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BREEDING STRATEGIES OF *EUTETRANYCHUS ORIENTALIS* (KLEIN) (ACARI: TETRANYCHIDAE) ON NEEM

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ABSTRACT
Development times of the citrus brown mite, *Eutetranychus orientalis* (Klein) was evaluated in the laboratory on excised leaf disc of Neem, *Azadirachta indica* A. Juss at 35 $\pm$ 2°C & 60 $\pm$ 5% relative humidity. Total development times from egg to adult stage were 9.48 $\pm$ 0.09 days. The pre-oviposition period, oviposition period, post-oviposition period were 0.5 $\pm$ 0, 7.7 $\pm$ 0.15 and 0.4 $\pm$ 0.07 days respectively. Fecundity averaged 30.1 $\pm$ 2.1 eggs and longevity, 8.6 $\pm$ 0.14 days.

KEY WORDS - *Eutetranychus orientalis, Azadirachta indica*, protonymph, quiescence, deutonymph, oviposition.

INTRODUCTION

The citrus brown mite, *Eutetranychus orientalis* (Klein), is a serious pest of a wide variety of agricultural, ornamental and medicinal plants (Rasmy, 1977; Dhooria, 1985; Gupta, 1985; Sangeetha and Ramani, 2011a). It inflicts heavy injury to the crop by sucking the sap from leaves, tender shoots, bark and fruits leading to heavy yield loss. *Azadirachta indica* A. Juss., the current host plant of *E. orientalis,* is a representative of family Meliaceae and is known by different names *viz.,* "Neem," "Sacred Tree," "Heal All," "Nature's Drugstore," "Village Pharmacy" and "Panacea for all diseases". Neem , a fast-growing tree that can reach a height of 15–20 metres (49–66 ft) has been distinguished as one of the world's most useful plants. The fruit, seeds, oil, leaves, roots and bark of the tree has amazingly high medicinal values. Neem products are believed to have antihelmintic, antifungal, antidiabetic, antibacterial, antiviral, contraceptive and sedative properties. Neem oil extracted from the seeds of the neem tree has medicinal and insecticidal properties due to which it has been used for thousands of years in medicines, cosmetics and. pest control. Even the humble little leaves have high medicinal value and are used to treat chickenpox and skin infection such as warts, scabies, acne and eczema, increase immunity of the body, reduce fever caused by malaria and for curing neuromuscular pains Twigs of neem are used as toothbrushes and commercial production of toothpastes. Neem extracts from barks and roots help in treatment of diabetis, AIDS, cancer, heart diseases, allergies, herpes, ulcers, hepatitis and several other diseases. Excellent oil derived from its seeds is used for healthy hair growth, improved liver functioning, detoxification of blood and balancing blood sugar levels with no side effects (Biswas *et. al.,* 2002). Neem extracts have been approved by the U.S. Environmental Protection Agencies for use on food crops. It has been proven in various research studies that neem is non-toxic to birds, benefical insects or human and protects crops from over 200 of the most costly pests. In spite of the ubiquitous nature of the tree, very little quantum of research has been carried out so far to save the tree from invasion of pests that affect its life and productivity. This prompted to undertake the present study on the biological aspects of one of the major mite pest infesting the tree viz., *E. orientalis.*

MATERIALS AND METHODS

Outdoor culturing of mites

Live cultures of *E. orientalis* were maintained on Neem in the field to ensure adequate supply of life stages as well as to observe closely the mode of infestation of the concerned species, on the host plant. To achieve this objective, two mite treatments (i) M^-, mite free plants and (ii) M^+, plants artificially infested with mites, were included in a randomized block design plots (3mx3m) which was replicated four times within a season. Cultivation of host plants was done by planting stem cuttings of Neem in enriched soils prepared for the study. The plots were irrigated regularly and the plants were made mite-free by spraying a broad-spectrum insecticide thiodicarb to eliminate mite pests and predators (Reddall *et al*., 2004). Artificial infestations of M^+ plants were done by stapling mite-infested leaf bits grown in the glass house 60 days after planting. The plots were covered with fine nets to ensure protection from pest attack and to reduce the risk of cross infestation between M^+ and M^- plots.

Indoor culturing of mites

Live cultures of different stages of the mites were maintained in the laboratory at 30 ± 2°C and 70 ± 5% relative humidity on fresh leaves of Neem, collected from the plots at an interval of 2 days or at the time of need. Mite culturing was carried out following the leaf flotation technique (Sangeetha and Ramani, 2007). Individual cultures of all life stages of the mite were maintained on leaf discs (16 cm^2) of neem placed in petridishes lined with water-saturated cotton pads and was treated as an experimental unit. Stock cultures of the mites were also maintained in the laboratory in the same manner so as to ensure constant supply of life stages.

Biological studies of mites

To determine the duration of sexual development, 10 colonies of newly moulted females were introduced along with 2-4 new males and the males were removed soon after the females laid their first set of eggs. Studies on parthenogenesis were initiated starting from 5-10 quiescent female deutonymphs that moulted to females and laid their first batch of eggs. Beginning with these eggs, the complete life cycle of the individual mites were traced. Adult mites of each colony were transferred to fresh leaf disc every 24hrs. Regular observations at 6h interval was done using Stemi DV4 stereozoom microscope to gather information on mating, oviposition, incubation and hatching, larval and nymphal stages, quiescence and moulting and total duration of F_1 generations. Values are expressed as mean ± SEM (Standard Error of Mean). 'n' indicates the number of trials.

RESULTS

The citrus brown mite, *Eutetranychus orientalis* passes through four developmental stages with resting (quiescent) and moulting phases at the end of larval and nymphal development. The duration of individual developmental stages of *E. orientalis* on Neem in different generations maintained in the laboratory is detailed below.

Oviposition

Adult females exhibited a general preference to the upper surface of the leaves though no specific selection of site for depositing eggs was noticed. Ovipositing females constructed silken webs prior to the deposition of the eggs. On several occasions, females were found depositing eggs at random on the webbing and secrete an adhesive coating over the egg. Freshly laid eggs were disc shaped and translucent. At the end of the first day of oviposition, a dark brown spot appeared on one side of the egg. On the following day, they turned to yellowish brown and later to orange on the third day. The eye spots appeared as a pair of dark red spots, few hours prior to hatching. The pre-oviposition period of *E. orientalis* on *A. indica* recorded was 0.5 ± 0 day. Oviposition period and post-oviposition period of the mite on neem comprised 7.7 ± 0.15 and 0.4 ± 0.07 days respectively (Table I).

TABLE I: Pre-oviposition, Oviposition and Post-oviposition periods of *E. orientalis* on *A. indica*

Pre-oviposition	Oviposition	Post-oviposition
0.5 ± 0	7.7 ± 0.15	0.4 ± 0.07

n = 35

Fecundity and Longevity

The eggs laid by a single female and the daily fecundity of *E. orientalis* are presented in Table II. Fecundity was recorded minimum during the 1st and 2nd days of oviposition under laboratory conditions. During the 4th or 5th days of oviposition, the fecundity attained the maximum level and it showed a gradual decline from the 8th day onwards, reaching the minimum on the final day of oviposition. This trend was common in both mated and virgin females. However, the average fecundity was found more in the case of mated females. The total number of eggs laid per female in her life time was 30.1 ± 2.0 eggs (Mated - 35.2 ± 1.9 eggs & Virgin - 25.0 ± 1.7 eggs). Longevity of the females averaged 8.6 ± 0.14 days (Mated - 8.6 ± 0.19 days & Virgin - 8.6 ± 0.24 days) (Table III).

TABLE II: Number of eggs laid by *E. orientalis* on *A. indica* in different days of oviposition

1	2	3	4	5	6	7	8	Total number of eggs laid
2.7±0.26	4.3±0.47	6.0±0.48	9.4±0.63	6.7±0.40	4.1±0.33	2.1±0.39	1.2±0.37	35.2±1.9 Mated female
2.1+0.26	3.2+0.47	5.1+0.48	7.2+0.63	4.4+0.40	2.3+0.33	1.4+0.39	1.0+0.37	25.0±1.7 Unmated female

n = 35

TABLE III: Longevity of *E. orientalis* on *A. indica*

Males	Mated females	Unmated females
8.6 ± 0.14	8.6±0.19	8.6±0.24

n = 35

The process of hatching was initiated by the formation of a semicircular slit at the equatorial region on the final day of incubation. Prior to the initiation of hatching, inflation of the egg and subsequent development of a hyaline area beneath the upper part of the egg shell could be noted. The slit formed continued to either side followed by wriggling movements of the larva. It was hastened by the active movement and thrusting action of the emerging larva which favored protrusion of its legs and mouth parts. Later, the larva struggled out of the egg shell through a backward kick using its hind legs. Since the division was not complete, the two halves of the egg shell were held together along a small length. Discarded egg cases could be located at the hatching sites. Hatching was completed in 12 – 15 minutes.

Duration of developmental stages (Table IV)

TABLE IV: Duration (in days) of development of *E. orientalis* on *A. indica*

Egg	Larva	1st Q	Proto-nymph	2nd Q	Deuto-nymph	3rd Q	Total duration
3.1±0.01	1.1±0.02	0.5±0	1.9±0	0.6±0.01	1.5±0	0.5±0	9.2 ± 0.04 Parthenogenetic Male
3.12±0.03	1.53±0.02	0.6±0.01	0.92±0.01	0.89±0.01	1.6±0.01	1±0	9.48 ± 0.09 Sexual Male
3.26±0.0	1.6±0.02	0.7±0.01	0.99±0	0.5±0	1.8±0	0.9±0.03	9.75 ± 0.06 Sexual Female

n = 35

Incubation period

The period of incubation of *E. orientalis* on *A. indica* took shortest duration of 3.1 days for parthenogenetic development and longest duration of 3.26 days for sexual development. However, the period averaged 3.12 ± 0.06days.

Larval period

The larva was small, hexapod and yellowish with slight sexual dimorphism especially at the hysterosomal region. The larva remained motionless for a short duration after hatching and then initiated feeding.. Feeding characteristics resembled that of the larva of other tetranychid representatives. As feeding proceeded, the colour of the larva turned reddish orange. The newly hatched larval active life averaged 1.33 ± 0.07 days. Like other tetranychid larvae, the larva of *E. orientalis* also passed through the first quiescent phase, which subsequently moulted into the 1st nymphal stage or the protonymph.

Protonymphal period

Protonymph or the first stage nymph differed from the larva by its slightly larger size, octopod nature and pale red coloration with greenish black spots on its dorsolateral region. The protonymph was more active than the larva. Feeding started at about 5 to 10 minutes after moulting from the quiescent stage as a result of which the colour of the protonymph turned darker Active feeding was followed by second quiescence and moulting phases respectively. The active protonymphal period on neem extended for 0.92 ± 0.03 day at the end of which the protonymph entered into the second quiescent phase, followed by the process of moulting into the deutonymph.

Deutonymphal period

Deutonymph or the second stage nymph represented the final instar before attaining the adult status. Deutonymphal stage showed marked resemblance with the adult stage, except for the smaller size, paler colour and difference in setation. Sexual dimorphism was quite obvious at this stage. The hysterosoma of the female was markedly robust due to ovarian development while that of the male tapered towards the anal region. Deutonymph exhibited voracious feeding which progressed till quiescence. The duration of deutonymphal period averaged 1.6 ± 0.04 day with the shortest duration of 1.5 days and longest duration of 1.75 days in thirty five generations studied

Adult Stages

Freshly moulted adult can be distinguished to their sexes with ease. Newly emerged male was slightly reddish in colour and characterized by elongate legs. Eye spots were prominent and red. Sexually mature male was smaller than the female with a tapering hysterosoma. Adult male was very active and often found moving in search of quiescent female deutonymphs. Adult female was larger with short legs and posteriorly rounded hysterosoma. The female was comparatively sluggish in nature and greenish-red in colour with black spots on the dorsum of the hysterosoma. Feeding was initiated soon after moulting and at this stage, they appeared light red in colour. As feeding progressed, the colour changed to dark red. The emergence of females after moulting immediately followed copulation and active feeding prior to the initiation of oviposition. Males either wandered actively, searching for females for mating or engaged in feeding activity. The average total duration from egg to adult stage was 9.48 ± 0.09 days.

Quiescent Periods

A period of total inactivity was noted in *E. orientalis* at the end of the active period of larva and subsequent nymphal stages. During this period, feeding and other life activities were found ceased followed by the settling down of the mite on a suitable site on the leaf blade. While in quiescence, all the legs were found withdrawn beneath the hysterosoma and the individual at this stage appeared oval in shape. After few hours, its body became turgid and shiny. Later the cuticle turned transparent. In the life history of *E. orientalis*, three quiescent phases, known as the first, second and third quiescence were noted, each at the end of the active larval, protonymphal and deutonymphal stages respectively. The respective durations recorded for the Ist, IInd and IIIrd quiescent stages of *E. orientalis* on *A. indica* were 0.6 ± 0.04 day, 0.89 ± 0.04 day and 1 ± 0 day

Moulting

The process of moulting followed every quiescent stage and resulted in the release of the subsequent instar. The process began with the development of a narrow slit across the dorsum at the propodosoma. Subsequently the slit got widened by the movements of the individual. At this stage, the anterior pair of legs and mouth parts of the emerging instar protruded out of the moulting skin. The active movements of the individual resulted in its emergence, leaving behind the exuviae on the leaf surface. However, the process required 10-15 minutes for completion.

Mating

Sexual reproduction by mating occurred in *E. orientalis* soon after the emergence of the female deutonumphs. Males were the sexually active partners who were found actively moving in search of receptive females. On several occasions fighting among males for a single female ready to emerge from quiescence were observed. The successful male mated with the emerging virgin female. During the process, the male crawled beneath the female and curved its hysterosoma towards the genitalia of the female and inserted its aedeagus to transfer its sperms. The femaleswere found mating only once while males copulated several times. Mating was completed in 2 minutes.

Total duration of development

The females of *E. orientalis* exhibited two types of reproduction – sexual and parthenogenetic. The eggs laid by mated females developed into both males and females with a sex ratio 1: 10 while those of virgin females developed only into males. The total duration of development showed variation with respect to the nature of reproduction. However, parthenogenetic development required comparatively shorter duration (9.2 $\pm$ 0.04 days) compared to sexual development (9.75 $\pm$ 0.06 days). The parthenogenetic development required comparatively shorter duration than sexual ones.

DISCUSSION

Information gathered on the developmental aspects of the spider mites studied depicted a common pattern of developmental processes as in other Tetranychid mite species. The development involved larval and 2 nymphal instars before attaining the adult status. Each of the instar after the larval stage was preceded by quiescent and moulting processes. Mites in general, exhibit certain degree of site selection for oviposition (Banu and Channa Basavanna, 1972; Sangeetha and Ramani, 2011b & c). However, *E. orientalis* showed no specific preference during oviposition. The eggs were laid in a very random fashion, all over the leaf lamina. The waxy secretion and sparse webbing in *E. orientalis* (Banu and ChannaBasavanna, 1972) probably served adequate protection to the eggs and the subsequent instars, which may be the possible reason for the randomised deposition of eggs. The pre-oviposition period, oviposition and post-oviposition periods of *E. orientalis* was recorded to be around 0.5, 7.5 and 0.4 days on *A. indica.* Similar observations on the life history of *T. evansi* were recorded by Bonato (1999) and Sangeetha and Ramani (2011c) on *O. biharensis*. The duration of egg laying period in the females of *E. orientalis* was 8 - 9 days. This was higher than those recorded by Dhooria (1985) when Lal (1977) and Rasmy (1978) noted no much variation in the oviposition periods for the mite. The number of eggs produced during the life time of a female tetranychid mite may vary greatly among species and also with hosts, temperature and relative humidity. It is not surprising to note that even individuals of the same species show variation in the number of eggs produced by them. In the present study, a maximum of 10 females to 1 male was found in the population of the mites reared on the *A. indica.* The number of females always outnumbered males. The hatching process involved the formation of an equatorial slit on the egg case and culminating in the separation of the case into two halves. The entire process averaged 10 – 20 minutes in duration. Moulting of the quiescent individuals was initiated by the formation of a horizontal slit at the dorsal region of the propodosoma followed by the slow backward movement of the individual. These findings are in support with earlier reports on tetranychid mites (Siddig and Elbadry, 1971; Banu and ChannaBasavanna, 1972; Gupta, 1985; Sangeetha and Ramani, 2007, 2008). Mating marks the success of reproductive potential, which is crucial to the survival of a species. The phenomenon of sperm transfer was direct, achieved through copulation. The males emerged earlier than the females and found guarding the quiescent female deutonymphs and copulating with the female immediately after the emergence of the latter. These observations are in agreement with the behavioural activities observed by Banu and ChannaBasavanna (1972). In depth studies on the mediation of the male attraction towards the female deutonymphs (Cone *et al.,* 1971; Hazan *et al.,* 1973; Penman and Cone, 1974) have attributed the role of sex pheromones in the process. Generally, a single copulation was reported in the case of females, while males were known to copulate many times (Banu and Channa Basavanna, 1972, Nandagopal and Gedia, 1995; Noronnha, 2006; Sangeetha and Ramani, 2007a, 2011b).

The instance of parthenogenesis has been reported in several spider mite species by Nandagopal and Gedia (1995). During the present study, mated females of all the species were found to lay eggs which developed into both males and females. However, the eggs deposited by virgin females always developed into males only. This has clearly indicated the occurance of normal sexual reproduction as well as parthenogenesis in *E. orientalis.* Probably, the occurrence of dual reproductive means may enhance the male population which is otherwise found low in field conditions.

Longevity of the mites was often found influenced by several factors, of which temperature, humidity and mating appear to be vital. The respective life span of the male as well as females of *E. orientalis* was 8-9 days. Longevity of the mite is in conformity with the observation of Ray and Rai (1981) on lady's finger. Further, mating has established a negative influence on the longevity of these individuals. Rate of daily egg production was another factor that influenced the longevity of these individuals. Results of the present study further suggest that high frequency of oviposition reduces the life expectancy of the females in this species. In addition infestation by this mite species further reduces the quality of the leaves which otherwise would exceedingly be used for preparation of Ayurvedic medicine and so on. The temperature-humidity combination of 35^{o}C & 60% RH was found to be best suited for the successful survival and development of the mite, *E. orientalis* on *A. indica*. Thus, the study elucidates the fact that warmer temperature and low relative humidity available during the summer months in our state would ensure ideal conditions for the rapid population build up of the citrus brown mite on neem. Being one of the most valuable crops of our medicinal concern, this has to be considered seriously, as this major mite pest would become a great threat to *A. indica.* Further, a cognitive aspect that emerged from the current study was the colonisation of the mite on a new, so far unrecorded host *A. indica* and its population build-up in alarming rates on the host so as to acquire the status of a major pest. Thus, the results of the present study enabled to extend the host range of the species, by adding a new host to the existing list of species of host plants.

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