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Diversity and Effectiveness of Insect Pollinators of *Jatropha curcas* L. (Euphorbiaceae)

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Seed of *Jatropha curcas* (Euphorbiaceae) is currently established as the source for biofuel Therefore, it is important to understand the diversity insects that pollinated *J. curcas* inflorescence yellow flowers. We also aimed to study the pollination effects on fruit set on *J. curcas*. Scan sampling method were carried out to explore the insect pollinators diversity from 07.00 up to 17.00 h in every 15 minutes. Visiting frequency of pollinators insects were observed by using focal sampling. Those information together with flowering periods, flower nectar volumes, and environmental factors were used as the basic data to determine the effectiveness of insect pollination both in covered and uncovered of seed set plants. Results showed that nine species of insect pollinators were from three order (Hymenoptera, Lepidotera, and Diptera) pollinated *J. curcas*. Four species of Hymenoptera i.e. *Prenolepis*, *Apis dorsata*, *Xylocopa confusa*, and *Apis cerana* showed the highest abundances. The highest abundance and species richness of pollinators occurred at 08.00-10.15 and 15.00-17.15 h. Bees of *X. confusa*, *A. cerana*, and *A. dorsata* of Apidae are effective as insect pollinators in *J. curcas* plantations, due to high visited frequencies. The insect pollinators also increased fruits and seeds set of *J. curcas* in the uncovered experiment plants. Thereby, enhancement the three pollinator insects as part of crop management have to be considered by farmers.

Key words: pollinator insects, diversity, visiting frequencies, fruit set, *Jatropha curcas* L.

INTRODUCTION

Nowadays we use many of edible sources (i.e. palm oil, soybean, corn, etc) as biofuel resources. *Jatropha curcas* L. showed the capability that also can be a resource for that purposes. Other important useful of the plant are used as traditional medicine, cattle woof resources, firewood, charcoal, paper pulp, fiberboard, and particles board (Nuryani 2007), also can reduce CO_2 pollution.

Flower of *J. curcas* is a small, odorless, and yellow inflorescence with dichasial chyme pattern. It classified as protandry or protogyny depends on type of sexes and amount of flower that first grown in one inflorescence. Flower transduction change protandry into protogyny at 40 $\pm$ 2 °C. There are 100 flowers in one inflorescence with ratio males and females are 29:1. Both sexes have five nectar glands produced nectar located at the base of flower. Male flower has ten stamens performed a circle patterns. Pollen is yellow, globular, in aperture shape with semitectate and perrucate axine. Diameters of pollen are between 81-89 µm, the number of pollen in lower and upper stamen are 220 and 435 grains, respectively. The female flowers has three cell ovary that terminated by three styles. During pollination, sepal and petal will protect the fruit development. Fruits will ripe after 40-50 days after pollination follows with fruit color change from green to yellow. A fruit contain 3-4 black seed (Raju & Ezradanam 2002; Bhattacharya *et al*. 2005).

Jatropha curcas were visited by bees (*Apis*, *Trigona* and *Ceratina*), ants (*Camponotus*, *Crematogaster*, *Solenopsis*, and *Pheidole*), thrips (*Scirothrips* and *thrips*), and fly (*Chrysomya*) (Raju & Ezradanam 2002). In Indramayu, West Java, *J. curcas* plantations were visited by bees, *Apis cerana*, *Ceratina* sp., and *Hylaeus* sp. (Atmowidi *et al*. 2008). However, lack of experimental reports regarding to effectiveness of insect pollinators of *J. curca*. Here, we further analyzed the diversity, visiting frequency, and effectiveness of insect pollinators in relation to seed set of *J. curcas*.

MATERIALS AND METHODS

Study Sites. Study was conducted at *J. curcas* field research plantation in Lulut Village, Kelapa Tunggal District, West Java ($\pm$ 30,000 ha, 153 m asl; 06 °30' 09.7'' S, 106 °55' 16.6" N) from March to October 2008.

Flower Phenology and Nectar Volume. The anatomies of *J. curcas* flowers were observed to understand the position of the nectar glands. Nectar volume from ten *J. curcas* plants were measured at 06.30, 08.30, 12.30, 14.30, and 16.30 h. Nectar volume were measured by using microcapiller pipette (*Dru USA by Drummond Sci. Co*. No.4 (1-5 µl) (Stout *et al*. 2006).

Insect Pollinators Diversity. Ten *J. curcas* plants were selected and diversity of insect pollinators was observed every 15 minutes from 07.00 to 17.00 h by using scan sampling method. Several insect flower visitors were collected for species identification purpose. Environment

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factors i.e. air humidity and temperature, light intensity, and wind velocity were measured in every insect observation.

Insect Pollinators Visiting Frequency. Observations of insect flower visiting frequency were conducted by scan sampling methods (Martin & Bateson 1993). The observations included foraging rate (number of flowers/minute), flower handling time (seconds/flower), and plant handling time (seconds/plant) (Dafni 1992).

Measurement of Insect Pollinators Effectiveness. Effectiveness of insect pollinators was measured by number of fruits produced by plants that exposed to pollinators compared with fruits produced by covered plants. Before flowering, ten plants were covered by using insect screen, while ten other plants were uncovered. After 30 days of fruiting period, the number of fruits per plant, fruits per bunches, fruit diameters, seeds per fruit, and seeds weight were counted. Seed viability (in percentage) was measured based on germinated seeds in plastic bag with three replications.

Data Analysis. Diversity of insect pollinators were analyzed based on Shannon diversity index (H'), Shannon evenness (E), Margalev species richness (d), and Sorensen similarity (Cs), using Primer e-5 Programme. Analysis of variance (ANOVA) and Tukey test at 95% level were implemented for differences insect visiting behavior. T-test was also conducted to compare the number of fruit produced by plants that covered and uncovered by insect screen.

RESULTS

Flower Phenology and Nectar Volume. Flower of *J. curcas* is small, odorless, yellow in color, and 4 mm in size. *J. curcas* was flowered daily during 06.00-08.00 h. The flower bloom in 20 days, which male flowers were bloom prior to the female and produce flowers daily until the male buds shed. The peak of flowering periods occurred in day 12 to 15th and decreased until day 20th. Ratio of male and female flowers was 33:1. There were five yellow oval-shape glands in flower base producing nectar. Male and female nectar volumes were 3.85 and 4.90 µl, respectively. The yellow color of flowers and the amounts of nectar assumed the insect to visit.

High nectar volume (4.09 and 3.85 µl for female and males, respectively) occurred at 06.00-09.00 am and flowers visited by six species of insect (total number 1,762 individuals). By afternoon, nectar volume decreased up to 0.42 and 0.37 µl for female and male flower. The abundances of insects were positively correlated with number of flowers, while opposite result occurred with nectar volume.

Insect Pollinators Diversity. Nine orders of insect visitors in *J. curcas* plantations were Odonata, Orthoptera, Mantodea, Homoptera, Thysanoptera, Coleoptera, Hymenoptera, Lepidoptera, and Diptera. From these observations we established nine species of insect pollinators (Table 1). Ants *Prenolepis*, always found in every observation, and has the highest abundance (1,772 individual) found at 07.00-07.15 h. Otherwise, three species of Apidae (*X. confusa*, *A. cerana*, and *A. dorsata*) have a high abundance in the morning and afternoon. Meanwhile, butterflies, i.e. *Junonia oritya*, *Graphium agamemnon*, *Ariadne ariadne*, and *Eristalis tenax* infrequently visited the flower. The diversity of insect pollinators was higher in the morning and afternoon compare to noon (Table 2). The insects which found in the morning versus afternoon was more similar (Cs = 77%) than both morning versus noon and noon versus afternoon (Cs = 46%) (Figure 1). The average of temperature during March until May 2008

Table 1. Number of species, individuals, and percentage of *J. curcas* insect pollinators diversity

Taxon	Species	Individuals	Percentage (%)
Hymenoptera			
Formicidae	*Anoplolepis*	11	0.19
	Prenolepis	4,409	75.16
Apidae			
Xylocopinae	*Xylocopa confusa*	523	8.92
Apinae	*Apis cerana*	348	5.93
	Apis dorsata	558	9.51
Lepidoptera			
Papiolidae	*Graphium agamemnon*	7	0.12
Nymphalidae			
Biblidinae	*Ariadne ariadne*	5	0.08
Nymphalidae			
Nymphalinae	*Junonia orithya*	3	0.05
Diptera			
Syrphidae	*Eristalis tenax*	2	0.03
Total	9	5,866	100

Table 2. Total number of *J. curcas* insect pollinators in time blocks for 20 days observation

Time blocks (h)	Insect pollinators										
	A	B	C	D	E	F	G	H	I	N	S
07.00-07.15	0	1,772	0	0	0	0	0	0	0	1,772	1
08.00-08.15	0	1,425	175	59	91	5	7	0	0	1,762	6
09.00-09.15	5	584	164	33	57	0	0	2	0	845	6
10.00-10.15	3	55	46	0	0	0	0	0	0	104	3
11.00-11.15	0	11	1	0	0	0	0	0	0	12	2
12.00-12.15	0	12	1	0	0	0	0	0	0	13	2
13.00-13.15	0	29	4	0	0	0	0	0	0	33	2
14.00-14.15	1	282	46	0	0	0	0	1	2	332	5
15.00-15.15	1	187	68	50	110	0	0	0	0	416	5
16.00-16.15	1	52	18	95	142	0	0	0	0	308	5
17.00-17.15	0	0	0	111	158	0	0	0	0	269	2

A: *Anoplolepis*, B: *Prenolepis*, C: *X. confusa*, D: *A. cerana*, E: *A. dorsata*, F: *A. ariadne*, G: *G. agamemnon*, H: *J. oritya*, I: *E. tenax*, N: Total individuals, S: Total species.

were 23.8-37.6 °C, while relative humidity were 36-88%, light intensity were 2,900-38,500 lux, and wind velocity were 0.6-1.6 m/s (Table 3).

Insect Visiting Frequency. The three highest foraging rate was showed by *X. confuse*, *A. cerana*, and *A. dorsata* for 20.86, 18.3, and 15.07 flowers/minute, respectively. Besides, the lowest foraging rate occurred by *E. tenax* (1.69 flowers/minute). The flowers handling time of *X. confusa*, *A. cerana* and *A. dorsata* were 2.88, 3.27, and 3.27 seconds/flower, respectively. The highest duration plant handling time were showed by *Prenolepis* (1060.90 seconds/plant), followed by *A. dorsata*,), *X. confuse*, and *A. cerana* respectively for 156, 69, and 60.46 seconds/ plant (Table 4).

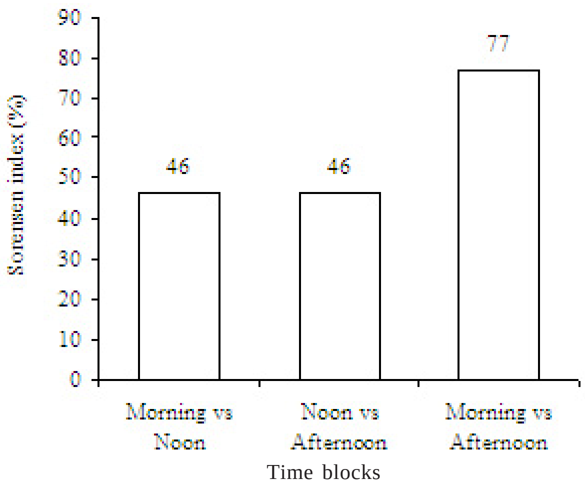

Figure 1. Sorensen similarity index of insect pollinators in three time blocks observations.

Measurement of Insect Pollinators Effectiveness. Number of fruit per bunch, seed per plants, and seed weight per plants in uncovered plants after each 30 days of observations were higher than that of covered plants. Fruit produced by uncovered plants increased in all three parameters, i.e. more than 241, 250, and 389% in the number of fruit per bunch, seed per plant, seed weight per plant, respectively (Table 5).

DISCUSSION

According to our observations, there are four conditions of insect pollinators (i) the visited activities have to be in flower, (ii) the insect morphology has to fit with their flower phenology (ecoevolution) (Raju & Ezradanam 2002), (iii) the visited duration frequency has to keep them movable from one flower to another in difference plantation, (iv) visited frequencies related to food sources (foraging behavior) and environmental factors (Faheem *et al*. 2004). Out of nine species of insect pollinators that collected in this study, not all of them

Table 5. Comparisons of covered and uncovered *J. curcas* fruit sets

Plant component	Field crop		Increase (%)
	Covered*	Uncovered*	
Fruit/bunch	2.77b	9.40a	23
Fruit/plant	8.30b	28.30a	241
Fruit diameter/plant	2.17b	2.30a	6
Seed set/plant	242b	846a	250
Seed weight/plant (g)	26.41b	129.39a	389
Seed germination	6.17b	8.83a	30

*The different alphabet in same row showed significant different with T-test level 95%.

Table 3. Insect diversity related to nectar volume and environmental factors

Time blocks (h)	S	N	d	E	H’	wv (m/s)*	li (x 100 lx)*	t (°C)*	h (%)*	F (µl)*	M (µl)*
07.00-07.15	1	1,772	0	0	0	0.75	68.20	26.68	86.35	4.09	3.85
08.00-08.15	6	1,762	0.67	0.39	0.71	0.79	221.45	30.45	73.95		
09.00-09.15	6	845	0.74	0.52	0.93	0.78	299.00	34.22	59.25	2.16	2.17
10.00-10.15	3	104	0.43	0.73	0.80	0.87	224.75	31.28	68.05		
11.00-11.15	2	12	0.40	0.41	0.29	1.14	294.95	34.06	50.00	1.13	1.13
12.00-12.15	2	13	0.89	0.39	0.27	1.17	333.15	33.78	50.20		
13.00-13.15	2	33	0.29	0.53	0.37	1.20	269.10	34.09	56.38	0.42	0.37
14.00-14.15	5	332	0.69	0.30	0.48	1.10	193.80	33.54	54.95		
15.00-15.15	5	416	0.66	0.79	1.28	1.21	133.75	34.93	48.45	0.26	0.26
16.00-16.15	5	308	0.70	0.75	1.21	1.31	138.30	35.25	44.20		
17.00-17.15	2	269	0.18	0.98	0.68	1.53	105.20	30.49	56.55	0.10	0.12

*Average values from 20 days observation. S: Total species, N: Total individual, d: Abundances index , E: Evenness index, H’: Shannon index, wv: Wind velocity, li: Light intensity, t: Temperature, h: Humidity, F: Female flower nectar volumes, M: Male flower nectar volumes.

Table 4. Visited frequencies of *J. curcas* insect pollinators

Species	N	visit/minute*	Visited duration/flower (s)*	Visited durations/plant (s)*
Anoplolepis sp.	11	2.17a	27.69a	22.22a
Prenolepis sp.	418	1.86a	32.2b	1060.99b
X. confusa	425	20.86b	2.88c	69a
A. cerana	256	18.33b	3.27cd	60.46a
A. dorsata	477	15.07b	3.98d	156c
G. agamemnon	5	3.16a	18.92e	9.46a
A. ariadne	7	2.95a	20.33e	14.23a
J. orithya	3	1.69a	35.43b	6.96a
E. tenax	2	1.89a	31.67ab	13.55a

*The different alphabet in same row showed significant different with T-test level 95%

were effective as *J. curcas* pollinators. Even though ants and flies have a body hair and size that fit into the flower tube, they can not classified as effective pollinators. This is due to, based on this study observations, ants visited a bunch of flowers within long duration without moving to the other flowers, and infrequently visited occurred in flies. However, ants performed as *Turnera ulmifolia* (Turneraceae) pollinators (Cuautle & Rico-Gray 2003) and flies can pollinate *J. curcas* in Indian plantations (Raju & Ezradanam 2002). Other insect such as butterflies has a large body size that cannot fit with *J. curcas* flower tube. There is a possibility that butterflies also performed as a pollinator, since when they visited flower to collect nectar, pollen were patched in their proboscis and legs. When they visit another flower, the pollen usually removes from their proboscis or legs and drop into pistil. However, *J. curcas* butterflies were infrequently visited, hence they were not effective as pollinators.

Besides ants, flies, and butterflies we also observed bees in this study. Bees generally have a heavy body hair, a long or short proboscis, and several have pollen baskets (honey bee) (Michener 2000). *X. confusa* has a large body size that not fit with *J. curcas* flower tube and they usually visit large size flower with long pistil and shaft anther (Momose *et al*. 1998). The observation showed *X. confusa* had the highest frequencies among visitor bees, the shortest visited durations per flower, and the highest visited durations per plant. The high abundances of *X. confusa* might also due to their close nest to the plantation (10 m in distance). To collect nectar and pollen from *J. curcas* flowers, they usually perched on petals hence pollen covered all over their body hair and ready to pollinated pistil in others. All of these reason assumed *X. confusa* as *J. curcas* classified as insect pollinators. The other two others bees, *A. cerana* and *A. dorsata* are well known as insect pollinator in many plantations (Momose *et al*. 1998; Kremen 2002; Neupane *et al*. 2006; Atmowidi *et al*. 2007). Indeed, Raju and Ezradanam (2002) and Bhattacharya *et al*. (2005) reported *Apis* as *J. curcas* pollinator in India plantation, Banjo *et al*. (2006) *J. curcas* Nigeria’s plantation. *Apis* has unique body structure to collect pollen called pollen basket in the hind tibia.

We observed a segregation times in visited frequencies between *A. cerana* and *A. dorsata*. These two species never visit in the same observation time, might be due to food competition. However, Steffan-Dewanter *et al*. (2002) reported that there were high competitions between *A. cerana* and *A. dorsata* in foraging behavior that might influence by nesting distance between the nest and the plantation. *A. cerana* nesting area located around village (100 m approximately) and *A. dorsata* nesting area commonly inside the forest (± 250 m from study site) (personal observations). Besides nesting areas, flower phenology and nectar volume also attract insect to visit flower. The amounts of flowers, sizes, shapes, colors, nectar volume, and number of pollens influenced the abundance of insect pollinators (Faheem *et al*. 2004). Barth (1991), reported bloomed flowers with inflorescence blue-yellow colors attracted Hymenoptera and Diptera. Bees can only see the light spectrum from 0-700 nm (ultraviolet-green) and 400-550 nm (blue-yellow).

During the study, we also observed that insect diversity in *J. curcas* plantation did not influence by temperature, light intensity, and wind velocity (Table 3). This result was incongruence with Hardwicke (2003) and Faheem *et al*. (2004) that reported the effectiveness of environmental factors of insect pollinator during foraging behavior. The contrary results of this study might be due to our site observation of *J. curcas* plantation was located in the cement ex-mining which is a highly polluted area. Besides, the plantation is located adjacent to an active mining and factory. Moreover, pesticide also subjected to *J. curcas* once a month to keep the plantation from pest infections (personal observation).

The role of effectiveness of insect pollinators in *J. curcas* had been reported by Raju and Ezradanam (2002), Bhattacharya *et al*. (2005) in India, Banjo *et al*. (2006) in Nigeria. Those were in agreement with our result that showed the number of fruit per bunch, seed per plants, and seed weight per plants were higher in opened plants than those in covered plants. The increase of these three parameters in uncovered plants has related to insect pollinator diversity in a cross pollination. Raju and Ezradanam (2002) reported that bees and flies were effective to pollinate within plant (geitonogamy) and between plants (xenogamy). Total fruit crop in covered plants (83 fruits/plant) was lower than uncovered plants (283 fruits/plant). Fruit set produced by xenogamy were resulted from 96% out of the total female flowers, while 77% in geitonogamy. All fruits were ripe from xenogamy, but 23% failed to ripe in geitonogamy (Raju & Ezradanam 2002). Insect pollinations leads earlier cessation of flowering and more synchronous pod and seed ripening, thereby possibly increase the weight of seed harvest.

The percentage of seed germinations from uncovered *J. curcas* (88.3%) was higher than the covered one (61.l7%). It showed that seeds set from xenogamy have a higher potential germination than from geitonogamy pollination. Xenogamy pollination enabled the mixed of genetics material from two different plants, resulted high genetics diversities and maximize its fitness. Mohr *et al*. (1995) reported genetics diversities result the hybrid vigor to maximize the growth and crops. Our result, concluded that bees pollination increasing the number of fruits per plant and fruits per bunch of *J. curcas*. Seed weights per plant indicate higher amount in uncovered plants than covered plants. The increased of fruit and seed set of *J. curcas* can also increased availability of biofuel resources from this plantation.

As a remark, this study gives important information about the insect pollinators in *J. curcas* plantation as non edible plantation biofuel resources. Our results, found three species that effective as pollinators of *J. curcas*, i.e. *A. cerana*, *A. dorsata*, and *X. confusa* that played a significant role in fruit set. Thereby, enhancement the three pollinator insects as part of crop management have to be considered by farmers. Reduced of pesticides and provided nesting sites for bees could increase the role of insect pollinators as well.

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