

BIOLOGICAL STUDY OF TWO-SPOTTED SPIDER MITE, *Tetranychus* sp. (ACARI: TETRANYCHIDAE) ON THREE LEAF PHASES OF MUNG BEAN AND ADZUKI BEAN FOR MITE MASS REARING

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ABSTRACT

Two-spotted spider mite (TSM) *Tetranychus* sp. is one of prey mites for phytoseiid mite mass rearing. Based on previous research that TSM population developed well on Mung bean (*Vigna radiata*) and Adzuki bean (*V. angularis*) as host plants, confirmed growth and development of TSM on both beans. Research was aimed to observe biology of TSM on three phases of Mung bean and Adzuki bean leaf such as at primary leaf unrolled completely (V1), at the 1st trifoliolate unrolled completely (V2), and at the 2nd trifoliolate unrolled completely (V3). Pre adult stadia, life cycle, male and female longevity, female fecundity of TSM, and trichome density were observed on petri dishes contained each leaf phase of beans. Result showed that three leaf phases of beans did not influence significantly in all biological variables, except fecundity. TSM fecundity was the highest on V3 phase of Mung bean (86.90). Trichome density on Mung bean leaf surface per mm² was lower than Adzuki bean. Trichome density of upper leaf surface of V3 phase of Mung bean (10.53) and Adzuki bean (3.07) were lower than V1 (13.20; 12.40) and V2 phase (6.20; 5.27). V3 phase of Mung bean was most suitable for TSM mass rearing.

Keywords: fecundity, mass rearing, trichome density, two spotted spider mite

INTRODUCTION

The Tetranychidae commonly known as spider mites and there are 1200 species in about 70 genera and more are yet to be discovered, especially in the Southern Hemisphere (Gerson *et al.*, 2003; Zhang, 2003). Bryobinae and Tetranychinae are two subfamilies in

Tetranychidae and most pest species belong to the Tetranychinae. They are named as spider mite because many members of this family produce silk webbing on host plants (Zhang, 2003). Spider mites are soft-bodied, medium-sized mites (about 400 μ m for an average adult female), and when alive, they are often red, green, orange or yellow in colour. All spider mites are phytophagous and several are major pests of crops (Gerson *et al.*, 2003). One of the important genus in this family is *Tetranychus* and its can be distinguished based on the shape and disposition of the aedeagus (Belloty *et al.*, 1986).

In Indonesia, two-spotted spider mite (TSM), *Tetranychus* sp. is one of the most important pests (Kalshoven, 1981) and can attack on every major food crop and ornamental plant (Zhang, 2003). TSM is a cosmopolitan species and common in greenhouses throughout the world, the most polyphagous species of spider mites (over 150 host plant species of some economic value). In addition, over 300 plant species in greenhouses are host of this mite (Zhang, 2003). Spider mites also produce toxin at meal times together with their salivary excretion. The toxin is involved in important process of host plant metabolism which results in reduction of fiber, fruits and seeds as well as causing yellowing and leaf defoliation, and even death of plant (Huffaker *et al.*, 1969). In dry season, tetranychid damage will further degrade plant health. Changes in concentration of sugars and amino acids in plants will provide better nutrition conditions for tetranychid growth. If the plant has shown severe attack, the mites will immediately move to a fresh leaf or other host plants (Crooker, 1985). In suitable environmental conditions, development and reproduction of spider mites is accelerated and the quality of host

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plant decrease quickly, in case of tetranichyd *Tetranychus urticae* Koch (Razmjou *et al.*, 2009).

Pesticide as single tactics can detract seriously from sustainability, and produced some problems such as pesticide resistance in target species and environmental degradation and contamination of food products. In Integrated Pest Management, biological control will be corrected option to solve some problems resulted pesticide application on agriculture (Koul and Cuperus, 2007). Since the 1960s, biological control of mites by means of beneficial insects and/or mites has seen a rapid development, in example: phytoseiid mite *Phytoseiulus persimilis* Athias-Henriot. Spider mites, in particular *T. urticae*, in a variety of greenhouse and outdoor crops was successfully controlled by phytoseiid mite (Anonymous, 2008).

In California, two species of spider mites infest grapevines: the Willamette mite, *Eotetranychus willamettei* (McGregor), and the Pacific mite, *Tetranychus pacificus* McGregor can be used as prey that enables the predator to build up and maintain its populations. Both two species of mites serve directly as natural enemies of weed pests, as alternate prey for predators (Gerson *et al.*, 2003). To support the mass rearing of predators, enough numbers of prey mites such as *Tetranychus* sp. has to be provided. Some species of legumes as food for mass rearing of TSM can be used to provide TSM in large numbers (Razmjou *et al.*, 2009)

Puspitarini *et al.* (2011) examined leaf phase of five legume varieties on population development of TSM. The result showed that TSM population was the highest in Mung bean (*V. radiata*), followed by Adzuki bean (*V. angularis*), snapbean (*Phaseolus vulgaris*) and soybean (*Glycine max*) (Willis and Anjasmoro cultivars). It seemed that the Mung bean contains more nutrient balance than others. In addition, Mung bean leaf surface was relatively smooth, and it had lower density of trichome. Mung bean leaf characteristics would make TSM easier to get their nutrient needed and to lay eggs. From the research, it can be concluded that Mung bean was the most suitable plant for the development of the TSM population, and Adzuki bean plants can be used as an alternative host.

To know leaf phases of crop appropriate for TSM development, observation on the biology of TSM on three leaf phases of the beans was required. In this research bean leaves of

V1, V2 and V3 phase were used as host for TSM. V1 is the phase of one week-old-plant leaves, V2 is the phase of two-week-old plant leaves and V3 is the phase of the three-week-old plant leaves. The objective of this research was to study pre adult and adult development of TSM and to analyze relationship of trichome density on three leaf phases of Mung bean and Adzuki bean such as V1, V2 and V3.

MATERIALS AND METHODS

Mass rearing of TSM (with body length of adult ca. 0.35 mm) and experiment used arena in laboratory. For a preparation, soaked rubber was placed on a Petri dish (d= 9cm). Then, a piece of synthetic cotton wool was placed on the wet rubber and a disc of leaf was placed on the wet cotton as the food for mite. The rubber and cotton were always kept wet to keep the humidity in arena. All of observation of this research was conducted under binocular microscope.

Mung bean and Adzuki bean were planted in polybag. Three leaf phases were used for each bean, such as V1 phase, a primary leaf unrolled completely; V2 phase, the 1st trifoliolate unrolled completely; and V3 phase, the 2nd trifoliolate unrolled completely. To get three leaf phases, which could apply on the same days, first group of bean seeds were planted on the first day to get a V3 phase. After one week, second group of bean seeds were planted for V2 phase. When V2 was one weeks and V3 was two weeks old, planting the last group of bean seeds for V1 phase took place. After V1 phase was one week old, V 2 and V 3 phase were obtained. Then, leaf phases V1, V2, and V3 were ready to use as TSM food on the arena, and each experiment could run simultaneously.

Development of Preadult Stadia and Adult of TSM on Three Leaf Phases of Mung bean and Adzuki bean

Growth and development of each pre adult stadium and life cycle of TSM on three leaf phases of Mung bean and Adzuki bean were observed by using Randomized Complete Block (RCB) design with six treatments. Each treatment was repeated ten times to obtain 60 units of experiment. The treatments involved three leaf phases of Mung bean and Adzuki bean, V 1, V2 and V3.

A pair of TSM adult was placed on each 10 Petri dishes for each treatment, so there were 60 petri dishes in the experiment. After 24 hours, female laid their egg(s), and they were removed. If more than one egg were laid in any arena, other eggs were removed. The egg was observed every three hours until they hatched and the observation continued until the adult emerged. Each phase and stadia of TSM were noted.

Fecundity of Female, Longevity of Female and Male, and Sex Ratio of TSM on Three Leaf Phases of Mung bean and Adzuki bean

The fecundity (number of eggs laid by a female) and the longevity of TSM on three leaf phases of Mung bean and Adzuki bean were observed by RCB design with six treatments. The treatments included three leaf phases of Mung bean and Adzuki bean, such as V1, V2 and V3.

The fecundity and longevity were observed by placing an adult female that emerged on the same day on each Mung bean and Adzuki bean leaf disc at certain phase in the arena. To obtain adult female mites on the same day, reared female mites were put on V1, V2 and V3 phases of Mung bean and Adzuki bean leaves lied on 18 arenas. Each phase leaf of the beans was repeated 6 times. After 24 hours, the female was removed. A female might lay 3-4 eggs per day. The eggs were observed until they hatched and it was continued until deutonymph emerged. A female deutonymph and an adult male were placed on each 10 arenas for each treatment, so there were 60 arenas. Most of the 60 adult females emerged on the same day. Observation on each arena was conducted everyday under binocular microscope to calculate number of eggs laid. After counted, the eggs were then removed using a needle-stemmed. Observation was conducted until the female died. The bean leaves were replaced every three days with fresh ones.

Longevity of TSM adult male on three leaf phases of Mung bean and Adzuki bean, V1, V2 and V3, were observed. RCB design was used to observe this variable. The longevity was observed by placing TSM adult males which appeared on the same day on arena experiment, and they were observed until they died. Adult male that emerged on the same day was obtained by putting 3 females of TSM from mass rearing on an arena with certain leaf disc. Each leaf was repeated 3 times, so there were 18

arenas. A female laid ca. 3 eggs per day. After 24 hours the females were removed. All eggs laid were observed from hatching to deutonymphs. Generally sex ratio of Tetranychid is 1:2, which is female biased. Then the female deutonymphs were moved to another arena with certain bean leaf. It was repeated 5 times for 30 arenas. One female was placed in each arena without any male. Eggs from virgin female developed to be male. Virgin deutonymph females were observed from starting of growing stage to starting time of laying eggs. After 48-hour adult emerged, it then was removed. When there were some eggs in every arena, there should be only one egg left in the arena. Observing the eggs was done from hatching to time of mite died. Death time of adult male was recorded. The bean leaves were replaced every three days with fresh ones.

TSM sex ratio on three leaf phases of Mung bean and Adzuki bean V1, V2 and V3 were observed and RCB design was adopted in this experiment. TSM sex ratio was observed by placing 10 TSM adult females from mass rearing in each of 10 arenas with certain leaf phase, so there were 60 arenas. The bean leaves were changed every three days with fresh leaves. After 24 hours, all the females were removed and the number of eggs was recorded. The eggs were observed from hatching to adult. The number of emerging males and females were then counted.

Trichome density on Three Leaf Phases of Mung bean and Adzuki bean

Trichome density on each leaf phase of beans were measured by cutting the leaves and counting the number of trichomes under microscope on 1 mm² of leaf area. Upper and lower leaf surfaces were counted and repeated five times for each leaf phase of beans.

RESULTS AND DISCUSSION

Development of Preadult Stadia and Adult of TSM on Three Leaf Phases of Mung bean and Adzuki Bean

The results showed that phase difference of Mung bean and adzuki bean plant was not significantly different on life cycle and preadult of TSM except deutonymph and teliochrysalis stadia (Table 1).

Preadult development and life cycle of TSM in three leaf phases of Mung bean and Adzuki bean had the same value. It seemed that

two beans have relatively same nutrient that was required by TSM. Although the leaf phases of beans were not significantly different on biology of TSM, but in general V3 phase gave good nutrient for TSM especially on Mung bean. Preadult stadia and life cycle of TSM on V3 phase tended to be shorter than V1 and V2. During this research, V1 and V2 phase leaves dried faster than V3. The dry leaves of V1 and V2 phase were followed by declining leaf nutrients which occurred as fast as they dried. It gave negative influence for TSM development. Actually leaf V1 and V2 phase had high nutrient for mite development. van de Vrie *et al.* (1972) reported that mites generally developed faster in younger leaves because it has higher protein content than older ones. Crooker (1985) stated that the preadult development, mortality, and fecundity are mainly influenced by the nutritional content of the protein in a host plant.

As TSM preadult development, three leaf phases of Mung bean and adzuki bean leaf gave same influence to adult development (Table 2).

Pre-oviposition, oviposition and post-oviposition periods of TSM female adult had the same period statistically, although TSM oviposition period on V3 phase of Mung bean and Adzuki bean tended to be longer than that on V1 and V2.

Study on others TSM adult development showed that three leaf phases of Mung bean and adzuki bean influenced the number of eggs laid by female (Table 3). The eggs laid by female on V3 phase of Mung bean was the highest. As the alternative food for TSM was leaf phase V3 of adzuki bean, leaf phase V3 was most the suitable for the development of TSM. Although leaf phases did not influence female and male longevity, also sex ratio, but they tended to be higher on leaf phase V3 than others.

Table 1. Average Periods of Preadult Stadia and Life Cycle of TSM on Three Phases of Mung bean and Adzuki bean

Stadia (periods)	Treatments						LSD
	Leaf Phase of Mung bean (M)			Leaf Phase of Adzuki bean (A)			
	V1	V2	V3	V1	V2	V3	
Eggs (hours)	70.79 a	70.25 a	69.96 a	72.37 a	69.76 a	71.91 a	-
Larvae (hours)	20.16 a	17.60 a	18.28 a	23.34 a	18.79 a	18.00 a	-
Protocrysalis (hours)	17.98 a	15.82 a	17.16 a	16.34 a	17.60 a	18.30 a	-
Protonympha (hours)	14.59 a	12.49 a	12.82 a	14.97 a	15.49 a	13.58 a	-
Deutocrysalis (hours)	16.09 a	16.61 a	14.67 a	16.42 a	16.52 a	16.02 a	-
Deutonympha (hours)	16.56bc	12.55 a	14.41ab	17.42 c	14.99 abc	17.56 c	2.71
Teliocrysalis (hours)	17.64 b	17.28 b	14.51 a	20.75 c	20.29 c	17.37 b	1.77
Preadult (hours)	173.81a	162.60a	161.80a	181.60a	173.45 a	172.75 a	-
Life Cycle (days)	9.01 a	8.51 a	8.46 a	9.41 a	8.97 a	9.00 a	-

Remarks: Numbers followed by different letters in the same row indicate significant difference at 5% error level

Table 2. Average periods of pre-oviposition, oviposition and post-oviposition of adult females of TSM on Three Leaf Phases of Mung bean and Adzuki bean

TSM Female adult (periods)	Treatments						LSD
	Leaf Phases of Mung bean (M)			Leaf Phases of Adzuki bean (A)			
	V1	V2	V3	V1	V2	V3	
Pre-oviposition (days)	1.77 a	1.74 a	1.72 a	1.85 a	1.74 a	1.80 a	-
Oviposition (days)	9.50 a	9.74 a	9.80 a	9.43 a	9.52 a	9.67 a	-
Post-oviposition (days)	2.74 a	2.78 a	2.81 a	2.79 a	2.82 a	2.88 a	-

Remarks: Numbers followed by different letters in the same row indicate significantly different at 5% error level

Fecundity of Female, Longevity of Female and Male, and Sex Ratio of TSM on Three Leaf Phases of Mung bean and Adzuki bean

Based on Table 3, TSM female longevity was longer than male longevity. It was also described on citrus red mite (CRM), *Panonychus citri* (McGregor) (Tetranychidae) that male longevity was shorter than female. Female and male longevity of CRM was around 10 and 9 days respectively, depending on citrus leaf age (Puspitarini *et al.*, 2007).

In general, the biology of TSM on Mung bean was better than that of on Adzuki bean. Proximate analysis on Mung bean and adzukabean showed that the content of Mung bean protein, water and fat were higher than that of Adzuki bean (Table 4). Mung bean leaves seemed to have balance nutrient; therefore, TSM could develop and grow optimally. The higher protein, fat content and water on Mung bean could be suspected positively affected the number of eggs laid by TSM. It was reported by

Wermelinger *et al.* (1985) that nitrogen, water, amino acid and sugar content of apple leaves were positively correlated with weight and egg production of *T. urticae*. Puspitarini *et al.* (2007) stated that fecundity of CRM that was reared on lemon citrus leaf was higher (32.30 eggs/female) compared that on sweet orange (26.20 eggs/female). It seemed to relate with protein content of lemon leaf that was higher (5.84%) than that of orange leaf (4.60%).

Trichome density on Three Leaf Phases of Mung bean and Adzuki bean

In addition, another factor that seemed to influence TSM development was leaf morphology, especially trichome density on leaf. Leaf trichome seemed to affect number of eggs laid by the mite. Trichome density of Mung bean leaf surface per mm² was lower than Adzuki bean. V3 phase had lower trichome density than V2 and V1 (Table 5).

Table 3. Adult development, fecundity and sex ratio of TSM on three leaf phases of Mung bean and Adzuki bean

Adult development (units)	Treatments						LSD
	Leaf Phases of Mung bean (M)			Leaf Phases of Adzuki bean (A)			
	V1	V2	V3	V1	V2	V3	
Female longevity (days)	14.01 a	14.26 a	14.34 a	14.06 a	14.08 a	14.35 a	-
Male longevity (days)	12.39 a	12.50 a	12.50 a	12.33 a	12.38 a	12.48 a	-
Fecundity (eggs/female)	56.80 a	71.20 d	86.90 e	56.43 a	59.13 b	65.83 c	1.59
Sex ratio (♂:♀)	1:1.81a	1:1.89a	1:2.07a	1:1.77a	1:1.83a	1:2.05a	-

Remarks: Numbers followed by different letters in the same row indicate significantly different at 5% error level

Table 4. Proximate content of Mung bean and Adzuki bean Leaves (Source: Puspitarini , 2011)

Species of beans	Protein (%)	Fat (%)	Carbohydrate (%)	Water (%)	Ash (%)
Mung bean	3.356	1.595	4.278	89.015	1.756
Adzuki bean	2.509	0.060	10.343	85.603	1.485

Table 5. Average of trichome density on upper and lower side leaf surface on three leaf phases of Mung bean and Adzuki bean

Species of Beans	Average of trichome density for both leaf surface sides of leaf phases (mm ²)					
	Upper side of leaf surface			Lower side of leaf surface		
	V1	V2	V3	V1	V2	V3
Mung bean	3.70 b	2.57 a	2.43 a	2.20 c	1.07 b	0.53 a
Adzuki bean	13.20 b	12.40 b	10.53 a	6.20 c	5.27 b	3.07 a

Remarks: Numbers followed by different letters in the same row for each leaf surface, indicate difference at 5% error level (LSD).

It caused smoother surface of Mung bean leaf compared to that of Adzuki bean, especially V3 phase. TSM preferred Mung bean, as their activity was relatively undisturbed by trichome. Density of leaf trichome correlated with plant resistance to herbivory. Population and fecundity level of herbivory is so high on leaf that it has low number of trichome (Dalin *et al.*, 2008). In addition, there was indication that trichome secretions on *Lycopersicon hirsutum* are likely responsible for potent repellency to two-spotted spider mites (Guo *et al.*, 1993). High acylsucrose content and high type-IV trichome density on *Solanum pimpinellifolium* increased mortality and repellence, and reduced oviposition of *T. urticae* (Munoz and Salinas, 2003; Alba *et al.*, 2008). In V3 phase leaf, TSM was easier to move around and laid more eggs on smooth leaf surface.

In general, based on biological variables of TSM on three phases and morphology characteristic of beans, V3 phase of Mung bean was the most suitable for TSM mass rearing. This result seemed to support research result of Puspitarini *et al.* (2011) that Mung bean was suitable plant for TSM. By using Mung bean as food for mass rearing, TSM could growth and develop faster; its population could be used for mite predator mass rearing, especially phytoseiid predator.

CONCLUSIONS

Three leaf phases of Mung bean and Adzuki bean, V1, V2, and V3 phases did not show significant differences to periods of preadult stadia, life cycle, male and female longevity, and sex ratio of TSM. The leaf phases of bean only influenced the fecundity of TSM. TSM fecundity was the highest on V3 phase of Mung bean (86.90). TSM fecundity was higher on V3 phase of Mung bean leaf (86.90) than that on V3 Adzuki bean (65.83). The number of eggs laid on V1, V2, and V3 phase of Mung bean leaf (56.80, 71.20, 86.90 eggs) were higher than all leaf phases of Adzuki bean, 56.43, 59.13, and 65.83 respectively.

Trichome density of Mung bean leaf surface per mm² was lower than that of Adzuki bean. Trichome density on upper leaf surface was higher than that of under leaf surface. Upper leaf surface Trichome density of V3

phase of Mung bean (10.53) and Adzuki bean (3.07) were lower than that of V1 (13.20; 12.40) and V2 phase (6.20; 5.27). Based on the result, we concluded that V3 phase of Mung bean was most suitable for TSM mass rearing.

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