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by Endang Mugiastuti

Submission date: 07-Feb-2023 03:35PM (UTC+0700)

Submission ID: 2008405226

File name: 18._Biodiversitas_2020_3_EM.pdf (286.13K)

Word count: 5594

Character count: 29255

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Organic control of *Bemisia tabaci* Genn. on *Capsicum annuum* with entomopathogenic fungi raw secondary metabolites

LOEKAS SOESANTO*, BISIRIL FATIHAH, ABDUL MANAN, ENDANG MUGIASTUTI,
NUR PRIHATININGSIH

Faculty of Agriculture, Universitas Jenderal Soedirman, Jl. Dr. Suparno No. 63, Purwokerto Utara, Banyumas 53123, Central Java, Indonesia.
Tel.: +62-281-638791, *email: lukassanto26@gmail.com

Manuscript received: 22 October 2020. Revision accepted: 25 November 2020.

Abstract. Soesanto L, Fatiha B, Manan A, Mugiastuti E, Prihatiningsih N. 2020. Organic control of *Bemisia tabaci* Genn. on *Capsicum annuum* with entomopathogenic fungi raw secondary metabolites. *Biodiversitas* 21: 5786-5791. Control of whitefly with entomopathogenic fungi raw secondary metabolites and their correlation was investigated under control condition. The in vitro test used randomized completely design consisted of five treatments (raw secondary metabolites of *Beauveria bassiana* B16 and B10, *Metarhizium anisopliae* M16, *Lecanicillium lecanii* L16, and no treatments) and repeated five times. The in planta one used Latin square design consisted of five treatments and repeated five times. Variables observed were the mortality of whitefly imago, chitinolytic index, incubation period, viral disease intensity, plant height and total leaves per plant. The results showed that all treatment of raw secondary metabolites of entomopathogenic fungi caused mortality in the *B. tabaci* imago by 77.8% (*B. bassiana* B16); 71.5% (*B. bassiana* B10); 66.7% (*M. anisopliae* M16); and 59.5% (*L. lecanii* L16). All raw secondary metabolites of entomopathogenic fungi reduce the intensity of viral diseases by 79.98% (*B. bassiana* B16); 74.00% (*B. bassiana* B10); 72.23% (*L. lecanii* L16); and 55.41% (*M. anisopliae* M16). The correlation between *B. tabaci* mortality due to raw secondary metabolites of entomopathogenic fungi with the viral disease intensity is very close and opposite. Every 1% increase in pest mortality causes decrease in viral intensity by 1.346%.

Keywords: *Bemisia tabaci*, entomopathogenic fungi, raw secondary metabolites, chitinase extracellular, viral disease

INTRODUCTION

Chili is an important commodity supporting the food sector and industry. Supriadi et al. (2018) explain that in Indonesia about 80% of chili production is allocated for household food needs, the remaining 20% is absorbed by the industrial sector. There are two types of chili plants that are generally cultivated in Indonesia, namely large red chili (*C. annuum* L.) and cayenne pepper (*C. frutescens* L.). Production constraints due to pests and diseases in the field still occur. Whitefly (*B. tabaci*; Hemiptera: Aleyrodidae) is a pest as well as a vector that causes viral diseases in large red chili commodities (Navas-Castillo et al. 2011; Moreno-Delafuente et al. 2013).

Virus control is currently emphasized on pest control which acts as a vector. According to He et al. (2013) and Rodríguez et al. (2019), periodic application of pesticides is carried out on *B. tabaci* to prevent further spread of the virus. The control of whitefly *B. tabaci* using chemical pesticides with the dosage and intensity of application has exceeded reasonable limits causing the danger of pest resistance (Legg et al. 2014). According to De Barro et al. (2011) and Macfadyen et al. (2018), *B. tabaci* has developed into many biotypes. Chemical pesticides to control whitefly must slowly turn to effective control. Expected control does not cause resistance to *B. tabaci* pests. The alternative is to utilize the role of entomopathogenic fungi. Mascarin et al. (2013) and Abdel-Raheem and Al-Keridis (2017) supported that entomopathogenic fungi as biological control agents have

the potential to control pests, replacing synthetic insecticides. In its application in the field entomopathogenic fungi have weaknesses such as abiotic stress factors, such as temperature, humidity and sunlight (Mustafa and Kaur 2008; Liu and Lin 2013; Han et al. 2014), the stability of conidia and blastopora production (Fernandes et al. 2012; Oliveira et al. 2018), and insect cuticle as a barrier for all entomopathogenic fungi to penetrate directly (Keyhani 2018).

Furthermore, the role of entomopathogenic fungi can be enhanced with the help of their raw secondary metabolites produces. The raw secondary metabolism of entomopathogenic fungi plays a role in increasing control effectiveness. According to Kim et al. (2013), entomopathogenic fungi bred in the filtrate (potato dextrose broth) can produce raw secondary metabolites in the form of hormones, enzymes (such as protease; lipase; chitinase), antibiotics, and other compounds that are insecticidal. The purpose of this research was to study the effect of raw secondary metabolites from four entomopathogenic fungi isolates on *B. tabaci* mortality, the intensity of viral diseases, and the correlation between *B. tabaci* and the viral diseases.

MATERIALS AND METHODS

Propagation of the four entomopathogenic fungi

The four entomopathogenic fungi were *B. bassiana* B16, *B. bassiana* B10, *M. anisopliae* M16, and *L. lecanii*

L16, collection of the Plant Protection Laboratory. Propagation of the entomopathogenic fungi was carried out using potato dextrose agar in a Petri dish and incubated for 7 days at room temperature (Yun et al. 2017).

Making secondary metabolites

The method for making secondary entomopathogenic fungi metabolites was carried out according to Kim et al. (2013), using potato dextrose broth. Medium PDB (200 g potato extract and 20 g sugar mixed into 1 L water) was prepared for sub-culture and activation of entomopathogenic fungi isolates. The cultured entomopathogenic fungi from the Petri dish was inoculated into an Erlenmeyer (500 mL) flask containing (100 mL) PDB medium, incubated at room temperature $28 \pm 2^\circ\text{C}$, and shaker at 155 rpm for one week.

Preparation of secondary metabolite solutions for application

Entomopathogenic fungi propagated using the PDB medium, the density of the conidia was calculated using a hemacytometer. According to Oliveira et al. (2018), conidia density was calculated by dripping 1 mL of entomopathogenic mushroom suspension which was propagated in the PDB medium onto the hemacytometer, conidia density was calculated with a 400 times magnification microscope. The density of conidia applied was 1×10^7 conidia / mL for each isolate. The filtrate mess around 3000 rpm for 5 minutes, then the filtrate was filtered again twice with Wattman filter paper No. 1 to then be applied by spraying (Rauf et al. 2018).

Exploration and propagation of whitefly infestation

Exploration is done by looking for pest imago in the agroecosystem around Banyumas. Propagation of whitefly infestation is carried out by breeding pests in chili plants that are covered by cage nets. Propagation of these pests is done until the pest population is sufficient for research activities.

Experimental design

The research design used for the Latin Square Design (RBSL) for in planta research and Randomized Group Design (RBD) for laboratory research. This study consisted of 5 treatments namely control and secondary metabolites of *B. bassiana* B16, *B. bassiana* B10, *M. anisopliae* M16, and *L. lecanii* L16. The conidia density used is 1×10^7 conidia / mL. Each treatment in the in vitro mortality test was repeated 5 times, the in-planta test was repeated 5 times, and the qualitative test of chitinase activity was repeated 4 times.

Large chili cultivation in the screen house

Large chili cultivation is carried out in a screen house consisting of seedlings carried out by preparing a medium of manure on a tray. Chili seeds are sown on the surface of the planting medium and watered until the planting medium is damp. Planting is done with a planting medium in the form of a mixture of manure and soil. Mixed planting medium was loaded into polybags as much as 5 kg/polybag. Large chili seeds were transferred to polybags.

Weed sanitation is done manually.

Making chitin agar medium

Chitin agar medium was prepared by mixing 0.5% colloidal chitin, K_2HPO_4 0.1 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.01 g, yeast extract 0.05 g, peptone 0.1 g, bacto tripton 0.1 g, NaCl, $(\text{NH}_4)_2\text{SO}_4$ 0.1 g, and so that 1 g into an Erlenmeyer containing 100 mL of distilled water, the solution is then homogenized with a magnetic stirrer and heated until dissolved, then sterilized in an autoclave at 121°C for 15 minutes at a pressure of 2 atm (Rachmawaty 2009). The method of making chitin colloid (Haedar et al. 2017), which is as much as 20 g chitin from shrimp shell powder, plus 400 mL concentrated HCl and then tightly closed and left for 24 hours at 4°C (all treatment stages in cold temperatures). Next filtered using glass wool and filtrate plus 200 mL of cold water and the pH of the solution neutralized with 10 N NaOH and spinning around 8000 rpm, temperature 4°C for 15 minutes. Pellet is washed with cold water and then mess around again. Pellet (colloidal chitin) is ready to use.

Qualitative test of entomopathogenic fungal chitinase enzyme

Chitinase enzyme qualitative test was performed by the method of Mubarik et al. (2010) modified, that is filter paper ($d = 5$ mm) soaked in a solution of secondary metabolites for 30 minutes and inoculated into chitin agar medium and incubated for 3 days. The clear zone is visualized by adding 0.1% congo red. The clear zone formed is measured based on the chitinolysis index formula (Rachmawaty 2009).

Metabolite infectivity test secondary to whitefly (in-vitro)

Large chili leaves are cut in a circle with a diameter of 3.5 cm; immersed in a solution of secondary metabolites for 5 seconds, and glued upside down (the upper surface of the leaf attaches to the top of the Petri dish) (He et al. 2013). Whitefly as much as 10 adults were transferred into a Petri dish which already contained large chili leaf pieces (Abdel-Raheem and Al-Keridis 2017). The treatment consisted of secondary metabolites of four entomopathogenic fungi isolates (1×10^7 conidia / mL) and control (without treatment). Each treatment was repeated 5 times. The total unit of the experiment was 25 Petri dishes so that the total imago *B. tabaci* needed was 250. Mortality of whitefly infestation in modified Bioassays insecticide method by Yao et al. (2017), calculated by the formula Putra et al. (2013).

Tests of metabolite infectivity secondary to disease variables due to virus carried by whitefly infestation in planta

This activity was started by transferring large chili seeds aged 15 DAP in a 20×25 cm polybag; the treatment consisted of secondary metabolites of four entomopathogenic and control fungi (without treatment) which were repeated 5 times; each trial unit has 3 units of plants, in total there are 75 large chili plants. After being placed, each plant is sprayed with a secondary metabolite

solution according to the treatment in the morning before the *B. tabaci* imago is infested. The virus transmission procedure from a large chili inoculum uses imago *B. tabaci*. Imago is transferred using the mouth apparatus suction into the containment of a large chili plant that has been sick for 30 minutes to get the virus (Acquisition Access period). Imago *B. tabaci* has moved again to each experimental unit (Putra et al. 2013). Imago is transmitted to each experimental unit as many as 10 imagoes so that the total needed was 750 imagoes. Leaf samples from plants indicate symptoms of mosaic, chlorosis, yellowing, stunting, mottle, necrosis, leaf deformation, and leaf bunching. The disease intensity is calculated every 12 days by the formula (Gashaw et al. 2014):

$$DI = \frac{\sum(n \times v)}{N \times Z} \times 100\%$$

Where; DI = disease intensity (%), n= Number of plants in each category, v = Numerical values of symptoms category, N= Total number of plants, and Z = Maximum numerical value of symptom category.

Numerical value used to assess the disease intensity is using a scale of 1-6 (Olawale et al. 2015) is: 1. No visible symptoms; 2. Mild mosaic/mottling/yellowing/mild necrosis on few leaves/branches of a plant (symptoms on less than 25% of the plant); symptom recovery; 3. Moderate mosaic/puckering/mottling/yellowing/necrosis on many leaves/plants and vein clearing (symptoms cover 25-50% of the plant); 4. Severe mosaic/puckering/mottling/yellowing/necrosis (symptoms on entire plant); 5. Severe mosaic/puckering/mottling/yellowing/necrosis and severe stunting (entire plant); and 6. Severe mosaic/puckering/mottling/yellowing/necrosis and severe stunting (entire plant), and death of the infected plants.

Data analysis

Observation data were processed with Microsoft Excel 2010 (DSAASTAT), so that the F test results (significance level of 0.05) and further tests with HSD (significant level of 0.05).

RESULTS AND DISCUSSION

Virulence of secondary metabolites of entomopathogenic fungi to adult *B. tabaci*

Observation of insect death results in dead insects drying out like mummies. Insect tissue looks dry and blackish in color. The results showed (Table 1) that all

secondary metabolites of entomopathogenic fungi were able to cause mortality against adult *B. tabaci*. The first observation showed that all secondary metabolites could cause low mortality. The second observation result showed that secondary metabolites of *B. bassiana* B16 (20.67%) and *L. lecanii* L16 (24.89%) were higher than *M. anisopliae* M16 (10.44%) and *B. bassiana* B10 (10.22%). The third and fourth observation result showed that *B. bassiana* B16 has highest mortality (37.33%; 45.28%) followed by *L. lecanii* (24.89%; 35.56%) and *M. anisopliae* M16 (24.89%; 35.33%), then *B. bassiana* B10 (18.67%; 28.33%). The fifth and sixth observation result showed that secondary metabolites of *B. bassiana* B16 have highest mortality (59.72%; 67.38%) followed by *B. bassiana* B10 and *M. anisopliae* M16 (each by 50.00%; 58.81% and 45.78%; 56.22%), and then *L. lecanii* L16 (44.72%; 51.94%). But, the secondary metabolites of *M. anisopliae* M16 and *L. lecanii* L16 did not significantly different. The last observation showed that secondary metabolites of *B. bassiana* B16, *B. bassiana* B10 and *M. anisopliae* M16 have highest mortality (each by 77.82%; 71.47%; and 66.67%) followed by secondary metabolites of *L. lecanii* L16 (59.52%).

This was found in all test insects, according to the explanation of Widariyanto et al. (2017). Each isolate has the ability to kill different test insects because of chitinase (Table 2). Internal factors that influence are isolate type (Soesanto 2017); propagule infectivity (Abdel-Raheem and Al-Keridis 2017); and the secondary metabolites it produces (Kim et al. 2013; Soesanto 2017). Analysis result showed that secondary metabolites of *B. bassiana* B16 have a higher rate of increase in mortality (12.63%) than other treatments. The lower rate of increase in mortality was shown sequentially by secondary metabolites of *B. bassiana* B10 (10.92%), *M. anisopliae* M16 (10.12%) and *L. lecanii* L16 (9.25%). This shows the ability of the secondary metabolites of *B. bassiana* B16 to have high effectiveness on deadly test insects (*B. tabaci* pests; imago). Each secondary metabolites of entomopathogenic fungi have different time causing mortality. Internal factors that influence are isolate type (Soesanto 2017) and its secondary metabolites (Kim et al. 2013; Soesanto 2017). Internal factors that cause differences in the mortality rate of *B. tabaci* for each isolate are the ability to produce secondary metabolites in the form of the chitinase enzyme to support the infection process into the tissues (Suryadi et al. 2016).

Table 1. Virulence of secondary metabolites of four entomopathogenic fungi against adult *Bemisia tabaci* in mortality in-vitro test.

Treatment (secondary metabolites of)	Mortality of adult <i>B. tabaci</i> (%)						
	12	24	36	48	60	72	84
<i>B. bassiana</i> B16	2 Fab	20.67 Fa	37.33 Ea	45.28 Da	59.72 Ca	67.38 Ba	77.82 Aa
<i>B. bassiana</i> B10	6 Ga	10.22 Fb	18.67 Ec	28.33 Dc	50.00 Cb	58.81 Bb	71.47 Aab
<i>M. anisopliae</i> M16	6 Fa	10.44 Fb	24.89 Eb	35.33 Db	45.78 Cb	56.22 Bbc	66.67 Ab
<i>L. lecanii</i> L16	4 Fab	18.67 Ea	24.89 Eb	35.56 Db	44.72 Cb	51.94 Bc	59.52 Ac
Control	0 Gb	0 Gc	0 Gd	0 Gd	0 Gc	0 Gd	0 Gd

Note: number in a row which followed by same capital alphabetical means not different (HSD value 5,238); and number in a column which followed by same small alphabetical means not different (HSD value 6,711)

According to Han et al. (2014), different pest death rates cause different Lethal Time (LT) values. LT is a value that represents the time required for insecticides, biopesticides, entomopathogenic fungi, and secondary metabolites to kill pests. According to Alavo et al. (2002), the term lethal time is also interpreted as 'killing time'. This term is related to the period required by active ingredients to kill test insects or target insects. LT50 value (Lethal Time 50), the time taken to kill insecticide 50% of the target pest population. According to Wang et al. (2004) and Han et al. (2014), the lower LT values showed more effective insecticides. As described by Han et al. (2014), *B. bassiana* B16 were most effective in causing mortality in *B. tabaci* imago base on its lethal time value.

Chitinolytic index of secondary metabolites of four entomopathogenic fungi

The qualitative test result showed (Table 2) the difference in the chitinolytic index for each secondary metabolites of entomopathogenic fungi. Secondary metabolites of *B. bassiana* B16 have the highest chitinolytic index by 3.5 then followed by secondary metabolites of *L. lecanii* L16 (2.00); *B. bassiana* B10 (1.68); and *M. anisopliae* M16 (0.73). This is in line with Ramanujam et al. (2011) and Mondal et al. (2016), that entomopathogenic fungi produce variant enzymatic activity of different enzymes. Chitinase enzyme is a secondary metabolite produced by entomopathogenic fungi and plays a role in increasing infectivity towards target insects (Suryadi et al. 2016). Chitinase enzyme degrades chitin and plays an important role in controlling harmful insect and fungal pathogens by catalyzing β -1 \rightarrow 4-linkages in chitin (C₈H₁₃O₅N)_n, a major polymer especially for building structural fungal cell walls (Hamid et al. 2013; Oyeleye and Normi 2018). Some entomopathogenic fungi tested indicated different chitinolytic index. The potency of entomopathogenic fungi depends on the capability of some enzymatic equipment such as chitinases and proteases (Mondal et al. 2016).

Virus disease intensity on chili

Virus disease intensity on chili in-plant test was observed according to the categories described by Arogundade et al. (2015). The in-plant test result showed

(Table 3) that all secondary metabolites of entomopathogenic fungi could decrease the disease intensity better than control from 24 to 33 DAP. But, on 37 to 41 DAP observation showed that the secondary metabolites of *B. bassiana* B16, *B. bassiana* B10 and *L. lecanii* L16 have lower disease intensity than *M. anisopliae* M16. Last observation result showed that the secondary metabolites of *B. bassiana* B16, *B. bassiana* B10 and *L. lecanii* L16 have the lowest disease intensity (each by 14.44, 18.75, and 20.03% or 79.98, 74.00, and 72.23% compared to control) then followed by *M. anisopliae* M16 (32.16% or 55.41% compared to control). The intensity of the disease in tested plants was observed according to the categories described by Arogundade et al. (2015). All secondary metabolites of entomopathogenic fungi can reduce the intensity of viral diseases, each by 79.98% (*B. bassiana* B16); 74.00% (*B. bassiana* B10); 72.23% (*L. lecanii* L16); and 55.41% (*M. anisopliae* M16).

The ability to reduce the intensity of different viral diseases is due to the ability to increase the mortality of *B. tabaci*. According to Gerling et al. (1980) and Putra et al. (2013), management of viral diseases can be done by reducing the population of imago *B. tabaci* as a vector pest. The development of viruses in plant tissue directly interferes with plant growth. Symptoms of viral diseases that cause leaves to turn yellow, mosaic, curly and curled, according to the explanation Arogundade et al. (2015), can cause leaf function to decrease. As a result, photosynthesis is not optimum, so that plant growth is disrupted. The treatment of *B. bassiana* B16 and *L. lecanii* L16 showed the highest increase in plant height and number of leaves when compared with other treatments, such as *B. bassiana* B10 and *M. anisopliae* M16.

Table 2. Chitinolytic index of secondary metabolites of four entomopathogenic fungi

Treatments (Secondary metabolites of)	Chitinolytic index
<i>B. bassiana</i> B16	3.50 a
<i>L. lecanii</i> L16	2.00 ab
<i>B. bassiana</i> B10	1.68 b
<i>M. anisopliae</i> M16	0.73 b
HSD Value	1.7956

Table 3. Virus disease intensity on chili in-plant test

Treatments (Secondary metabolites of)	Disease intensity (%)				
	24	29	33	37	41
<i>B. bassiana</i> B10	6.93 Ba	7.10 Ba	9.41 ABa	10.37 ABa	18.75 Aa
<i>B. bassiana</i> B16	3.45 Aa	4.25 Aa	5.53 Aa	5.41 Aa	14.44 Aa
<i>L. lecanii</i> L16	3.38 Ba	3.97 Ba	6.56 Ba	6.62 Ba	20.03 Aa
<i>M. anisopliae</i> M16	6.95 Ba	13.14 Ba	15.76 Ba	28.03 Ab	32.16 Ab
Control	30.71 Db	44.05 Cd	45.35 BCb	55.56 Bc	72.12 Ac

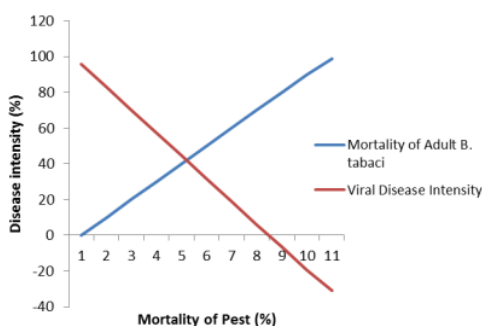
Note: number in a row which followed by same Capital alphabetical means not different (HSD value 11,022); and number in a column which followed by same small alphabetical means not different (HSD value 11,022)

Table 4. Secondary metabolites of entomopathogenic fungi effect on chili in-plant test which infested by adult *Bemisia tabaci*

Treatments (Secondary metabolites of)	Increasing plant height (%)	Increasing total leaf per plant (%)
<i>B. bassiana</i> B16	173.35	69.66
<i>L. lecanii</i> L16	172.56	91.70
<i>B. bassiana</i> B10	90.82	54.55
<i>M. anisopliae</i> M16	105.49	49.20
Control	-	-

Table 5. Pearson value and regression analysis between mortality and disease intensity

Treatments (Secondary metabolites of)	Pest mortality (%)	Virus disease intensity (%)
Control	0	72.12
<i>M. anisopliae</i> M16	66.7	32.16
<i>L. lecanii</i> L16	59.5	20.03
<i>B. bassiana</i> B10	71.5	18.75
<i>B. bassiana</i> B16	77.8	14.44
Pearson =	-0.965319379	
	Y = 95.642 -1.287X	

**Figure 1.** Correlation between adult whitefly mortality and virus disease intensity

Plant growth

The development of viruses in plant tissue interferes with plant growth (Arogundade et al. 2015). The result of in-planta test showed (Table 4) that the secondary metabolites of entomopathogenic fungi could be increasing plant height better than control plant by 173.35% (*B. bassiana* B16); 172.56% (*L. lecanii* L16); 105.49% (*M. anisopliae* M16); and 90.82% (*B. bassiana* B10). Also, secondary metabolites of entomopathogenic fungi could be increasing total leaf per plant by 91.70% (*L. lecanii* L16); 69.66% (*B. bassiana* B16); 54.55% (*B. bassiana* B10); and 49.20% (*M. anisopliae* M16). The treatment of *B. bassiana* B16 is thought to be able to influence growth (plant height and number of leaves) because it causes the highest pest mortality so that the potential for the virus is reduced. According to Yin et al. (2003), the sub-phase of plant growth and development can be divided into three growth stadia, namely rapid growth in the beginning (vegetative); linear growth; and the saturation phase for aging or fruit ripening. According to El-bassiony et al. (2010), increasing vegetative growth of plants was carried out to obtain plants with good vigor. The effort to keep plants from becoming infected with disease is also a step to maintain vegetative growth of plants. Therefore, the treatment of secondary metabolites of entomopathogenic fungi can increase vigor in the form of plant height and a higher number of leaves because it can reduce the intensity of disease.

Relationship between pest mortality and intensity of disease

Calculation of the correlation value and regression between pest mortality and disease intensity can be seen in Table 5.

The Pearson analysis result showed a correlation between pest mortality (adult whitefly) and disease intensity (virus vector) by -0.9653. Also, the regression analysis resulted in $Y = 95.642 - 1.287X$.

Graph of the relationship between pest mortality and disease intensity (Figure 1), it is known that the inverse nature between pest mortality and pest intensity is proven (negative correlation value results). Based on the results of calculations with the Pearson formula in Table 5, it is known the value of correlation between pest mortality and disease intensity is (negative) -0.965. This value can be categorized high or very closely with the negative nature which means the opposite. This is in accordance with the explanation of Gerling et al. (1980), the population of *B. tabaci* whitefly infestation greatly influences the intensity of disease in an agroecosystem, causing significant loss of yield if not managed properly. Regression calculations are performed to find out further relationships between the two variables. Based on the graph of the relationship between pest mortality and disease intensity, it is known that the inverse nature between pest mortality and pest intensity is proven. That is if there is an increase in mortality in *B. tabaci* will cause a decrease in the intensity of the disease due to viruses carried by vector pests. According to Horowitz et al. (2011) increasing the vulnerability of active ingredients to control *B. tabaci* populations can reduce the potential of viruses that can infect plants.

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