THE EFFECT OF MILK FERMENTED WITH PROBIOTIC BACTERIA SUPPLEMENTATION ON HAEMATOLOGIC CONDITION OF BROILER

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ABSTRACT

The effect of probiotic supplementation in fermened milk on broiler hematologic condition, (the number of erythrocyte, hemoglobin and hematocryte value),used 100 broilers, and used Completely Randomized Design, with four treatments:R0=as control, R1= 0,5% probiotic, R2 = 1,25% probiotic, R3= 2,00% probiotic, which was five times repeated. The resultsshowed that theprobiotic supplementation up to1,25 percent, had no significantcy(p>0.05) on hematologic condition, but 2 percent probiotic supplementation has significantly increased the hematologic condition.

Key Words :*Probiotic, Haematologic, Erythrocyte, Haemoglobine, Haematocrite Value*

INTRODUCTION

Probiotic is classically defined as a microbial dietary supplement that beneficially affect the host through its effects in the intestinal tract . This definition however, was initially intended for use with animal feed. Probiotic microorganisms that have a favourable influence on physiological processes of the host by their effect on the intestinal flora may play a role in improving human health(Ericson and Nail, 2000). Erythrocytes contained hemoglobin that carries oxygen from the lungs and will be released into the tissue, and also binding carbon dioxide in tissue that will be brought to the organ secretions. Hemoglobine functions such as respiration and blood pigment as a buffer in the blood system, which is closely related to the ability of blood to carry oxygen. Non-pathogenic microbes such as *Lactobacillus bulgaricus*and *Streptococcus thermophillus* are capable of producing lactic acid and some amino acids and vitamins produced by microbes, and also as a precursor for the formation hemoglobin.

Microbes that classified as lactic acid bacteria (LAB) have a high microbial activity due to the resulting product will inhibit the growth of pathogenic bacteria that can damaged the cell membrane permiability and ended with the destruction of the cell wall, resulting in the release of hemoglobin from the cell. Fe has a large influence on the formation of blood hemoglobin. Fermented milk contains complete mineral,and will absorbed in the small intestine,and because has a low molecular weight, the compounds are soluble in water. Calcium and phosphorus are essential for the growth of bones and teeth, while iron in building muscle, skin and eggs of red blood cells, stimulates nerves, maintaining muscle elasticity and maintain osmotic pressure (Surono, 2004). Organic acids produced by lactic acid bacteria, mainly lactic acid and acetic acid to help stimulate activities of gastric the rate of passage become slower, and cause increased absorption of nutrients as a result of metabolic processes in the body and substance formation of red blood cells and hemoglobin will increase.

Oxidation reactions can occur in body cells and damage the body, especially the cell membrane. One of the important body of the cell membrane is the erythrocyte membrane. Oxidation reaction on erythrocyte membranes will result in damaging erythrocytes, will eventually lower the body resistance and response to disease. Erythrocyte damage would cause metabolic disorders at least will affect the productivity of livestock. The damage can be inhibited by the ability of lactic acid bacteria which can suppress the growth of various gram-positive bacteria and gram negative. Suppression process is influenced by the production of hydrogen peroxide inhibits the growth of pathogenic bacteria through the powerful influence of oxide on bacterial cell or through the destruction of the basic molecular structure of nucleic acids and proteins of cells and production of special proteins called bacteriocin.

Previous studies in rabbit which are given yoghurt containing *Lactobacillus bulgaricus and Streptococcus thermophillus* 2.00% of body weight, can increase the number of erythrocytes, hemoglobin, and hematocrit values significantly. Studies conducted on rats using of yoghurt containing *Lactobacillus bulgaricus, Streptococcus thermophillus, Lactobacillus acidophilus, and*

Bifidobacterium of 1.25% and 2.00% of body weight can increase the number of erythrocytes, hemoglobin and hematocrit values.

MATERIALS AND METHODS

Bacteria Strain: Two bacteria used in this research, are *Lactobacillus bulgaricus* and *Streptococcus thermophilus* as yoghurt culture starter.

Animal : one hundred broiler cp 707, day old chick, the treatment given from day old chicks until six weeks.

Experimental Design, broiler were randomly devided into 4 treatments groups with 5 replications. The treatments consisted of probiotic yoghurt, R0=control , R1= control + 0,5% probiotic, R2 = control + 1,25% probiotic, R3= control + 2,00% probiotic

Data were analysed in a Completely Randomized Design (CRD). Further analysis for significantly was conducted using Duncan Test, the parameter observed were : the number of erythrocyte, hemoglobin, and hematocrit value.

RESULT AND DISCUSSIONS

Table 1, The results of the effect of treatment with addition of probiotic (Lactobacillus bulgaricus, Streptococcus thermophillus) on the number of erythrocyte, hemoglobin, and haematocrit.

Tabel 1. Effect of the treatment on total erythrocyte,haemoglobine, hematocryte value

Treatment	Erythrocyte	Hemoglobine	haematocryte
	(x 10^6) alls/mm^3	g/ml	%
Ro	2,27	8,10	31,4
R1	2,32	8,38	31,6
R2	2,88	9,01	32,4
R3	3,04	9,22	35,5

Notes :

R0 : control

R1 : control +0,5% from body weight

R2 : control +1,25% from body weight

R3 : control +2,00% from body weight

Based on Table 1, R3 treatment has total erythrocyte, significantly higher compared with the treatment R0 and R1, while R2 and R3 has not significancy. The highest hemoglobin levels achieved in R3, that significantly higher than R0 and R1, while R2 and R3 showed no significancy. The highest hematocrit value achieved by the R3 treatment was significantly; compared with the treatment of R0, R1 and R2.

Lactobacillus produce metabolite on increased the size of new cells,and will influence the body weight gain, and also improved metabolism*Lactobacillus bulgaricus* and *Streptococcusthermophillus, Lactobacillus Acidophilus*it can increase the elasticitythe cellular membranes thereby improving cell membranes, which in turn will improved the ability of erythrocytes to maintain the integrity of the membranes.

According, to 1997 Lovita, 2005,the organic acids produced by lactic acid bacteria such as lactic acid and acetic acid can protect the cell membranes and damaged other subcellular oxidation reaction by the peroxide bond.

Lactic acid bacteria which can increase the elasticity of cell membranes so that it will produce better cell membrane, which in turn will improve the ability in maintaining erythrocyte membrane integrity (Ganong, 1985). Other factors that affect their ability to both bacteria, will survives in the lower acid to base environment (Fuller, 1992). The addition of yoghurt are still within the criteria of normal erythrocytes. Acetic acid , some amino acids and vitamins produced by microbes, is a precursor to the formation of hemoglobin.

Acetic acid is inferred that changing in the cycle become keto glutarat kreb-alpha acid, and then binded the ketoglutarat two-alpha acid with one molecule of glycine to form pyrole compounds. The next four pyrole compounds to form compounds protoporfirin. One compound, known as n protoporfirin III, when binded with iron will form a molecule hem. Finally, four molecules bind one end of the globin molecule, the formation of hemoglobine, which in turn will increased the amount of hemoglobin (Guyton, 1985).

According to Swenson (1970), increasing in hemoglobin and erythrocyte number will be in line with the increase in hematocrit value because there is a

positive relationship between the three components.The overall results indicated that administration of probiotic (Lactobacillus bulgaricus and Streptococcus thermophilus) on the dose up to 2% of body weight, will increase the amount of value erythrocyte, hemoglobin and hematocrit, still in the normal range.

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THE EFFECT OF CLOVE OIL DOSAGE ON POPULATION OF *Callosobruchus maculatus* F., Seed VIABILITY AND VIGOR OF TWO SOYBEAN CV AFTER THREE MONTHS STORAGE DURATION[1]

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ABSTRACT

The objective of the experiment was to study the effect of clove oil dosage on controlling storage weevil *C maculatus* F., and maintaining seed quality including seed viability and seed vigor of two soybean cv afer three months storage duration. The experiment was conducted in Laboratory of Seed Technology ,Faculty of Agriculture Padjadjaran University, Jatinangor, Sumedang, West Java, from March until May 2009. The experiment design used was Split Plot Design consist of two factors and replicated three times. The main factor was cultivar of soybean, were Anjasmoro (big size seeds) and Sindoro (medium size seeds). The sub factor was bio protectant of clove oil dosage, which consist of five levels, were : without clove oil, 0.5 mL kg^{-1} soybean seed, 1.0 mL kg^{-1} , 1.5 mL kg^{-1} soybean seed, and 2.0 mL kg^{-1} soybean seed. All of the experiment consist of 45 units experiment , where every experiment unit used 75 g of seed. Seeds and five pairs of storage weevil were fill in plastic cup and placed in plastic container during three months storage. The result of experiment showed that there were interaction effect between cultivars and dosage of clove oil on seed damage percentage after three months storage, but there were no intercation effect on another parameters was observed. Sindoro has better storability than Anjasmoro, as indicated by weevil population, seed damage percentage, germination capacity, vigor index and seed moisture content. Dosage of clove oil 5 mL/kg soybean seed showed better affected on suppression of storage weevil and maintaning seed viabilty and vigor.

Key words : Clove oil, *C maculatus*, Seed viability and Seed Vigor.

INTRODUCTION

Soybean is one of the third important food crop in Indonesia after rice and corn. Soybean seed contain approimately 400 g kg^{-1} protein and 200 g kg^{-1} oil (Fehr,1987 cited by Egli and Crafts- Brandener, 1996) and is used as a source of edible vegetable oil, forage enrichment , and many traditional food product. Most of Indonesian feeds of traditional food product made from soybean seeds.

Soybean needs seriously handling for full fil domestic consumption, because has economic strategic value. Demand of soybean grain showed indicated increasing paralel with population growth . Until now domestic production still lower than national demand. To full fill domestic demand, Indonesia government policy imported soybean grain until 750 metric ton – 1 million ton per year from several country (BPS, 2010).

One of the effort to full fill domestic demand for increasing soybean yield productivity used was high quality seeds following by cultivated technology application and extensification cultivated area. Since 90's decade Indonesian Agriculture Department has strategic issue to support food strengthening program particularly for increasing used high quality seeds. Which government policy recognized with the six of seed program to solved the problem in seed quality stock during planting time. The six of seed program government policy include the approriate number of high seed quality , appropriate seed quality, appropriate specific variety , appropriate planting time, approriate agroclimat zone ,and approapriate price properly. But , until now a lot of farmer used non certified seed, because in several region has gap between planting time with stock of seed. Other reason , farmer considered seed price still expensive.

Seed storage is one of solution to solve the problem lack of high quality stock during planting time, usually during dry season. But some of weakness of soy bean seed is has short longevity in bad storage system . Soybean food reserve are rich of protein and lipids could generate fast to deteriorate if seed placed in bad storage . To avoid this problem needs appropriate storage technology , particularly in pest storage cotrol without decreased seed viability or vigor.

Seed deterioration during storage duration affected by seed properties characters and environment storage condition include biotic and abiotic factors. Seed properties were seed coat characteristic, food reserve chemical composition, seed viability and vigor , moisture content , and genetics (ortodox or recalsitrant) . Abiotic factor were temperature, relative humidity and light intensity during storage, while biotics factor affected seed viability during storage

is insect and fungi (Šimic *et al* ., 2006 ; Copeland and McDonald,2004 ; Justice and Bass,2002) .

One of the alternative way to maintaning seed viablity and vigor during storage is to controlling growth of pest storage. Seed moisture content adjustment could effective to protect pest and microorganism invasion. Moisture content 8-9 % inhibit insect and microorganism invasion, while moisture content between 12 % - 18 % not safe for seed storage , seed is easy invasion by insect and fungi (Imdad, 1999). Moisture content safe for storage of food crop storage and horticulture is 9 – 12 % (Direktorat Jendral Tanaman Pangan dan Hortikultura, Direktorat Perbenihan, 2000 ; Interntional Seed Testing Association, 2003) .

In tropical and sub tropical region were three species of *Callosobrochus i.e. C maculatus* F. is one of the storage pest usually invasion soybean seed during storage, *C chinensis* invasion mung bean seed and *C analis* (F) invasion tunggak bean (Anwary *et al.*, 2004). Imago active in day with life cycle between 21-30 days average at room temperature 30 ^{o} C and Relative Humidity 80 -85 % (Kalshoven, 1981). Primary food reserve was destroyed by *C maculatus* caused significant decreasing of seed viability and vigor capacity due to germination inhibition and produce abnormal seedling. Pest population development can be inhibit by sintetic protectant, but had negative impact on environment safety. One of effort to solve this problem used natural protectant or bio protectant made from clove oil. Clove oil is volatile oil contain 70-85 % eugenol , which oil vapour have long period aromatic (Guenther, 1990). Kardinan statement (2000) eugenol from clove oil was effective to control *Stegobium paniceum.* The importance of bio protectant not only capable to control pest storage , but able to maintanance seed viability and vigor.

Several experiment result showed proved bio protectant made from clove oil can to supressing pest storage. Sri Dewi Kartika *et al* (2006) and Sumadi *et al* (2009) proven clove oil 2.5 mL kg $^{-1}$ good effect to controlling *Sitophillus zeamais* and maintaining corn seed viability until three months storage. Other experiment study about the effect of clove oil on soybean seed Wilis cultivar , showed 1.5 mL kg^{-1} seed had good effect. Rika Meilasari (2000) proven was used clove oil 1 mL 100^{-1} g seed capable to supress *C*

maculatus develepment without decreasing mung bean seed viability after 4 months storage period.

Negative effect on seed viability can occurred if use higher concentration of clove oil. Seed storage experiment by Rita Enggreni (2000) showed that clove oil 5 mL kg^{-1} seed were reduced soy bean seed viability until 50 % compare with control treatment (without clove oil) after two months storage. How ever storage insect population can reduce until 0 %, while at control treatment insect population increase until 40 % . Other experiment about negative effect used higher dosage clove oil 20 mL kg^{-1} sorghum seeds caused severe seed viability after three months storage (Julia Wingantini, 2005). Also, experiment by Sri Dewi Kartika *et al* (2006) showed that > 5 mL kg^{-1} with direct application caused corn seed deterioration .

Seed response to protectant factor depend on dosage and specific characteristic of seed coat. Seed with thicker seed coat more resist than seed with thinner seed coat. Each soy bean variety have specific chemical composition , seed coat physical properties , seed size and seed coat colour (Tekrony *et al.*, 1987). Variation of seed size depend on cultivar. In Indonesia , soybean seed size classification consist of three categories based on 100 grains weight , ie . small size (6-10 g), medium size (10 – 12 g) and large size (> 13 g). According to cultivar description , Anjasmoro as large size and Sindoro as medium size categories. Seed coat of larger seed size thinner and more permeable than the small one (Monorahardjo *et al* , 1993 cited by Sumadi , 1997) . While seed large size have better vigor (Tekrony *et al* .,1987).

According to explanation some information coud be conclude that the effect of clove oil for controlling storage weevil and maintaining seed viability and vigor after three months storage duration depend on clove oil dosage and soybean seed cultivar . While, soybean seed viability and vigor could be maintanance after three months storage if application by appropriate dosage of clove oil. So, the result of experiment could be done to completed information about seed bank strategic.

MATERIAL AND METHODS

The experiment was conducted at Seed Technology Laboratory, Faculty of Agriculture, except for determine protein content at Chemical Analysis Laboratory , Faculty of Mathematics and Science, Padjadjaran University , Jatinangor , since March 2009 until May 2009.

Two seed cultivar of *Glycine max* L. were used, one from the Balitbiogen and the other from IPB (Bogor Agriculture Institute) , Clove oil were use from P.T. Indrasari Jakarta which contain 70 -85 % eugenol. *C maculatus* imago obtain from SEAMEO , Bogor, and *kertas merang* for germination substrat, plastics sheet with size 30 x 20 cm.

The experiment was arranged in a split plot design with three replications. Two levels of soybean cultivar i.e Anjasmoro (large size seed) and Sindoro (medium size seed) were place in the main plot and five levels of clove oil dosage i.e no clove oil application as control , 0.5 mL kg $^{-1}$ seed , 1.0 mL kg $^{-1}$ seed , 1.5 mL kg $^{-1}$ seed , and 2.0 mL kg $^{-1}$ seed as sub plot. Data were analyzed statistically and mean were separated by DMRT.

All of the experiment consist of 2 x 5 x 3 x3 packet. Each a packet of used 75 g seed was fill in plastic cup and covered by aluminium foil , and then were placed in each container and store under room temperature for one, two and three months respectively. Before storage, seed protect by clove oil with indirect application use small cotton and place at site corner of plastic cup (Sumadi, 2006) and were invested five pairs of *C maculatus* in plastic cup.

One week prior storage were tested seed quality .ie. germination percentage, seed moisture content , vigor index , 100 grains seed weight, and protein content . Each month after storage seed quality was measured. Parameter will be measured after storage include pest population, seed damage by insect, moisture content,weight of 100 seeds , germination rate, electric conductivity value and vigor index.

Seed analysis . Seed quality testing based on ISTA procedure with some modification :

a. Seed moisture content measured by basic or oven methods which seeds drying at 130 ^{o}C during 50 menits . Five grams seed weighing (Fresh weght), followed by drying used oven at 130 ^{o} C during 50 menits and weighing with electrical balance (dry weight). Seed moisture content = FW – DW /FW x 100 %. Each treatment replicated three times.
b. Germination rate was tested by rolled paper test and placed in germinator cabinet. Seedling measured at First Day Count (FDC) at 5th and Last Day Count (LDC) at 8th . Calculation and expression of result are expressed as percentage by Sum of normal seedling divide seed number was tested by 100 %.
c. Seed vigor were determined by vigor index and electric conductivity value.

   IV = G5/D5 + ...G8/D8. Vigor Index showed that speed of germination, which value close to maximum (10) as good quality.

RESULT AND DISCUSSION

The physical and physiological state of seeds as internal factor was greatly influence their life span or seeds storability (Copeland and McDonald, 2004). Seeds that have been broken, cracked, or even bruised deteriorate more rapidly than undamage seeds (McDonald, 1985 and Prietley, 1986). Other seed physical properties affected rapid of deterioration was moisture content and seed size. Even without physical symptoms, seed may be physiologically impaired and become susceptible to rapid deterioration. For instance , seed vigor state before storage affected deterioration rate.

The means of seed quality including moisture content, germination rate or germination capacity, and vigor before storage presented in Table 1. According to result of prior test seed quality, Anjasmoro and Sindoro cultivar seeds have good physical and physiological standard. Prior of storage, the moisture content , germination capacity, and vigor index both of cultivars seed as good quality based on certitifcation standard. Harrington (1972) cited by Copeland and McDonald (2004) have recomendation which moisture content for long duration storage ortodox seed does not above 14 or below 5 %. Seeds

store at moisture content above 14% begin to exhibit increased respiration, heating and fungal invasion that destroy seed viability more rapidly , while below 5 % cause seed membran structure hasten seed deterioration.

Table 1. Mean of Seed Quality before Storage

Seed Properties Characters	**Anjasmoro**	**Sindoro**
Moisture Content (%)	9.62	8.5
Weight of 100 seeds (g)	13.7	8.73
Germination Rate (%)	96.0	98.0
Value of Vigor Index	9.6	9.68
Protein Content (%)	40.31	39.30

Note : maximum value of Vigor index in this case is 10

Protein content as one of the internal factor were influence seed storability. One of protein properties is hydrophilic to vapour water arround of seed. Where soybean seed as one of the legume family with rich protein content usually have short storage longevity. Exception under well storage control , while sealed storage methods at low temperature and low relative humidity able to maintain seed quality.

The analysis of variance result showed that no significant effect of interaction between soybean cultivar and clove oil dosage on weevil population, weight of 100 seeds and moisture content after three months storage. Both of treatment between cultivar and clove oil dosage showed that was independent effect respectively (Table 2).

Table 2. Weevil Population, and 100 grains weight , and moisture content after three months storage duration

Treatment	Weevil Population	Weight of 100 seeds (g)	Moisture Content (%)
Soybean Cultivar			
Anjasmoro	0.33 b	14.40 b	10.36 a
Sindoro	0.00 a	8.91 a	10.66 a
Clove oil dosage			
m_0 (0.0 mL kg^{-1})	0.33 b	11.50 a	10.66 a
m_1 (0.5 mL kg^{-1})	0.00 a	11.61 a	9.62 a
m_2 (1.0 mL kg^{-1})	0.00 a	11.40 a	10.23 a
m_3 (1.5 mL kg^{-1})	0.00 a	12.28 a	11.00 b
m_4 (2.0 mL kg^{-1})	0.00 a	11.49 a	11.05 b

After storage weevil population indicated decrease compare when fisrt invested before storage. Even some of them all of weevil was invested are died. Probably died of weevil in all of treatment are stress in the new place. Anjasmoro seed size bigger than Sindoro seed size .Seed size significantly affected on weevil population , which *C maculatus* like invasion the big one. Development of weevil at place contain the bigger seed size like Anjasmoro cultivar were condusive. The bigger size showed that more succeptable to *C maculatus* invasion than the small one. How ever no significant affected on moisture content , although increasing moisture content was occurred compare before storage. After three months storage duration moisture content both of

seed was increase between 0.74 % - 2.16 % . Although,increasing of seed moisture content still under limited for safe storage.

Seed damage was affected by cultivar and clove oil dosage together. Infleunce of cultivar character particularly seed coat thickness depend on clove oil dosage (Table 3). Increasing of clove oil application showed supressing of pest invasion on seed both cultivar.

Table 3. Seed damage percentage

Kultivar	**Seed damage percentage (%)**			
	m_0	**m_1**	**m_2**	**m_3**
Anjasmoro	**4.00 b**	**2.67 b**	**0.00 a**	**0.67 a**
	D	**C**	**A**	**B**
Sindoro	**2.00 a**	**0.00 a**	**0.00 a**	**0.00 a**
	B	**A**	**A**	**A**

Effect of clove oil dosage on seed damage percentage by weevil depend on cultivar properties. While size of Anjasmoro seed bigger than Sindoro was suspected have thinner seed coat than the small one .Sukarman dan Raharjo (2000) were report their experiment soybean seed with small size have well storability than seed with medium size. Performance indicator as implication of cultivar properties and clove oil application showed in physiological character (Table 4).

Table 4. Seed Germination Rate, Vigor Idex and Electtic Conductivity

Treatment	Seed Germination (%)	Vigor Idex	Electric Conductivity ($\mu S\ g^{-1}$)
Soybean Cultivar			
Anjasmoro	87.07 a	8.55 a	3.35 a
Sindoro	96.67 b	9.57 b	5.15 b
Clove oil dosage			
m_0 (0.0 mL kg^{-1})	92.00 ab	9.03 b	4.32 bc
m_1 (0.5 mL kg^{-1})	88.00 a	8.71 a	3.53 a
m_2 (1.0 mL kg^{-1})	90.00 ab	8.82 ab	4.07 b
m_3 (1.5 mL kg^{-1})	93.33 b	9.24 bc	4.56 cd
m_4 (2.0 mL kg^{-1})	96.00 b	9.50 c	4.78 d

Influence on seed physiological of cultivar character and clove oil are independent. According to value of germination rate and vigor index , sindoro have storability potential better than Anjasmoro.How ever both of physiological character still good quality, germination rate more than 80 % as ISTA standard for certified seed. Also, vigor index is high category . In this case some inconsitency result showed EC value. Good seed have membrane integrity better than bad seed quality with low value , while EC Sindoro seed higher than Anjasmoro. Integrity membrane can determine by measure of electrolite leakage after soaking (Viera *et al*, 2001 ; Copeland and MacDonald, 2004). EC value was measured by EC meter, value of EC indicate degree of membrane destroyed as high of fatty acid content (Tatipata *et al*, 2004 : Tatipata, 2010).

Influence of clove oil on germination rate, vigor index and EC depend on dosage. Which dosage increasing until 1.5 mL kg^{-1} gave germination rate and vigor was increased compare with the lower one , but no significant increase of germination rate and vigor index if dosage add until 2.0 mL kg^{-1} . Like cultivar character, some inconsistent result showed at EC data was observed. Even EC data inconsistency , but all of value from seed testing showed that seed quality still high after storage. This indicate application of clove oil as bio protectant until 2 mL kg^{-1} able to control pest invasion and maintain seed viability and vigor.

Several experiment used clove oil was proved by Rika Meilasari (2000) on control of *C maculatus* development without decreasing mung bean seed viability after 4 months storage period, Sri Dewi Kartika *et al* (2006) and Sumadi *et al* (2009) on control of *Sitophillus zeamais* and maintaining corn seed viability until three months storage. Other result experiment report by Zainal *et al* (2010) that is clove oil abble to inhibit growth of *Clavibacter michiganensis* on tomato seed.

CONCLUSSION

Interaction between soybean cultivar and clove oil dosage had significant effect on seed damage, while there were no sigirificant effect on another variable that were observed. Sindoro has better storability than Anjasmoro seed, it has indicated by weevil population, seed damage percentage, germination rate, vigor index and seed moisture content. The clove oil 1.5 mL kg^{-1} soybean seed indicated better influence on decreased weevil population, seed damage percentage, maintaining germination rate of soy bean seed, and vigor index.

According conclussion result was sugested following to study about the effect of weevil number, kind of packaging material, and longer storage duration under variation room condition .

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