Characterization and Biocontrol Potential of Entomopathogenic Bacteria in *Jatropha curcas* Rhizosphere Against *Spodoptera litura*

Erfan Dani Septia[1], Faza Abdurahman Fiddin[1], Maftuchah[1,2,]

[1]Department of Agrotechnology, Faculty of Agriculture and Animal Science, University of Muhammadiyah Malang, Jl. Raya Tlogomas no 246 Malang 65145, East Java, Indonesia

[2]The Center of Biotechnology Development, University of Muhammadiyah Malang

Corresponding author Email erfandani@umm.ac.id

DOI: https://doi.org/10.36077/kjas/2026/v18i1.18329

Received date: 1/1/2025

Accepted date: 31/12/2025

Abstract

Spodoptera litura causes significant damage to plants and disruption the production process of *Jatropha curcas* seeds up to 70% if not controlled in attacks above the economic threshold. *S. litura* control is now beginning to be constrained by *S. litura* resistance. The suspected resistance is due to the usage of synthetic pesticides that not controlled. entomopathogenic bacteria are important biocontrol of *S. litura* pests that support sustainable plantations. *J. curcas* plant is an alternative biodiesel producer with a potential seed oil content of 47.58%. Currently, the exploration of local entomopathogenic bacteria from the rhizosphere *J. curcas* hasn't been done. This study uses exploration and descriptive methods by analyzing data characterization of entomopathogenic bacteria through analysis using the website PBSTAT-CL 2.1.1 https://apps.pbstat.com/reports/pbstat-cl/. The effectiveness of bioassay in analysis using IBM SPSS Statistics 26 software, with probit analysis (lethal time 50) LT_{50}. This research obtained 34 isolates of entomopathogenic bacteria rhizosphere *J. curcas* with 6 groups of characterization. The maximum cophenetic distance at 0.7 from isolates. Effectiveness from all isolates reached 100% mortality and the analysis results of LT_{50} probit fastest for 31,645 hours.

Keywords: Entomopathogenic bacteria, *Spodoptera litura*, *Jatropha curcas*, Biocontrol

Introduction

Spodoptera litura is an important pest in *Jatropha. curcas*, if not controlled at its economic threshold will result in a decrease in seed production of up to 70%. The infestation of S. litura, which damages both the young leaves and developing seeds of *J. curcas*, results in substantial economic losses for *J. curcas* plantations. *S. litura* allegedly experienced resistance to synthetic insecticidal materials from the results of surveyed area due to unawareness use by farmers in Garut, West Java, Indonesia [1]. Besides, in Jember, East Java, Indonesia adjacent to the plantation *J. curcas* Pasuruan reported *S. litura* has a resistance ratio value of 1.46 in the botanical active ingredient azadirachtin [2]. Information from China occurred evaluation of resistance of 21 active ingredients of insecticides, including conventional and new chemical insecticides, with resistance values of 183.3 to 234.1 [3], *S. litura* was also reported to be resistant to bioinsecticides of active pyrethroids in China with resistance ratios ranging from 11.5 to 9123.5 times [4]. Therefore, China made modifications to its phytochemical capabilities by inhibiting the biosynthesis pathway of glutathione using 0.5% indole-3-methanol (I3C), xanthotoxin, and rotenone (ROT) to inhibit the growth of *S. litura* larvae.

J. curcas is currently one of the important plants to produce biodiesels as a development of renewable energy sources. It is a potential for Indonesia to develop *J. curcas* with the ability of plants to grow in various climates and can improve marginal or dry land. Indonesia has about 78 million hectares of marginal and degraded land. The content of 100 dried seeds *J. curcas* from Pasuruan garden can produce crude oil 47.58%, saponification 230.01 mg KOH g^{-1}, and esterification 226.14 mg KOH g^{-1} [6]. *J. curcas* from Ethiopia produces crude oil content between 47.10-59.32%, saponification 180.9-202.0 mg KOH g^{-1}, oleic acid (34.2–42.2%) and linoleic acid (34.8– 41.8%). With this, *J. curcas* will produce biodiesels with quality that does not differ much if planted in various regions [7]. India conducted emissions tests produced by diesel oil mixed with *J. curcas* biodiesel that can reduce nitrogen of oxides (NOX) emissions by 12.39%, smoke emissions by 6.98%, and CO2 by 6.67% when compared to diesel oil.

Based on the problems in *S. litura* and the potential of *J. curcas*, existing required control of *S. litura* by using microbial biocontrol, which at the same time can support sustainable agriculture. One biocontrol is an entomopathogenic bacterium that has a specific toxin to the target pest even some entomopathogenic bacteria have antimicrobial peptides (AMPs) that serve to help inhibit the growth of pathogenic bacteria. Even some countries have developed an increase in the ability of toxic bacteria, entomopathogenic to be genetically modified entomopathogenic bacteria (GM-EPB) and already some genera of bacteria are modified but still under study for environmental impacts that will occur [10]. The ability of Lepidoptera pest mortality results will be influenced by the food eaten by the pest because certain leaves have a resistance to toxic entomopathogenic bacteria [11].

The toxic mechanism of entomopathogenic bacteria in attacking pest immunity through the homesite, with a decrease in homesite by 34.9% after 12 hours of infection. Then the density of homesite decreased drastically to less than 35% of control in the 12 hours before *S. litura* death [12]. The best biocontrol is obtained from around the plant environment, which will be carried out through pest control [13]. The purpose of this study was to find out the diversity of character of entomopathogenic bacteria rhizosphere of *J. curcas* Pasuruan and the level of effectiveness of biocontrol against *S. litura* by in vitro.

Material and Methods

Location Exploration of Entomopathogenic Bacteria

Soil samples were collected from the rhizosphere of six crossbred Jatropha curcas genotypes, namely JC5, JC6, JC7, JC18, IP3A, and IP3P [14]. Sampling was conducted in the J. curcas experimental garden located in Krajan, Kedung Pengaron, Kejayan, Pasuruan, East Java, Indonesia (7°45’54.8"S, 112°50’37.4"E). All genotypes (G1: JC18; G2: JC6; G3: JC5; G4: JC7; G5: IP3P; G6: IP3A) were grown within the same site; therefore, a single geographic coordinate was used to represent the sampling location. For each genotype, soil was collected from the rhizosphere of three individual plants. This study employed exploratory and descriptive methods to characterize entomopathogenic bacterial isolates obtained from the six J. curcas genotypes.

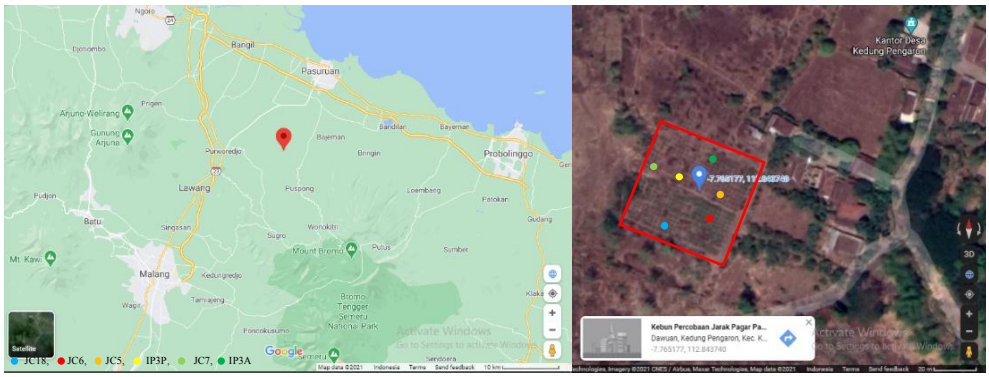

Fig 1. Ground sample breeding point around six genotypes of *J. curcas* crosses. https://www.google.co.id/maps/place/7%C2%B045'54.8%22S+112%C2%B050'37.4%22E/@-7.7657085,112.8428212,390m/

Exploration of Entomopathogenic Bacteria

Soil sampling was conducted at six points around the rhizosphere of each *Jatropha curcas* plant. Excavation was performed at a depth of 20–30 cm from the topsoil. The isolation of entomopathogenic bacteria was carried out using the baiting or “*fishing*” method with *Tenebrio molitor* larvae serving as biological traps. Approximately 250–300 g of rhizosphere soil was placed into sterile plastic containers, and ten healthy T. molitor larvae were gently introduced and allowed to burrow naturally. The containers were sealed with perforated lids to maintain airflow and incubated at room temperature (25–28°C). Larval mortality was monitored daily for up to seven days.

Dead larvae were removed immediately, surface-sterilized with 70% ethanol for 1 minute, and rinsed twice with sterile

distilled water. Each larva was dried on sterile filter paper and dissected longitudinally under aseptic conditions. Hemolymph and internal tissues showing signs of bacterial infection were streaked onto nutrient agar (NA) plates. After incubation at 28°C for 24–48 hours, bacterial colonies with distinct morphology were selected and purified using quadrant streaking. The purified isolates were subsequently maintained for further characterization [15].

Gram Staining Test of Entomopathogenic Bacteria

Gram staining of the entomopathogenic bacterial isolates was performed following standard microbiological procedures. A drop of sterile distilled water was placed on a clean glass slide, and a loopful of the bacterial culture was transferred onto the drop using an inoculating loop. The suspension was spread to form a thin smear, which was then air-dried and heat fixed by briefly passing the slide through a flame.

The fixed smear was flooded with crystal violet for 1 minute, rinsed gently with running water, and blotted dry. The slide was then covered with iodine solution for 1 minute, followed by another gentle rinse. Decolorization was carried out by applying 95% ethanol for approximately 10 seconds, then rinsing again with water. The smear was counterstained with safranin for 30 seconds, rinsed, and left to air-dry. The stained cells were examined under a light microscope at 1000× magnification using immersion oil. The Gram reaction (positive or negative) and cell morphology were recorded and documented [16].

Identification of Entomopathogenic Bacteria

Identification of the entomopathogenic bacterial isolates was carried out using both macroscopic and microscopic observations. Macroscopic characterization included an assessment of colony morphology, such as shape, colour, margin, internal structure, elevation, and respiratory type. Microscopic examination was performed using a binocular light microscope at 1000× magnification to determine Gram reaction and cellular morphology based on the results of Gram staining [18].

The characteristics obtained from macroscopic and microscopic observations were compared with the descriptions provided in Bergey's Manual of Systematic Bacteriology and relevant taxonomic literature. The characterization data were then converted into binary matrices and analyzed using the PBSTAT-CL 2.1.1 software (https://apps.pbstat.com/reports/ pbstat-cl/, accessed February 18, 2024) to generate phenotypic similarity profiles and cluster groupings.

Growth Test of Entomopathogenic Bacteria

The growth of the entomopathogenic bacterial isolates was evaluated using nutrient broth cultures incubated for 24 hours. Optical density (OD) measurements were recorded every 2 hours from 0 to 24 hours. Absorbance was measured using a spectrophotometer at an optimum wavelength of 570 nm, following the procedure described by [19].

Bioassay Test of Entomopathogenic Bacteria

The bioassay was conducted using third-instar *Spodoptera litura* larvae obtained from the second laboratory reared generation. Bacterial cultures were prepared by inoculating a loopful of each isolate into 10 mL of nutrient broth (NB) and incubating the tubes on a shaker (150 rpm) for 24 hours at room temperature to obtain actively growing suspensions. For each treatment, ten *S. litura* larvae were

placed in a sterile plastic bioassay container (300 mL volume) and provided with fresh *J. curcas* leaves cut into 5 × 5 cm pieces. A total of 1 mL of the bacterial suspension was sprayed evenly onto the leaf surface using a micropipette-fitted sprayer. Negative controls received sterile distilled water, while positive controls were treated with a commercial systemic insecticide containing carbosulfan 200 EC diluted to 20% of the recommended concentration.

Fresh leaves were replaced every 12 hours, coinciding with larval observations. Monitoring was carried out at 12-hour intervals for a total duration of 10 days. The parameters recorded in the bioassay included: (i) feeding inhibition, (ii) time to infection, (iii) average mortality rate, and (iv) corrected mortality percentage.

1. Inhibition of Eating Ability After Application of Entomopathogenic Bacteria

Feeding inhibition was measured by comparing the area of leaf consumed in the treated groups with that of the negative control. Each leaf had an initial surface area of 25 cm² (5 × 5 cm). Percent feeding inhibition was calculated using the formula described by [20]:

$$PM = \frac{(Lk-Lp)}{(Lk+Lp)} \times 100\%$$

Note: PM: inhibition of eating (%); Lk: area of control leaves eating; Lp: extensive leaf treatment that eating.

2. Time of Entomopathogenic Bacteria Infection and Average Speed of Death *S. litura*

The time of entomopathogenic bacterial infection is observed based on data of the initial time of the day of death of the caterpillar. Average speed of death *S. litura* is observed based on the number of deaths every 12 hours. that occur until the caterpillars test the overall death during the observation time within 10 days. The data calculated the average speed of death by using the following formula:

$$V = \frac{TIN2 + T2N2 + T3N3 + \cdots TnNn}{n}$$

Note: V: Speed of death; T: Observation time; N: Number of dead insects; n: Number of insects tested [21].

3. Mortality Percentage *S. litura*

Calculates the percentage of test and corrected pest deaths from the value of existing control deaths as data analysis, Mortality correction calculation analysis using Schneider-Orelli's formula method, source: http://www.ehabsoft.com/ldpline/onlinecontrol.htm follows:

$$\text{corrected \%} = \frac{(\text{mortality \% in treated plot} - \text{mortality \% in control plot})}{(100 - \text{mortality \% in control plot})} \times 100$$

Mortality data in the analysis using IBM SPSS Statistics 26 software. With analysis probit (*lethal time* 50) LT_{50} [21].

Results and Discussion

Exploration of Entomopathogenic Bacteria from *J. curca* Rhizosphere

Based on the exploration of entomopathogenic bacteria from the *J. Curcas* rhizosphere, a total of 34 entomopathogenic bacteria isolates were obtained. Macroskopic and microscopic observation, followed by characterization analysis, grouped these isolates into six distinct of entomopatogenic bacteria. The clustering analysis showed a maximum cophenhetic distance of 0.7, as illustrated in figure 2.

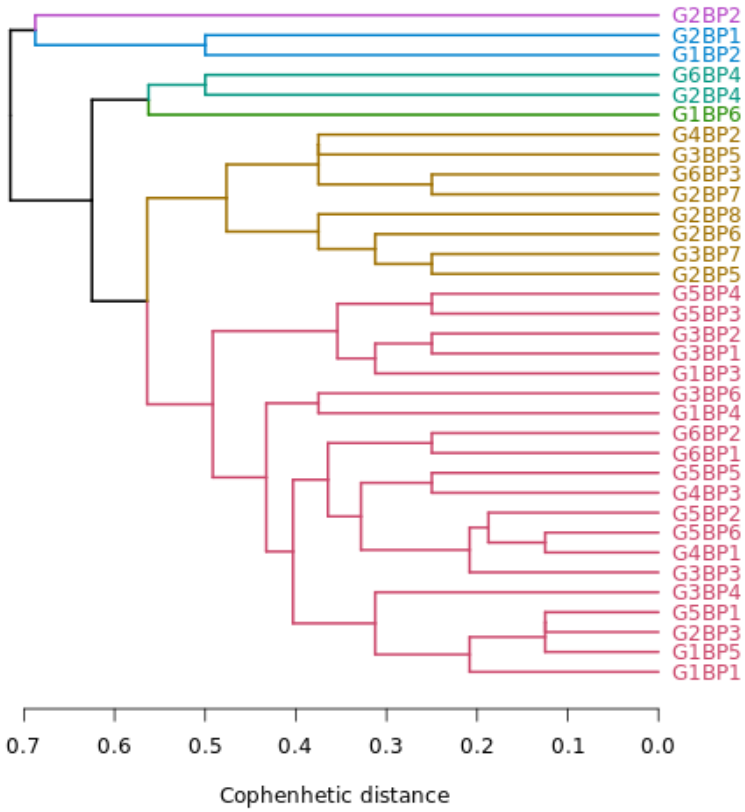

Fig 2. Dendrogram phylophenotype characterization of entomopathogenic bacteria rhizosphere *J. curcas.*

The characterization of entomopathogenic bacteria isolated from the *Jatropha curcas* rhizosphere in Pasuruan, Indonesia, revealed that approximately 88.2% of the isolates shared similar characteristics. The dominant colony morphologies were circular and irregular, with colony colors ranging from white to red and yellow. Most isolates exhibited flat edges, smooth internal structures, and convex to low-convex elevations. The bacteria were aerobic, displayed cocci and bacilli cell forms, and were predominantly Gram-negative, as shown in Figure 3. Nevertheless, further evaluation of the entomopathogenic potential of each isolate is required to determine their effectiveness as biological control agents against *Spodoptera litura*.

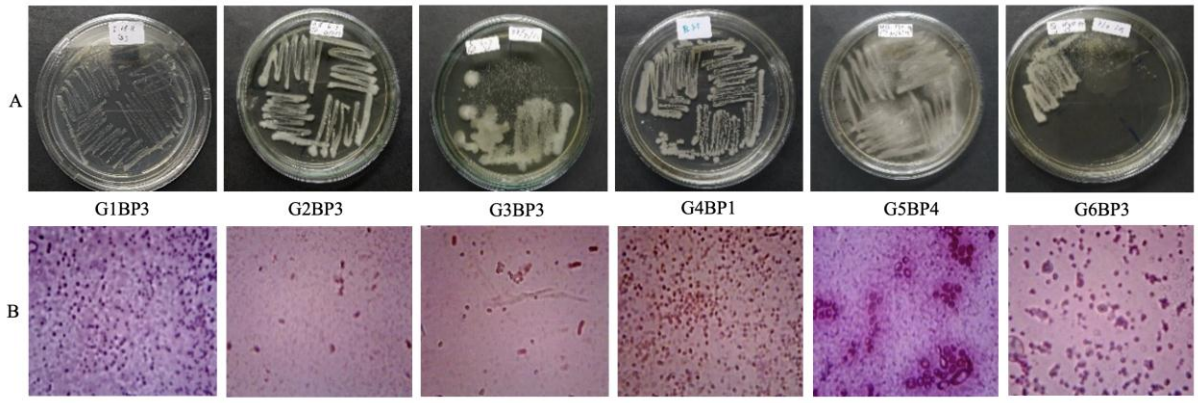

Fig 3. Characteristic entomopathogenic Bacteria colonies a: Macroscopic and b: Microscopic.

Entomopathogenic Bacteria Growth Test Results

The highest absorbance values were observed in isolates G1BP3 and G4BP1, each reaching an absorbance of 1.485 λ. These isolates were still in the exponential growth phase and had not yet entered the stationary phase. In contrast, the lowest absorbance value was recorded for isolate G6BP3, with an absorbance of 0.893 λ, indicating that the culture had reached the stationary phase. The mean absorbance values suggest that the bacterial isolates exhibited distinct growth patterns and varying capacities to utilize nutrients from the growth medium to support cell division. These differences in growth dynamics indicate that bacterial cell division occurred at different rates among the isolates, ultimately influencing the total number of bacterial cells produced [22].

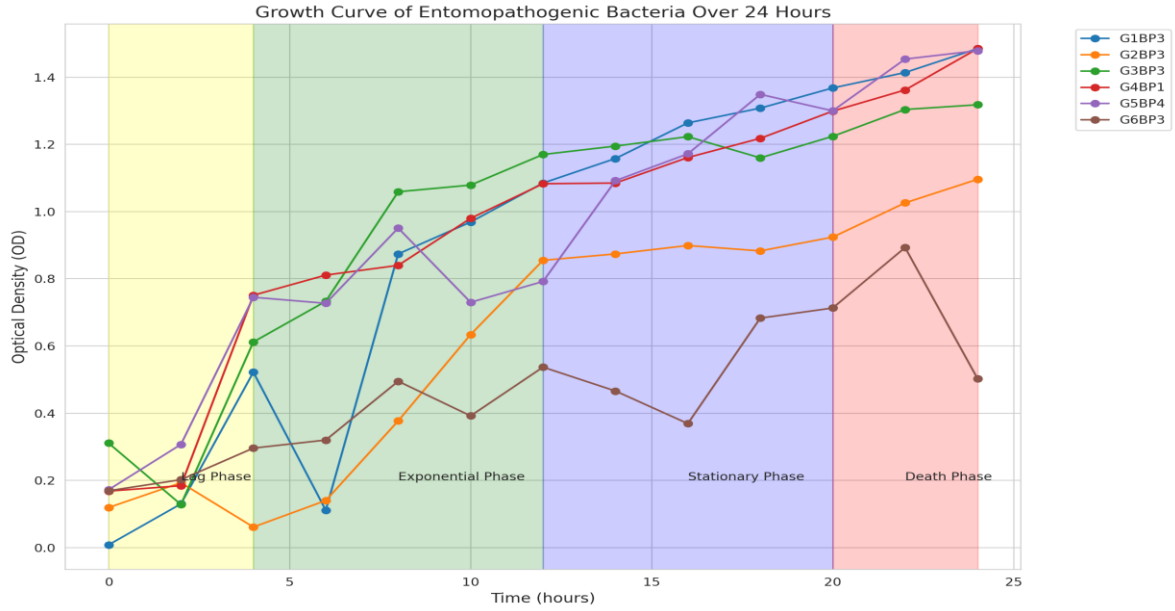

Fig 4. Entomopathogenic bacteria growth.

Based on the overall genus grouping, the entomopathogenic bacteria exhibited predominantly positive linear growth models. This suggests that the explored bacterial isolates were capable of rapid growth within 24 hours and maintained relatively consistent growth patterns. The positive linear relationships indicate that

the growth pattern of entomopathogenic bacteria isolated from the *Jatropha curcas* rhizosphere had a stronger influence on absorbance values than bacterial age.

In contrast, several isolates showed negative linear models, indicating that bacterial growth patterns were less influential than the duration of bacterial viability during the observation period. This result suggests that changes in absorbance values were primarily associated with increases in bacterial cell biomass. Variations in linear model parameters were reflected in the development of bacterial cell biomass, while intercept values may represent corrections or biases affecting both biomass estimation and absorbance measurements.[19].

Entomopathogenic Bacteria Bioassay Test Results

Inhibition of Feeding Activity Following Application of Entomopathogenic Bacteria

The percentage of feeding inhibition in *Spodoptera litura* caused by toxic entomopathogenic bacteria isolated from the *Jatropha curcas* rhizosphere was evaluated beginning 12 hours after application, as shown in Figure 5. All entomopathogenic bacterial isolates exhibited an average feeding inhibition of 19% relative to the negative control, whereas the positive control resulted in only 6% inhibition.

The initial percentage of feeding inhibition did not necessarily correspond to the fastest progression toward complete (100%) feeding inhibition. Based on the linear model presented in Figure 5, rhizospheric entomopathogenic bacteria from *Jatropha curcas* demonstrated strong positive effectiveness in suppressing the feeding activity of *S. litura* compared to the positive control.

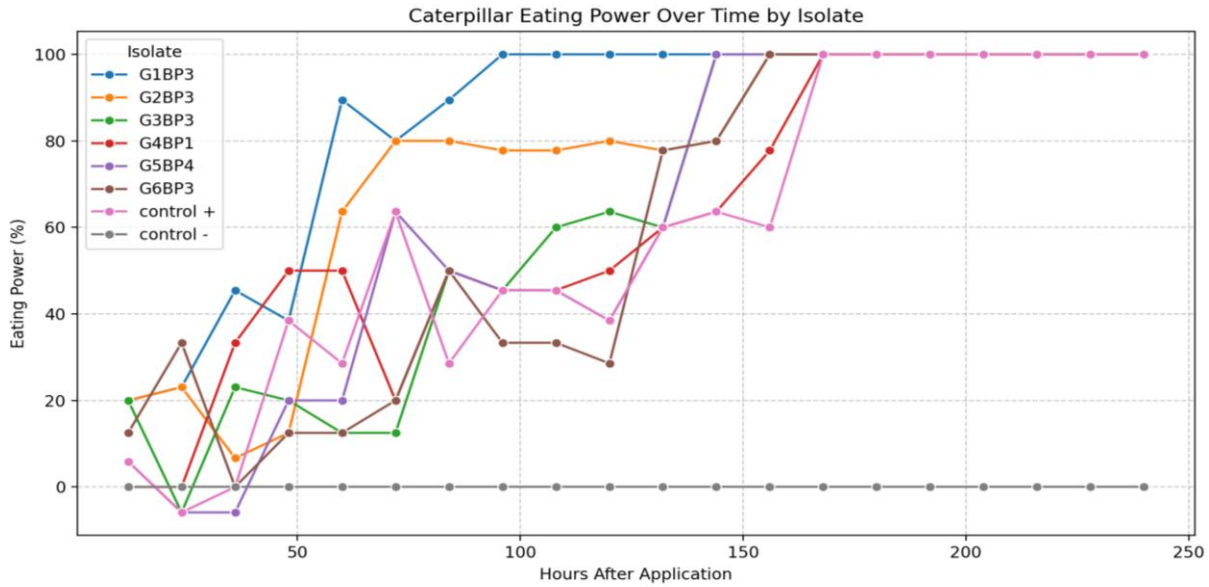

Fig 5. Inhibition of eating ability *S. litura*.

A higher percentage of feeding inhibition corresponds to an increased likelihood and faster onset of mortality in *Spodoptera litura* larvae. According to [21], elevated feeding inhibition significantly increases the probability of pest mortality, as larvae enter a prolonged non-feeding phase that ultimately leads to death. The toxic action of entomopathogenic bacteria begins with damage to the epithelial cells of the insect midgut, allowing bacterial toxins to penetrate the membrane system and form

pore-like structures that disrupt both the digestive system and the integument of the host insect [23].

The feeding activity of *Spodoptera litura* varied markedly among bacterial isolates, as illustrated in the boxplot analysis. Isolates GLBP3 and G2BP3 exhibited consistently high median feeding activity, exceeding 80%, with narrow interquartile ranges, indicating stable and uniform responses. In contrast, isolate G3BP3 showed a wider distribution of values, with a median of approximately 50%, suggesting substantial variability in its effect on larval feeding behavior. Isolates G4BP1 and GSBP4 demonstrated moderate variability, with median values ranging between 70% and 80%. The control treatments provided clear reference points: the positive control maintained consistently high feeding activity, comparable to GLBP3 and G2BP3, whereas the negative control effectively suppressed feeding activity to near-zero levels.

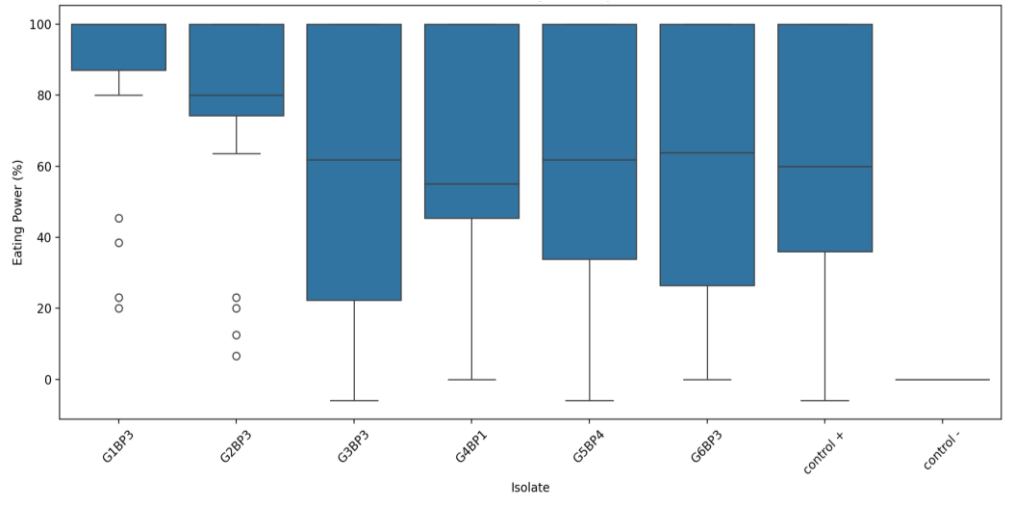

Fig 6. Distribution of feeding activity (%) of Spodoptera litura among entomopathogenic bacterial isolates.

These findings highlight the differential efficacy of bacterial isolates in modulating the feeding behavior of *Spodoptera litura*. The consistently high and stable feeding activity observed in isolates GLBP3 and G2BP3 indicates that these isolates were less effective in suppressing larval feeding, suggesting a limited inhibitory or potentially neutral biological effect. In contrast, isolate G3BP3 exhibited substantial variability in feeding responses, which may reflect differences in larval susceptibility or isolate-specific biological activity rather than experimental inconsistency. The strong suppression of feeding observed in the negative control confirms its suitability as a benchmark for evaluating feeding inhibition in *S. litura*. This comparison provides valuable insight into the relative performance of entomopathogenic bacterial isolates and supports their potential application in integrated pest management strategies. Further studies are required to elucidate the mechanisms underlying isolate-specific variations and to optimize their efficacy for biological pest control.

Time of Entomopathogenic Bacteria Infection and Average Speed of Death S. litura

The infection rate, as shown in Figure 6a, indicated that the positive control began to exhibit infection symptoms within 12 hours after application. At this time point, approximately 83.3% of the entomopathogenic bacterial isolates

demonstrated higher infection effectiveness compared to the positive control, suggesting a rapid onset of pathogenic activity during the early stages of exposure.

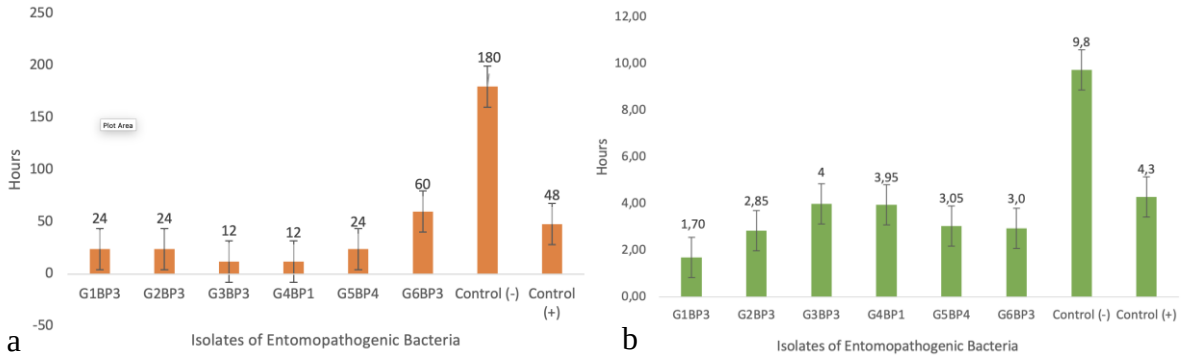

Fig 7. a: Time of infection entomopathogenic bacteria, and b: Average speed of death *S.litura*.

The average death rate of 10 test caterpillars in one entomopathogenic Bacteria isolate treatment based on figure 6b, resulting in the fastest death occurred at G1BP3 in the 4th morning observation time with an average mortality rate of 1,7 days. There are differences in yield trends between the average rate of infection and death, but all isolates have effective results and show the overall mortality data.

The analysis of the average speed of death across bacterial isolates and infection times reveals key insights into their pathogenic effectiveness. In the left chart, GLBP3 demonstrates the shortest average speed of death, indicating high virulence, while G6BP3 and the negative control exhibit the longest durations, suggesting limited pathogenic effects or neutral behavior. The remaining isolates, including G2BP3, G3BP3, G4BP1, and GSBP4, display moderate durations, highlighting their intermediate levels of pathogenicity. The positive control, as expected, shows a shorter average speed of death compared to the negative control, serving as a reliable reference for assessing isolate efficacy.

The right chart correlates the speed of death with time of infection, showing that infections at 24 hours result in the fastest average death, with a tightly distributed dataset, implying a strong and consistent bacterial impact at this interval. Infections at later time points (48–180 hours) result in longer death durations, with broader distributions, suggesting reduced bacterial efficacy or host resilience over time. These findings underscore the importance of both bacterial isolate selection and timing of infection in determining pathogenic outcomes. Further research is needed to explore the molecular mechanisms behind these variations and optimize bacterial use in pest or disease management strategies.

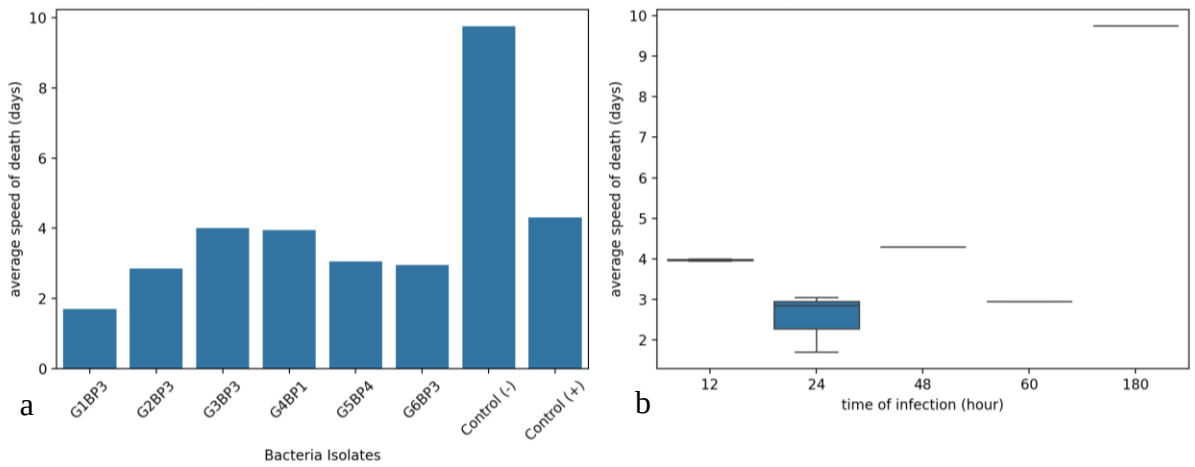

Fig 8. a: Average Speed of infection entomopathogenic bacteria and b: Speed of death *S. litura* vs time of infection.

The average speed of death is determined by the type of protein toxin owned by each entomopathogenic bacteria. One of the commonly known protein toxins is cry protein crystals from the genus *Bacillus* spp., cry proteins will form crystals that are toxic to pests. The cry is easily soluble in water and belongs to the group of δ-endotoxin bacteria. The parasporal crystals from *Bacillus* spp. will enter the body of test insects and pass through the digestive tract of insects until in digestive damage [24].

3.2.3 Mortality Percentage of S. litura

All isolates of entomopathogenic bacteria have good effectiveness because they can kill the entire caterpillar until it reaches a value of 100% mortality seen from figure 7. The time difference is required to achieve maximum mortality of each isolate of entomopathogenic bacteria *J. curcas*.

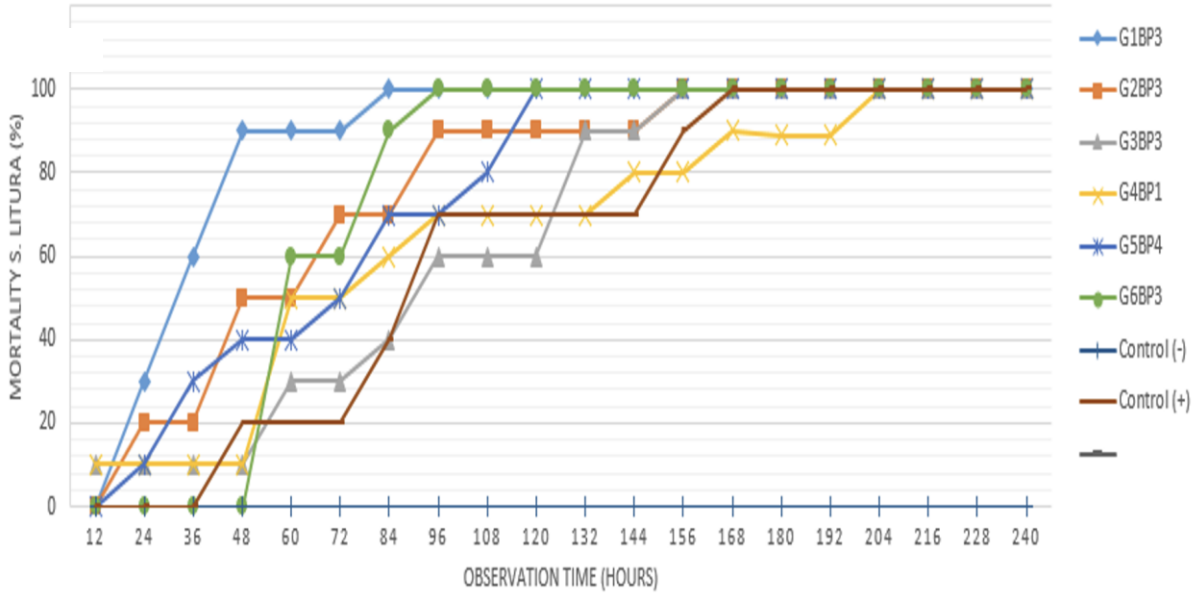

Fig 9. Mortality Percentage *S. litura* by using various isolates of entomopathogenic bacteria.

The difference is due to the ability to produce enzymes and toxic during the processing of infection in insects after contact with the cuticle in the hemocoel. The bacteria have a mechanism in killing caterpillars, one of which is the bacteria will attack the hemocoel by multiplying the Bacteria cells in the insect hemolymph and then producing toxins to kill insects [25].

Table 1. Probit LT_{50} Analysis.

Bacteria Isolates	Average (hours)	Lower Bound (hours)	Upper Bound (hours)
G1BP3	31.645	18.070	42.652
G2BP3	52.128	5.807	77.885
G3BP3	96.409	74.329	109.209
G4BP1	84.087	64.023	100.040
G5BP4	66.347	13.767	85.082
G6BP3	63.447	54.368	71.929
Control (-)	532.377	-	-
Control (+)	88.521	32.676	111.766

Noted: 95% Confidence Limits for Hours

Smaller LT_{50} values can kill larvae faster than higher LT_{50} values. According to [26], biological agents can be said to be good if they can cause the death of the target pest in a short time. Lethal time is the time it takes to kill test insects up to 50%. On positive control has a probit value of LT_{50} 88,521 hours. The 6 isolates of entomopathogenic bacteria *J. curcas* have better effectiveness capabilities compared to positive control.

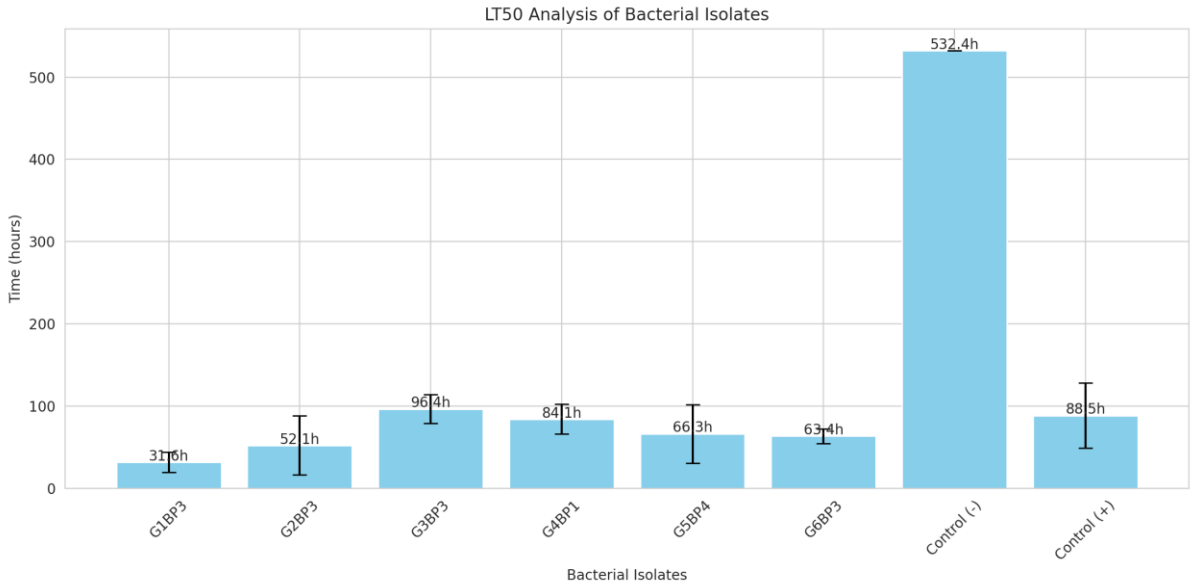

Fig 10. The LT50 analysis of entomopathogenic bacterial.

The LT50 analysis of bacterial isolates reveals substantial differences in the time required to achieve 50% mortality in the tested population. Among the isolates, GLBP3 demonstrates the shortest LT50 at approximately 37.6 hours, indicating its high virulence and rapid action. G2BP3, G3BP3, G4BP1, GSBP4, and G6BP3 exhibit intermediate LT50 values ranging from 52.1 to 96.4 hours, reflecting moderate effectiveness. The negative control (532.4 hours) shows an exceptionally prolonged LT50, signifying its minimal pathogenic impact. Conversely, the positive control records an LT50 of 88.5 hours, providing a benchmark for moderate effectiveness against the target organism.

The observed variations in LT50 suggest differential pathogenic mechanisms among the bacterial isolates. GLBP3's rapid action implies a potent and efficient mechanism, making it a strong candidate for biocontrol applications. The higher LT50 values for other isolates indicate variability in their efficacy, potentially influenced by environmental factors or intrinsic properties of the isolates. The stark contrast between the negative control and the isolates underscores the active role of the bacteria in inducing mortality. Future investigations should focus on elucidating the molecular mechanisms underlying these differences to optimize the use of bacterial isolates in pest management strategies.

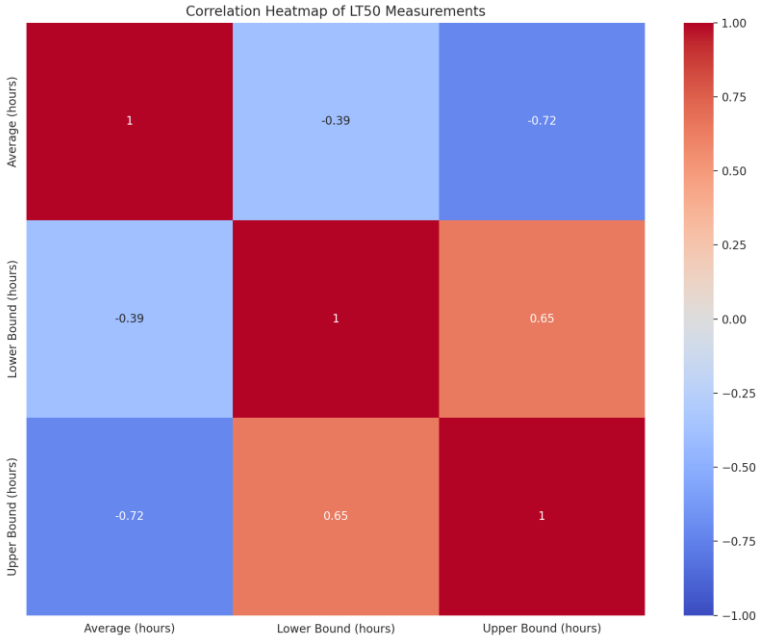

Fig 11. Correlation Heatmap of LT50 Measurements.

The high low value of LT_{50} produced depends on the toxicity level of the type of entomopathogenic bacteria of successfully explored. The effectiveness or not level of killing test insects depends on the nature of toxicity since toxicity will determine the effectiveness of the bioinsecticides used [27]. The difference in LT_{50} values of each isolate of bacteria can be influenced by several other factors including how bacteria can enter the target insect's body, toxic amounts, and target pest resistance power [28].

The impact of rhizosphere entomopathogenic bacteria *J. curcas* on *S. litura*

Symptoms caused by dead caterpillars infected with entomopathogenic bacteria begin with caterpillars removing liquid feces and removing yellow liquid from the mouth. When viewed from the appearance of the color on the caterpillar appears *S. litura* dark brown to black. The state of the body of the dead caterpillar is very easily destroyed by the touch that hits the body of the caterpillar because the skin of the caterpillar becomes very thin, the legs of the caterpillar are deformed like crushed, *S. litura* die produces a distinctive smell such as a sweet smell to sting.

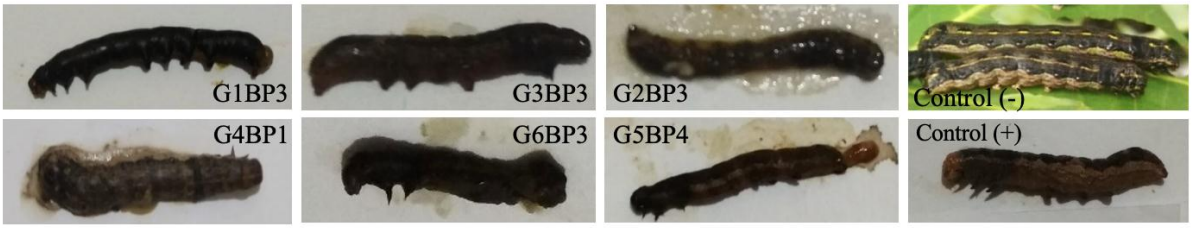

Fig 12. The impact of rhizosphere entomopathogenic bacteria *J. curcas* on *S. litura*.

This corresponds to the symptoms of *S. litura* dying in the study [25], which used the rhizosphere entomopathogenic bacteria of corn crops, starting with the larvae infected with bacteria showing symptoms of larvae losing appetite. The larva has a decreased diet, its movement becomes slow, and the body turns black. Also, the body is curved, soft, and has a foul smell.

Conclusion

From the results of this study, activity obtained 34 isolates of entomopathogenic bacteria rhizosphere of *Jatropha cucas*, which is divided into 6 groups, with a maximum cophenetic distance at 0.7. The effectiveness shown throughout the isolation of entomopathogenic bacteria reached 100% mortality with the fastest LT_{50} probit analysis results for 31,645 hours.

Acknowledgments

The award will be presented to the chairman of the University of Muhammadiyah Malang has provided research resources, through a Developing *J. curcas*. Thank you to the chairman and all staff at the Center of Biotechnology Development, University of Muhammadiyah Malang, for the support of equipment and analysis assistance.

Conflict of interest

The authors declare no conflict of interest.

References

1. R. A. M. Ramadhan and D. Dono. *Pendugaan Peristiwa Resistensi* Spodoptera litura L. *Berdasarkan Survey dan Wawancara di Desa Mekarjaya Kecamatan Cikajang Kabupaten Garut*. Prociding Conference National PEI. (Bandung, Indonesia, 2017). [In Bahasa Indonesia] https://www.researchgate.net/publication/338045691
2. A. Rahmawati. Resistensi Hama Ulat Grayak (*Spodoptera litura* F.) terhadap Insektisida Botani Azadirachtin dan

Pemanfaatanya sebagai Buku Ilmiah Populer. [*Grayak Caterpillar Pest Resistance* (Spodoptera litura F.) *to Azadirachtin Botanical Insecticide and Its Utilization as a Popular Scientific Book*] [Thesis] Pendidiikan Biologi, Universitas Jember (2016). [in Bahasa Indonesia]. p. 95. http://repository.unej.ac.id/handle/123456789/78117

3. X. Wang, L. Lou, and J. Su, J. of Asia-Pacific Entomology, **22**,3:728–732(2019). https://doi.org/10.1016/j.aspen.2019.05.009
4. L. Xu, Y. Mei, R. Liu, X. Chen, D. Li, and C. Wang, J. Pesticide Biochemistry and Physiology, **169**:104649 (2020). https://doi.org/10.1016/j.pestbp.2020.104649
5. Y. Cen, X. Zou, L. Li, S. Chen, Y. Lin, L. Liu, and S. Zheng, Pesticide Biochemistry and Physiology, **168:**104632 (2019). https://doi.org/10.1016/j.pestbp.2020.104632
6. Maftuchaha, A. Zainudin, A. Winayaa, Y. Rahmadesi, J. Energy Reports, **6**,8:345–350 (2020). https://doi.org/10.1016/j.egyr.2020.11.160
7. G. A. Ewunie, O. I. Lekang, J. Morken, and Z. D. Yigezu, J. Energy Reports, **7**:439–452(2021). https://doi.org/10.1016/j.egyr.2021.01.007
8. U. Rajak, P. K. Chaurasiya, P. Nashine, M. Verma, T. R. Kota, and T. N. Verma, J. Energy Conversion and Management, **224**:113362(2020). https://doi.org/10.1016/j.enconman.2020.113362
9. J. Xu, X. Luo, G. Fang, S. Zhan, J. Wu, D. Wang, and Y. Huang, J. Insect Biochemistry and Molecular Biology, **127**:103487(2020). https://doi.org/10.1016/j.ibmb.2020.103487
10. U. Azizoglu, G. S. Jouzani, N. Yilmaz, E. Baz, and D. Ozkok, J. Science of the Total Environment, **734**:139169(2020). https://doi.org/10.1016/j.scitotenv.2020.139169
11. G. Vengateswari, M. Arunthirumeni, and M. S. Shivakumar, J. Toxicology Reports, **7**:1428-1437(2020). https://doi.org/10.1016/j.toxrep.2020.10.005
12. C. Liaoa, M. Zhenga, Y. Chena, M. Wanga, and B. Lia, J. Process Biochemistry, 81:28-32(2019). https://doi.org/10.1016/j.procbio.2019.03.021
13. H. Sukorini, F. W. Aigahayunindy, E. D. Septia, and N. Khewkhom, E3S Web of Conferences, **226**,00022:1-9(2021). https://doi.org/10.1051/e3sconf/202122600022
14. Maftuchah, A. Zainudin, B. Heliyanto, H Sudarmo,M. Mel, and L. K. Kuan. *Combining Ability in* Jatropha curcas L. *Genotypes*. Proceedings of the Pakistan Academy of Sciences: B. Life and Environmental Sciences, (Pakistan, 2017). 54,3:259–261(2017). https://www.paspk.org/wp-content/uploads/2017/09/Combining-Ability.pdf
15. Hasyim, Nuraidi, and Trizelia, J. Horticultura, **19**,3:334-343(2009). [in Bahasa Indonesia] http://hortikultura.litbang.pertanian.go.id/jurnal_pdf/193/hasyim_kubis.pdf
16. A.Frediansyah and I. M. Sudiana, J. Widyariset, **16**,2: 201-210 (2013). [in Bahasa Indonesia] https://widyariset.pusbindiklat.lipi.go.id/index.php/widyariset/article/view/108/99
17. Departemen Pertanian: Badan Karantina Pertanian. Pedoman Diagnosis (OPTK) Golongan Bakteri. (2008). p. 79 [in Bahasa Indonesia] https://karantina.pertanian.go.id/fileman/Uploads/Documents/pusat%20KT%20dan%20KHN/Pedoman%20Diagnosis%20OPTK%20Gol%20Bakteri.pdf

18. E. D. Septia and F. Parlindo, Agriprima, J. of Applied Agricultural Sciences, **3**,1:1–14 (2019). [in Bahasa Indonesia] https://doi.org/10.25047/agriprima.v3i1.159
19. Zamani, P. Neviaty, and M. Muhaemin, Maspari J, **8**,1:39–48(2016). [in Bahasa Indonesia] https://www.neliti.com/publications/151598/
20. Hariri, and M. Agus, J. HPT Tropika, 1**2**,2:119–28(2012). [in Bahasa Indonesia] https://doi.org/10.23960/j.hptt.212119-128
21. Setiawan, N. Agus, and S. Achmad, Planta tropika J. Of Agro Science, **2**,2: 99–105(2014). https://doi.org/10.18196/pt.2014.029.99-105
22. D. Rajonovic, Denis, X. Muñoz-Berbel, and J. Mas, J. PLoS ONE, 1**4**,5: 1–14 (2019). https://doi.org/10.1371/journal.pone.0216292
23. M. E. C. Sousa, F. A. B. Santos and V. Wdanerley-Teixeira, J. of Insect Physiology, **56**,12: 1913–1919(2010). https://doi.org/10.1016/j.jinsphys.2010.08.014
24. R. Schunemann, N. Knaak, and L. M. Fluza, ISRN Microbiology, **2014,**135675: 1-12(2014). https://doi.org/10.1155/2014/135675
25. N. Nelly, U. Khairul, A.Y. Putri, H. Hamid, and M.Y. Syahrawati, J. Biodiversitas, **21**,2: 753-758(2020). [in Bahasa Indonesia] https://doi.org/10.13057/biodiv/d210243
26. Y. Effi, Master's thesis, (2016). http://scholar.unand.ac.id/id/eprint/22617
27. Adam, Triani, J. Rina, and T. Rosdah. *Bioesai Bioinsektisida Berbahan Aktif* Bacillus thuringiensis *Asal Tanah Lebak Terhadap Larva* Spodoptera litura. Paper Presented in Prosiding Conference National Lahan Suboptimal. (Palembang, Indonesia,2014). [in Bahasa Indonesia] https://123dok.com/document/y62pmo7z-welcome-eprints-sriwijaya-university-unsri-online-repository.html
28. M. S. Rini, R. Rahadian, M. Hadi, and D. Zulfiana, J. Biologi, **5**,2:1-10(2016). [in Bahasa Indonesia] https://ejournal3.undip.ac.id/index.php/biologi/article/view/19485