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The modification of organic fertilizer and planting technique of mustard (*Brassica juncea* L.) response to arthropod diversity

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Abstract

Based on the results of the 2015 author's survey, it was found that farmers to control pest attacks in mustard production centers in the Landasan Ulin Utara Subdistrict, Banjarbaru City always use synthetic chemical pesticides with frequency of use, average dosage, and high average concentrations ranging. This can result in the death of natural enemies of these pests and a decrease in the population of natural enemies as natural control of pests in the field; the occurrence of pest resistance to these pesticides; the occurrence of pest resurgence and the emergence of residues that have a negative impact on the environment, so it is necessary to do agroecosystem management by providing organic fertilizer, how to plant and use of *B. thuringiensis*. This study aims to study the interaction and combination of organic fertilizer, intercroping plants and using of *B.thuringiensis* on mustard planting to the diversity of arthropods. The research method used was a factorial design of a split plot design with three replications consisting of 3 factors, namely organic fertilizer (chicken manure and water hyacinth compost); intercroping plants (leeks and basil), and *B. thuringiensis*.. The conclusions from the results of this study are no interaction between organic fertilizer, intercroping plants and *B. Thuringiensis* on arthropod diversity. The mustard plantations have high arthropod diversity which indicated by diversity index number about 3.357, 3.307 and 3.291 on intercropping treatment between mustard with basil, chicken manure and water hyacinth compost without being applied *B.thuringiensis,* respectively.

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Introduction

Mustard (*Brassica juncea* L.) is one of horticulture commodities classified into important vegetable type which is very popular in Indonesia (Zulkarnain, 2010). Mustard plant is a family cruciferae (brassicaceae) with cabbage, broccoli and radishes or rades, so the morphological properties of the plants are almost identical to root systems, stems, flowers, fruits (pods) and seeds In Indonesia the mustard may grow in low-temperature, lowlands and high temperatures. Mustard contains complete nutrition and qualifies for people's nutritional needs (Edi *et al*., 2010), it can be consumed raw as vegetables or processed in various forms of cuisine. Mustard has a very promising prospect and high economic value, because the harvest of mustard always sells in the market, both local and regional markets and even exported to foreign countries.

During mustard cultivation, the one of the problems is attack of pests such as tritip caterpillars (*Plutella xylostella* Linn), crocodile (*Crocidolomia binatalis* Zell), grayak worm (*Spodoptera litura* Fabricius), ground worm (*Agrotis ipsilon*) and others (Gazali, 2011; Dosdall,*et al*., 2011; Loganayagi, 2014; Munir, *et al*., 2015). It is interesting to develop technique of planting to overcome the problem. The one way to managed agro ecosystems such as providing organic matter (Shekhawat, *et al*., 2012), intercropping technique which can increase arthropod diversity (Plaza and Oilseed, 2010), and use of bioinsecticide (*B. thuringiensis*) which has high selectivity and its effect as a new poison can be seen if ingested by insect pests, so relatively safe for other insects that do not eat parts of plants containing poison of *B. thuringiensis* (Novizan, 2002; Federici *et al*., 2010). The advantage of agro ecosystems management couldbe more resistant to pest explosions. The goal of agro ecosystem management is to create a balance inthe environment, sustainable yields, biologically managed soil fertility and pest population regulation through biodiversity and low input use (Altieri, 1999).The higher the ecosystem biodiversity, the more stable the ecosystem is, while the simplification of ecosystems causes ecosystem instability (Untung, 1993a).Previous studies were observed the diversity index of equity and index of arthropod wealth on intercropped agricultural land of apples and broccoli resulted in a diversity index value of 2.73 which was included in the medium category; evenness index of 0.82 which approaches the value of 1 which means it has evenly distributed evenness and stable conditions; and richness index of 4.72, (Amin *et al*., 2016). However, there is little study to investigate the combination of intercropping treatment, use of types of organic fertilizer (chicken manure and water hyacinth compost) and the application of *B. thuringiensis* to diversity of arthropods. This study focuses more on interactions between treatments for the diversity of arthropods in mustard greens.

This research is expected to be found a sustainable agriculture system and environmentally friendly form of agro ecosystem management that can increase the diversity of arthropods. The pattern of agro ecosystem management is using organic fertilizer, intercropping plant and bioinsecticide in the form of pesticide which is active from *B. thuringiensis* which final goal isprofitable economically and ecologically sustainable. This study aims to investigate the interaction between organic fertilizers, intercropping and *B. thuringiensis* on arthropod diversity and to evaluate the combination of organic fertilizer, intercropping and *B thuringiensis* on arthropod diversity.

Materials and methods

Place and time

This research was carried out in the center of mustard plants in organic wetlands in Sub-District of Landasan Ulin Utara and in the Entomology Laboratory of the Faculty of Agriculture, Lambung Mangkurat University, Banjarbaru. The research was conducted in June - August 2015.

Materials and tools

The materials used in this study was Kumala mustard seeds, Tidore superior basil seeds, Fragran leek seeds, chicken manure, water hyacinth compost, alcohol and *B. thuringensis* under the trade name B-Tox.The tools

used in the study are soil processing equipment, maintenance tools, stationery, sweep net (insect nets), pittfall trap, yellow trap, light trap, binocular microscope, killing bottle, and camera.

Statistical analysis

Technique of variable using Factorial Design of Split Plot Design with 3 replicates consist of 3 factors and each factor consists of 3 levels.

Factor 1 is organic material consisting of:
O_0: Without organic fertilizer;
O_1: Add chicken manure for 20 ton/ha;
O_3: Add water hyacinth compost for20ton/ha.
Factor 2 is intercropping plant consists of:
I_0: Crop monoculture of mustard;
I_1: Intercropping of mustard –leeks;
I_2: Intercropping mustard – basil
Factor 3 is the *B. thuringiensis* application consisting of:
B_0: Without application *B. thuringiensis*;
B_1:Applied*B.thuringiensis* as recommended;
B_2:Applied*B.thuringiensis*2timesthe recommended dosage.

For this design variable, could be illustrated the influence of each factors
in equation below:

$$Y_{ijkn} = \mu_{ijkl}+K_i+B_j+E(a)_{ij}+O_k+E(b)_{ik}+(BO)_{jk}+E(c)_{ijk}+I_l +(BI)_{jl}+(OI)_{kl}+(BOI)_{jkl}+E(d)_{ijkl} \ldots\ldots(Eq.\ 1)$$

Where:

Y_{ijkl} = Observation value
μ_{ijkl} = Average treatment
B_j = Effect of vertical factor treatment B
$E(a)_{ij}$ = Influence of error a
O_k = Effect of horizon factor treatment O
$E(b)_{ik}$ = Influence of error b
$(BO)_{jk}$ = Effect of interaction B with O
$E(c)_{ijk}$ = Influence of error C
I_l = Effect of horizontal factor treatment I
$(BI)_{jl}$ = Influence of interaction I with B
$(OI)_{kl}$ = Effect of interaction O with B
$(BOI)_{jkl}$ = The effect of interactions I, O and B
$E(d)_{ijk}$ = Influence of error d

The number of experimental was 81 plots with a combination of treatments (Table 1), the size of plot 2 × 5 m and the distance between plots about1 m.

Seeding of basil seeds

The basil seed was seeded in the nursery plot. After 1 month, the basil seedlings ready to plant in the experimental plot according to treatment with 20 x 20 cm spacing. The number of basil seeds is 20% of the number of mustard per plot of 43 basil seeds per plot.

Seeding of mustard seeds

The mustard seeds a same treatment with basil seeds. After 15 days, the mustard ready to planted according to treatment to the experimental plot with a spacing of 20 x 20 cm.

Seeds of leeks

Seed of leeks was obtained from farmers which have growth for 2.5 months. The leeks seed was planted in experimental plots according to treatment with 20 x 20 cm spacing and 43 leeks seeds per plot.

Soil processing

Soil processing was done by digging the soil becomes loose and made plot treatment with the size of 2 x 5 m plot with the distance between plots in 1 m group.

Concentration of organic fertilizer

Chicken manure and water hyacinth compost was mixed with land 15 days before the mustard seedlings, the concentration was 20 kg per plot. Then planted 15 day's old mustard seeds as much as 215 plants per plot experiment.

Intercropping

Leeks seed and basil seed, each contains of 43 seeds were planted randomly among the mustard plants. One of experimental plot is containing of 172 plants. For all treatment in one plot, it was need 215 seeds of mustard.

Application of *B. thuringiensis*

The concentration of *B. thuringiensis*is1liter of water and 10 g B-tox per plot for recommended dosage, in order hand the treatment also using twice the for the dosage.

The *B. thuringiensis* was applied to spray every week, on 7 days to 21 days of plants growth before harvest or maximal have 3 times spraying. There is have different harvest time of mustard (25-30 days) and intercropping plant (basil and leeks, 2 month) so that the planting of intercropping plant earlier for one month and the followed by planting of mustard.

The object of observed for those treatments were the number and types of arthropods found in the experimental plots.

The investigation was conducted for 7 days, 14 days and 21 days. Arthropods was captured by a pitfall trap of 5 pieces per plot with placed diagonal for 24 h. Light trap 1 fruit per trial, placed in the square at night for 6 h. In addition, a yellow trap tool is placed in the middle of the trial plot each one yellow trap per trial is installed during the day for 6 h, besides it is also used sweep net with 10 double swing.

Arthropods caught during the study were included in the bottles for each plot and were given a plot code experiment then grouped according to the species, and performed a computational calculation.

Identification of parasitoids and predators, pest insects and other arthropods was conducted based on identification keys made by Boucek (1988).

Data analysis for the three stages of the study were conducted as follows:

Index of diversity (H) according to Shannon - Wiener (South wood, 1978; Ludwig and Reynold, 1988).

$$H = -\sum_{i=1}^{Sobs} pi\ lnpi$$

Where:

pi:Σni / N

H:Shannon-Wiener Diversity Index

pi:The number of individuals of a species / total number of species

ni:Number of individual species i

N: Total number of individuals.

Index of Evennes(E) according to Pilou(Ludwig and Reynold, 1988).

$$E = \frac{H}{\ln S}$$

Where:

H:Diversity index

S: The whole type

Index of Species Richness (R) according to Margalef (Ludwig andReynold, 1988).

$$R = \frac{S-1}{\ln N}$$

Where:

R = Index of species richness

S = Number of species

N = Number of individual species

Index of Domination (D) according to Simpson (South wood, 1978, Ludwig & Reynold, 1988)

$$D = \sum_{i=1}^{Sobs} (ni/N)^2$$

Where:

D = Index of domination

ni = Number of individuals per species

N = Number of individuals of all species

The effect of combination of treatments (organic fertilizer, intercropping and *B. thuringiensis*) on the above indexes for each plot experiment was using Randomized Block Design Variant Group with Strip-Plot Design pattern.The effect of treatment was

determined from the F value and the mean value is differentiated by different standards using Duncan's Multiple Range Test (DMRT) by using Statistical Product and Service Solutions (SPSS) 17.0.

Results and discussion

The results of arthropod capture using pitfall trap, yellow trap, light trap and sweep net in the first phase of the study were found 9 orders, 31 families and 39 species. The results showed that all mustard plants that were given organic fertilizer, intercropping and *B. thuringiensis* applications had higher natural enemy populations (predators and parasitoid) than the pest population. The treatment that had the highest natural enemy population was found in the intercropping treatment between mustard and basil given chicken manure and without the application of *B. thuringiensis*. Mustard greens which were not treated with organic fertilizer, intercropping and the application of B. thuringiensis population were higher than their natural enemy populations (predators and parasitoid).

Table 1. The Combination Treatment.

Factor		B_0	B_1	B_2
	I_0	$O_0I_0B_0$	$O_0I_0B_1$	$O_0I_0B_2$
O_0	I_1	$O_0I_1B_0$	$O_0I_1B_1$	$O_0I_1B_2$
	I_2	$O_0I_2B_0$	$O_0I_2B_1$	$O_0I_2B_2$
	I_0	$O1I_0B_0$	$O_1I_0B_1$	$O_1I_0B_2$
O_1	I_1	$O_1I_1B_0$	$O_1I_1B_1$	$O_1I_1B_2$
	I_2	$O_1I_2B_0$	$O_1I_2B_1$	$O_1I_2B_2$
	I_0	$O_2I_0B_0$	$O_2I_0B_1$	$O_2I_0B_2$
O_2	I_1	$O_2I_1B_0$	$O_2I_1B_1$	$O_2I_1B_2$
	I_2	$O_2I_2B_0$	$O_2I_2B_1$	$O_2I_2B_2$

This is because predators are polyphagic so that they never lack food for breeding and predators also have high search capacity so they can reduce pest populations. This is supported again by a system of farming with intercropping resulting in the availability of pests as prey from predators and hosts of parasitoid. According to Price *et al.* (1980) and Wallner (1987) the population of many phytophagic insects is largely determined by predators. Predators are often generalists that eat various prey species, so that predators are easy to survive especially in multipelcroping farming systems.

The variance analysis with significance level of 0.05 using Statistical Product and Service Solutions (SPSS) 17.0 found that there is no interaction between type of organic fertilizer with intercropping plant, organic fertilizer with the use of *B. thuringiensis*, the concentration of *B. thuringiensis* with intercropping plants, and also there is no interaction between the types of organic fertilizer, intercropping plants and using *B. thuringiensis*. Another result, there is no single factor effect of *B. thuringiensis* application on arthropod diversity, whereas single factor intercropping, and organic fertilizer significantly affect arthropod diversity (Table 2.).

Based on the DMRT test, it was found that the highest index of arthropod diversity was obtained in experimental plots by using chicken manure and water hyacinth compost, intercroping between mustard and basil of 3.307, 3.291 and 3.357, respectively. All of indexes are including the criteria of high diversity level.

The lowest index of arthropod diversity was found in mustard plantation that it was no treatment by organic fertilizer with the index value of diversity 2.933 and in the treatment of traditional mustard, with value of diversity index of 2.964, both indexes include the criteria of moderate diversity (Table 3 and Table 4).

Table 2. Summary Analysis Of The Range Of Interactions Between The Treatment Of Organic Fertilizer Application, Application Of Intercropping Plant Types And *B. Thuringiensis* Application To The Diversity Index.

Treatment	Significance (Sig)
Types of Organic Fertilizers	0,038*
B. thuringiensis	0,097*
Types of intercropping plants	0,000*
Interaction between organic fertilizer with B. thuringiensis	0,850
Interaction between organic fertilizer and intercropping plants	0,290
Interaction between B.thuringiensis and intercropping plants	
Interaction between organic fertilizer, B.thuringiensis with	0,800
intercropping plants	0,837

Note: Significance (Sig) <5% means there is interaction.

The diversity index is depend on species and density level of arthropod population. The diversity is consist of richness and evenness (Odum, 1996 and Mchart*et al.*, 2012). The index of richness is the number of species in the community, while the level of evenness is the distribution of all individual within a community.In other hand, there is no interaction between organic fertilizer and intercropping, organic fertilizer *and B. thuringiensis,* types of organic fertilizers, intercropping plants and the use of *B. thuringiensis* against arthropod diversity. Interestingly, it has interaction between doses of *B. thuringiensis* with intercropping plants. This interaction appear because arthropods influenced by organic fertilizers are ground or surface arthropods. The arthropods play a role as decomposer arthropods which are very useful in the process of food webs and their results are exploited by plants as organic fertilizers (Odum, 1996). While intercropping influenced arthropods are plant-eating arthropods (plant pests), predatory arthropods and plant pest parasitoids. Plant-eating arthropods can cause harm because they are pests that attack cultivated plants.

Predatory arthropod and parasitoids that act as natural enemies of plant-eating arthropods (Mahmoud, *et al.*, 2013; Tonfack *et al.*, 2009; and Xu, *et al.,* 2011).

Table 3. Arthropod Diversity Index, Richness Index, Evenness Index on Planted Mustard with Organic Fertilizer.

Type of Organic Fertilizer	Diversity Index	Richness Index	Evenness Index
Without organic fertilizer	2.933[a]	4.413 [a]	0.964 [a]
Water hyacinth compost	3.291[b]	5.478 [b]	0.986 [b]
Chicken manure	3.307[b]	5.478 [b]	0.987 [b]

The mean value of the diversity index of the type followed by the same letter is not significantly different based on the DMRT test at the 5% level.

Planting of mustard given single factor treatment of organic fertilizer and single factor intercroping effect on arthtropoda of richness index. The highest treatment by chicken manure and water hyacinth compost, which have high level of richness index up to 5.478. Low species richness was found in the treatment without organic fertilizer about 4.413, this number is medium level of richness. The influence of single factor of organic fertilizer to Index of arthropod evenness is shown the higher number of 0.987 and 0.986 which treatment by chicken manure and water hyacinth compost, respectively. Index of arthropod evenness values approaching to 1 means the spreadi of the number of individuals of each species is not

much different or evenly distributed. The lowest number of arthropod evenness index was treated without organic fertilizer about 0.964 (Table 3.). The Single factor intercroping, and organic fertilizer significantly affect the diversity of arthropods, it is due to intercropping and organic fertilizer treatment that can increase species richness, and the evenness index of the species causing an impact on diversity. The influence of single intercropping factor on arthropod richness index in the mustard plant and basil is shown high number of 5.695, it give higher number about 20.2% than that planting of mustard without intercropping (Table 4).

Table 4. Mean of Arthropod Diversity Index, Richness Index, Evenness Indexon Planted Mustard with Intercropping.

Intercropping	Diversity Index	Richness Index	Evenness Index
Monoculture of mustard	2.964 [a]	4.545 [a]	0.965 [a]
Intercropping mustard with leeks	3.211[b]	5.202[b]	0.985[b]
Intercropping mustard with basil	3.357[c]	5.695[c]	0.986[b]

The mean value of the diversity index of the type followed by the same letter is not significantly different based on the DMRT test at the 5% level.

The high index value of arthropod diversity wasobtained in type of experimental plots of chicken manure, water hyacinth compost, intercropping of mustard with basil. The lowest biodiversity index was found in monoculture treatment of mustard greens which were not given organic fertilizers. According to previous researcher that polyculture using attractant plants randomly represents a cropping pattern that tends to determine the diversity of the highest predator arthropods (Wardani and Nazar, 2002).

In addtition, the richness of insect species and insect diversity are positively correlated with plant species richness(Haddad, *et al.*, 2000). The C-organic content of compost and chicken manure can be a source of food for soil insects and soil surface insects, so that the index of species richness in mustard crops treated with chicken manure and compost could be increased. The C-organiccontent in chicken manure is 31.80% higher than that compost fertilizer only about 24,%, by using of poultry fertilizer has the highest index value of arthropods species diversity (Shanker and Padmavathi, 2011).

The effects of organic farming system have a positive influence on species richness, evenness, and abundance compared with conventional farming system(Tuck *et al.*, 2014; Bengtsson and Weibull, 2005) and also organic farming system can increase biodiversity(Letters *et al.*, 2010;Pfiffner and Balmer, 2011). The effects of organic farming contributes to the conservation of biodiversity, especially for insects (Montañez and Amarillo-Suárez, 2014).The maximum abundance and total individuals are found on organic land. Organic farming practices could bring in natural enemies and pollinators from the outside. (Anbalagan and Ignacimuthu, 2015). In order hand, the effect of treatment type also effect to the mustard itself.

As shown in Fig. 1, the mustard fertility clearly observe by development and number of leaf growth. Intercropping of mustard with basil treatment by chicken manure (Fig.1a) and water hyacinth compost (Fig.1b) almost have same leaf growth. In the case of monoculture of mustard without organic fertilizer (Fig.1c) shown the lack of number of leaf, it because there is no additional supply nutrient for growth.

The diversity will increase because addition of a species and it has same density distribution (Ludwig and Reynold, 1988). The diversity and abundance of insects in general will also be determined by environmental factors. Each type of insect has a certain suitability to the environment. Therefore, the environmental phenomenon is very influential such

as temperature and humidity. Temperature is an environmental factor that determines or regulates insect life activities. At a certain temperature, the activity ofinsect life is high very active, while at other temperature insect activity is low (less active). The air humidity plays a very big role on the water content of the insect body, and the life cycle of insects so that organisms organize and the spread of insects (Khaliq, *et al.*, 2014).

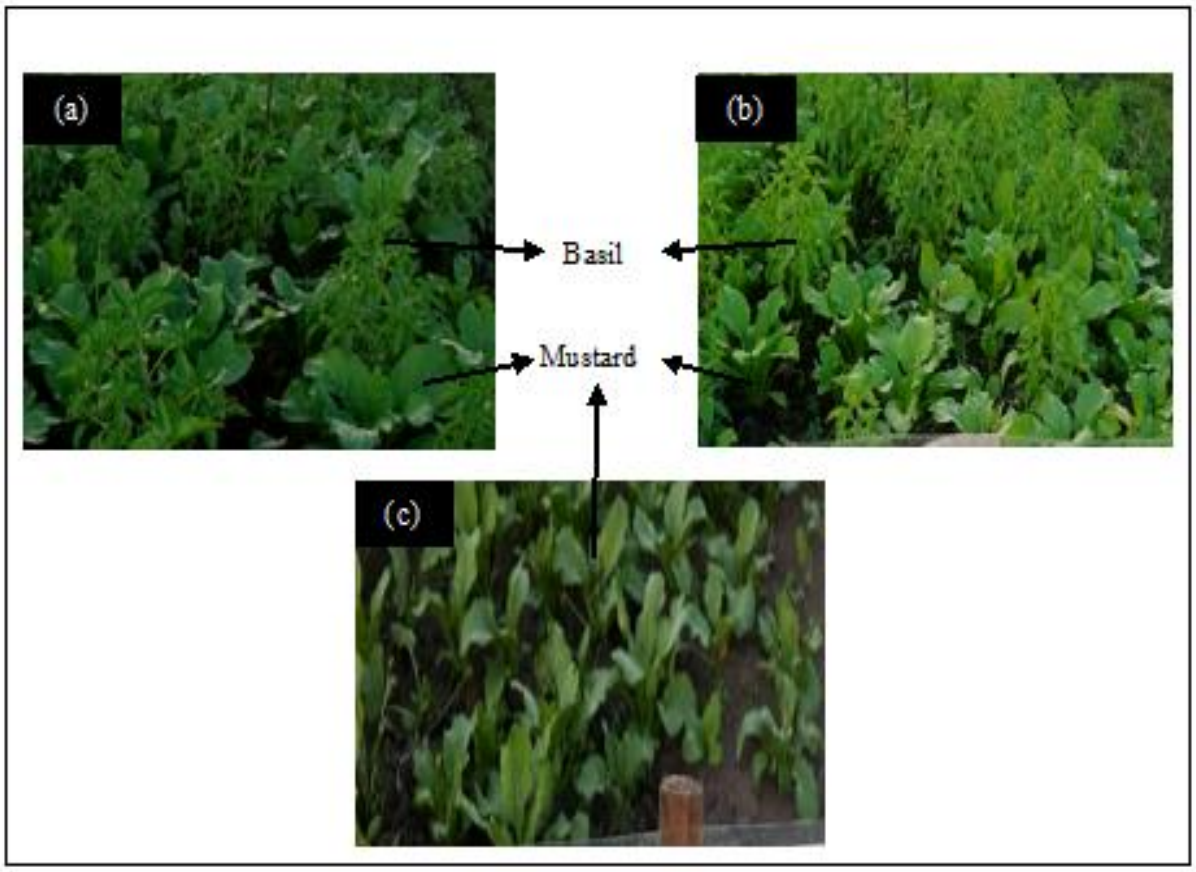

Fig. 1. Intercropping of mustard with basil treatment by chickenmanure (a); water hyacinth Compost (b); Monoculture of mustard without organic fertilizer (c).

The single factor of *B. thuringiensis* application also has no effect on arthropod diversity, it shown by no effect to number and size of species, because*B. thuringiensis* application is densely packed with pest populations, so that it does not reduced number of species (Gazali, *et al.,* 2015)). Single factor of intercropping and organic fertilizer significantly affect to arthropods diversity, it shown by interaction between organic fertilizer application and *B. thuringiensis* application. Another result is shown that the*B. Thuringiensis* application and interaction between *B. thuringiensis* application and intercropping treatment did not affect to index of arthropod richness. The influence of a single factor on the dominance index on organic fertilizer treatment, intercropping, and application of *B. thuringiensis* did not affect the dominance index. This is due to fertilizer use of organic, intercropping and application of *B. thuringiensis* does not cause a decrease in one or several species of arthropods so that there is no dominance of one or several species of arthropods against other arthropod species (Hongjiao, *et al.*, 2010; Montañez and Amarillo-Suárez, 2014; Sisterson *et al.*, 2004).

Conclusion

Based on the results, it can be concluded that no interaction between organic fertilizer, intercropping plants and *B. thuringiensis* on arthropod diversity. The mustard plantations have high arthropod

diversity which indicated by diversity index number about 3.357, 3.307 and 3.291 on intercropping treatment between mustard with basil, chicken manure and water hyacinth compost without being applied *B.thuringiensis,* respectively.

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