

**LIFE HISTORY TRAITS OF *Neoseiulus californicus* IN THE MONTHS FROM
SEPTEMBER TO DECEMBER WHILE FEEDING *Tetranychus urticae***

A THESIS

BY

MD. RIAD SULTAN

Student No. 1701026

Session: 2022-2023

Semester: January-June, 2024

MASTER OF SCIENCE (MS)

IN

ENTOMOLOGY



**DEPARTMENT OF ENTOMOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY
DINAJPUR, BANGLADESH**

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JUNE 2024

DEDICATED
TO MY
BELOVED PARENTS

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The Author

ABSTRACT

Tetranychus urticae (Acari: Tetranychidae), Koch is regarded as a destructive pest of many crops all over the world and *Neoseiulus californicus* is one of the most important predators of *T. urticae*. The aim of this study was to investigate the life history traits of *N. californicus* in the months of September, October, November and December when fed on *T. urticae* as prey. The immature development and adult performance of *N. californicus* were significantly differed during the studied months. Male developmental times were shorter than females in each month. The longest adult female life span, oviposition period and female fecundity observed in December whereas the shortest in September. In sex ratio, utmost number of females (0.73) was counted in October and the lowest (0.64) in December. Survivorship varied from 88.89 to 100% during immature development in October, November and December but all immatures were survived in September. From life table analysis, the highest net reproductive rate ($R_0 = 33.91$) was found in November while the intrinsic rate of natural increase ($r_m = 0.39$) and generation time ($T=15.30$) was estimated the highest in December. The finite rate of increase was found maximum as $\lambda=1.74$ whereas the gross reproductive rate was minimum (GRR=29.66) in September. Based on these results, we cautiously expect that the biological traits of *N. californicus* are effective to control *T. urticae*.

Key words: *Neoseiulus californicus*, *Tetranychus urticae*, month, development, fecundity, life table, sex ratio.

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CHAPTER 1

INTRODUCTION

The phytoseiid mite, *Neoseiulus californicus* McGregor (Acari: Phytoseiidae) is considered as a promising candidate for biological control of spider mites in various field crops (McMurtry and Croft, 1997), specifically greenhouse crops (Calvitti and Tsolakis, 1992; Smith *et al.*, 1993). The predatory mite, *N. californicus* is distributed over wide geographical range, both in arid and humid areas of sub-tropical and temperate zones and is reported from the USA, Australia, Africa, Europe and Asia (McMurtry 1977; Ferreira and Carmona 1994). It was first introduced in 1954 in California where it was collected from lemon trees (de Moraes *et al.*, 2004). Besides, they were also collected from Texas (USA), Mexico, Brazil, Guatemala, Peru, Argentina, Chile, Cuba, Colombia, Japan, Taiwan (introduced), Portugal, Italy, France and Spain (McMurtry, 1977; Ferreira and Carmona, 1994; Rencken and Pringle, 1998; de Moraes *et al.*, 2004). Different indigenous strains of *N. californicus* like US, Korean, Japanese strains are explored which successfully control the phytophagous mites and small arthropod pests.

It has a broader food range and a higher capability to reside in patches with low pest density than other phytoseiids (Wei-Lan and Laing, 1973; Croft and Slone, 1997; Pratt *et al.*, 1999). The broader food range may provide advantage to facilitate permanent establishment of *N. californicus*, which can survive and reproduce on alternative food sources including different mites, insects, and even pollen (Swirskii *et al.*, 1970; Castagnoli and Falchini, 1993; Croft *et al.*, 1998), even though it prefers spider mites (McMurtry and Croft, 1997). It has been described as one of the main natural enemies of spider mites (Greco *et al.*, 2005; Gerson and Weintraub, 2007), feeding on all stages of *Tetranychus urticae* Koch, and other pest mites (McMurtry, 1997; Castagnoli *et al.*, 1999

b; Ragusa *et al.*, 2009), and that associate with their dense webbing as it can cut through with its chelicerae (McMurtry *et al.*, 2013). This predatory mite can decrease spider mite population below economic threshold in the greenhouse and field, e.g. on gerbera (Schausberger and Walzer, 2001), strawberry (Sato *et al.*, 2007), sweet pepper (Weintraub and Palevsky, 2008), cucumber (Alzoubi and Cobanoglu, 2010); clementine (Abad-Moyano *et al.*, 2010), apple (Pringle and Heunis, 2006), and citrus (Katayama *et al.*, 2006).

On the other hand, Two-spotted spider mite (TSSM), *T. urticae*, is a cosmopolitan and harmful phytophagous herbivore pest of agricultural crops (20-45% lost of the yield according to the season) as growth, chlorophyll contents and fruit size as well as quality are affected in severe mite infection (Rhodes *et al.*, 2006; Premalatha *et al.*, 2018) in Bangladesh and throughout the world. It attacks a wide range of plants including ornamentals, fruit crops and vegetables and feeds on more than 1100 species of plants in 140 families of economic value (Vassiliou and Kitsis 2013). This mite attacks mainly the underside of leaves, causing the formation of bleached spots, abundant web, and significant production loss (Ferla *et al.*, 2007). The available *Tetranychus* species are such as two-spotted spider mite *T. urticae* (Helle and Sabelis, 1985), the Kanzawa spider mite *T. kanzawai* Kishida (Hamamura *et al.*, 1976), the Pacific spider mite *T. pacificus* (Amano and Chant, 1977; Takashi and Chant, 1994), the tomato spider mite *T. evansi* (Moraes and McMurtry, 1985) or the gorse spider mite *T. lintearius* (Dufour) (Pratt *et al.*, 2003). For the control of TSSM, farmers mainly depend on synthetic acaricides or pesticides (Sato *et al.*, 2007). Insecticidal control is expensive as well pollutes the environment and leads to many health problems due to high residual effects. Because of their rapid developmental rate, short generation time and high net reproductive potential, TSSM rapidly develops resistance to various miticides only after a few applications (Van

Leeuwen *et al.*, 2010). Using predatory mites of the family Phytoseiidae as biological control agents can be quite effective against *T. urticae* when done correctly to reduce pest damage below the level of economic injury (Afifi *et al.*, 2015; Chong, 2022). *N. californicus* is able to survive in the absence of *T. urticae*, by feeding pollen, other mites, aphids and thrips (Moraes and Flechtmann, 2008), or even perform cannibalism (Escudero and Ferragut (2005), as generalist predator (McMurtry and Croft, 1997). Tightly associated with *T. urticae* (Taj and Jung, 2012) and the use of alternative food source and the ability to resist to higher temperatures than other species allow *N. californicus* to remain for a longer period in the environment by preemptively acting. Mites may have a great genetic variability and its species specialize in environments where they remain for a long time (Magalhães *et al.*, 2007). To control mites using natural enemies is a reality in the biofactories of many countries where they produce natural enemies and sell them to farmers, mainly in Europe and North America (Moraes, 2002). There are about 30 species of predatory mites commercially produced as biological control agents such as *Phytoseiulus persimilis* Athias-Henriot, *Neoseiulus cucumeris* (Oudemans), *N. californicus* (McGregor) and *Amblyseius swirskii* Athias-Henriot that have been successfully applied in many countries (van Lenteren, 2012; Liu *et al.*, 2017).

The predator *N. californicus*, a native species of Bangladesh, was first reported on eggplant in northern and southern Bangladesh (Gapud, 1981). Recently, this predator was found in the vegetable fields of HSTU campus. But the life table parameters and predation efficiency of this predatory mite is still unexplored. Life processes are closely related to environmental factors which play a crucial role in developmental and reproduction rates of arthropods (Gotoh *et al.*, 2010; Shi and Ge, 2010). Knowing the abiotic requirements of the different stages of the life of a target species can be used to

forecast its possible distribution and population dynamics. The intrinsic rate of natural increase value (r_m) is a good indicator to describe and evaluate the adaptation and growth of a population of arthropods to distinct environmental situations (Birch, 1948) and the predator efficiency is strongly influenced in the months of September, October, November and December. So, the environmental requirements for the development of *N. californicus* as vital information for using this predatory mite in biological control program. This study investigates the effects of the studied months on the major biological parameters of the predator including development, reproduction and fecundity and important life table parameters under laboratory conditions and also compares the Bangladeshi *N. californicus* with the most frequent and commercially successful spider mite biocontrol agent in the world *Phytoseiulus persimilis* (McMurtry and Croft, 1997). If the indigenous origin *N. californicus* prove to be the promising one as compared to other strains, then it can be produced commercially and used to control the pests biologically throughout the country which can be safe for both the environment and human health.

Objectives

1. To investigate the biological traits of the predatory mite *N. californicus*.
2. To establish *N. californicus* as a potential biological control agent of *T. urticae*.

CHAPTER 2

REVIEW AND LITERATURE

Leguminous plants (Fabaceae) are considered of a great economic importance for local consumption and exportation in Bangladesh. Legumes play a vital role in agro-ecosystems based on their ability to form a symbiosis with soil rhizobia that fix atmospheric nitrogen. Soybean, *Glycine max* (L.), is considered as a good source of oil and protein for human and livestock consumption.

Tetranychus urticae (Acari: Tetranychidae), Koch is regarded as a destructive pest of beans, eggplants, tomatoes, cucumbers, potatoes, strawberries, horticultural crops etc. In Bangladesh, the infestation of two-spotted spider mites has been exacerbated by several factors. High temperatures and low humidity, common in many regions of the country, create ideal conditions for these mites (plantpropagation.org). Farmers in Bangladesh often rely heavily on chemical control methods, which can lead to the development of pesticide resistance in spider mite populations. This makes it increasingly difficult to manage infestations using traditional chemical treatments alone (MDPI). Due to the excessive use of chemicals, the natural enemies of the spider mites are decreased and thus recently, TSSM become an important pest of vegetable crops in Bangladesh.

2.1. Biology of *Tetranychus urticae* (Koch)

The two spotted spider mite *Tetranychus urticae* Koch (Acarina: Tetranychidae) is a serious pest on many crops and ornamental plants. This mite feeds on more than 150 species of host plants, including most deciduous fruit trees and vegetables (Jepson *et al.*, 1975; Krips *et al.*, 1998; Sabelis, 1981; van de Vrie *et al.*, 1972). The spider mite has a wide host range includes deciduous bushes and trees belonging to the family Rosaceae,

but it is in association with fruit trees such as apple, pear, plum, peach, prune and cherry that it reaches economic importance.

Species in the Tetranychidae undergo three immature stages; a six-legged larval stage, and eight-legged protonymph and deutonymph stages. Each of these stages is followed by a quiescent stage known as the protochrysalis, deutochrysalis and the teleiochrysalis respectively (Van de Vrie *et al.*, 1972). Mated females will produce either female or male offspring, while unmated females produce males only (Cagle, 1946). The eggs of the two-spotted spider mite (*Tetranychus urticae*) are small, spherical, and typically range from transparent and colorless to opaque straw yellow. They are generally found on the underside of leaves, where the mites prefer to lay them. (Penn State Extension) (NCIPMHORT) The eggs' incubation period is highly dependent on photoperiod, temperature and nutritional status of the female as summer females exposed to cool conditions lay diapausing eggs (Van de Vrie *et al.*, 1972). *T. urticae* development, survival, and reproduction are driven mainly by temperature and precipitation with lesser effects from food supply and host nitrogen status (Wermelinger *et al.*, 1992). Rainfall can have a significant negative impact on spider mites, and, also, that may be exacerbated by high temperatures (Van de Vrie *et al.*, 1972; Simpson and Connell, 1973). Dispersal is largely by wind and they can also readily crawl within trees (Garman and Townsend, 1938; Smitley and Kennedy, 1985).

Females are capable of laying over 100 eggs during their lifespan, up to 19 eggs per day in optimal conditions. This rapid reproductive rate allows for quick population growth and significant plant damage within a short period (NCIPMHORT). Two-spotted spider mites cause damage to plants by piercing leaf cells with their needle-like mouthparts and extracting cell contents, leading to chlorotic spots on leaves. Severe infestations can result in extensive leaf discoloration, stippling, and even complete defoliation. The mites

also produce fine webbing, which can cover the plants and further impede their growth (Entomology and Nematology Department) (Koppert BioCrop). Mainly, damage to the host is done by both immature and adult mites. This damage can result in leaf abscission and small fruit with poor color (Garman and Townsend, 1938), caused by *Tetranychus* spp. (Youngman *et al.*, 1986, Mobley and Marini, 1990). Several workers have reported effects on growth, yield, fruit colour and fruit bud production of apple trees which they have attributed to infestations of *T. urticae* (Chapman *et al.*, 1952; Van de Vrie, 1956; Gould, 1965; Briggs and Avery, 1968; Avery and Briggs, 1968; Coghill, 1969). In commercial orchards, the potential of spider mites to cause severe economic damage necessitates chemical control several times a year (Croft, 1975). However, it is considered the most destructive mite in apple orchards where it has been shown to affect fruit size, color, number of fruits the following season, and tree vigor (Croft and McGroarty, 1977).

Many mite species overwinter in diapause (Veerman, 1992) in protected habitats. Shorter day length and cool temperatures are the main inducers of diapause in spider mites (Van de Vrie *et al.*, 1972). In early spring the overwintering eggs hatch and the young larvae move to the foliage and start feeding (Pfeiffer and Schultz, 1986; Jeppson *et al.*, 1975). TSSM overwinter in facultative adult diapause (Veerman, 1992) and use tree trunk barks as a major overwintering habitat (Lee, 1990; Kim and Lee, 2003) and also in ground vegetation or on fallen leaves (Kim *et al.*, 2005).

Spider mites have a tendency to develop their resistance to many classes of pesticides (Brader, 1977, Dennehy *et al.*, 1983, Herron *et al.*, 1994). Bowie *et al.* (1988), and Collyer and Geldermalsen (1975) observed the development of resistance in the *T. urticae* against some pesticides, e.g. organotin miticides, Azocyclotin, dicofol. Many authors investigated the pesticides resistance in the TSSM and reports on the occurrence

of acaricide resistant *T. urticae* as well as on the failure to control *T. urticae* using different pesticides (Edge and James, 1986; Fergusson-Kolmes *et al.*, 1991; Herron *et al.*, 1993; Cho *et al.*, 1995; Beers *et al.*, 1998; Stumpf and Nauen, 2001; Sato *et al.*, 2004; Suh *et al.*, 2006). TSSM has a relatively high possibility of developing pesticides resistance due to haplo-diploid parthenogenesis, short life cycle and high reproductive potential (Boudreaux, 1963; Huffaker *et al.*, 1969, 1970; Cranham and Helle, 1985).

2.2. Taxonomy of *T. urticae*

Kingdom: Animalia

Phylum: Arthropoda

Sub- phylum: Chelicerata

Class: Arachnida

Sub-class: Acari

Super-order: Acariformes

Order: Prostigmata

Family: Tetranychidae

Genus: *Tetranychus*

Species: *T. urticae*

(Koch, 1836)

2.3. Biology of *Neoseiulus californicus* (McGregor)

Mites belonging to the family Phytoseiidae are generally known as predators of phytophagous mites and are regarded as the most important natural enemies of spider mites (Hell and Sabelis, 1985). The generalist predatory mite of the Phytoseiidae *Neoseiulus californicus* (McGregor) has the potentials to feed on spider mites,

tarsonemid mites, other small arthropods and pollen (Castagnoli *et al.*, 1999). For this reason, this predatory mite has been studied extensively with respect to its basic biology and potential application as biological control agents (Hoy, 1982; Helle and Sabelis, 1985).

2.3.1. Identification

2.3.1.1. Morphological characteristics

N. californicus adults are relatively small, typically around 0.3 mm to 0.5 mm in length. The body is oval and somewhat flattened. Adults are typically pale yellow to tan, but the color can vary depending on their diet. They have eight legs (as all adult mites), with the first pair often being slightly longer and more robust. They have a distinctive pattern of dorsal setae which are relatively short and smooth also crucial for identification. The chelicerae (mouthparts) are adapted for piercing and sucking, indicative of its predatory nature. The peritreme (a structure associated with the respiratory system) extends forward to the level of the second pair of legs, which can help differentiate it from other similar species (Chant and McMurtry, 2007; Hoy, 2011).

2.3.1.2. Molecular Identification

DNA barcoding using mitochondrial DNA, particularly the cytochrome c oxidase I (COI) gene, is a reliable method for distinguishing *N. californicus* from other closely related mite species. This technique involves sequencing a standard region of the genome and comparing it to a reference database (Tixier *et al.*, 2008; Kreiter and Tixier, 2007).

2.4. Sexual dimorphism

Females are generally larger than males often exhibit a more rounded abdomen when carrying eggs, while males tend to be more slender. There are minor differences in the

arrangement and length of setae (sensory hairs) on their bodies, particularly on the dorsal shield and legs, which can be observed microscopically.

2.5. Regional distribution and natural habitat of *N. californicus*

Neoseiulus californicus, a predatory mite widely used in biological control, has a diverse regional distribution. This species is known for its adaptability to various climates and is found all over the world, both in arid and humid areas of sub-tropical and temperate zones. The predatory mite naturally occurred in USA, Mexico, Brazil, Guatemala, Peru, Argentina, Chile, Cuba, Colombia, Japan, Taiwan (introduced), Portugal, Italy, France and Spain (de Moraes *et al.*, 2004; McMurtry, 1977; Rencken and Pringle, 1998; Ferreira and Carmona, 1994). Jung *et al.* (2006) reported a natural population of *N. californicus* distributed in citrus orchards with ERM in Jeju Island for the first time from the outdoor field in Korea.

Table 1. Distribution of *N. californicus* throughout the world

Continents	Distributed Countries
Africa	South Africa
Asia	Japan, South Korea, Taiwan, parts of China
Europe	Portugal, Italy, France and Spain, Mediterranean areas
North America	Mexico, USA, Cuba, Guatemala
South America	Brazil, Argentina, Chile, Peru, Colombia
Oceania	Australia

- The natural habitat of *N. californicus* is very diverse. The predatory mite is found on several natural habitats such as apple, peach grape Strawberries, Avocado, Cassava, Wheat, Pine Mallow, Citrus, Alfalfa, Poplar, Lemon fruit, Vegetables and Ornamental plants.

The mites can even be found between ground cover such as dry leaves (Castagnoli and Simoni, 2003).

2.6. Taxonomy of *N. californicus*

Kingdom: Animalia

Phylum: Arthropoda

Class: Arachnida

Sub-class: Acari

Super- order: Parasitiformes

Order: Mesostigmata

Family: Phytoseiidae

Genus: *Neoseiulus*

Species: *N. californicus*
(McGregor, 1954)

2.6.1. Synonyms

- *Amblyseius californicus* (McGregor, 1954)
- *Cydnodromus californicus* (Athias-Henriot, 1977)
- *Neoseiulus chilensis* (Dosse, 1958)
- *Neoseiulus mungeri* (McGregor, 1954)
- *Typhlodromus californicus* (McGregor, 1954)
- *Typhlodromus mungeri* (McGregor, 1954)

The information was compiled from resources like the Global Biodiversity Information Facility (GBIF) and the University of Florida's Entomology and Nematology Department database.

N. californicus was first described by McGregor in 1954 from lemon in California as *Typhlodromus californicus* (de Moraes *et al.*, 2004). In 1958, this species was renamed as *Typhlodromus chilensis* (Dosse, 1958). After 1958, it was moved to the genus *Amblyseius* as *Amblyseius chilensis* (Ehara, 1964) and later to the genus *Neoseiulus*, and *Neoseiulus chilensis* is also considered a synonym of *N. californicus* (Moraes *et al.*, 1986; Chant and Mcmurtry, 2003; Ehara and Amano, 2004). Ehara and Amano (1998) considered this species as *Amblyseius californicus*.

2.7. Host range and specificity

N. californicus feeds on Tetranychids (e.g. TSSM, ERM) and on Tarsonemids. Adults eat all stages of spider mites; larvae eat mainly eggs, while nymphs eat eggs, larvae and nymph. *N. californicus* shows a feeding preference for the larval and nymphal stages of the TSSM when the pest is present at low densities (Malais and Ravensberg, 2003). However, *N. californicus* can survive for a few days without eating a prey by feeding solely on a diet of pollen (Malais and Ravensberg, 2003). Castagnoli and Simoni (2003)

recorded successful development and reproduction in laboratory trials on the following prey species:

2.7.1. Mites

2.7.1.1. Tetranychidae: *Tetranychus pacificus* (McGregor), *Panonychus ulmi* (Koch), *Tetranychus cinnabarinus* (Boisduval), *Eotetranychus orientalis* (Klein), *Mononychellus tanajoa* (Bondar) eggs, *M. progresesivus* Doreste, *Oligonychus pratensis* (Banks), *O. perseae* Tuttle, Baker et Abbatiello, *O. ilicis* (McGregor)

2.7.1.2. Tarsonemidae: *Polyphagotarsonemus latus* (Banks) and *Phytonemus pallidus* (Banks)

2.7.1.3. Eriophyidae: *Aculops lycopersici*, *Aculus schlenchtendali* (Nalepa) and *Aceria dioscoridis* (Solimati and Abou-Awad).

2.7.1.4. Tenuipalpidae: *Brevipalpus phoenicus* (Geijskes)

2.7.1.5. Pyroglyphidae: *Dermatophagoides farinae* (Hughes)

In laboratory trials nymphs were not able to mature on the Eriophid mite *Phyllocoptruta oleivora* (Swirskii)

2.7.2. Insects

N. californicus accepted *Frankliniella occidentalis* (Pergande) as alternative food, although their oviposition dropped dramatically. Limited maturation was found on eggs of the moth *Prays citri* Milliere, but females did not produce eggs. On a diet of crawlers of the red scale *Aonidiella aurantii* (Mask.) nymphs matured quite well and females produced few eggs (Castagnoli and Simoni, 2003). In laboratory trials, nymphs of *N. californicus* could not mature on eggs of *Spodoptera littoralis*, *Ectomyelois ceratoniae*

and *Ceroplastes floridensis*, nor on *Bemisia tabaci* or *Retithrips syriacus* (Swirskii *et al.*, 1970).

Table 2. Host Plants of *T. urticae*

Host Plant	Common Name	Pest Targeted	References
<i>Citrus spp.</i>	Citrus plants	Spider mites (<i>Tetranychus spp.</i> , <i>Panonychus citri</i>)	Zhang, Z.Q. (2003).
<i>Fragaria spp.</i>	Strawberry plants	Two-spotted spider mite (<i>Tetranychus urticae</i>)	McMurtry, J.A., and Croft, B.A. (1997).
<i>Vitis spp.</i>	Grape vines	European red mite (<i>Panonychus ulmi</i>)	McMurtry, J.A., and Croft, B.A. (1997).
<i>Solanum lycopersicum</i>	Tomato	Two-spotted spider mite (<i>Tetranychus urticae</i>)	Zhang, Z.Q. (2003).
<i>Pyrus spp.</i>	Pear trees	Pear rust mite (<i>Epitrimerus pyri</i>)	McMurtry, J.A., and Croft, B.A. (1997).
<i>Rosa spp.</i>	Rose plants	Spider mites (<i>Tetranychus spp.</i>)	McMurtry, J.A., and Croft, B.A. (1997).
<i>Phaseolus spp.</i>	Beans	Two-spotted spider mite (<i>Tetranychus urticae</i>)	McMurtry, J.A., and Croft, B.A. (1997).
<i>Cucumis sativus</i>	Cucumber	Two-spotted spider mite (<i>Tetranychus urticae</i>)	Zhang, Z.Q. (2003).
<i>Capsicum annuum</i>	Pepper	Broad mite (<i>Polyphagotarsonemus latus</i>)	Zhang, Z.Q. (2003).
<i>Solanum melongena</i>	Eggplant	Broad mite (<i>Polyphagotarsonemus latus</i>)	Zhang, Z.Q. (2003).

2.8. Life cycle and description of *N. californicus*

N. californicus is an oblong, tiny, mobile, predatory mite. It has five life stages: the egg, a six-legged larva, eight-legged protonymph and deutonymph stages, and the adult (Sabelis, 1985). The life stages of *N. californicus* are described below:

2.8.1. Egg

The eggs of *N. californicus* are oval-shaped and translucent. Initially, they appear shiny white but may turn cream-colored as they mature. Approximately 0.2 mm in diameter. Typically laid on the underside of leaves, often near veins where humidity is higher, providing a favorable microenvironment for development. Duration: 2-3 days (McMurtry and Croft, 1997; Gerson *et al.*, 2003).

2.8.2. Larva

The larval stage is the first active stage after hatching. Larvae are translucent, pale in color, and possess six legs. Approximately 0.2-0.3 mm in length. Larvae are relatively inactive and do not feed aggressively. This stage lasts only a short period before molting into the protonymph stage. Duration: 1-2 days (Helle and Sabelis, 1985; Gerson *et al.*, 2003).

2.8.3. Protonymph

Protonymphs emerge from the larval stage and are characterized by having eight legs. They are more active than larvae and begin to exhibit predatory behavior. Approximately 0.3-0.4 mm in length. Semi-translucent to whitish, with possible yellowish or greenish tints depending on diet. Protonymphs start to feed on prey mites, becoming more mobile and seeking out food sources. Duration: 2-3 days (McMurtry and Croft, 1997; Gerson *et al.*, 2003).

2.8.4. Deutonymph

The deutonymph stage follows the protonymph. Deutonymphs are larger and more robust, continuing the trend of increased activity and predation. Approximately 0.4-0.5 mm in length. Similar to protonymphs but may appear slightly darker or more opaque. Deutonymphs are highly active predators, feeding on various stages of pest mites, including eggs, larvae, and nymphs. This stage is crucial for the growth and development of the mite before reaching adulthood. Duration: 2-3 days (Helle and Sabelis, 1985; McMurtry and Croft, 1997).

2.8.5. Adult

Adult females are larger than males, measuring approximately 0.5-0.6 mm in length, whereas males are slightly smaller, around 0.4-0.5 mm. Both males and females are translucent and can be pale orange or pink in color. Mating occurs shortly after the deutonymph stage molts into an adult. Males are often seen actively searching for newly molted females. Females are prolific egg layers, with the capacity to lay several eggs per day under optimal conditions. The eggs are laid singly or in small clusters on the underside of leaves. Duration: Several weeks (Helle and Sabelis, 1985; McMurtry and Croft, 1997).

2.9. Environmental requirements for development and reproduction of *N. californicus*

2.9.1. Temperature

N. californicus is active over a wide range of temperatures. They can be tolerated much colder temperatures for short periods of time. They reproduce and develop well where the upper and lower temperature limits ranging from 10 to 33°C (50-91°F) (Malais and

Ravensberg, 2003). The reproduction rate is optimal at 23°C (Castagnoli and Simoni, 1991).

2.9.2. Relative humidity

Humidity strongly affects egg survival in *N. californicus*, while the larvae are slightly more tolerant than eggs to the same temperature-humidity regimes. They can tolerate a wide range of humidity (40 - 80% relative humidity), but prefer humidity at the upper end of this range. A relative humidity of less than 60% retard the developmental period. The higher the level of relative humidity within the range, the better the predator can maintain themselves in a crop. High temperature (between 29 and 33°C) and high humidity (95 and 100%) minimized mortality and considerably shortened the developmental time of non-feeding stages (Castagnoli and Simoni, 2003; Rott and Ponsonby, 2000).

2.9.3. Photoperiod

Photoperiod significantly affects the developmental time of *N. californicus*. A photoperiod of 16:8 (L) hours shortened the developmental time of *N. californicus* compared to a 12:12 (L) hours photoperiod. The extended light periods enhance metabolic activities, thereby accelerating growth and development. (Riahi *et al.*, 2007). Females of *N. californicus* exposed to longer photoperiods (16:8 L) laid more eggs than those under shorter photoperiods (8:16 L). The increased reproductive output under longer light conditions could be attributed to enhanced photosensitivity and higher metabolic rates, leading to increased energy allocation towards reproduction. (Gotoh *et al.*, 2004). Short day lengths (8:16 L) induced diapause in a significant proportion of the population, which is a survival strategy during unfavorable conditions. (Kasap, 2009).

2.10. Biological control of spider mites by phytoseiids

Phytoseiids have been used in biological control programs in a wide variety of agricultural systems (McMurtry, 1982). These crops include apple (Croft and MacRae, 1992; Steinburg and Cohen, 1992; Croft and Slone, 1997), grape (Kinn and Douth, 1972; Duso, 1992; Duso and Pasqualetto, 1993), nursery citrus (Grafton-Cardwell *et al.*, 1997), strawberry (Croft and Coop, 1998), peppermint (Morris *et al.*, 1999) and hops (Strong and Croft, 1995). These experiments used a variety of phytoseiids and had varying degrees of success.

The use of pesticides in orchards is one of the main obstacles to establishing a successful phytoseiid settle down. A strategy that has been used to overcome this problem in other systems is the development and release of resistant strains of phytoseiids to various pesticides (Hoy, 1982, 1985). Pyrethroid-resistant strains of *Typhlodromus pyri* have been successful in controlling ERM in Nova Scotian apple orchards (Hardman *et al.*, 2000; Moreau *et al.*, 2000). Croft and Hoying (1975) released strains of *N. fallacis* that were resistant to organophosphates, carbaryl, or both into Michigan orchards. The predators were easily established, but only the organophosphate resistance persisted in the populations. Some strains of *N. californicus* are highly resistant to pesticides (Croft, 1990; Castagnoli *et al.*, 2005; Escudero and Ferragut, 1998, 1999) allowing the use of *N. californicus* in integrated pest management systems.

2.11. Potential of *N. californicus* as a biological control agent

Biological control has been practiced in entomology and acarology more than in any other field. The field of biological control has been defined as the study, importation, augmentation and conservation of beneficial organisms for the regulation of population densities of other organisms (Debach, 1964). When implementing a biological control

program, there are certain desirable characteristics to be considered. The natural enemy needs to be adapted biologically, physiologically and ecologically to the host (Doutt and Debach, 1964). Common desirable attributes include: high powers of dispersal and searching capacity, prey specificity, the ability to survive at low prey densities, reproductive potential, a power of increase greater than the prey, voracity, synchronous distribution with prey, and the ability to resist pesticides (Messenger *et al.*, 1976; Gerson and Smiley, 1990; McMurtry, 1982). McMurtry and Croft (1997) have suggested that although most studies on the efficacy of phytoseiids as control agents have focused on the rapid rate of increase in predators, the ability of the predators to regulate spider mites at a low density may be just as important. Control agents that regulate mites at these low densities generally have the ability to survive when starved, and can use alternative food, cannibalism, or predation on other phytoseiid species as means of survival during times when the favored prey are scarce (McMurtry and Croft, 1997). *N. californicus* possesses many of the characteristics described above. According to a classification of predatory mites based on feeding specialization developed by McMurtry and Croft (1997), *N. californicus* is a type II predator. As a type II generalist, *N. californicus* has a broad diet range that includes not only various arthropods, but also plant sap, honeydew, and pollen (McMurtry and Croft, 1997). *N. californicus* can adapt to fluctuations in prey populations, providing stable pest suppression over time (Croft *et al.*, 1998; Castagnoli *et al.*, 1999; Escudero and Ferragut, 2005; Greco *et al.*, 2005). The ability of *N. californicus* to survive on a broad array of food sources contributes to its stability and may mitigate its effect on community structure and other beneficial arthropods (Jones, 1976; Powers and Mc-Sorley, 2000; Cross *et al.*, 2001; Rhodes *et al.*, 2006). *N. californicus* thrives in a wide range of environmental conditions. So, It can tolerate varying temperatures and humidity levels, which makes it suitable for both greenhouse and open-field applications

(Gerson *et al.*, 2003). On the other hand, the rapid development from egg to adult (approximately one week) allows them for quick population increases in response to prey availability (Helle and Sabelis, 1985; Gerson *et al.*, 2003). *N. californicus* can be integrated into IPM programs alongside other biological control agents and selective pesticides. Its ability to adapt to various pest management strategies enhances the overall effectiveness of IPM programs (Helle and Sabelis, 1985; Knapp and Henter, 2018). By effectively controlling spider mite populations, *N. californicus* reduces the need for chemical pesticides. This not only lowers production costs but also mitigates the risks of pesticide resistance and environmental contamination (McMurtry and Croft, 1997; van Lenteren, 2000).

N. californicus can be released using various methods, including broadcast release, placement of sachets, and blower devices. In greenhouses, banker plants that provide alternative food sources can sustain predator populations. Preventative releases at the start of the growing season and curative releases in response to pest infestations are common strategies (McMurtry and Croft 1997; Knapp and Henter, 2018). Regular monitoring of pest and predator populations is essential for successful implementation. Adjusting release rates based on monitoring data ensures that *N. californicus* populations are maintained at effective levels (van Lenteren, 2000; Gerson *et al.*, 2003). Furthermore, *N. californicus* is successfully used to control spider mites both in the field and in protected crops (Castagnoli and Simoni, 2003). It is able to persist on plants at low or negligible spider mite densities (Schausberger and Walzer, 2001).

So, *Neoseiulus californicus* is an effective biological control agent against spider mites, offering numerous benefits such as environmental safety, reduced reliance on chemical pesticides, and compatibility with integrated pest management programs. Its adaptability,

high reproductive capacity, and predatory efficiency make it highly potential biological agent for controlling spider mites.

2.12. Overwintering potential

2.12.1. Diapause induction

Diapause is a critical physiological adaptation that allows organisms to survive unfavorable environmental conditions. During winter conditions, phytoseiid mites generally undergo reproductive diapause; females cease laying eggs. This reproductive pause ensures that offspring are not born into an environment with insufficient resources for survival (Overmeer, 1985; Tauber *et al.*, 1986). Diapausing females also tend to be less active than non-diapausing mites, feed rarely (Hoy and Flaherty, 1970; Rock *et al.*, 1971; Wysoki, 1974; Van Houten *et al.*, 1988; Morewood and Gilkeson, 1991), and are much more resistant to starvation when in diapause (Croft, 1971; Ivancich, 1990). Females are inseminated before they enter hibernation sites (Putman, 1962); males and juveniles fail to reach the overwintering stage before the onset of the adverse season, and bound to perish (Chant, 1959b; Putman, 1959, 1962). Day length, rather than temperature, has been shown to be the factor influencing diapause induction in *N. californicus* (Castagnoli *et al.*, 1996). The phytoseiid mites show a Type I response (Danks, 1987), entering diapause only when exposed to short day lengths. Reported critical photoperiods range from 11.2 hour (Hoy, 1975a) to 15.3 hour (Croft, 1971) and vary among populations as well as among species. Within a species, critical photoperiods are longer for populations from high latitudes or high elevations (Croft, 1971), and this trend is common among insects (Danks, 1987).

Temperature also has a strong influence on diapause induction. The length of the critical photoperiod may be somewhat different at different temperatures (Hoy, 1975a) and

diapause is often prevented completely if temperatures remain above 20°C or so (Sapozhnikova, 1964; Rock *et al.*, 1971; Hoy, 1975a; Morewood and Gilkeson, 1991). Temperature alone, in the form of a thermoperiodic cycle, can induce diapause when mites are reared in constant darkness and this thermoperiodic response corresponds very closely to the photoperiodic response (Van Houten *et al.*, 1987, 1988).

The ability to diapause is not universal in phytoseiid mites; rather, some species and some strains or populations within a species have been shown to lack a diapause response or to overwinter without diapausing (Wysoki and Swirskii, 1971a, b; McMurtry *et al.*, 1976; Overmeer, 1985). Hart *et al.* (2002) mentioned that the *N. californicus* can lay eggs during the UK winter and may therefore be able to overwinter as an egg stage and also reported that the US strain of *N. californicus* survived over three months without entering diapause outdoor in winter under sheltered condition in the UK, where the temperature did not fall below 0°C.

An investigation was carried out by Jolly (2000) into the ability to diapause for three strains of *N. californicus*: from Spain, the USA and one collected from the UK. This study was conducted at 21°C under a short day length (8L: 16D) provided TSSM as food. As a result, the US strain did not show any diapausing symptom whereas Spanish strain exhibited limited diapausing ability (16.1%) and the UK strain was able to diapause about 95.7%. Castagnoli *et al.* (1996) also investigates whether the alternative diets successfully used for mass-rearing of central Italian strain of *A. californicus* affected their responses to diapause inducing stimuli, when the species kept at temperatures and photoperiods close to native geographical areas. At temperatures between 17 and 21°C the length of day affected diapause induction more than temperature. With a ten-hour photoperiod at the tested temperatures, the number of diapausing females of both species exceeded the critical threshold of 50%. With an

increase of two hours of light at 17°C the incidence of diapause dropped when fed on TSSM or on pollen.

Diapause is an adaptive strategy that enables *N. californicus* to endure periods of adverse environmental conditions, such as winter or drought. By entering diapause, populations can survive until favorable conditions return, ensuring long-term persistence (Denlinger, 2002). The timing of diapause can be synchronized with the life cycles of prey populations. *N. californicus* emerges from diapause when prey availability increases, optimizing predation efficiency. This synchronization enhances the effectiveness of *N. californicus* as a biological control agent (Veerman, 1985; Tauber *et al.*, 1986).

2.13. Dispersal of phytoseiid mites

Dispersal is an adaptive process that permits individual organisms to colonize new habitats and escape unfavorable conditions (Price, 1984). The dispersal of *Neoseiulus californicus* involves both active and passive mechanisms, influenced by environmental conditions, prey availability, and plant structure. Two types of dispersal are as follows

2.13.1. Active dispersal

2.13.1.1. Walking

N. californicus primarily disperses by walking short distances. It moves actively in search of prey or more favorable environmental conditions. Walking is typically effective for local distribution within a plant or between closely spaced plants (Sabelis and Van Rijn, 1997).

2.13.1.2 Climbing and Dropping

These mites can climb to elevated positions on plants and drop, using silk threads to descend to lower foliage or neighboring plants. This behavior helps them avoid unfavorable conditions or locate new prey (Gerson and Smiley, 1990).

2.13.2. Passive dispersal

2.13.2.1. Phoretic dispersal

N. californicus can hitch rides on other animals, such as insects or birds, to disperse over longer distances. This form of dispersal, known as phoresy, allows mites to colonize new areas rapidly (Gerson and Smiley, 1990).

2.13.2.2. Wind dispersal

Wind can facilitate the passive dispersal of *N. californicus*. Mites may be blown from one plant to another, especially in open field conditions. While less directed, wind dispersal can cover significant distances (van Houten *et al.*, 1995).

Effective dispersal enhances the mite's ability to manage pest populations, contributing to sustainable pest management program.

CHAPTER 3

MATERIALS AND METHODS

3.1 Experimental site

The research work was carried out in the Entomology laboratory (25.64° N, 88.64°E) of Hajee Mohammad Danesh Science and Technology University, during the period of August 2023 to January 2024. The biological traits of the predatory mite were examined in the months of September, October, November and December and the ambient conditions of those months are shown in the table 3.

The materials and methods used in the experiments are described in details here:

3.2 *Tetranychus urticae* rearing

The seeds of country bean, *Lablab purpureus* were sown in pots filled with soil and cow dung mixture and allowed to germinate by providing adequate sunlight, air and water. After getting the seedlings, two spotted spider mites (TSSM) were inoculated from the field and maintained on bean seedlings outside of the laboratory. Leaflets infested with spider mites were provided as prey for the colonies of *N. californicus*.

3.2.1 Duration: June, 2023 to January, 2024

3.3 *Neoseiulus californicus* rearing

N. californicus were collected from the eggplant of the vegetable gardens of the HSTU campus and made colonies provided *T. urticae* as prey reared in plastic boxes during the months from August to December in the laboratory of Entomology, HSTU. The rearing arena (Figure 1) was composed of two small plastic boxes inserted by glue gun to a bigger box (17.5× 10.4cm) with gaps half-filled with soap water. Inside of the inner box, a mixed stages of *T. urticae* infested country bean leaflets (7× 6.3cm) were supplied frequently as food of *N. californicus*. On the upper inner side of the inner box, sticky

material from glue-pot was mounted to prevent mite escape. On top of the bigger box, a lid with cotton clothes (5cm× 5cm) was placed to control the moisture level as well as prevent contamination.

3.3.1 Duration: August, 2023 to January, 2024.

3.4 Immature development and adult performance of *N. californicus*

To determine the developmental time of *N. californicus*, 25 gravid females were taken randomly from rearing units for each treatment and transferred to *T. urticae* infested bean leaf which was placed on wet cutting tissue paper in a small petri dish (9cm ×2cm). The females were allowed to lay eggs for 24 hrs and then transferred to their main colonies. Individual egg was transferred by fine hairbrush to each bean leaf disc (2.8 cm diameter) supplied with mixed stages of *T. urticae* on a wet cutting tissue paper in a small Petri dish (5cm× 1.5cm) containing $70 \pm 5\%$ R.H. The Petri dishes were then kept in the trays by numbering set in the months of September, October, November and December with $12 \pm 0.5L:12 \pm 0.5D$, $11.5 \pm 0.3L:12.5 \pm 0.3D$, $11 \pm 0.2L:13 \pm 0.2D$ and $10.7 \pm 0.1L:13.3 \pm 0.1D$ photoperiod, respectively. Thirty replications were maintained for each treatment. As the leaf discs became aged, the mites were replaced to new, freshly cut discs until the female and male died. Water was added daily to the arenas to keep the tissue paper moist for preventing their escape and maintaining the inner moisture.

The duration of developmental stages egg to adults of the predator was recorded at 12-hour intervals to determine stage-specific development time and mortality under the trinocular Zoom Stereo Microscope (Model: SM-2TZ LED, AmScope Microscope, Irvine, CA 92606, USA). Since the inactive period of the immature were short, the active and inactive period of larval and nymphal stages were combined. After adult emergence, each female was coupled with a male for mating and fed with the same diet. If needed,

an additional male was added to ensure successful mating. Oviposition was recorded at 24-hour intervals. The number of eggs oviposited by each female were recorded daily. The newly laid eggs of each female from five petri dishes were counted daily until the female died and transferred the eggs to fresh leaf discs where they were reared up to adulthood to determine the sex ratio of the offsprings. The experiments were continued until the death of all individuals. Mixed stages of *T. urticae* were supplied as food.

3.5 Life table analysis

Observations on life history, including adult fecundity and longevity were used to construct a time-specific life table for *N. californicus* when maintained under laboratory conditions. The actual death occurred in the egg and immature stages were taken under consideration when the female survival rate in each month was determined. The life table parameters for females held at different temperatures were estimated using the Birch (1948) equation:

$$\sum e^{-r} l_x m_x = 1$$

Where, x = age in days, l_x = the age-specific survival rate, m_x = the age-specific fecundity, the intrinsic rate of natural increase ($r_m = \ln (\sum l_x m_x) / T$), the finite rate of increase ($\lambda = e^r$), the net reproductive rate ($R_0 = \sum l_x m_x$), the mean generation time ($T = \sum x l_x m_x / R_0$), and the gross reproductive rate ($GRR = \sum m_x$). Doubling time [$DT = (\ln 2) / r$] was calculated as described by Mackauer (1983).

3.6 Statistical analysis

Treatment effect on the traits (i.e. developmental time, survival, pre- oviposition period, longevity and fecundity) were determined by using analysis of variance (ANOVA), and means were separated by using Tukey's Honest Significant Difference test (Tukey's HSD test) (SAS Institute, 2005). The jackknife technique was used to estimate mean

demographic parameters of l_xm_x of the life table and their SE. This method was first applied to life table analysis as proposed by Meyer *et al.* (1986). The jackknife procedure estimation standard errors for life table parameter estimates by sequentially removing one female and her offspring from the original data set and recalculating each life table parameter from the truncated data set. The method can estimate R_0, T, r_m, λ, DT and GRR and means were compared by ANOVA (Tukey HSD test) using SAS. All graphical works were done through Microsoft excel program.

Table 3: Environmental factors in the tested months

Months	Temperature range (°C)	Humidity range (%)	Photoperiod (hour)
September	30-34 °C	80-85%	12 ± 0.5L:12 ± 0.5D
October	26-30 °C	75-80%	11.5 ± 0.3L:12.5 ± 0.3D
November	22-26 °C	70-75%	11 ± 0.2L:13 ± 0.2D
December	18-22 °C	65-70%	10.7 ± 0.1L:13.3 ± 0.1D

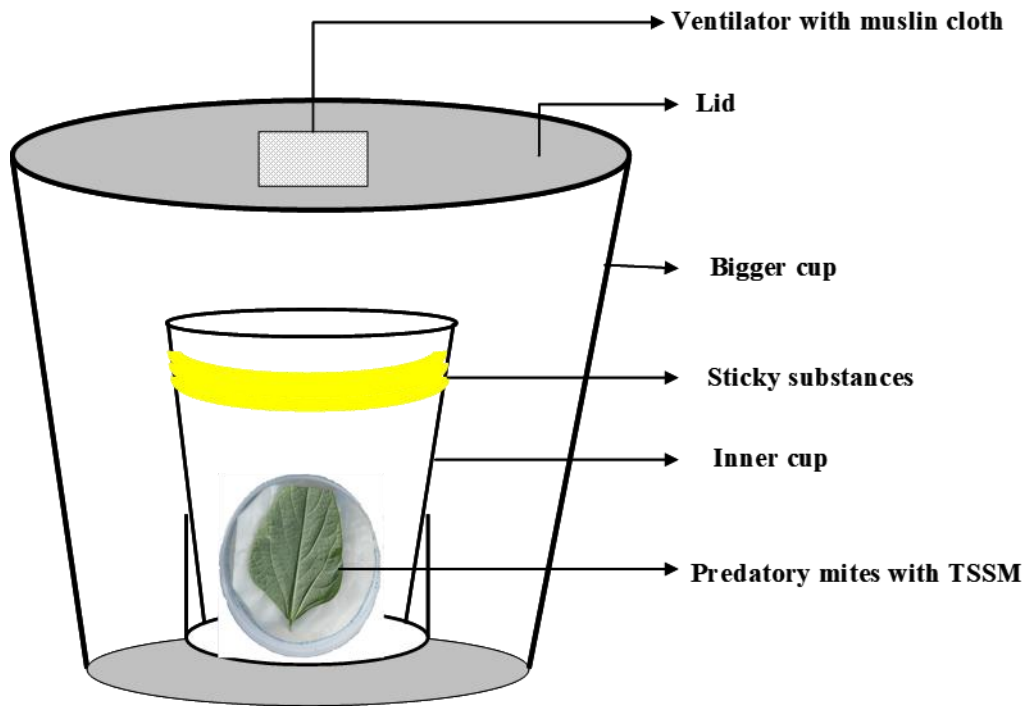
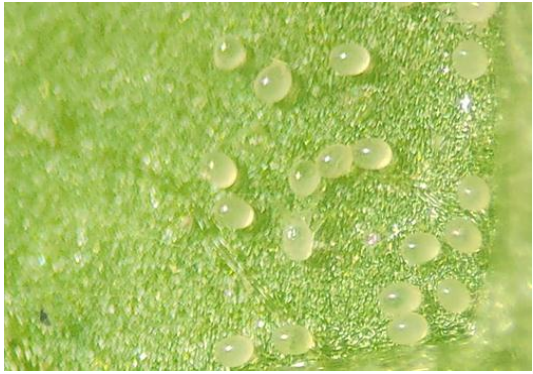


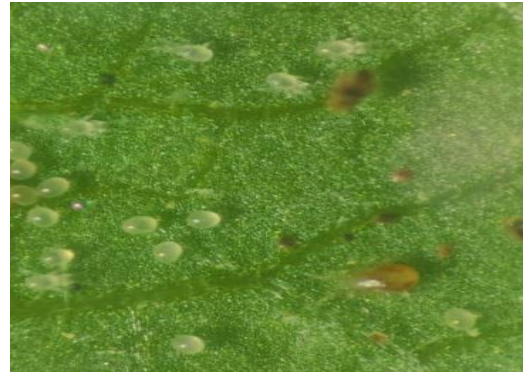
Figure 1. Rearing apparatus of the predatory mite



Plate 1. Rearing media of *N. californicus*



Eggs



Larvae



Protonymph



Deutonymph



Adults

Plate 2. Different stages of *N. californicus*



Bean seedlings



T. urticae infested leaf

Plate 3. Mass culture of *T. urticae* on bean plants



Plate 4. Camel hairbrush for picking different stages of *N. californicus*



Plate 5. Stereo Microscope (Model: SM-2TZ LED, AmScope Microscope, Irvine, CA 92606, USA) for observing different stages and counting eggs

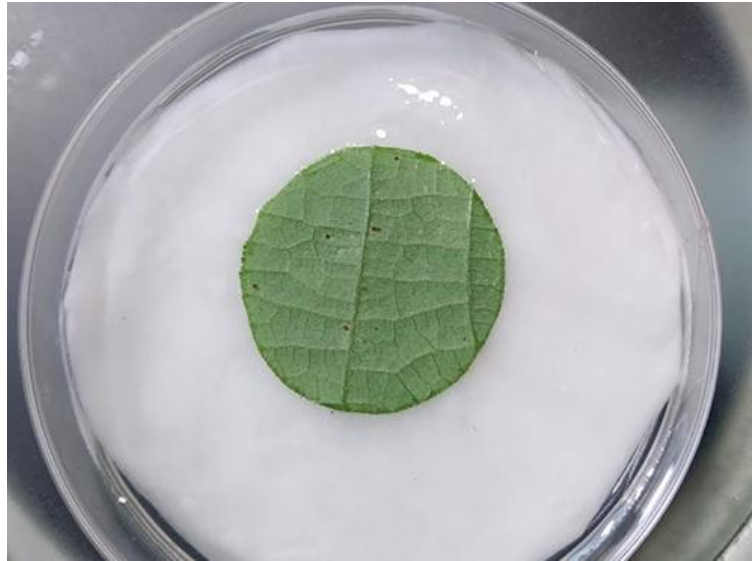


Plate 6. Experimental arena of *N. californicus* with food



Plate 7. Observation of experimental arenas under the stereomicroscope

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Immature development

The egg development periods of *N. californicus* were significantly different during the tested months. The duration of the egg stage ranged from 0.79 to 1.79 days in females while 0.86 to 1.55 days in males observed from September to December. In December, female egg development time was the longest (1.79 days) where only 0.79 and 1.61 days were taken in September and November (Table 4). Significant differences were found in October and December (Female: $df = 3, 63; F = 61.75; P < 0.05$ and male: $df = 3, 36; F = 13.73; P < 0.05$) but no difference was observed from September to October and November to December.

The newly hatched larvae of *N. californicus* were white in color with three pairs of legs (Plate 2). The larval periods were estimated in four consecutive months and showed no variation (Table 4). The developmental times of larval stage in both the sexes showed no significant differences (Female: $df = 3, 63; F = 1.62; P > 0.05$ and male: $df = 3, 36; F = 0.12; P > 0.05$).

The oval-shape, white color and 8 legged protonymphs (Plate 2) were developed immediately after larval molting. Upon feeding, they turned into reddish color. The development time of protonymph gradually increased in relation to cooler months showing no significant differences (Female: $df = 3, 63; F = 1.10; P > 0.05$ and male: $df = 3, 36; F = 0.10; P > 0.05$).

Female and male deutonymphs developed within the range of 1.06-1.35 and 0.95-1.15 days in four studied months prior to molting to the adult stage. No significant difference was observed in both the sexes from September to December. (Female: $df = 3, 63; F = 2.26; P > 0.05$ and male: $df = 3, 36; F = 0.85; P > 0.05$).

Development from the egg stage to adulthood of *N. californicus* occurred during the four individual months (September to December) is shown in Table 4. The immature development influenced by abiotic factors prevailed in studied months where the total development time for completion of the life cycle increased significantly with decreasing temperature during September, October, November and December, respectively. Direct estimates of development time derived from monthly developmental studies were significantly different (Female: $df = 3, 63; F = 81.15; P < 0.05$ and male: $df = 3, 36; F = 17.05; P < 0.05$).

4.1.2 Adult performance

Adult longevity: Female and male longevity usually increased with consecutive cooler months during September to December. Analysis of variance showed that mean adult longevity, the length of time from adult emergence to natural death, was significantly different among the months in both the sexes (Female: $df = 3, 63; F = 42.52; P < 0.05$ and male: $df = 3, 36; F = 9.43; P < 0.05$) and was greatest with a value of 21.41 days in female and 15.95 days in male in December, declining to a low of 13.59 days in female and 10.09 days in male in September (Table 4).

Reproduction: The month wise reproduction as the per capita total egg production of *N. californicus* was varied significantly ($df = 3, 63; F = 5.04; P < 0.05$) (Table 5). The total number of eggs was the highest (49.18 ± 2.22) in December, but it was the lowest (40.47) in September. The mean daily egg production, calculated by dividing the total

egg production by oviposition period, was varied significantly in different studied months ($df = 3, 63; F = 30.06; P < 0.05$). Daily oviposition was found the highest (3.41 ± 0.09 eggs) in September while it was the lowest (2.15) in October.

After adult emergence, the females were mated with released males. Examined months had a significant effect on the duration of the preoviposition ($df = 3, 63; F = 101.34; P < 0.05$) and oviposition periods ($df = 3, 63; F = 9.20; P < 0.05$). The duration of the preoviposition period was significantly longer in December than other studied months (Table 5). The duration of the oviposition period was significantly longer in December than other months. The longest preoviposition and oviposition period (2.24 ± 0.06 and 15.47 ± 0.52 days) were observed in the month of December whereas they were shortest (1.11 ± 0.05 and 11.94 ± 0.33 days) in November and September, respectively. The duration of the post-oviposition period was significantly longer (3.71 ± 0.39) in December than other months (Table 5; $df = 3, 63; F = 10.54; P < 0.05$). The age-specific fecundity rate (m_x) was peaked at earlier ages and the fecundity period was the shortest in September than other tested months. Daily reproductive rate declined earlier in September than other months (Figure 2).

4.1.3 Survivorship and sex ratio

The survival rate of the *N. californicus* from the immature stages to adult emergence ranged from 88.89 to 100%, which was found the lowest in the month of October. There were no statistically significant differences in immature survival among the tested months (Table 5; $df = 3, 116; F = 3.50; P > 0.05$). The curve of age-specific survival rate (l_x) started to decline at earlier ages in September than other studied months. In September to December, female adults started to die on days 13, 17, 14 and 17,

respectively (Figure 2). The sex ratio of the phytoseiid species was female biased and varied significantly among the four months. (Table 5; $df = 3, 16$; $F = 5.32$; $P > 0.05$).

4.1.4 Life table parameters

Demographic growth parameters were estimated by a jackknife analysis of $l_x m_x$ life table data during September to December (Table 6). These parameters were significantly varied in the studied four months. The net reproductive rate (R_0) ($df = 3, 63$; $F = 37.38$; $P < 0.05$), intrinsic rate of natural increase (r_m) ($df = 3, 63$; $F = 4671.39$; $P < 0.05$) and finite rate of increase (λ) ($df = 3, 63$; $F = 4292.25$; $P < 0.05$) were all significantly different and was the highest for *N. californicus* reared in November, December and September, respectively. Mean generation time, T , was significantly different and was the lowest in September ($df = 3, 63$; $F = 9936.14$; $P < 0.05$). Population doubling times, DT , showed a statistically significant difference ($df = 3, 63$; $F = 11697.63$; $P < 0.05$) and were the lowest when the predators reared in September. The prevailed weather condition of October had the highest gross reproductive rate (GRR) showing significant differences ($df = 3, 63$; $F = 106.65$; $P < 0.05$).

Table 4. Developmental time and adult longevity (day) of *N. californicus* during the months from September to December

Months	Sex*	N*	Egg	Larva	Protonymph	Deutonymph	Egg to Adult	Adult longevity
September (30-34°C)	F	17	0.79 ± 0.07 B	0.88 ± 0.05 A	1.00 ± 0.07 A	1.06 ± 0.09 A	3.74 ± 0.10 C	13.59 ± 0.30 C
	M	11	0.86 ± 0.10 b	0.82 ± 0.08 a	1.05 ± 0.08 a	0.95 ± 0.11 a	3.68 ± 0.12 b	10.09 ± 0.94 b
October (26-30°C)	F	15	0.80 ± 0.07 B	0.90 ± 0.07 A	1.07 ± 0.05 A	1.07 ± 0.10 A	3.83 ± 0.11 C	17.57 ± 0.28 B
	M	10	0.90 ± 0.07 b	0.85 ± 0.11 a	1.10 ± 0.10 a	1.00 ± 0.11 a	3.85 ± 0.13 b	15.20 ± 0.91 a
November (22-26°C)	F	18	1.61 ± 0.06 A	1.00 ± 0.10 A	1.11 ± 0.08 A	1.19 ± 0.07 A	4.92 ± 0.08 B	18.81 ± 0.69 B
	M	9	1.44 ± 0.10 a	0.89 ± 0.14 a	1.11 ± 0.11 a	1.11 ± 0.11 a	4.56 ± 0.15 a	15.22 ± 0.70 a
December (18-22°C)	F	17	1.79 ± 0.06 A	1.12 ± 0.10 A	1.21 ± 0.11 A	1.35 ± 0.10 A	5.53 ± 0.10 A	21.41 ± 0.54 A
	M	10	1.55 ± 0.12 a	0.90 ± 0.12 a	1.13 ± 0.10 a	1.15 ± 0.08 a	4.70 ± 0.08 a	15.95 ± 1.00 a

N* = Number of individuals tested, F*^A = Female, M*^a = Male.

All values are means ± SE. Means for each stage of the same sex in the same column followed by different letters are significantly different at $P < 0.05$ (Tukey HSD test).

Table 5. Reproductive phases (day), oviposition, survival of immature stages and sex ratio of *N. californicus* during the months from September to December

Months	Preoviposition period	Oviposition period	Postoviposition period	Daily fecundity /female	Lifetime fecundity /female	Survival to adulthood (%)	Sex-ratio
September (30-34°C)	1.12 ± 0.05 B	11.94 ± 0.33 B	0.53 ± 0.21 C	3.41 ± 0.09 A	40.47 ± 1.06 B	100 ± 0.00 n.s.	69.41 ± 1.53AB
October (26-30°C)	1.17 ± 0.06 B	14.80 ± 0.54 A	1.60 ± 0.41 BC	2.15 ± 0.06 B	46.00 ± 1.40 AB	88.89 ± 5.85 n.s.	72.61 ± 0.89 A
November (22-26°C)	1.11 ± 0.05 B	15.17 ± 0.69 A	2.56 ± 0.56 AB	3.12 ± 0.12 A	46.44 ± 1.59 AB	96.67 ± 3.33 n.s.	66.90 ± 2.26 AB
December (18-22°C)	2.24 ± 0.06 A	15.47 ± 0.52 A	3.71 ± 0.39 A	3.18 ± 0.10 A	49.18 ± 2.22 A	93.33 ± 4.63 n.s.	63.43 ± 1.76 B

Means in the same column followed by the different letters are significantly different at $P < 0.05$ (Tukey HSD test).

ns- Not significantly different at $P < 0.05$ (Tukey HSD test)

Table 6. Life table parameters (\pm SE) of *N. californicus* during the months from September to December

Months	R_0	r_m	T	λ	DT	GRR
September (30-34°C)	26.59 \pm 0.11C	0.35 \pm 0.0004B	10.08 \pm 0.007C	1.74 \pm 0.0008A	1.25 \pm 0.0009D	29.66 \pm 0.05C
October (26-30°C)	29.39 \pm 0.17B	0.29 \pm 0.0005D	11.55 \pm 0.013B	1.34 \pm 0.0007 D	2.37 \pm 0.0042A	33.70 \pm 0.05A
November (22-26°C)	33.91 \pm 0.61A	0.33 \pm 0.0025C	10.19 \pm 0.073C	1.39 \pm 0.0034C	2.08 \pm 0.0159B	33.54 \pm 0.49A
December (18-22°C)	29.76 \pm 0.13B	0.39 \pm 0.0003A	15.30 \pm 0.015A	1.49 \pm 0.0005B	1.73 \pm 0.0015C	31.71 \pm 0.08B

Means in a column followed by different letters are significantly different at $P < 0.05$ (Tukey HSD test).

R_0 , net reproductive rate; r_m , intrinsic rate of natural increase per day; T , mean generation time in days; λ , finite rate of increase; DT , population doubling time in days; GRR , gross reproductive rate.

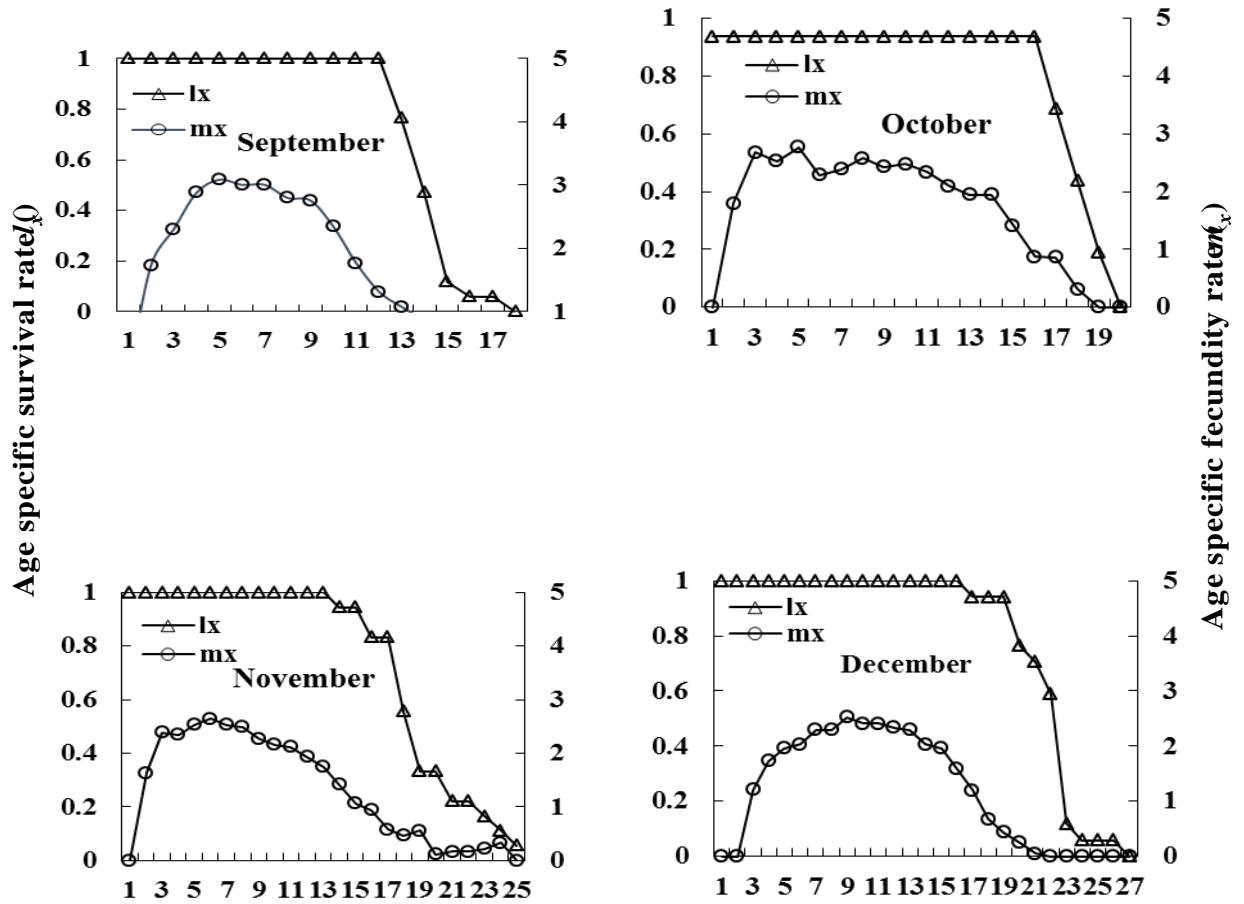


Figure 2. Age-specific survivorship (l_x) and age specific fecundity (m_x) of *N. californicus* in the tested months. l_x = proportion of females alive at age x . m_x = (proportion of females) \times (age-specific oviposition).

4.2 Discussion

Results indicated that the predatory mite, *N. californicus* is able to complete their development in the months of September to December while provided *T. urticae* as prey. The developmental period ranges from 3.74 days to 5.53 days in the experiment while the prevailed temperature was 18°C to 34°C in the four studied months. Castagnoli and Simoni (1991), and Hart *et al.* (2002) also recorded the egg to adult development period 5.8 and 8.1 days, respectively at 25°C while provided prey *T. urticae*. But Gotoh *et al.* (2006) observed the development period 3.79 to 3.88 days at 25±1°C in the commercial Spical strain while using the prey *T. urticae* which is slightly different from our result because we considered a vast range of temperature in the four studied months. Kim *et al.* (2009) recorded the development period of *N. californicus* was 8.73 days at 20°C provided *T. urticae* while the current observation was 5.53 days in the month of December when the prevailed temperature was 18°C to 22°C. Castagnoli and Simoni (1991) examined the Italian strain of *N. californicus* and found that this mite species develops and reproduces successfully at the temperature ranges from 13 to 33°C. The population used in this study developed from egg to adult at a faster rate (3.74 days during the month of September when the temperature ranges between 30°C to 34°C) compared with that studied by Gotoh *et al.* (2006) using the prey *P. ulmi* (4.33 - 4.39 days at 25±1°C). The faster rate of development observed in this study may be attributable to strain/population differences, or differences in rearing conditions and the prey (*T. urticae*). These findings suggest that *N. californicus* has a potential to develop over a wide range of temperatures in the tested months and on the pest mite *T. urticae*.

The lifetime fecundity per female (49.18 eggs) was higher in the month of December while the temperature ranges from 18 to 22°C than the Spical strain (46.2-46.67 eggs, Gotoh *et al.*, 2006), Riverside strain (43.3 eggs, Ma and Laing 1973; 54.5 eggs, Croft *et al.*, 1998) and the indigenous Japanese strain (41.6 eggs, Gotoh *et al.*, 2004) at 25±1°C

when fed on *T. urticae*. Furthermore, Castagnoli and Simoni (1991) also reported the optimal reproduction rate of the Italian strain at 23°C.

The oviposition rate of *N. californicus* was 3.03-3.17 eggs at 25±1°C while the current study showed 3.12 eggs/day in the month of November when thermal range was 22°C to 26°C (Gotoh *et al.*, 2006). At 25°C with a diet of *T. urticae*, the egg production rate of the Riverside strain (3.1 eggs per day, Ma and Laing, 1973) and the Japanese strain (3.31-3.42 eggs per day, Gotoh *et al.*, 2006) was almost similar to our present study.

Life table parameters are good indices of population growth under a given set of conditions. The r_m -value of the studied population was 0.29 during the month of October (Temperature range 26 - 30 °C) provided mixed stages of prey, which is lower than Spical strain (0.31, Gotoh *et al.*, 2006) at 25 ± 1°C when feeding on only eggs of *P. ulmi*. The reason for the lower r_m -value in the present study might be the prey stages because the r_m - values tend to be higher when fed on eggs than when fed on all prey stages (Ma Laing 1973; Mesa *et al.*, 1990; Castagnoli and Simoni 1991; Castagnoli *et al.*, 1999a; Gotoh *et al.*, 2004). Moreover, working with the same strain and temperature, Canlas *et al.* (2006) observed a lower r_m - value (0.206) using all stages of *T. urticae* as prey than the value (0.27) found by Gotoh *et al.* (2004) when fed solely with *T. urticae* eggs in the same Japanese strain. On the contrary, the r_m - value found higher in the studied population than the Columbian strain (0.19, Mesa *et al.*, 1990, used preys were all stages of *T. urticae* and *Mononychellus progresivus*) the African strain (0.227, Rencken and Pringle 1998, provided prey was all stages of *T. urticae*), the Italian strain on tomato plant (0.118, prey was *T. urticae* but stages not mentioned, Castagnoli and Simoni 1991), and the value was similar with the Italian strain (0.259, Castagnoli and Simoni 1991, prey was *T. urticae* but stages not specified).

The mean generation time (T) of studied population was higher (15.30days) during the month of December (18 to 22 °C) provided mixed stages of *T. urticae* compared to Spical strain (11.01-11.30days) at 25 °C when fed egg stage of *P. ulmi* (Gotoh *et al.*, 2006). The Columbian strain (16.7days, Mesa *et al.*, 1990) and the Japanese strain (17.5 days, Canlas *et al.*, 2006) showed longer period than the present population because they used all stages of *T. urticae* as prey. However, the number of female progenies produced per female (R_0) was 33.91 in the research population. Castagnoli *et al.* (1991) also reported the R_0 value 36.6 when the Italian strain reared on mixed stages of *T. urticae*. The variation of the R_0 may be due to the strain characteristics, because working with the Japanese and African strains, provided mixed stages of *T. urticae*, Canlas *et al.* (2006), and Rencken and Pringle (1998) found the small number of female progeny (22.9 and 11.2, respectively). Xia *et al.* (1998) reported the R_0 value (42.9) quite larger than the present study while working with *Amblyseius orientalis* supplied *P. ulmi*, honey and water. Takafuji and Chant (1976) and Blommers (1976) also found the R_0 values 50.9 and 50.2 while they were working with *Amblyseius degenerans* (Prey was immatures of *T. pacificus*) and *A. bibens* (All stages of *Tetranychus* as food). Life table statistics, such as those presented here, provide a valuable tool in the evaluation of a potential biological control agent in an area considering the seasonal variations and this approach has demonstrated utility (Bernal and González, 1997).

So, considering the potential characters of a predator, this study explored some prospective characteristics of *N. californicus* for the successful biological control of pest mites. As compared to other strains or species of *Neoseiulus* in the world, particularly the higher fecundity and R_0 , short generation time and moderate r_m of the studied *N. californicus* seems to be effective for the biological control of the two spotted spider mite, *Tetranychus urticae* successfully.

CHAPTER 5

SUMMARY AND CONCLUSION

The present study was conducted in the laboratory of the Department of Entomology, Hajee Mohammad Danesh Science and Technology University (HSTU) during June 2023 to January 2024.

Neoseiulus californicus, a predatory mite, is widely used in biological control programs to manage populations of the two-spotted spider mite, *Tetranychus urticae*. The life history traits of *N. californicus* varied significantly depending on the environmental conditions and the quality of prey available. When fed on *T. urticae* during the months of September, October, November, and December, several key life history traits were observed including development time, survival rate, reproductive performance, longevity, net reproductive rate, the intrinsic rate of natural increase, generation time, gross reproductive rate, doubling time etc.

The developmental time from egg to adult ranged from 3.74 days to 5.53 days while the lowest in the month of September and highest in December. The survival rate of *N. californicus* was greater (100%) in the month of September due to favorable conditions but lower (93.33%) in the cooler month of December. Daily fecundity of the female was higher (3.41) in the month of September compared to other tested months. Adult longevity of the female was maximum (21.41) in the month of December while minimum (13.59) in the month of September. Net reproductive rate varied in the studied months where longer (33.91) in November but shorter (26.59) in September. In the tested months the intrinsic rate of natural increase was found the highest (0.39) in December while the lowest (0.29) in October. Generation time was maximum (15.30) in the month of December whereas minimum (10.08) in September month. Both of the doubling time

of the generation and gross reproductive rate was found the longest (2.37, 33.70) in October but the shortest (1.25, 29.66) in September, respectively.

From the above results of the experiment in the tested months showed significant variation. The high fecundity, short generation time, and moderate r_m of population of *N. californicus* indicate significant potential as an effective biological control agent of *T. urticae*.

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