analytical test

The hemolysis test

The purpose of this study was to assess the pathogenicity or toxicity of isolate *S. marcescens* strain NPKC3_2_21 through the hemolysis test. The materials used were *S. marcescens* strain NPKC3_2_21, blood agar plate (BAP) medium, and aquades. While, the tools used were Petri dish, tubes, vortex, bunsen and incubator. The pathogenicity of *S. marcescens* strain NPKC3_2_21 was assessed through hemolysis test, the isolate was inoculated in blood agar medium and observed whether the pattern occurred α-Hemolysis, β-Hemolysis or γ-Hemolysis.

The acute oral tests on white rats

The purpose of this study was to determine the oral toxicity of *S. marcescens* strain NPKC3_2_21 in humans through white rats. The materials used were S. *marcescens* strain NPKC3_2_21 10^8 cfu/gram and white rats (Sporague-Dawley rat). In this test, a dose of 5000 mg/ kgBB was used and control was tried on 5 Sparague Dawley male rats. Male body weight of Sprague Dawley rats used in this study was 165-180 gram for treatment of 5000 mg/KgBW and 160-183 grams for control. Before being treated, the rats were fasted for 24 hours. After being treated, observations of behavioral changes and physiological reactions were observed at 1 hour, 2 hours, 3 hours, 4 hours and 24 hours after treatment. Observations in the form of weighting, counting the number of dead rats and visible clinical symptoms were carried out until the 14^{th} day after treatment. On the 14^{th} day all living rats mutilated and performed macroscopic (anatomical pathology) to observed on visceral organs.

Assessment of acute dermal tests on white rats

The purpose of this study was to determine the dermal toxicity of *S. marcescens* strain NPKC3_2_21 in humans through the white rats. The materials used were *S. marcescens* strain NPKC3_2_21 10^8 cfu/gram and white rats (Sporague-Dawley rat). In this test, a dose of 2000 mg/KgBW was used. Treatment and control doses were tried in 5 male rats. Rat body weight used between 160-183 grams for control and 165-178 g treatment. The rats that be treated were shaved their hair on the back of an area of 3 cm x 3 cm using a shaver, then left for 24 hours before being treated. Provision of test material is carried out by dripping the test material on the part of the shaved skin. The treated part of the skin is covered with plastic so that the liquid does not evaporate and the outer layer is covered with gauze and bandaged. The observations of behavioral changes and physiological reactions were carried out at 1 hour, 2 hours, 3 hours, 4 hours, and 24 hours after treatment. After 24 hours of observations, the bandage was opened, the skin was cleaned with water, and observed changes in the skin. Observations include weighting, calculating the number of dead mice, and clinical symptoms that appeared to be carried out until the 14^{th} day after treatment. On the 14^{th} day all living rats were mutilated and performed macroscopic (anatomical pathology) to observed on visceral organs.

RESULTS AND DISCUSSION

According to the results of a laboratory test on the effectiveness of *S. marcescens* strain NPKC3_2_21 bacteria in dissolving P compared to different types of P solvent microbes in Pikovskaya medium culture, after 14 days of incubation and then measured spectrophotometrically at 693 nm, *S. marcescens* strain NPKC3_2_21 bacteria has potential to accumulate phosphate up to 2987 ppm in 13 ml Pikovsaya medium culture. This result showed the highest accumulated value as compared to different types of P solvent microbes, such as *Bacillus amyloliquefaciens* strain 50, *Pseudomonas fluorescens* strain YLSS3, *Trichoderma harzianum* strain MGQ2 and *Streptomyces thermovulgaris* strain 09924-c8Ka-50-3 (Table 1).

Table 1. The ability comparison of *Serratia marcescens* strain NPKC3_2_21 to different types of P solvent microbes through a quantitative measurement using spectrophotometric method

Group	Isolate code	Dissolved phosphate measurement with microplate reader		Accumulation of P concentration (ppm) in 13 ml Pikovsaya medium culture using spectrophotometric method
		Absorbance λ = 693 nm	Final absorbance (absorbance value - blanko)	
-	Blanko for bacteria and *Actinomycetes*	0.187	-	-
-	Blanko for fungi	0.195	-	-
Bacteria	*Serratia marcescens* strain NPKC3_2_21	1.681	1.494	2987
	*Bacillus amyloliquefaciens*strain 50	1.612	1.425	2849
	Pseudomonas fluorescens strain YLSS3	0.731	0.544	1087
Fungi	*Trichoderma harzianum* strain MGQ2	0.55	0.355	1099
Actinomycetes	*Streptomyces thermovulgaris* strain 09924-c8Ka-50-3	0.46	0.273	545

Serratia marcescens NPKC3_2_21 in dissolving P have a correlation with organic acids production. According to Satyaprakash (2017), *S. marcescens* may release several organic acids that are products of the microbial metabolism. Organic acids that solubilize phosphates are primarily citric, lactic, gluconic, 2-ketogluconic, oxalic, glyconic, acetic, malic, fumaric, succinic, tartaric, malonic, glutaric, propionic, butyric, glyoxylic, and adipic (Kumar 2018; Satyaprakash 2017; Walpola and Yoon 2012; Selvi et al. 2017; Yousefi et al. 2015; Ahmed and Shahab 2011; Kalayu 2019). Organic acids produced by phosphate solubilizing microbes dissolve the insoluble soil P by chelation of cations. The groups of hydroxyl and carboxyl from the acids chelate the cations which bound to P and convert it into soluble forms. These acids may complete for fixation sites of Al and Fe insoluble oxides, on reacting with them, stabilize them, and are called "chelates" (Whitelaw 2000; Walpola and Yoon 2012).

From Tables 2 and 3, we assessed the ability of *S. marcescens* in solubilizing P from three different P sources ($Ca_3(PO_4)_2$, $FePO_4$, and $AlPO_4$), assessed the organic acid produced by *S. marcescens* as well as measured their growth ability at various pH conditions (alkaline and acidic pH conditions). From the study, we observed that *S. marcescens* strain NPKC3_2_21 was proven to solubilize P at acidic pH conditions 4.5 and 4.0, but indeed it was much better to solubilize P at alkaline pH conditions. From the results, it was known that the more alkaline the pH, the more effective *S. marcescens* strain NPKC3_2_21 in solubilizing P. Besides, if we observed from the aspect of organic acids production, it was proven that *S. marcescens* strain NPKC3_2_21 was producing malic and citric acids to solubilize P. In line with the P solubilization and the total of organic acids produced by *S. marcescens* strain NPKC3_2_21 at various pH conditions, this also correlated with the total bacteria population, where we observed that the bacterial populations of *S. marcescens* strain NPKC3_2_21 showed the same optimum growth in the presence of alkaline, neutral, and extremely low pH condition, but showed the best of growth at alkaline pH conditions. This result is according to the study done by Gong (2022) which stated that *S. marcescens* strain Pt-3 is an efficient phosphate-solubilizing bacterium due to producing organic acids (Paul & Sinha 2013; Maheswar and Sathiyavani 2012; Mohamed 2018).

The another advantages that must be underlined of this *S. marcescens* strain NPKC3_2_21, besides, this bacteria has a potential to solubilize P by producing organic acids, this *S. marcescens* strain NPKC3_2_21 has endophytic properties, so in the end of the application of *Serratia* as phosphate solubilizing microbe is more effective.

In order to prove the characteristics of *S. marcescens* strain NPKC3_2_21 bacteria as endophytic bacteria, the test is done by inoculating bacteria on the roots of rice plants that have been sterilized. The results showed that *S. marcescens* strain NPKC3_2_21 bacteria are found in the stem base tissue of rice plants after 48 hours of the first application on the first day (A), in the stem base tissue after 14 days of the second application on the first day (B) and in the stem base after 14 days of the second application on the 7th day (C) (Figure 1). While the control does not detect any bacterial growth in the media (D).

In further observation, it is seen that this *Serratia* strain bacteria is proven to grow and develop in plant tissues. As seen in Figure 2, on the second day of incubation (A), the growth of *Serratia* red bacteria is only in the base tissue of rice stalks. However, on the seventh day of incubation (B), the red color derived from the prodigiosin pigments has filled all the rice stem tissue, indicating this *S. marcescens* strain NPKC3_2_21 are endophytic that can live and develop in whole plant tissues. It also appears that the rice plant tissue inoculated by *S. marcescens* looks dense and green. There are no symptoms that appear in the form of necrosis on rice leaves. This shows that *S. marcescens* strain NPKC3_2_21 are not potentially pathogenic for plants (C).

Table 2. The ability of *Serratia marcescens* as a phosphate solvent, organic acid production and their growth ability at alkaline and neutral pH conditions

The ability of *Serratia marcescens* strain NPKC3_2_21				
pH	**Total bacteria cultured in Pikovskaya medium**	**P-solubilizing and organic acids production from alkaline soil insoluble P form**		
		$Ca_3(PO_4)_2$		
		Total of P (ppm)	**Malic acid (mL)**	**Citrate acid (OD)**
8.0	1.46 x 10^{10}	4365	15.56	0.042
6.75	9.9 x 10^9	4115	18.38	0.024

Table 3. The ability of *Serratia marcescens* as a phosphate solvent, organic acid production and their growth ability at acidic pH condition

The ability of *Serratia marcescens* strain NPKC3_2_21							
pH	**Total bacteria cultured in Pikovskaya medium**	**P-solubilizing and organic acids production from acidic soil insoluble P form**					
		$AlPO_4$			**$FePO_4$**		
		Total of P (ppm)	**Malic Acid (mL)**	**Citrate Acid (OD)**	**Total of P (ppm)**	**Malic Acid (mL)**	**Citrate Acid (OD)**
pH 4.5	5.5 x10^6	1140	0.83	0.043	3725	23.99	0.21
pH 4.0	3.9 x 10^6	325	1.69	0.047	2265	11.26	0.12

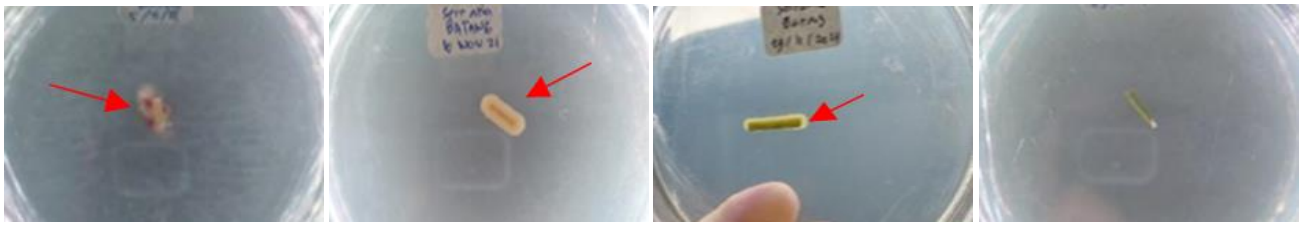

Figure 1. *Serratia marcescens* bacteria are found at the base of rice plant stem after 48 hours (A) and on the seventh day (B) after the first application, and are rediscovered after 14 days of the second application (C) Control (D)

Figure 2. Endophytic assay of *Serratia marcescens* strain NPKC3_2_21 bacteria. A. Bacteria growing at the base of tissue after 3 days of incubation, B. Bacteria growing throughout the tissue after 7 days of incubation, C. Rice plant with *S. marcescens*, C. Microscopic appearance of *S. marcescens*

Next, the isolate colonies were taken and examined under microscopic observation to prove the shape and color of the bacterial cells. Based on the results of microscopic observations, bacterial colonies that grow in the plant tissue were confirmed to be *S. marcescens* bacteria because their cells were rod-shaped and gram negative (D). It is in accordance with the opinion of Khanna (2103) that *S. marcescens* is gram-negative rod-shaped bacteria from the family of *Enterobacteriaceae.*

In line with its function as endophytic P-solubilizing bacteria, where these bacteria can enter the root tissues, residing inside the plant, associate with the roots of plant, enhance the availability of P by solubilizing P from insoluble P compounds, *S. marcescens* strain NPKC3_2_21 bacteria is also entomopathogen proved by preliminary study of entomopathogenic test applied in *Spodoptera litura.*

Based on the results preliminary study of entomopathogens test of *S. marcescens*, it is observed that the death rate of larvae after applying these bacteria is effective in killing *S. litura* larvae. In total, there are 3 dead larvae out of the total of 3 *Spodoptera litura* larvae killed after 48 hours of *Serratia* application, while in control (without *Serratia* application), there are no dead larvae found after 48 hours (all 3/3 larvae are alive) (Figure 3). According to several previous study, *S. marcescens* bacteria are pathogenic bacteria to insects and larvae because they can produce several hydrolytic enzymes, such as protease (Jupatanakul et al. 2020), chitinase (Martinez-Zavala et al. 2020), and lipase (Kumar et al. 2012) which are toxins.

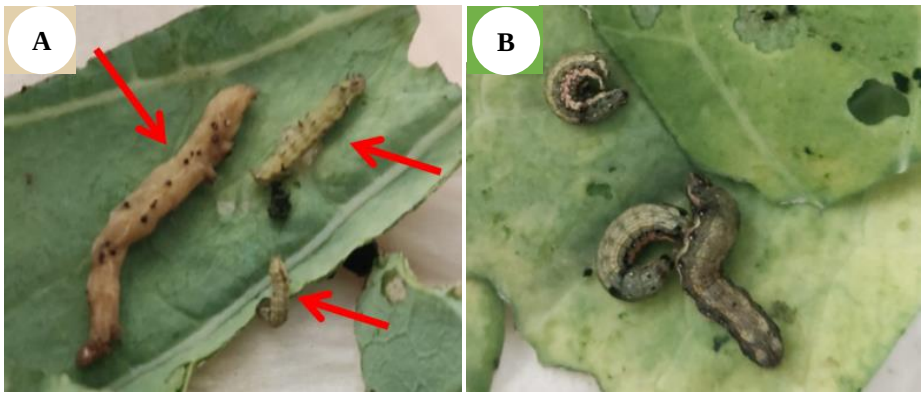

Figure 3. The result of *Serratia marcescens* application in *Spodoptera litura* larvae after 48 hours. A. Treatment: 3/3 of the caterpillars die. B. Control: 3/3 of the caterpillars are all alive

In further observation of field trials, the following is a comparison of rice stem borer attack development that occurs in an outbreak condition after control with the application of *S. marcescens* strain NPKC3_2_21. According to Figure 4 and Figure 5, *S. marcescens* strain NPKC3_2_21 is very effective in reducing the attack level of stem borer pests. It can be seen that the attack percentage with the severity level reaching 75% at the beginning, which is almost close to crop failure in the age of 25 DAC (Figure 4) can slowly decrease. At 6 days after application, the attack decreased to only 65%, and at 18 days after application, the attack rate decreased quite drastically because the attack only left 25% of the symptoms.

The reason why *S. marcescens* strain NPKC3_2_2 is a strong biocontrol for rice because *S. marcescens* is a facultatively-anaerobic bacterium (Marin 2017) with antibacterial activity (Clements et al. 2019). Most wild-type strains of *S. marcescens* produce a characteristic secondary metabolite, the red pigment prodigiosin (PDG). The prodigiosin has no defined role in the physiology of producing strains but have been reported to have antifungal, antibacterial, algicidal, antiprotozoal, antimalarial, immunosuppressive, anticancer, and antiproliferative activities (Castro 1967; Boger and Patel 1988; Williams and Quadri 1980; Demain 1995; Han et al. 1998; Cerdeno et al. 2001; Furstner 2003; Montaner and Pe´rez-Toma´s 2003; Samrot et al. 2011; Rakh 2017). Besides, *S. marcescens* are facultatively-anaerobic so that this species get lived easily on rice farming in aerobic condition by using oxygen for the respiration or anaerobic condition by switching to fermentation or anaerobic respiration if oxygen is absent.

Furthermore, the effect of *S. marcescens* strain NPKC3_2_21 bacteria on humans was also studied. *Serratia marcescens* is associated with urinary and respiratory infections, endocarditis, osteomyelitis, septicemia, wound infections, eye infections, and meningitis. Transmission is by direct contact (Buckle 2015). However, the pathogenicity of this strain is not yet fully understood. In this study, it also learned about the effect on mammals through hemolysis tests, acute dermal tests, and acute oral tests on white rats.

Hemolysis can significantly affect the reliability of test results and occur in the pre-analytical phase (Febryani 2019). The aim of this study is to reveal the correlation of hemoglobin levels on hemolysis samples. There are three patterns of hemolysis that occur: (i) α-Hemolysis: growth on blood plates causes incomplete destruction of blood cells. This produces dark green discoloration around bacterial colonies, reflecting the presence of biliverdin and other hemoglobin breakdown products; (ii) β-Hemolysis: growth on blood plates causes complete destruction of blood cells, resulting in transparency of the region surrounding bacterial colonies; (iii) γ-Hemolysis: no observable destruction of blood cells surrounding bacterial colonies (Slater 2007). Based on hemolysis test results, it is shown that *S. marcescens* strain NPKC3_2_21 is not pathogenic to humans, as proved by the results of gamma hemolysis (Figure 6).

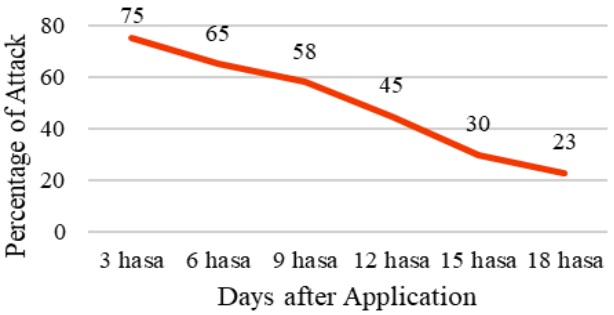

Figure 4. Graph of Stem Borer Attack development after *Serratia marcescens* strain NPKC3_2_21 application

A **B** **C**

Figure 5. *Serratia marcescens* strain NPKC3_2_21 application results: A. 6 days after application, B. 12 days after application, C. More than 18 days after application

In addition to hemolysis tests, toxicological effects on mammals are also performed on white rats to examine the effect of *S. marcescens* strain NPKC3_2_21 bacteria on oral and dermal tests. Acute oral and dermal toxicity tests on white rats were carried out to determine the toxicity and its side effects in humans. In oral testing, a dose of 500 mg/kgBW was used, while dermal testing uses a maximum dose of 2000 mg/kgBW. The test results showed the observations on rat body in oral at a dose of 500 mg/kgBW and dermal at a dose of 2000 mg/kgBW are presented in Table 4, oral treatment at a dose of 5000 mg/kgBW does not cause death, during behavioral observations showed pale, weak and standing hair (Table 5), and in macroscopic observation of the visceral organs of rat treated with *S. marcescens* showed changes in the anatomical pathology, namely lung pneumonia, hemorrhagic liver, blunt heart apex, cloudy ventricular dilatation (Table 6). In dermal treatment, a dose of 2000 mg/kgBW does not cause death, during behavioral observations showed rats experiencing weakness, and standing hair (Table 7), in macroscopic observation of the visceral organs of rats traeted with *S. marcescens* showed changes in the anatomical pathology, namely blunt heart apex, ventricular dilatation, cloudy diaphragm (Table 8). Based on oral and dermal acute toxicity concluded that *S. marcescens* strain NPKC3_2_21 is classified as non-pathogenic or non hazardous if used in accordance with the recommendations.

Table 4. Average body weight of the Sporague-Dawley rat group that got acute oral and dermal toxicity treatment after being given *Serratia marcescens* with a dose of 5000 mg/kgBW and 2000 mg/kgBW

Day	Rat Weight (grams)		
	Control	Dosage of 5000 mg/kgBW	Dosage of 2000 mg/kgBW
0	174.55	175.68	172.35
3	182.30	185.38	168.90
6	189.04	192.35	160.78
9	198.25	201.25	165.36
14	214.58	214.56	178.90

Table 5. Clinical symptoms of the group of Sprague Dawley rats treated with acute oral toxicity, after given *Serratia marcescens* strain NPKC3_2_21 with a dose 5000 mg/KgBW

Time	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5
1 hour	Hair standing, pale weak	Hair standing, pale weak	Hair standing, weak	Hair standing, weak	Hair standing, weak
2 hours	Hair standing, pale weak	Hair standing, pale weak	Hair standing, weak	Hair standing, weak	Hair standing, weak
3 hours	Hair standing, pale weak	Hair standing, weak	No	No	Weak
4 hours	No	No	No	No	No
24 hours	No	No	No	No	No
Day 2 - 14	No	No	No	No	No

Table 6. Anatomical pathology findings of Sprague Dawley rats treated with acute oral toxicity, after given *Serratia marcescens* strain NPKC3_2_21 with a dose 5000 mg/KgBW

Organ	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5
Lungs	No	Hemorrhagic	Hemorrhagic	No	No
Heart	No	No	No	No	No
Kidney	No	No	No	No	Weak
Brain	No	No	No	No	No
Intestine	No	No	No	No	No
Stomach	No	No	No	No	No
Heart	Blunt apex	Dilated	Blunt apex	Blunt apex dilated	Blunt apex dilation of right ventricular
Diaphragm	Murky	Ventricles	Murky	No	No
Spleen	No	Right	No	No	No

Table 7. Clinical symptoms of the group of Sprague Dawley rats treated with acute dermal toxicity, after given *Serratia marcescens* strain NPKC3_2_21 with a dose 2000 mg/KgBW

Time	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5
1 hour	Hair standing, weak	Hair standing, weak	Hair standing, weak	Hair standing, weak	Hair standing, weak
2 hours	Hair standing, weak	Hair standing, weak	Hair standing, weak	Hair standing, weak	Hair standing, weak
3 hours	No	Hair standing, weak	Hair standing, weak	Hair standing, weak	Hair standing, weak
4 hours	No	Hair standing, weak	Hair standing, weak	Hair standing, weak	Hair standing, weak
24 hours	No	Hair standing, weak	Hair standing, weak	Hair standing, weak	Weak
Day 2 - 14	No	No	No	No	No

Table 8. Anatomical pathology findings of Sprague Dawley rats treated with acute dermal toxicity, after given *Serratia marcescens* strain NPKC3_2_21 with a dose 2000 mg/KgBW

Organ	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5
Lungs	No	No	No	No	No
Heart	No	No	No	No	No
Kidney	No	No	No	No	Weak
Brain	No	No	No	No	No
Intestine	No	No	No	No	No
Stomach	No	No	No	No	No
Heart	Ventricle dilation	Ventricle dilation	Ventricle dilation	Ventricle dilation	Ventricle dilation
Diaphragm	No	No	Murky	Murky	Murky
Spleen	No	Right	No	No	No

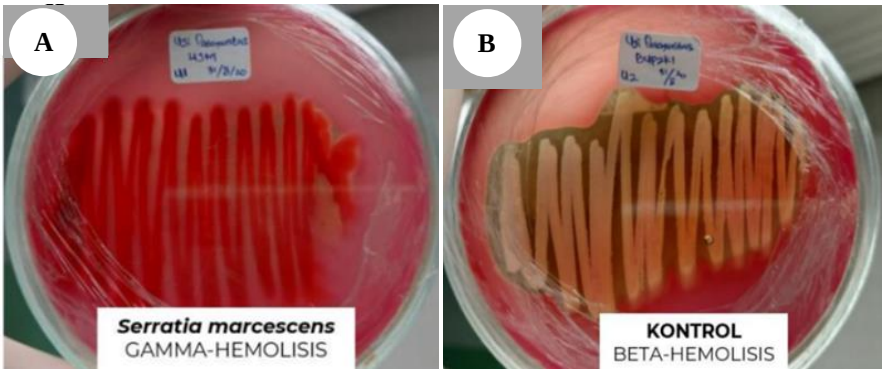

Figure 6. *Serratia marcescens* strain NPKC3_2_21 bacteria is a non-pathogenic bacteria proved by pathogenicity test in human (Hemolysis Test). A. *Serratia* showed Gamma Hemolysis means non-destruction of red blood cells or non-toxic to humans. B. Beta Hemolysis means destruction of red blood cells (control)

In conclusion, research findings that *S. marcescens* strain NPKC3_2_21 might be a promising bio-agent as endophytic P-solubilizing bacteria for use in an innovative strategy for the integrated management of soil. Besides, *S. marcescens* strain NPKC3_2_21 acts as a biocontrol against rice stem borer (*Scirpophaga innotata*) and *Spodoptera litura*. Furthermore, *S. marcescens* strain NPKC3_2_21 can be applied in acidic soil and was proven safe to human.

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