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Morphology and Biology of *Phyllocoptes azadirachtae* Chandrapatya (Acari : Eriophyidae)

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ABSTRACT

Phyllocoptes azadirachtae Chandrapatya belongs to Family Eriophyidae, Suborder Actinedida. The scanning electron photographs of dorsal shield, genitalia, microtubercle and featherclaw are presented. This mite is able to feed and reproduce on 3 neem plants; *Azadirachta indica* Juss. *siamensis* Val. (Sadao-Thai), *Azadirachta indica* Juss. (Sadao-India) and *Azadirachta excelsa* (Jack) Jacobs (Sadao-Chang). Sadao-Thai is proved to be the most suitable host for *P. azadirachtae* judging from low mortality rate during development (7%), short life cycle (7.8 days) and high fecundity (21.8 eggs/female).

Key words: *Phyllocoptes azadirachtae*, neem, eriophyid mite, morphology, biology

INTRODUCTION

Eriophyoids are the smallest phytophagous mites, ranging in length from 80 to 300 μm. Most eriophyoids are host specific, causing galls, erinea, russetting and leaf or shoot deformation, and many species are leaf vagrants (Keifer, 1952; Jepson *et al*., 1975; Chandrapatya and Baker, 1986). Three species of eriophyoid mite were recorded feeding on Sadao India (*Azadirachta indica* Juss.) namely *Phyllocoptes azadirachtae* Chandrapatya, *Diptilomiopus azadirachtae* (Boczek) and *Calepitrimerus azadirachtae* Channabasavanna (Channabasavanna, 1966; Boczek and Chandrapatya, 1992, 1993). In 1966 Channabasavanna reported that *C. azadirachtae* lived as a vagrant on the tender shoot not on the leaves. In addition, premature leaf falling was probably induced by this mite. Ten years later, *C. azadirachtae* was one of the most serious pest of neem in India (Uthamasamy *et al.,* 1973).

In Thailand, *D. azadirachtae* was reported as a vagrant mite where *P. azadirachtae* caused russetting on the lower leaf surface (Boczek and Chandrapatya, 1992, 1993). Sombatsiri *et al*. (1995) reported that young neem leaves infested by eriophyoid mites might become malformed and dried up while old leaflets became yellowish and dropped. Preliminary surveys of the neem mite in several parts of Thailand revealed that *P. azadirachtae* was commonly found on 3 varieties of neem, *Azadirachta indica* Juss. *siamensis* Val. (Sadao-Thai), *Azadirachta indica* Juss. (Sadao-India) and *Azadirachta excelsa* (Jack) Jacobs (Sadao-Chang). Heavy mite infestation on the old leaves induced russetting on both leaf surfaces whereas malformation usually occurred mainly on the young shoot.

Eriophyoids develop from egg through two nymphal instars to adult. The immature stages are sometimes referred to as larval or nymphal, or the first instar may be called a larva and second instar

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a nymph (Lindquist, 1996). However, information on biology of the neem mite has not been available in the literature.

Studies on the external structures of eriophyoid mite began with the remarkable work of Nalepa (1887) where he produced his observations on these minute mites with the light microscope available at that time. With modern day phase contrast and differential interference phase contrast microscopes, more detailed morphological information has been accumulated on eriophyoids (Keifer, 1952, 1959; Krantz, 1973). However, some structures still require higher resolution, so scanning and transmission electron microscopy are being used to reveal both external and internal morphology of eriophyoid mites (Nuzzaci, 1979; Chandrapatya and Baker, 1986; Petanovic and de Lillo, 1992; Huang, 2001). Unfortunately morphological characters of *P. azadirachtae* have never been investigated under these types of microscope.

The external morphology of adult *P. azadirachtae* emphasising on the dorsal shield, cuticular sculpturing, genitalia and leg were investigated along with biology, longevity and fecundity of this mite under laboratory conditions.

MATERIALS AND METHODS

External morphology study

Adult mites were fixed overnight in calcium formaldehyde at 4°C. After washing in distilled water, the specimens were post-fixed in 1% osmium tetroxide for 24 h at 4°C. After the specimens were rinsed 3 times with distilled water, they were placed in plastic containers made from BEEM capsules. The BEEM capsules were cut about halfway to form a 5×7 mm cylinder. Each open end was then covered with filter paper held in place by BEEM capsule lids with the center removed. This facilitated liquid exchange during dehydration and critical point drying and also prevented the loss of specimens during processing. Specimens in the plastic containers were dehydrated in a graded series of ethanol (30, 50, 70, 90, 100 and 100%) and then transferred to a Balzers Union CPD 020 Critical Point Dryer. The dried specimens were attached to brass stubs with double-sided sticky tape and coated with gold-palladium in Balzers Union SCD040 Coater. The specimens were viewed with a Jeol JSM-5410 LV Scanning Electron Microscope at 15 KV and images were recorded on Kodak VP 100 film.

Life history study

The life history study of *P. azadirachtae* was conducted in the laboratory on leaves of *A. indica siamensis*, *A. indica* and *A.excelsa* collected at Kasetsart University. Each leaflet (1 cm in diameter) was placed upside down on moisten cotton pads in a 10×24×2 cm plastic box, with 14 equal cells of 5.5×5×1 cm, to maintain the vitality of the leaf. Any leaf that showed signs of deterioration was replaced with a new one. The plastic boxes were kept in the incubator set at 28±2°C and 58±4% R.H. Ten female mites were introduced on each individual leaf. All mites were removed after 24 h and only 1 egg was allowed to stay on each leaflet. The observation was made every 6 h until all mites reached adulthood. The number of stages and time required for each developmental stage was recorded.

Longevity and fecundity of unmated female

A female imagochrysalis was placed on each neem leaflet resting on moisten cotton pad. The mites were subjected to temperature 28±2°C and 58±4% R.H. Each leaflet was checked every 6 h for adult eclosion and leaf surfaces were checked daily for egg deposited during the previous 24 h until adult death. Three neem varieties were also tested in this experiment.

Data from biology, longevity and fecundity studies were subjected to ANOVA using SAS program and Lsd was employed to separate the treatment means.

RESULTS AND DISCUSSION

External morphology of *Phyllocoptes azadirachtae* (Figure 1 and 2)

The body of *P. azadirachtae* is fusiform and exhibits the three standard acarine body regions: the gnathosomal region with the mouthparts, the podosoma with only 2 pairs of legs and the opisthosoma with the genital region situating on the anterior part (Figure 2a). A pair of typical antapical setae is located on gnathosoma. Dorsal shield of *P. azadirachtae* is a flattened, subtriangular with net-like pattern (Figure 1a). The anterior portion of the dorsal shield forms a broad-based lope over rostrum. Numerous granules are located on the underneath of dorsal shield that

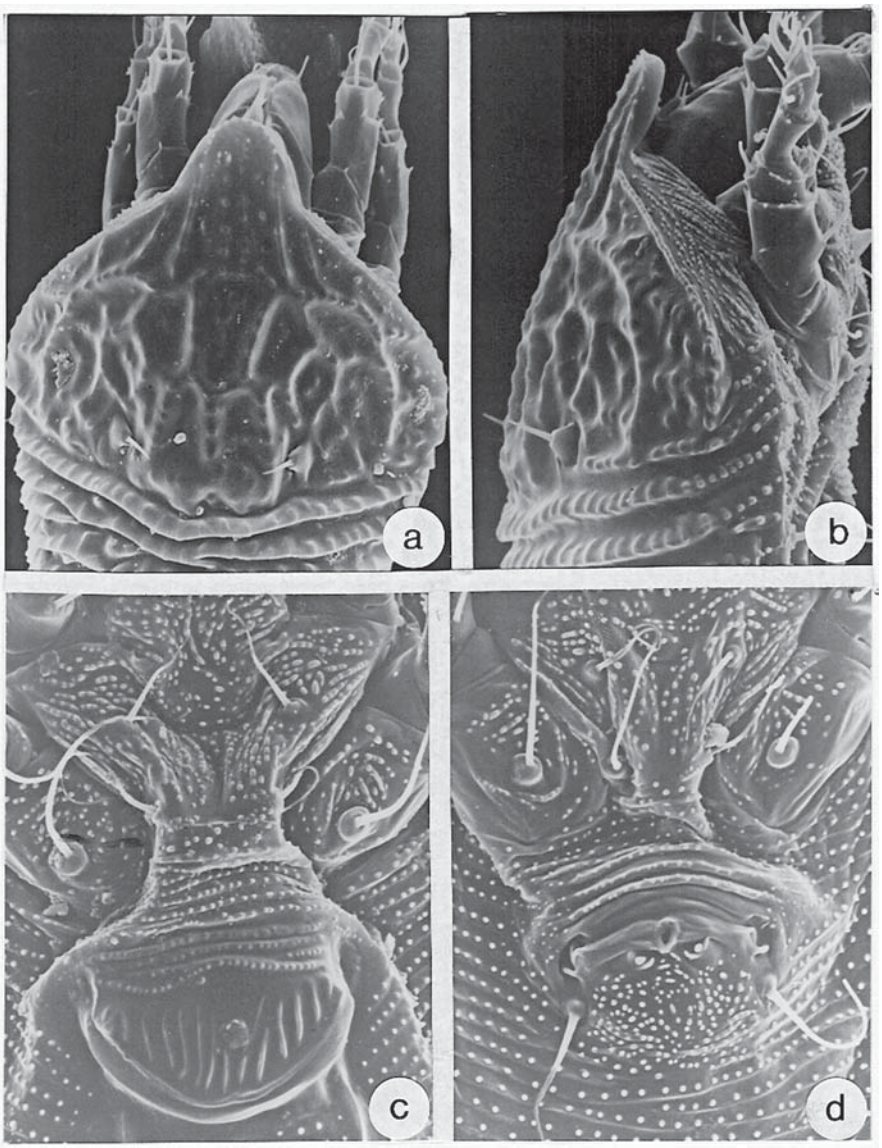

Figure 1 External structures of *Phyllocoptes azardirachtae* Chandrapatya a) dorsum of dorsal shield, b) lateral of dorsal shield, c) female genitalia and d) male genitalia.

can be seen clearly on lateral view (Figure 1b). Dorsal tubercles situate slightly ahead of rear shield margin. Scapular setae are typical in shape, short and point up.

Both female and male genitalia protrude from the anterior part of opisthosoma, close but not appress to the second coxae. Female genital coverflap covers the genital opening which is a transverse slit. The coverflap open posteriorly and approximately 12-14 distinct longitudinal lines are presented on each flap (Figure 1c). The male genitalia has a genital shield open anteriorly. A pair of peg-like structure are situated on the anterior margin of the genital shield. The proximal part of this shield, posterior to these structures, is covered with granules (Figure 1d). Legs of *P. azadirachtae*

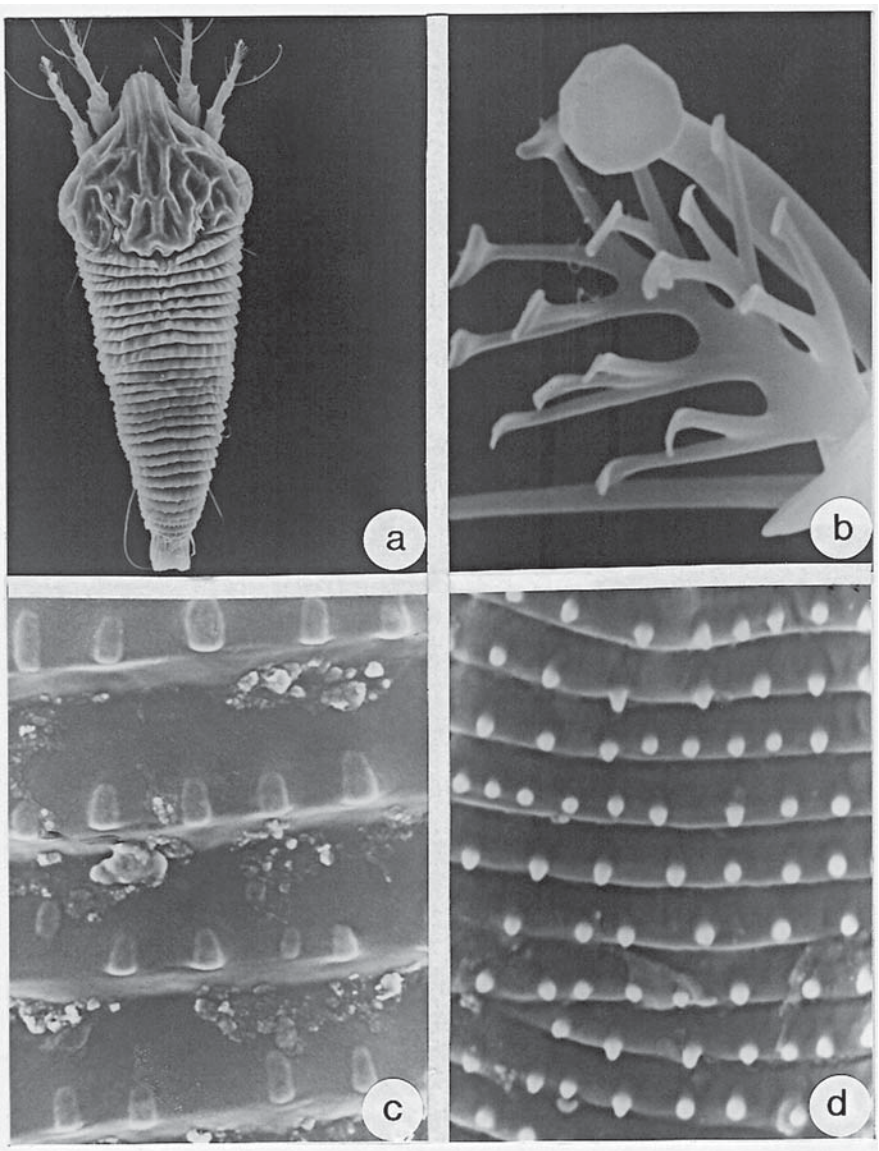

Figure 2 External structures of *Phyllocoptes azardirachtae* Chandrapatya a) dorsum of female , b) featherclaw and solenidion, c) dorsal microtubercle and d) ventral microtubercle.

consist of 6 segments. Both coxal fields are covered with granules. Three pairs of coxal setae and all leg setae are presented. The featherclaw is a four-rayed structure and the solenidion ended in a bulb-like structure (Figure 2b).

The opisthosoma is composed of 28-30 tergites and 62-69 sternites. The dorsal sculptural elements, microtubercles, are indistinct, oval in shape and situated on the ring margin (Figure 2c). The ventral microtubercles are triangular or pointed and locate on the posterior margin of the sternites (Figure 2d).

All external features examined under Scanning Electron Microscope are similar to the work of Boczek and Chandrapatya (1992) but the details of dorsal shield, coxal field and microtubercles can be seen more clearly in the SEM photographs.

Life history of *Phyllocoptes azadirachtae*

Phyllocoptes azadirachtae was reared on leaflets of 3 neem species at 28±2°C, 58±4%R.H. Each mite passed through 3 developmental stages consisting of the egg, larval and nymphal stages before adult emergence. Two quiescent stages known as the nymphochrysalis and imagochrysalis preceeded the nymphal and adult stages. Developmental periods of *P. azadirachtae* on each host plant are presented in Table 1.

Female mites deposited eggs singly on leaf surface, preferably near the midrib. Newly laid eggs were light cream in color and gradually turned to light red or red before hatching. The incubation period of *P. azadirachtae* on *A. excelsa* (3.69 ± 0.65 days) was significantly shorter than those on *A. siamensis* and *A. indica* (4.00 ± 0.31 and 4.11 ± 0.75 days). A newly-hatched larva was light red in color with 2 pairs of legs. Feeding usually occurred immediately after hatching, consequently, the body color changed into dark red. The larval periods significantly differed on 3 neem species, with the longest on *A indica* (1.82 ±

Table 1 Duration of each developmental stage of *Phyllocoptes azadirachtae* Chandrapatya on 3 different neem species under laboratory conditions (28±2°C and 58±4%R.H.).

Developmental stages	Mean±S.D* (days)		
	A. indica siamensis (Sadao-Thai)	*A. indica* (sadao-India)	*A. excelsa* (Sadao-Chang)
Egg	4.00±0.31 a	4.11±0.75 a	3.69±0.65 b
n	104	48	60
Larva	1.23±0.18 c	1.82±0.56 a	1.38±0.33 b
n	102	31	43
Nymphochrysalis	0.78±0.24 a	0.88±0.56 a	0.79±0.20 a
n	99	31	39
Nymph	1.05±0.33 c	2.38±0.71 a	1.34±0.37 b
n	98	25	37
Imagochrysalis	0.74±0.22 b	0.93±0.22 a	0.76±0.09 b
n	97	23	36
Life cycle (egg-adult)	7.80±0.64 b	9.38±1.12 a	7.91±0.83 b
n	97	23	36

* Means in the same row not followed by same letters are significantly different ($p<0.05$) as determined by Lsd.

0.56 days) followed by 1.38 ± 0.33 days on *A. excelsa* and 1.23 ± 0.18 days on *A. indica siamensis*. The leaflets of *A indica* and *A. excelsa* were not found to be suitable for *P. azadirachtae* larval development since 35.42 and 28.33% larval mortalities were recorded as compared to only 4.59% observed on *A. indica siamensis* leaflets.

The full grown larvae then entered the resting period called the nymphochrysalis in which larva stopped feeding and the new cuticle was formed under the old cuticle. The shiny surface on the old cuticle clearly indicated the time of ecdysis. This resting stage lasted approximately 0.78-0.88 days on the 3 neem species before molting to nymph. The second instar or nymph was relatively large and red in color. This stage was more active and prefered to move along both sides of midrib. Approximately 19% and 5% of nymphs died on *A. indica* and *A. excelsa* leaflets. *P. azadirachtae* developed significantly faster on *A. indica siamensis* (1.05 ± 0.33 days) than those on *A. excelsa* (1.34 ± 0.37 days) and *A. indica* (2.38 ± 0.71 days). The last resting stage, imagochrysalis, of *P. azadirachtae* lasted 0.74 - 0.93 days.

Adults emerged from imagochrysalis were fusiform, active, and red in color. They gradually increased in size and their color changed to deep red. *P. azadirachtae* was able to complete its development on 3 neem species. The life cycle of *P. azadirachtae* reared on *A. indica* was significantly longer (9.38 ± 1.12 days) than those on *A. excelsa* (7.91 ± 0.83 days) and *A. indica siamensis* (7.80 ± 0.64 days). A total of 89% of *P. azadirachtae* completed their life cycle on *A. indica siamensis* leaflets where only 42 and 58% of those mites reared on *A. indica* and *A. excelsa* leaflets developed successfully into adult stage.

Percentage of egg hatch was considerably high (95-96%) when the mites were reared on *A. indica siamensis* and *A. excelsa* leaflets where only 88.89% of egg was hatched on *A. indica* leaflets. The egg of *P. azadirachtae* gradually changed to deeper color as recorded on other mite species, such as *Eriophyes laevis* Nalepa (Shevchenko, 1975) and *Tegolophus artocarpi* K (Ghosh and Chakrabarti, 1989). *P. azadirachtae* eggs on 3 neem species hatched within 3.69- 4.00 days at 28°C, which was quite longer than 2.39 and 3.04 days of *Coptophylla caroliniani* Chandrapatya & Baker and *Aceria mississippiensis* Chandrapatya & Baker at 29°C (Chandrapatya and Baker, 1986). This could be due to the incubation period which increased as temperature decreased (Dobrivojevic and Petanovic, 1985; Westphal *et al.*, 1990; Shi, 2000). However, egg period of *Aculus fockeui* (Nalepa & Trouessart) and *T. artocarpi* required 3-5 days at 17-19°C and 4.29 days at 24°C (Huang *et al*., 1994; Ghosh and Chakrabarti, 1989) which was similar to this finding.

Larvae fed on cell contents immediately after hatching which was similar to *C. caroliniani* and *A. mississippiensis* (Chandrapatya and Baker, 1986), but differed from *T. artocarpi* that remained inactive for about 6 h (Ghosh and Chakrabarti 1989). The study indicated that the larval periods of *P. azadirachtae* averaged 1.23 – 1.82 days which was longer than 0.78 and 1.08 days of *C. caroliniani* and *A.mississippiensis* at 29°C (Chandrapatya and Baker, 1986) However, Westphal *et al*. (1990) reported that larvae of *Aeria cladophthirus* (Nalepa) required 2-3 days to develop at 18-24°C and *T. artocarpi* larvae required 2.95 days at 24°C (Ghosh and Chakrabarti, 1989) which was contrast with this finding due to lower temperature.

Immature stages are usually similar to adults in appearance, but are smaller in size and lack external genitalia (Lindquest, 1996). The nymph of *P. azadirachtae* developed successfully within 1.05-2.38 days which was faster than 3.03 days of *T. artocarpi* at 24°C (Ghosh and Chakrabarti, 1989) and 4-5 days of *A. cladophthirus* at 18-24° C (Westphal *et al.*, 1990). However, nymphs of *C. caroliniani* and *A. mississippiensis* needed only 0.54-0.80 days at 29°C to complete their development (Chandrapatya and Baker, 1986).

P. azadirachtae completed its development within 7.8 – 9.3 days at 28°C which was longer than 4.81 and 6.64 days of *C. caroliniani* and *A. mississippiensis* at 29°C (Chandrapatya and Baker, 1986) and shorter than 10-12 days of *T. artocarpi* at 24°C (Ghosh and Chakrabarti ,1989) and 11-15 days of *A. cladophthirus* at 18-24°C (Westphal *et al.*, 1990).

Longevity and fecundity

Longevity and fecundity of *P.* females on 3 neem species are shown in Table 2. *P. azadirachtae* reared on *A. indica* lived significantly longer (15.24 days, ranging 10-21 days) than on *A. indica siamensis* (13.06 days) and on *A. excelsa* (12.06 days). Male mites lived considerably shorter than females in all treatments (averaged 9.55 – 10.36 days). Female mites on *A. indica siamensis* laid the first egg after being adult for 0.63 ± 0.39 days while those reared on *A. excelsa* and *A. indica* needed 0.81 ± 0.49 and 1.68 ± 0.95 days, respectively. The longest oviposition period (11.28 ± 1.55 days) was observed on *A. indica siamensis* where the highest egg production of 21.81 ± 4.18 eggs/female and 1.95 ± 0.41 eggs/female/day were noted. This results clearly indicated that *A. indica siamensis* was the most suitable host plant for rearing *P. azadirachtae.* Mites cultured on *A.*

Table 2 Longevity and fecundity of *Phyllocoptes azadirachtae* Chandrapatya on 3 different neem species under laboratory conditions (28±1°C and 58± 4%R.H.).

Parameters	Mean±S.D* (days)		
	A. indica siamensis (Sadao-Thai)	*A. indica* (Sadao-India)	*A. excelsa* (Sadao-Chang)
Pre-oviposition Period	0.63±0.39 b	1.68±0.95 a	0.81±0.49 b
range	(0.5 – 2.0)	(0.5 – 4.0)	(0.5 – 2.0)
n	16	17	18
Oviposition Period	11.28±1.55 a	8.53±2.99 b	8.94±1.98 b
range	(8.5 – 14.0)	(3 – 13.0)	(5.5 – 13.0)
n	16	17	18
Post-oviposition Period	1.22±1.00 b	5.03±2.61 a	2.31±2.11 b
range	(0.5 – 3.0)	(0.5 – 10.0)	(0.5 – 7.0)
n	16	17	18
Egg/female	21.81±4.18 a	7.65±3.46 c	12.44±2.85 b
range	(12 – 25)	(3 – 15)	(8 – 17)
n	16	17	18
Egg/female/day	1.95±0.41 a	0.92±0.30 c	1.41±0.26 b
n	16	17	18
Female longevity	13.06±1.65 b	15.24±3.13 a	12.06±3.19 b
range	(10 – 15)	(10 – 21)	(7 – 17)
n	16	17	18
Male longevity	10.36±2.01 b	11.89±2.85 a	9.55±2.82 b
range	(8 – 15)	(8 – 16)	(7 – 15)
n	6	9	20

* Means in the same row not followed by same letters are significantly different (p<0.05) as determined by Lsd.

indica lived considerably longer on the host plant, but the egg production was very low (only 0.92 ± 0.30 eggs/female/day) and the egg-laying period was also short (8.53 ± 2.99 days). Therefore, total egg production was lower than those mites on the other 2 host plants. In addition, mites on *A. indica* leaflets also had long post-oviposition period which in turn affected the number of egg production in total as well.

Many workers reported that temperature affected mite longevity. Chandrapatya and Baker (1986) reported that *A. mississippiensis* female had a longevity of 30.4 days as compared to 34.9 days for *C. caroliniani* at 25°C. The pre-oviposition period was also depended on the temperature. Shi (2000) indicated that *E. gibbosus* had a pre-oviposition period of 2.85 days at 22.6°C (2.85 days), Boczek *et al.* (1984) found that the oviposition period of *A. fockeui* was 28 days at 28° C which was considerably higher than all experiments in this study. Moreover, *E. gibbosus* at 22.6°C laid only 8.4 egg/female (Shi, 2000) where *Phyllocoptruta oleivora* (Ashmead) at 27° C produced 15.4 eggs/female (Allen *et al.*, 1995) which was in the same range as this study. However, Wahba *et al.* (1985) reported that *Aceria tulipae* K laid 44 eggs/female at 26°C which was much higher than those of *P. azadirachtae* in all treatments.

CONCLUSION

Phyllocoptes azadirachtae is a pest of neem in Thailand. Field observation revealed that this mite was frequently encountered on Sadao-Thai leaf (*A. indica siamensis*) than the other neem species. Biological observation also agreed with this statement since the mite showed the highest survival percentage, the shortest life cycle and the highest egg production on *A. indica siamensis*. This mite distributes in many parts of the country, causing leaf deformation and subsequently leaf defoliation. Hence, more works are needed to be investigated, especially life table and proper control measures in order to keep *P. azadirachtae* under control.

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