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Botanical insecticide of chili and ginger extract on *Nilaparvata lugens*, brown planthopper

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Abstract. Invasion of *Nilaparvata lugens* or brown planthopper (BPH) in paddy fields has led to the use of excessive chemical insecticides due to its effectiveness. However, excessive applications has contributed several negative effects on the environment, farmers as well as non-targeting organisms. As an alternative, a mixture of a botanical insecticide containing mixture of chili and ginger extracts have been studied. Three parameters were studied namely the extract concentration, time of exposure and temperature of exposure. The results showed that 90% of BPH mortality was found at 40% extract concentration with 72 hr duration time of exposure at optimal temperature 30°C. HPLC analysis proved the presence of capsaicin and gingerol at peaks of 4.502 min and 11.046 min respectively. From repellency analysis, the BPH showed selective repel action against the treated paddy. This is due to the pungent odor contributed from compound of gingerol, shogaol and capsaicin presented in the mixture. Meanwhile, contact toxicity studies have successfully give 100% of mortality of BPH. Microscopic analysis have shown BPH deformities was increased by the extract concentration.

1. Introduction

Rice is known as a staple food in Asia. However the planting is affected due to invasion of 800 species of pests every years resulted in low yield of rice production. *Nilaparvata lugens*, brown planthopper (BPH) is one of the serious pest in paddy field plantation. It was found at lowland in China, Cambodia, Vietnam, Malaysia, Indonesia, Japan, India, Korea, Thailand, Sri Lanka, Bangladesh, and Philippines [1]. BPH are small insects but move in big colony. Its population peak during dry season and commonly found at irrigated fields like paddy field. They consumed on the plant, indirectly causing damage in terms of quality and quantity of paddy produced per season [2]. The leaves turn color from green to orange-yellow before coming to brown and dry known as hopperburn, which eventually kills the plant. The close canopy of rice plants, densely seeded crops, excessive nitrogen used and early insecticide spraying had contributed to the BPH development. Its high fecundity and migratory ability resulted in vast damage of rice plant [3]. Synthetic chemical pesticides such as carbofuran is preferable however resulted in environmental drawbacks, pest resurgences [4] and toxic to human. Thus, it is necessary to investigate the use of bioinsecticide which serves as greener alternatives towards chemical insecticide.

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The application of bioinsecticide through botanical extract application serves as an alternative over chemical insecticide as they possess avoidance in chemical insecticide and environmental friendly pesticide. Chili and ginger have pungent odor as well as warming effect. Both have been reported as a potential bioinsecticide [5]. However the studies on the application of the mixture between ginger and chili towards BPH is under discovered. In this studies, mixture extracts of chili and ginger will be investigated due to its high pungent principle against insect especially BPH. The toxicity effect, physical and chemical changes of BPH in terms of mortality rate, repellent action, contact toxicity, HPLC analysis and morphology will be analyzed.

2. Methodology

2.1 BPH breeding and bioinsecticide preparation

The adult BPHs used in the studies measured ranging from 3.5-4.5 mm in length were supplied from the Malaysian Agricultural Research and Development Institute, MARDI, Bertam Pulau Pinang. The BPH were breded in a suitable cage at 25 ± 1 °C, supplied with paddy plants as their food source. Fresh chili fruits of *Capsicum annum* and ginger rhizome of *Zingiber officinale* from local market in Perlis State, Malaysia were used in the study. The samples were washed and dried at 40° C for 2 days [6]. About 10g of samples was placed into a 250ml conical flask and soaked with 60 ml of ethanol subjected to solvent extraction in sonicator bath (Branson, United States) for 30 minutes. After that, the mixture were filtered prior for evaporation process using rotary evaporator at 70 °C until all solvent evaporated [7]. The mixture were kept for mortality, repellency and contact against BPH analysis.

2.2 Mortality analysis of BPH against the prepared bioinsecticide

Ten adult BPH in plastic bottles were exposed to the prepared bioinsecticide based on three factors; bioinsecticide concentration (10-40 v/v%), time duration of exposure (24-72 h) and temperature of exposure (24 - 40 °C). These three factors were used to optimize the mortality of BPH. Response Surface Methodology (RSM) was used in the analysis. Total run number of experiment was 39 run.

2.3 Repellency analysis of BPH against the prepared bioinsecticide

For repellency analysis, 2 month old of paddy seedlings were sprayed by three different solutions namely commercial pesticide, NEXUS 18.3SL) (Agrofarm Sdn Bhd, Jejawi), prepared bioinsecticide and control respectively. The three solutions were diluted to 25% prior for spraying on the paddy seedlings. The paddy seedlings were placed in a small pots and sprayed with the prepared solutions before air dried at room temperature and transferred into a plastic container. Ten adult BPH were placed into the plastic container and then were sealed by another plastic container to avoid BPH from hopping outside. Small holes were punched on the plastic container. Ten replicates were prepared and ran for 24h. For control, the paddy was treated using a mixture of tap water and Tween 80. Tween 80 was used as a surfactant to lower down the surface tension between the distilled water and paddy leaves as the paddy leaves was covered with wax. The number of BPH on the treated and control paddy were counted and recorded after 24h [9].

2.4 Contact toxicity and morphological analysis of BPH against the prepared bioinsecticide

Contact toxicity test has been conducted as was done by Xu *et al* (2015). A filter paper was immersed for 5 s into 40% of prepared bioinsecticide and tap water as for control before drying at room temperature. The filter paper were shaped into a cylinder and placed into a glass tube. After that, ten adult of BPH were placed into the glass tube and sealed with cloth to avoid BPH hopping outside [9]. Three replicates were conducted for this treatment. The number of mortality BPH were counted and recorded after 24 h.

Morphological analysis of dead BPH was observed under 40X magnification by light microscope. The image of the BPH morphology under bioinsecticide and control treatment were observed.

3. Results and Discussion

3.1 Mortality analysis of BPH after exposure to the prepared bioinsecticide.

Table 1 shows the mortality result of BPH at highest and lowest reading out of 39 runs. It shows that the highest mortality rate of BPH were 100 % at 1st and 19th run with concentration of prepared bioinsecticide were 46.21 % and 40 % after 48 and 72 hours exposure time at temperature of 30°C respectively. Meanwhile, the lowest mortality rate of BPH was 10 % at 20th run with concentration of prepared bioinsecticide was at 25% after 14.06 hours exposure at temperature of 25°C. The result shows that the prepared bioinsecticide had potential in order to reduce population of BPH with consideration of concentration of extract, time and temperature. No mortality was observed for control sample. The optimized condition to obtain high mortality of BPH is obtain after statistical validation analysis.

Table 1. Result for the response mortality

Run	A: Concentration of extract [%]	B: Time [h]	C: Temperature [°C]	Response 1 Mortality [%]
20	25.00	14.06	25.00	10
1	46.21	48.00	30.00	100
19	40.00	72.00	30.00	100

The mortality results was analyzed by using ANOVA analysis as shown in Table 2. The model F-value obtained from the analysis is 13.10 that implies that the model is significant with R^2 0.8421. The sources with p-value <0.005 give significant mortality effect to the BPH namely terms A, B, C, AB, AC and A^2 . All factors (concentration, time, and temperature) are significant contribute to the mortality of BPH. Parameter A appears as the highest F-value which represent that concentration of prepared bioinsecticides contributes to the most significant effect to BPH rather than temperature and time. Lack of fit is statistically not significant with F-value of 2.58, implies to the pure error.

Table 2. Analysis of Variance (ANOVA) of mortality BPH

Source	Sum of squares	Degree of freedom	Mean square	F-value	P-value
Model	12623.60	11	1147.60	13.10	<0.0001*
A	8019.09	1	8019.09	91.51	<0.0001*
B	1224.39	1	1224.39	13.97	0.0009*
C	758.97	2	379.49	4.33	0.0234*
AB	675.00	1	675.00	7.70	0.0099*
AC	875.69	2	437.85	5.00	0.0143*
BC	312.38	2	156.19	1.78	0.1875
A2	575.22	1	575.22	6.56	0.0163*
B2	105.65	1	105.65	1.21	0.2819
Residual	2366.14	27	87.63		
Lack of fit	1806.14	15	120.41	2.58	0.0524

R²=0.8421

* Significant value (p<0.005)

The verification was conducted at the predicted conditions suggested from RSM analysis as shown in Table 3. The experimental value obtained (90 %) is close to the predicted value (99.67 %), represent for 9.71 % difference, hence prove the adequacy and validity of predicted model. From the analysis, the optimum condition to produce 99.67 % of BPH's mortality is at concentration of 40% with 72 hrs exposure period and temperature of 30 °C.

Table 3. Experimental and predicted values of BPH mortality at an on optimal conditions

	Predicted	Experimental
Concentration bioinsecticide (%)	40	40
Time (h)	72	72
Temperature (°C)	30	30
Mortality rate of BPH (%)	99.67	90.00

3.2 Repellency analysis of BPH against the prepared bioinsecticide

Based on Figure 1, about 60% BPH choose to stay at paddy stem that treated with tap water (control plant) while 20% BPH were stayed on the stem treated with the prepared bioinsecticide and only 5% BPH were on the commercial pesticide. The repellent activity is in line with the previous research where only 8.4% of BPH were stayed on the treated paddy stem with methyl euganol as a botanical pesticide while 76.6% were stayed on the control paddy stem [9]. It shows that the combination of chili and ginger have special characteristic that can repel BPH from the paddy stem due to its pungent odor. The finding is in line with previous research which showed that several botanical extracts namely ginger, shamar and chili pepper had contributed for the insecticidal action for pest control [13]. Nguyen, 2014 showed that application of capsaicin in green chilies gave burning effect to the pests [14].

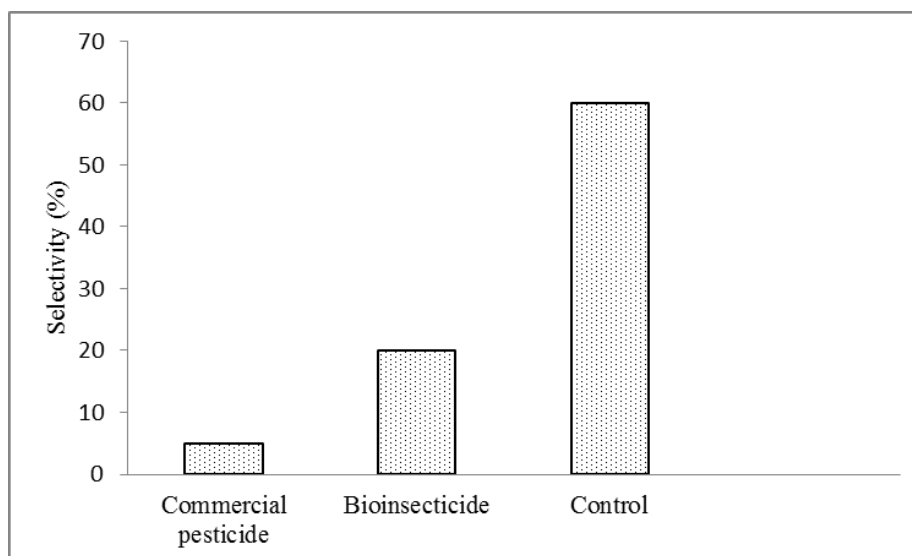


Figure 1. Repellent effect against BPH by the prepared bioinsecticide

3.3 Contact toxicity and morphological analysis of BPH against prepared bioinsecticide

From contact toxicity studies, it shows that 100% of BPH are died within 24 h after exposure to the treated filter paper with the prepared bioinsecticide. However, only 10% of BPH are died in the control treatment (Figure 2). The contact effect is positively correlated with the bioinsecticide concentration and is in line with the mortality results in Table 3.

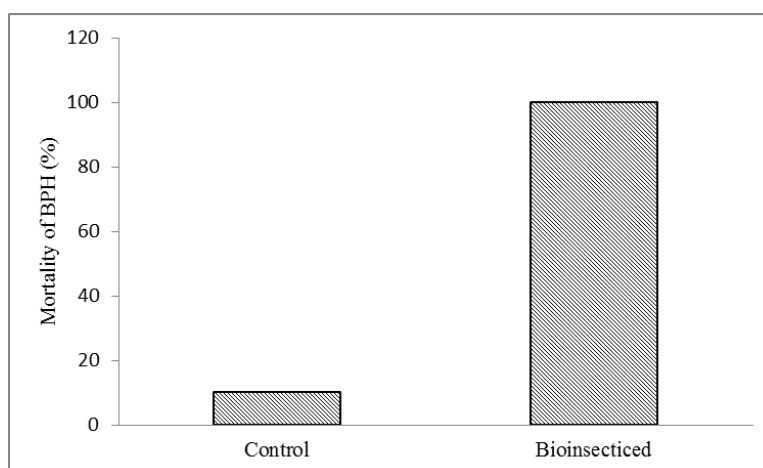


Figure 2. Contact toxicity using filter paper

From the morphological analysis, BPH treated with the prepared bioinsecticide shows deformation effect (Figure 3). The deformation of BPH are depends on the concentration of the bioinsecticide, where the higher the concentration, the higher the deformation and support the mortality studies.



Figure 3. Morphology of BPH after treatment. A: BPH treated with tap water and Tween 80, B: BPH treated with 10% of bioinsecticide, C: BPH treated with 25% of bioinsecticide, D: BPH treated with 40% of bioinsecticide

The control treatment of BPH (Figure 3A) shows no sign of body's deformation as the mixture between tap water and Tween 80 are tolerable by the BPH. Meanwhile in Figure 3B-D, it shows that the level of deformation becomes more obvious than Figure 3A and in line with the bioinsecticide concentration increment. The morphological analysis is in line with the mortality result as shown in Table 3, where the higher the concentration, the higher the mortality of BPH. Senthil Nathan *et al.*, (2006) showed that application of neem pesticide resulted in size reduction of BPH based on the concentration applied [15].

4. Conclusion

From the findings, it shows that the BPH successfully bred in a suitable cage in a room temperature with paddy plants as the food sources. The result shows that the optimum mortality rate of BPH is 90.00% after exposure to 40.00% of the prepared bioinsecticide for 72 hr at 30°C. The studies showed that time, temperature and concentration are significant parameters to the mortality of BPH. The mortality of BPH might be due to the presence of chemical compounds namely capsaicin and gingerol in the prepared bioinsecticide. These chemical compounds resulted in pungent odor and warm effect, consequently resulting in BPH's mortality. The prepared bioinsecticide gives an alternative choice over chemical insecticide for controlling BPH invasion. It is an efficient, safe and low in cost of bioinsecticide for commercialization.

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