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Biocontrol of Moler Diseases (*Fusarium oxysporum* f. sp *capae* (Snyder and Hans)) on Shallot with Endophytic Bacteria

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Abstract. *Fusarium Basal Root (FBR) disease locally known as "moler", caused by Fusarium oxysporum f.sp cepae (FOCe), poses a significant threat to Indonesian shallot cultivation, leading to yield losses of approximately 50%. Therefore, this research aims to identify endophytic bacteria with optimal capabilities to combat FOCe infection while improving shallot growth and yield. Randomized Completely Block Design (RCBD) consisting of seven treatments was used, including six different strains of endophytic bacteria (Bacillus cereus P14, Bacillus cereus Se07, Bacillus sp. HI, Bacillus sp. SJI, Serratia marcescens ULG1E2, and Serratia marcescens JB1E3) and a control, each treatment was replicated three times, and three plant units in each replication. The introduction of endophytic bacteria was performed through the bulb soaking method before planting, while FOCe suspension was inoculated to shallot growing media 4 weeks after planting. Parameters Variables such as moler disease development, plant growth, and shallot bulb weight were monitored. The results showed that endophytic bacteria effectively suppressed moler disease and boosted shallot growth and yield compared to the control. Specifically, S. marcescens JB1E3 showed 52.25% effectiveness in reducing moler disease severity, while S. marcescens ULG1E2 increased shallot yield by 65.50%.*

Keywords: *Endophytic bacteria; Fusarium basal rot; Fusarium oxysporum f.sp cepae; moler disease.*

Type of the Paper: Regular Article.

1. Introduction

Shallot (*Allium ascalonicum* L.) is a popular horticultural product widely used by the population in Indonesia. In addition to being used as a cooking spice, red shallot is also used as a traditional medicinal ingredient [1,2]. From 2019 to 2022, the shallot yield is approximately 9.93 tons/ha, 9.71 tons/ha, 10.48 tons/ha, and 10.75 tons/ha, respectively [3]. The productivity of shallot has experienced fluctuations influenced by several factors, which include plant pathogens [4].

Pathogens present in shallot can lead to various diseases, including *Alternaria porri* [5], which causes purple blotch disease, *Colletotrichum gloeosporioides* causing anthracnose disease [6], *Peronospora destructor* which leads to false mildew disease [7]. Other diseases caused by pathogens present in shallot also include *Stemphylium vesicarium*, the causal agent of *Stemphylium* leaves blight disease [8], *Cercospora duddiae*, the cause of leaves spot disease [9], *Xanthomonas*

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axonopodis pv. *allii*, responsible for bacterial leaves blight (BLB) disease [10], and *Fusarium oxysporum* f.sp *cepae* (FOCe), the primary agent causing Fusarium basal rot disease, also known as moler disease [11–13].

Moler disease can damage roots and bulbs of shallot with symptoms of root rot, color changes, and necrosis at the base of bulb layers. Other characteristics of the symptoms appear on leaves, such as abnormal leaves growth, twisted leaves due to elongated pseudo stems, pale green or yellowish leaves color, and wilting [14]. The losses caused by this disease are approximately 50%, which can lead to crop failure [15].

Several efforts have been made to control the moler disease, including fungicides which are frequently used, as well as the gathering and removal of afflicted plants. In addition, crop rotation, the adoption of resistant cultivars, and soil solarization are further management strategies [16]. Biological control using *Bacillus subtilis* B1 and non-pathogenic *Fusarium oxysporum* T14a can suppress this disease and improve the growth of shallot [17,18]. The use of this method offers an eco-friendly substitute for plant disease management, including the application of endophytic bacteria [19,20].

Endophytic bacteria possess the capacity for both biological control and improving plant growth. This is attributed to the capability to safeguard plants, by proving more resistant to both biotic and abiotic stressors, leading to an increase in crop yields [21,22]. Endophytic bacteria can inhibit pathogen growth by inducing systemic resistance in the host plant [23]. Induced Systemic Resistance (ISR) refers to the interaction between particular bacteria and plants, leading to the plant's increased resistance against pathogens [24]. Additionally, endophytic bacteria can create phytohormones including IAA (Indole Acetic Acid), gibberellin, and cytokinin, and supply iron through siderophores [25].

Several investigations [26–28] have shown the potential of endophytic bacteria as biocontrol agents against various plant pathogens. For example, some endophytic bacteria can combat *Fusarium oxysporum*, the culprit behind wilt disease in oil palm, tomato, and chickpea, as well as *Ralstonia solanacearum* causing chili wilt disease, *Pantoea stewartii subsp. stewartii* leading to Stewart's wilt disease in corn, *Curvularia oryzae* inducing black grain disease in rice, and *Xanthomonas axonopodis pv. glycines* provoking bacterial pustules in soybeans [29–32]. Additionally, Resti et al. [33] found that six specific isolates of endophytic bacteria can improve shallot yields while suppressing *Xanthomonas axonopodis pv. allii*, the agent of bacterial leaves blight in shallot. These isolates include *Bacillus cereus* P14, *Bacillus cereus* Se07, *Bacillus sp*. HI, *Bacillus sp*. SJI, *Serratia marcescens* ULG1E2, and *Serratia marcescens* JB1E3, with disease suppression and yield improvement ranging from 28.32% to 64.30% and 50.65% to 214.85%, respectively. This research examined the effectiveness of six endophytic bacteria as potential

biological control agents in the management of moler disease in shallot. The objective of the present investigation is to identify Indigenous endophytic bacteria that are effective in suppressing moler disease and increase growth and yield traits in shallot.

2. Materials and methods

This research was carried out in the experimental station of the Faculty of Agriculture, Universitas Andalas Padang, and at the Microbiology and Phytopathology Laboratory of the Department of Plant Protection from January to May 2023. Randomized Completely Block Design (RCBD) consisting of seven treatments and three replications was used, and each replication had three plant units. The following endophytic bacterial strains were introduced into shallot seeds as part of the treatments:

A = *Bacillus cereus* P14
B = *Bacillus cereus* Se07
C = *Bacillus* sp. HI
D = *Bacillus* sp. SJI
E = *Serratia marcescens* ULG1E2
F = *Serratia marcescens* JB1E3
G = Control

2.1. Preparation of FOCe

FOCe pathogenic fungus is part of the laboratory collection of the Department of Plant Protection, Universitas Andalas. The fungus was cultured on Potato Dextrose Agar (PDA) (Merck) and incubated for seven days at room temperature.

FOCe was propagated on a sterilized rice medium. After 15 minutes of soaking in clean water, 700 g of rice was dried and cleaned. A heat-resistant plastic bag containing 100 g of rice was autoclaved for 15 minutes at 121°C and 1 atm of pressure. Once the rice was cooled, 5 mm-diameter fungal mycelium was added to the rice medium and incubated for 30 days at room temperature [34].

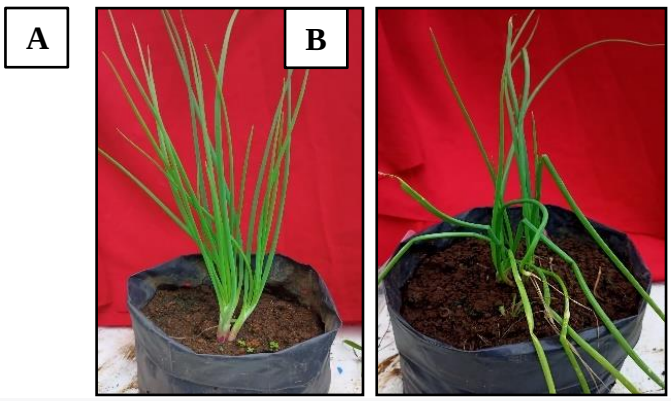

Fig. 1. Pathogenicity test on shallot; A. Control, B. Shallot inoculated with FOCe (21 DAI).

2.2. Pathogenicity Test of FOCe

In this research, a pathogenicity test was conducted using a 4-week-old shallot of Bima

Brebes variety, cultivated in polybags containing sterile soil. The inoculation process involved burying 10 g of rice substrate, containing FOCe culture, into the soil at a depth of 3 cm around the roots of the shallot. A pathogenicity test was carried out until initial symptoms appeared. The initial symptoms included leaves that did not grow upright and appeared twisted, with leaves color turning pale green or yellowish (Fig. 1) [35].

2.3. Preparation of Endophytic Bacteria

The six different endophytic bacterial species extracted from healthy shallot root tissues and used in this investigation were obtained from earlier research [33]. Using the quadrant streak method, endophytic bacteria were cultivated on Nutrient Agar (NA) media (Merck) and purified using the same technique.

Single bacterial colonies were propagated in 25 mL of Nutrient Broth (NB) media (Merck) in culture bottles with a 50 mL capacity, and the bacteria were then incubated for 24 hours at 150 rpm on a rotary shaker (preculture). Following propagation, 1 milliliter of the endophytic bacterial suspension obtained from the preculture was added to 199 milliliters of sterile coconut water in culture bottles. The main culture was then incubated for 48 hours at 150 rpm on a rotary shaker. The population density was determined by comparing the turbidity of the culture suspension with Mc. Farland scale 8 solution (approximately 10^8 cells/mL) [36].

2.4. The Ability of Endophytic Bacteria in Controlling Moler Disease in Shallot

2.4.1. Introduction of Endophytic Bacteria and Shallot Planting

The planting medium used consisted of a blend of sterilized soil and compost in a 2:1 (v/v) ratio, placed in 10 kg-volume polybags as outlined by Syawal et al. [37]. The introduction of endophytic bacteria occurred prior to shallot planting. Bulbs of Bima Brebes variety were partially cut from the top, followed by sterilization through a 30-second immersion in 70% alcohol, and subsequent rinsing with sterile distilled water. These bulbs were then immersed in an endophytic bacterial suspension with a density of 10^8 cells/ml, based on the treatment, for 15 minutes [33]. The control treatment involved soaking in sterile distilled water for 15 minutes.

2.4.2. Inoculation of FOCe

Inoculation was performed on a 4-week-old shallot by burying 10 g of rice substrate containing FOCe fungus around the shallot roots. After inoculation, the plants were observed and maintained until harvest. Observations were made on disease development, plant growth, and bulb weight.

2.4.3. Observation of Disease Development

The incubation Period (Days) was observed daily after inoculation until the plants exhibited chlorosis symptoms on leaves followed by necrosis at the leaves' tips. The effectiveness of incubation period suppression was calculated using Formula (1) [38].

$$E = \frac{P - K}{K} \quad (1)$$

Note:
E: Effectiveness
P: Treatment
K: Control

To calculate the disease incidence, observations were carried out from the appearance of symptoms until the plants were 60 days old after planting, with a 7-day interval. Formula (2) was used to calculate the incidence of disease.

$$I = \left(\frac{a}{b}\right) \times 100\% \quad (2)$$

Note:
I: disease occurrence
a: Number of plants affected.
b: Number of plants observed.

The following Formula (3) was used to determine the effectiveness of suppressing disease incidence.

$$E = \frac{K - P}{K} \quad (3)$$

Note:
E: Effectiveness
P: Treatment
K: Control

The severity of the disease was observed every 7 days from the onset of symptoms until the plants reached 60 days old. The formula (4) below was used to calculate the severity of the disease, and (3) and its effectiveness.

$$KP = \frac{\sum(n \times v)}{N \times V} \times 100\% \quad (4)$$

Note:
KP: Disease severity (%)
n: Number of plants from each attack category
v: Score value for each attack category
N: Number of plants were observed
V: Highest attack score

The disease severity for Moler disease was scored according to the criteria presented in Table 1.

The weight of rotting bulbs from individual plants was observed after harvesting by separating the rotting bulbs from the healthy ones. The effectiveness of suppressing rotting bulbs was calculated using Formula (3).

Table 1. Severity score of Moler disease in shallot

Score	Information
0	There are no symptoms of Moler disease
1	> 0 – 20% Number of symptomatic leaves
2	> 21– 40% Number of symptomatic leaves
3	> 41– 60% Number of symptomatic leaves
4	> 61– 80% Number of symptomatic leaves
5	> 81– 100% Number of symptomatic leaves

Source: Prabowo et al. [39]

2.4.4. Observations of growth and yield

Plant height and number of leaves were counted 1 week after planting and continued every 7 days until the plant reached 60 days of age in order to record observations on plant growth and yield. The fresh weight of bulbs was measured after harvest by cleaning the soil adhering to bulbs and cutting roots and leaves. The dry weight of bulbs was measured after drying in the air for 2 weeks. The effectiveness of improving growth and bulb weight was calculated using Formula (3).

2.4.5. Data Analysis

Data analysis was carried out using analysis of variance (ANOVA). If the analysis results were significantly different, a further test was carried out using Duncan's New Multiple Range Test (DNMRT test) with a significance level of 5%.

3. Results and Discussion

3.1. Disease Development

The presence of endophytic bacteria in shallot has an impact on the duration of the incubation period, the occurrence of disease, the severity of the disease, and the weight of rotting bulbs. The incubation period for the endophytic bacteria treatment ranged from 16.33 to 30.00 days post-inoculation (DAI), while the control had an incubation period of 16.33 DAI. Introducing S. marcescens JB1E3 bacteria as a treatment extended the incubation period of FOCe with an effectiveness of 83.71%. The incidence of Moler disease ranged from 93.33% to 46.67%. The introduction of *S. marcescens* JB1E3 was the most effective treatment, suppressing disease incidence by 49.99%. Disease severity in the endophytic bacteria treatment ranged from 14.22% to 29.78%. *S. marcescens* JB1E3 treatment exhibited the least severity of the disease at 14.22%, showing a disease suppression effectiveness of 52.25%. (Table 2).

Introducing endophytic bacteria had a significantly greater effect in suppressing disease development compared to the control. The endophytic bacteria with the highest ability to suppress Moler disease was *S. marcescens* JB1E3, which extended the incubation period of Moler disease to thirty days with an effectiveness of 83.71%. The longer appearance of symptoms due to the extended incubation period led to a decrease in disease incidence of about 49.99% and a reduction in disease severity to 52.25% (Table 2).

The introduction of biocontrol agents such as endophytic bacteria can affect the physiological response of plants, leading to induced resistance [40]. According to Resti et al. [41], the introduction of *S. marcescens* JB1E3 has been shown to elicit resistance in shallot against bacterial leaves blight disease. This resistance is evidenced by increased peroxidase enzyme activity observed in both roots and leaves after the inoculation with the endophytic bacteria. Additionally, Someya et al. [42] reported that *S. marcescens* B2 induces resistance in cyclamen plants against *Fusarium* wilt through the production of exo and endo chitinase enzymes. Various biocontrol agents, such as *Pseudomonas fluorescens*, *Trichoderma (spp.?), Bacillus subtilis*, and other *Bacillus* species, have proven effective in reducing disease severity [18,43]. Wulan et al. [44] found that the use of *Bacillus spp*. not only inhibits moler disease in shallot but also improves the levels of jasmonic acid and salicylic acid, contributing to the induction of resistance.

Table 2. Development of moler disease on shallot introduced with endophytic bacteria and inoculated with FOC (52 days after inoculation)

Treatment	IP (hsi)	E (%)	DI (%)	E(%)	DS (%)	E(%)	BR (gr)	E (%)
B. cereus P14	18.11 ± 0.69 b	10.90	80.00 ± 0.00 ab	14.28	23.11 ± 2.04 ab	22.40	3.22 ± 0.84 b	75.02
B.cereus Se07	18.78 ± 3.10 b	15.00	68.89 ± 19.25 b	26.19	20.00 ± 4.62bc	38.84	4.89 ± 0.38 b	62.06
Bacillus sp. HI	19.89 ± 1.39 b	21.80	73.33 ± 6.67 b	21.43	21.33 ± 4.62 bc	28.37	4.33 ± 2.19 b	66.41
Bacillus sp. SJI	18.67 ± 1.00 b	14.33	77.78 ± 3.85 ab	16.66	23.55 ± 2.04 ab	20.92	2.89 ± 1.02 b	77.58
S. marcescens ULG1E2	19.34 ± 0.58 b	18.43	80.00 ± 0.00 ab	14.28	19.56 ± 1.54bc	34.32	5.33 ± 0.33 b	58.65
S. marcescens JB1E3	30.00 ± 7.80 a	83.71	46.67 ± 17.64 c	49.99	14.22 ± 6.16 c	52.25	2.67 ± 2.33 b	79.29
Control	16.33 ± 0.33 b	0.00	93.33 ± 0.00 a	0.00	29.78 ± 3.36 a	0.00	12.89 ± 2.34 a	0.00

*Numbers that followed share the same letter within the identical column do not exhibit significant differences as per the DNMRT test at a 5% significance level

Note: IP: Incubation Period, DI: Disease incidence, DS Disease Severity, BR: Bulbs Rot, E: Effectiveness

In addition to leaves symptoms, there is also rotting in shallot bulbs affected by FOCe (Fig. 2), with the weight of the rotting bulbs ranging from 2.67 to 12.89 g. The impact on the weight of rotting bulbs varied significantly among treatments involving endophytic bacteria. The lowest weight of rotting bulbs was observed in *S. marcescens* JB1E3 treatment at 2.67 g with an effectiveness of 79.29% (Table 2). This is due to the ability of *S. marcescens* JB1E3 to suppress both disease incidence and severity, leading to fewer rotting symptoms in shallot bulbs. Meanwhile, the ability of other endophytic bacteria in the same test was not comparable to reducing the incidence and severity of disease.

Symptoms of rotting bulbs in shallot showed an advanced phase of moler disease, a condition that affects roots and bulbs of shallot caused by FOCe. The manifestations of moler disease consist

of the rotting of bulbs, initiated from the stem's base. If the symptoms worsen, rotting occurs at the bottom of the bulbs and spreads in. White fungal mycelium is sometimes found on the rotted parts of bulbs [43].

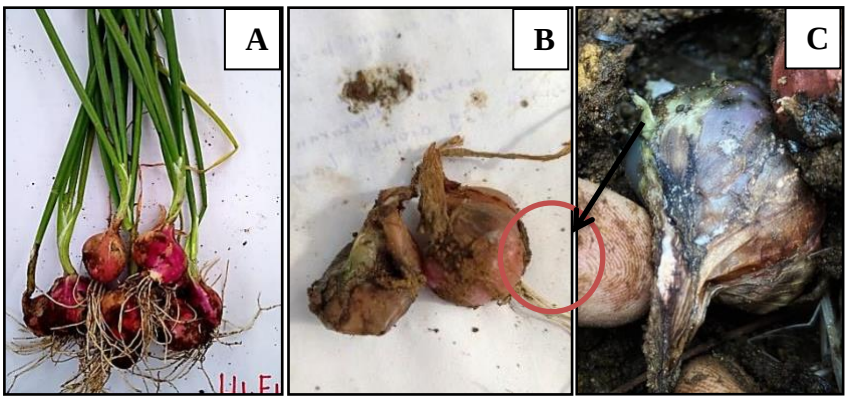

Fig. 2. Symptoms of rot in shallot bulbs; A. Healthy bulbs, B. Rotten bulbs, C. Fungus mycellia on rotten bulbs.

3.2. Plant Growth

Introducing endophytic bacteria to the shallot affects the plant's stature and the number of leaves on the shallot (Table 3).

Table 3. Growth of shallot introduced by endophytic bacteria and inoculated with *F. oxysporum* f.sp *cepae* (49 days old)

Treatment	Plant Height (cm)	Effectiveness (%)	Number of Leaves (Pieces)	Effectiveness (%)
B. cereus P14	40.55 ± 2.43	1.53	21.45 ± 2.41 b	9.66
B. cereus Se07	41.89 ± 1.90	4.88	25.00 ± 4.51 ab	27.81
Bacillus sp. HI	41.61 ± 2.87	4.18	21.33 ± 1.46 b	19.07
Bacillus sp. SJI	40.58 ± 1.84	1.60	20.33 ± 1.20 b	3.97
S.marcescens ULG1E2	43.83 ± 1.32	9.74	29.22 ± 4.84 a	49.41
S.marcescens JB1E3	42.11 ± 1.67	5.43	21.00 ± 2.08 b	7.39
Control	39.94 ± 1.36	0.00	19.56 ± 0.96 b	0.00

* Numbers that followed share the same letter within the identical column and do not exhibit significant differences as per the DNMRT test at a 5% significance level.

According to the statistical analysis, the treatment with endophytic bacteria did not yield a statistically notable impact on the height of the plants. In addition, the plant height, both in the treatment and control groups, varied between 40.55 and 43.83 cm. Introducing endophytic bacteria to the same shallot cultivar did not affect plant height, because these bacteria cannot promote growth in plant height. Regarding leaves count, *S. marcescens* ULG1E2 treatment exhibited the highest at 29.22 leaves, showing an effectiveness of 49.40%.

Besides inhibiting disease progression, the introduction of endophytic bacteria can also increase shallot growth. The treatment involving *S. marcescens* ULG1E2 exhibited the most notable efficiency in the improvement of both plant growth and bulb yield. This is attributed to the bacterium's capacity to solubilize phosphate essential for plant growth, thereby stimulating both growth and yield. This result is in line with the investigation conducted by Resti et al. [45] that *S. marcescens* produces IAA, and siderophores, and can solubilize phosphate to improve plant

growth. Matteoli et al. [46] also reported that *S. marcescens* UENF-22GI can solubilize P and Zn and produce IAA (Indole Acetic Acid) to promote the growth of corn plants.

3.3. Weight of Shallot Bulbs

The treatment with endophytic bacteria exhibited a statistically significant influence on the weight of shallot bulbs compared to the control group. The fresh weight of shallot bulbs varied between 41.22 and 68.22 g. The highest fresh weight of bulbs was observed in *S. marcescens* ULG1E2 treatment, which was 68.22 g with an effectiveness of 65.50% (Table 4). The dry weight of shallot bulbs ranged from 35.44 to 57.11 g. The highest dry weight of bulbs was found in *S. marcescens* ULG1E2 treatment, which was 57.11 g with an effectiveness of 61.14% (Table 4).

Table 4. Weight of shallot bulbs after introduction of endophytic bacteria and inoculation with *F. oxysporum* f.sp *cepae* (80 days after)

Treatment	Fresh Weight (gr)	Effectiveness (%)	Dry Weight (gr)	Effectiveness (%)
B. cereus . P14	50.33 ± 3.76 bc	22.10	43.44 ± 3.56 bc	22.57
B. cereus Se07	52.89 ± 5.80 bc	28.31	46.33 ± 4.93 abc	30.73
Bacillus sp. HI	51.22 ± 10.84 bc	24.26	44.33 ± 8.08 bc	25.08
Bacillus sp. SJI	44.78 ± 1.71 bc	8.64	37.78 ± 1.64 bc	6.60
S. marcescens ULG1E2	68.22 ± 9.90 a	65.50	57.11 ± 10.91 a	61.14
S. marcescens JB1E3	56.33 ± 10.74 ab	36.66	49.33 ± 7.31 ab	39.19
Control	41.22 ± 1.33 c	0.00	35.44 ± 3.24 c	0.00

* Numbers that followed share the same letter within the identical column and do not exhibit significant differences as per the DNMRT test at a 5% significance level.

This research shows that the inclusion of endophytic bacteria can improve shallot yields by increasing both the fresh and dry weights of bulbs. Resti et al. [33] similarly reported that endophytic bacteria derived from *Bacillus cereus* Se07 (SN2E2), *S. marcescens* PPM4 (ULG1E2), and *Bacillus sp*. SJI (PU2E2) contributed to increased shallot yields with effectiveness percentages of 281.66%, 230.94%, and 121.50%, respectively. Based on this research, the introduction of endophytic bacteria into shallot can suppress the development of moler disease and increase shallot production.

4. Conclusions

In conclusion, endophytic bacteria showed the capacity to inhibit the progression of moler disease (FOCe) and improved the growth and yield of shallot compared to the control. Among endophytic bacteria, *S. marcescens* JB1E3 stands out for its remarkable ability to suppress moler disease, achieving a disease severity suppression effectiveness of 52.25%. Meanwhile, *S. marcescens* ULG1E2 showed the most effective endophytic bacterium in terms of improving yield, in which bulbs weight increased effectiveness by 65.50%.

Abbreviations

ISR Induced Systemic Resistance
BLB bacterial Leaves Blight
FOCe *Fusarium oxysporum* f.sp *cepae*
IAA Indole Acetic Acid
RCBD Randomized Completely Block Design
DAI Days after Inoculation.

Data availability statement

Data will be shared upon request by the readers.

CRediT authorship contribution statement

Zurai Resti: Conceptualization, Methodology, Resources, Formal analysis, Investigation, Data curation, original draft. Darnetty: Validation, Data curation, Formal analysis. Ujang Khairul: Supervision, Validation, Funding acquisition. Reflin: Validation, Project administration, Sri Lestari Kurnia Siregar: Investigation. Data curation, Project administration.. Farah Nabila Tores: Formal analysis, Writing – Review & editing

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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