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Research Article
Biological Control of *Spodoptera frugiperda* Pests with a Combination of *Mirabilis jalapa* Nanoemulsion and *Beauveria bassiana*

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Abstract
Background and Objective: *Spodoptera frugiperda*, a major pest of corn crops, causes significant losses for farmers. Biological control approaches offer a sustainable and environmentally friendly solution to address this issue. This study examines the effectiveness of *Mirabilis jalapa* and *Beauveria bassiana*, both individually and in combination, on the mortality of 3rd instar *S. frugiperda* larvae. **Materials and Methods:** This study tested various treatments on 3rd instar larvae of *S. frugiperda*, including *M. jalapa* extracts at different concentrations (0.1-0.8%), *B. bassiana* alone and combinations of both. Larval mortality was measured over 72 hrs and the data were analyzed using linear regression to determine the efficacy of each treatment. **Results:** The *M. jalapa* alone resulted in mortality rates ranging from 27 to 40%, while *B. bassiana* alone caused 20% mortality. The combination of *M. jalapa* and *B. bassiana* showed a significant increase in mortality, ranging from 27 to 60%, with the highest synergistic effect observed in the combination of *M. jalapa* F2 0.4 and *B. bassiana* (60%). Linear regression analysis showed a strong positive relationship between treatment levels and mortality percentage with an R^2 of 0.734. **Conclusion:** The combination of *M. jalapa* and *B. bassiana* is effective in increasing the mortality of *S. frugiperda* larvae, demonstrating great potential as a sustainable biological control method. This approach not only reduces reliance on chemical pesticides but also mitigates the risk of pest resistance, making it an environmentally friendly and sustainable solution for managing corn crop pests.

Key words: Biological pest control, synergistic effect, maize pest management, *Spodoptera frugiperda*, *Mirabilis jalapa* nanoemulsion, *Beauveria bassiana*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Spodoptera frugiperda is an insect from the Lepidoptera family Noctuidae, which is a significant pest in tropical and subtropical regions of America. The infestation of this insect has been reported in several countries, including Indonesia. The population and symptoms of *S. frugiperda* attacks in Indonesia were first discovered in Pasaman Barat Regency, West Sumatra, in March, 2019. Subsequently, from April, 2019 to January, 2020, its presence has been reported throughout Indonesia[1]. Currently, the population and symptoms of *S. frugiperda* attacks have been found in all provinces of Indonesia. The population and attacks have been observed since the early growth phases of corn plants, starting from the formation of the third leaf, followed by the plant's apex, male flower buds, male flowers and the ears of corn. If the larvae damage the apex, young leaves or the plant's growth point, they can kill the plant, causing up to 100% damage and significant yield losses. The government tends to limit the eradication of *S. frugiperda* through chemical means, favoring environmentally friendly approaches. Biopesticides using plant extracts have recently shown great potential due to their low cost, lack of residual effects and environmental friendliness[2].

However, the use of biopesticides in insect pest control remains low due to their relatively low effectiveness, the need for repeated applications and slow mode of action[3]. Therefore, to enhance their efficacy, botanical pesticides can be combined with biological agents for pest control. For instance, combining biopesticides with entomopathogenic fungi can improve effectiveness. One example is the combination of *Alternaria tenuissima* plant extract with *B. bassiana* fungus, which can be used as a new biological control tool for *Marasmia frugiperda* pests. Test results showed that after application, the life cycle and survival rate of rice leaves were longer, while the fecundity of the insect pests decreased[4]. Botanical insecticides often provide rapid knockdown effects but are unstable and short-lived, whereas biological pesticides like entomopathogenic fungi (EPF) are more persistent but act more slowly. To address these limitations, combinations of entomopathogenic fungi with botanical extracts have been explored. Fernández-Grandon *et al.*[5] demonstrated that combining *Metarhizium anisopliae* with pyrethrum enhances the mortality of the pea aphid, *Aphis fabae*, providing a more effective approach compared to using each agent separately. Similarly, Touhidul Islam *et al.*[6] found that combining *B. bassiana* with neem extract was highly effective in controlling *Bemisia tabaci* on eggplant, achieving maximum nymph mortality with the right mixture.

Moreover, Mohan *et al.*[7] reported that the combination of *B. bassiana* and neem extract had a synergistic effect on insect mortality, though antagonistic effects were observed in some sensitive isolates. Deb *et al.*[8] showed that *B. bassiana* is biologically compatible with various botanical extracts such as neem, garlic and certain fungicides, supporting its use in Integrated Pest Management (IPM) programs. Ahmad *et al.*[9] further demonstrated that combining *B. bassiana* with botanical extracts like *Acacia nilotica* and *Azadirachta indica* resulted in synergistic effects on *Musca domestica*, highlighting the potential of these integrated strategies for sustainable pest management. Based on these findings, this study aims to investigate the effects of combining *B. bassiana* with *M. jalapa* extract against *Spodoptera frugiperda*, with the goal of identifying more effective and environmentally friendly pest control strategies. However, currently, there has been no research on nanoemulsion-based nanopesticides combined with biological agents such as entomopathogenic fungi. Specifically, there is a lack of research on *M. jalapa* nanoemulsions combined with entomopathogenic fungi, particularly *B. bassiana*. This study aims to combine *M. jalapa* and *B. bassiana* to increase insect mortality, thereby making the *M. jalapa* nanoemulsion and *B. bassiana* more effective and efficient when applied on a field scale.

MATERIALS AND METHODS

Study area: This study was conducted from December, 2023 to March, 2024 at the Agricultural Pest Laboratory, Universitas Hasanuddin and Laboratory Biology and Chemistry Makassar State University.

Research procedure: The insects used in the study were the 3rd instar larvae of *S. frugiperda*. Insect samples of about 50 larvae were collected from the corn plantations in the Polombangkeng Utara Area, Takalar, South Sulawesi. The larvae were fed organic pickled corn during rearing. The materials and equipment used in this study include a rotary evaporator, stirrer, ultrasonicator, hemocytometer, 96% ethanol, surfactant (tween 80) and PDA (potato dextrose agar). The materials used in this study, including 96% ethanol, surfactant (tween 80) and PDA (potato dextrose agar), were purchased from CV Citra Persada and CV Intraco, both located in Makassar, South Sulawesi, Indonesia. Utilizing various instruments, this research used a microscope binocular XsZ-107BN, magnetic stirrer benchmark scientific H4000-hs, rotary evaporator RE 201D, hemocytometer type Neubauer Improved 301614, SK7210HP-Shanghai Kudos

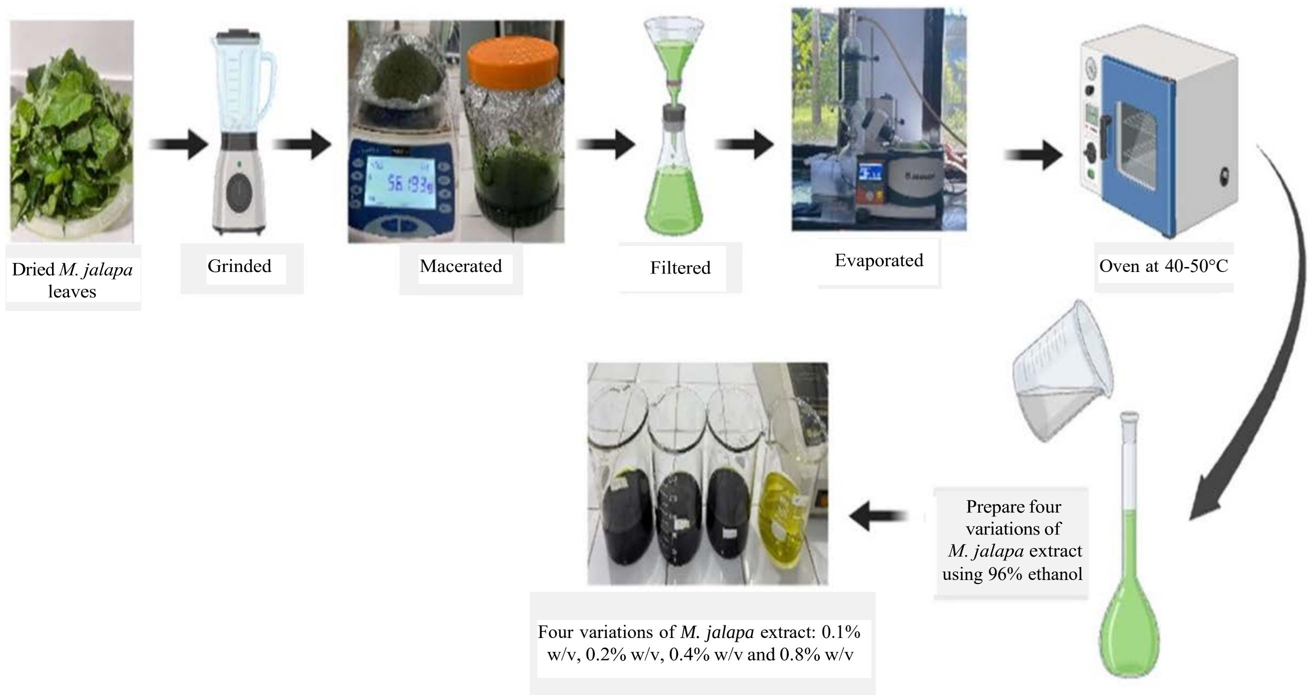

Fig. 1: Preparation and extraction of *M. jalapa*
Source: Suryani *et al.*[10]

ultrasonic, a Shimadzu Type IRPrestige-21 Fourier Transform Infrared Spectrophotometer (FTIR), a Microtrac Nanotrac Wave II particle size analyzer (PSA) and a Varian Cary 50 Conc. The UV-VIS Photometer and a JEOL JCM 6000 Scanning Electron Microscopy (SEM). All tools and materials come from the City of Makassar, South Sulawesi Province, Indonesia.

The process of preparation and extraction of *M. jalapa* leaves is detailed as follows. The method for creating *M. jalapa* nanoemulsion refers to previous research by Suryani *et al.*[10]. Figure 1 shows the steps involved in the preparation and extraction process of *M. jalapa*. The process likely includes the collection, drying and extraction of active compounds, which are then formulated for further experimental or application purposes. This preparation method is essential for obtaining the nanoemulsion or bioactive components used in studies involving *M. jalapa*.

Figure 2 illustrates the process starting with the preparation of *M. jalapa* nanoemulsion and *B. bassiana* isolated from infected insects. The nanoemulsion is dried and then applied to the larvae. Simultaneously, *B. bassiana* spores are diluted, counted under a microscope and also applied to the larvae. The combination of these treatments aims to increase larval mortality in *S. frugiperda*.

Multiplication of *B. bassiana* fungus on potato dextrose agar (PDA) medium: The *B. bassiana* fungus is cultured on potato dextrose agar (PDA) medium, which is incubated for approximately 2 weeks at room temperature. After 15 days, the fungus that has formed spores can be harvested and made into a suspension. The effective spore density used is 10^7 [11].

Observation of mortality: Mortality observations are conducted 24 hrs after the application of various treatments. There are a total of 15 treatments, including a control treatment, several formulations of *M. jalapa* nanoemulsion, *B. bassiana* with a spore density of 2.68×10^7 and combinations of *M. jalapa* nanoemulsion and *B. bassiana* spores at the same density. Details of the treatments were provided in Table 1 in the treatment description column.

Data analysis: The data from the mortality observations 24 hrs after application were analyzed as follows, calculate the percentage mortality of *S. frugiperda* larvae for each treatment group, including the control group, *M. jalapa* nanoemulsion alone, *B. bassiana* alone and the combination of *M. jalapa* nanoemulsion and *B. bassiana* then compare the mortality percentages between the different

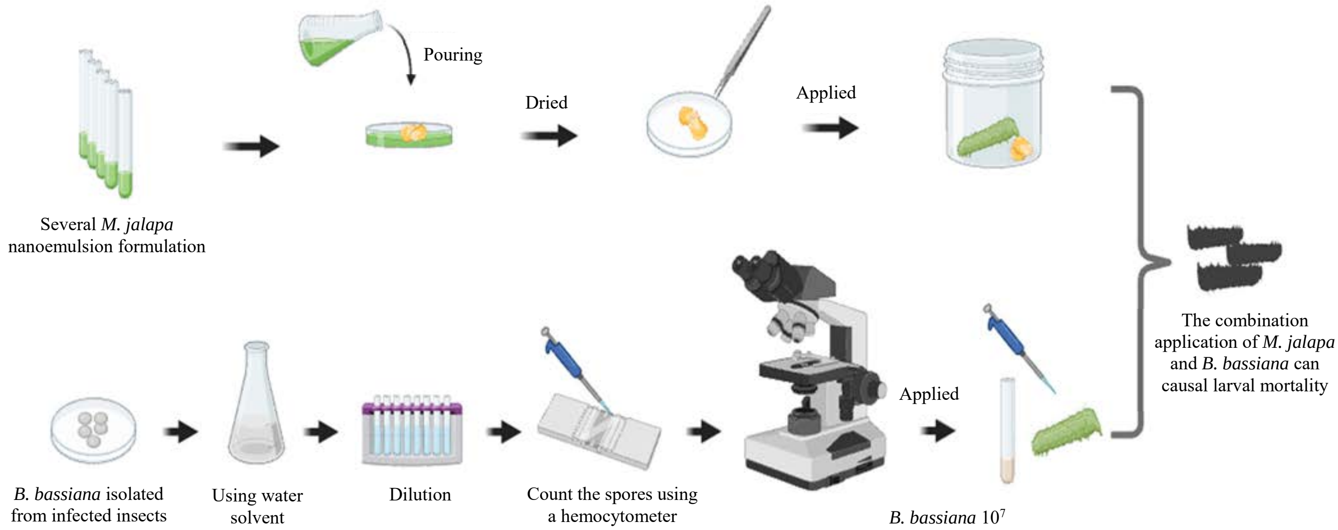

Fig. 2: Application of *M. jalapa* nanoemulsion and *B. bassiana* fungi combination to 3rd instar larvae of *S. frugiperda*

Table 1: Mortality rates of *S. frugiperda* larvae after 24 hrs of treatment

Treatment	Description of treatment	Percentage
1	Control (distilled water)	7
2	Positive control (ethanol 96%)	20
3	*M. jalapa* nanoemulsion (F1 0.1)	27
4	*M. jalapa* nanoemulsion (F1 0.8)	33
5	*M. jalapa* nanoemulsion (F2 0.1)	27
6	*M. jalapa* nanoemulsion (F2 0.2)	33
7	*M. jalapa* nanoemulsion (F2 0.4)	40
8	*M. jalapa* nanoemulsion (F2 0.8)	33
9	*B. bassiana* (spore density 2.68×10^7)	20
10	Combination (*M. jalapa* F1 0.1+*B. bassiana*)	53
11	Combination (*M. jalapa* F1 0.8+*B. bassiana*)	27
12	Combination (*M. jalapa* F2 0.1+*B. bassiana*)	33
13	Combination (*M. jalapa* F2 0.2+*B. bassiana*)	47
14	Combination (*M. jalapa* F2 0.4+*B. bassiana*)	60
15	Combination (*M. jalapa* F2 0.8+*B. bassiana*)	47

treatment groups to evaluate the effectiveness of each treatment. After that, analysis of linear regression. Perform linear regression analysis to determine the relationship between the mortality percentage and the various treatments. Use the mortality percentages as the dependent variable and the different treatment groups as independent variables with a significance level α 0.05. The regression equation helps identify the strength and significance of the relationship between treatment types and mortality rates. Analyzed the regression coefficients to understand the impact of each treatment on the mortality rate, then determined the R-squared value to assess the proportion of variation in mortality percentage explained by the treatments.

RESULTS AND DISCUSSION

Based on the research results, the percentage mortality rate of *S. frugiperda* after 24 hrs of application varied among the different treatments (Table 1). The *M. jalapa* nanoemulsion caused mortality ranging from 27 to 40%. The application of *B. bassiana* resulted in a 20% mortality rate after 24 hrs. This lower rate can be attributed to the time required for the fungus to make contact, germinate and penetrate the insect's body. Therefore, when this fungus is combined with a botanical pesticide, in this case, *M. jalapa*, it is expected that its effectiveness will increase. This was evident from Table 1, where the combination treatment of *M. jalapa* nanoemulsion and *B. bassiana*, specifically treatment 14, which includes

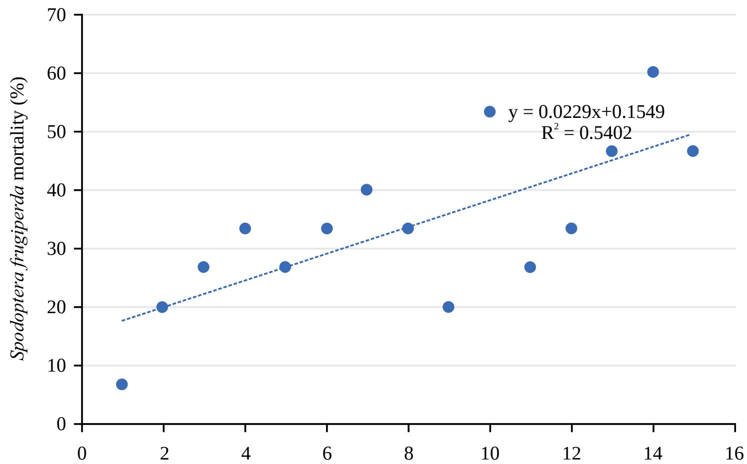

Fig. 3: Regression equation of the relationship between several treatment variations and the percentage of *S. frugiperda* mortality

M. jalapa nanoemulsion and *B. bassiana* with a spore density of 2.68×10^7, resulted in a 60% mortality rate of S. *frugiperda* larvae after 24 hrs of application. This indicates that the efficacy of botanical pesticides can be enhanced when combined with other biological agents, in this case, *B. bassiana* fungus.

Several studies support this finding, such as the combination of *Ocimum gratissimum* L. extract and cassava compost applied to *Capsicum annuum* L. plants, which resulted in a 93 and 87% increase in chili yield. Additionally, this combination improved fruit safety by reducing pesticide residue content on *C. annuum* L. plants[12]. The data indicate that the combination treatments have significantly higher mortality rates compared to the individual applications of *M. jalapa* nanoemulsion or *B. bassiana*. This synergy suggests that integrating botanical pesticides with biological agents can effectively improve pest control outcomes, providing a sustainable and environmentally friendly solution for managing *S. frugiperda* infestations.

Figure 3 displays the regression equation showing that there is a positive correlation between treatment variation and the percentage of *S. frugiperda* larval mortality. The regression equation is:

$$y = 0.0229x+0.1549$$

with a coefficient of determination value of $R^2 = 0.5402$.

The $R^2 = \sqrt{0.5402} = 0.74$ that indicating 74% of the variation in mortality can be explained by this model. The regression equation illustrated that the higher the treatment variation, the greater the mortality rate of *S. frugiperda* larvae, with an R square of 0.74, indicating a high strength of the relationship.

The appearance of *S. frugiperda* larvae that experienced mortality with the application of a combination of *M. jalapa* and *B. bassiana* formulations was shown in Fig. 4.

As shown in Fig. 4a, the untreated *Spodoptera frugiperda* larvae exhibit normal morphology. Following the application of *M. jalapa* and *B. bassiana* formulations, Fig. 4b illustrates the initial signs of infection, while fungal growth is evident on the larvae's surface in Fig. 4c. Complete colonization by fungal mycelium was observed in Fig. 4d.

Several studies have explored the effectiveness of combining botanical pesticides with other biological agents. For example, a combination of *M. jalapa* plant extract and the entomopathogenic fungus *Metarhizium anisopliae* was found to increase the mortality of *S. frugiperda* larvae and reduce the insect's immune system, as evidenced by decreased hemocyte counts and lectin protein levels, which are crucial for insect immunity[13]. Similarly, research combining botanical pesticides, such as the combination of caraway, orange peel and wintergreen oils extracts, showed that within 24 hrs, this formulation combination caused a mean mortality rate of 80 and 100% of desert and migratory locusts, respectively[14]. The combination of botanical pesticides of lemongrass and kenikir effects the parameters of attack intensity, percentage of attacks and leaf area index[15]. The results of other studies showed that the combination of betel leaf extracts showed the best inhibitory power of 20% against stem rot disease and 13.33% against *Fusarium* corn cob rot disease[16]. The current study's results on the synergistic effects of *B. bassiana* combined with various botanical extracts on pest mortality are consistent with several recent publications. For instance, Shakarami *et al.*[17] reported that a blend of essential oils from *Citrus vulgaris* and *B. bassiana* exhibited a synergistic effect against *Ephestia kuehniella* larvae,

Fig. 4(a-d): Morphology of dead larvae with the application of a combination of *M. jalapa* and *B. bassiana* formulations, (a) Healthy *Spodoptera frugiperda* larvae before treatment, (b) Larvae showing signs of infection post-treatment, (c) Larvae with fungal mycelium developing on the surface after death and (d) Complete fungal coverage of larvae
Source: Personal documentation

mirroring the current study's findings of enhanced pest mortality through combination treatments. Similarly, Habib-Ur Rehman *et al.*[18] found that a binary mixture of *B. bassiana* and neem extract resulted in the highest larval mortality rates, reinforcing the efficacy of combining entomopathogenic fungi with botanical insecticides. Moreover, the study by Tesari *et al.*[19] demonstrated that combinations of *B. bassiana* with several botanical pesticides, such as *Dioscorea hispida* and *Zingiber officinale*, not only reduced relative consumption but also had synergistic effects on *Spodoptera litura*, aligning with the current findings that combinations can enhance pest control efficacy. Afandhi *et al.*[20] also observed a maximum mortality rate of 44% for *S. litura* when combining *B. bassiana* with chinaberry extract, supporting the effectiveness of integrated pest management approaches similar to those investigated in this study. Ribeiro *et al.*[21] highlighted that botanical insecticides like neem extracts are compatible with *B. bassiana*, which is crucial for integrated pest management in organic farming systems. This compatibility was consistent with the observed potential for combining *B. bassiana* with other botanical extracts. Fernández-Grandon *et al.*[5] reported additive effects when combining *Metarhizium anisopliae* with pyrethrum, further supporting the idea that combining biological agents with botanical insecticides can enhance pest control outcomes. Lastly, the study by Touhidul Islam *et al.*[6] showed that combining neem with *B. bassiana* achieved a maximum nymph mortality rate of 92.3%, which aligned with the current research's focus on leveraging such combinations for effective pest management. Overall, these recent findings corroborate the current study's results, highlighting that combining *B. bassiana* with various botanical extracts can provide enhanced pest control and suggests promising avenues for sustainable and effective pest management strategies. Based on these findings, it can be concluded that the combination of botanical pesticides in the form of *M. jalapa* nanoemulsion and *B. bassiana* fungus is effective in increasing the mortality of *S. frugiperda* larvae. This indicates a significant potential for these methods as sustainable biological control strategies. This approach not only reduces reliance on chemical pesticides but also minimizes the risk of pest resistance, making it an environmentally friendly and sustainable solution for managing rice pests.

CONCLUSION

Overall, this study demonstrates that both *M. jalapa* and *B. bassiana*, as well as their combination, are effective in increasing the mortality of *S. frugiperda* larvae. This combination can serve as a highly effective strategy in biological pest control, offering a more sustainable and environmentally friendly alternative compared to chemical pesticides. The results highlight the potential of integrating botanical pesticides with biological agents to enhance pest management practices while reducing reliance on synthetic chemicals and minimizing environmental impact. Future research should explore optimizing the dosage and application methods of the *M. jalapa* and *B. bassiana* combination to maximize effectiveness and sustainability. Additionally, field trials could further validate these findings and assess long-term impacts on pest populations and ecosystem health.

SIGNIFICANCE STATEMENT

This research tackles the pressing need for sustainable pest management by exploring the effectiveness of *Mirabilis jalapa* and *Beauveria bassiana* in controlling *Spodoptera frugiperda*, a major pest in corn crops. When combined, these treatments significantly increased larval mortality compared to their individual use, reaching a maximum of 60%. The results underscore the potential of this integrated biological control strategy as an environmentally friendly alternative to chemical pesticides, providing a promising method to reduce pest resistance and support sustainable farming practices.

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