FUNCTIONAL RESPONSE AND EVALUATION OF *Neoseiulus californicus* AS BIOLOGICAL CONTROL AGENT OF *Tetranychus urticae*

A THESIS

BY

DOLON RANI DAS
Student No. 1701106
Semester: January-June, 2024
Session: 2022-2023

MASTER OF SCIENCE (MS)
IN
ENTOMOLOGY

DEPARTMENT OF ENTOMOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY
DINAJPUR-5200

JUNE 2024

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DEDICATED

TO MY

BELOVED PARENTS

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The Authoress

ABSTRACT

The research work was conducted in the laboratory of the Department of Entomology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur during May to December 2023. The functional response of *Neoseiulus californicus* (Mesostigma: Phytoseiidae) to five densities of TSSM *Tetranychus urticae* (Trombidiformes: Tetranychidae) was investigated in the laboratory conditions. The research work was carried out on leaf disc in petridishes with 5 replications. The rate of searching efficiency and handling time of predator were estimated as 0.588 d^{-1} and 0.0052 min for egg, 0.593 d^{-1} and 0.0052 min for larva, 0.626 d^{-1} and 0.0197 min for protonymph, 0.0504 d^{-1} and 0.0283 min for deutonymph. The handling time increased as the prey progressed to the next stage. The model forecasts a maximum prey consumption on egg and larval stages were 192.30 while minimum on deutonymph 35.33 based on the functional response. Biological control of two spotted spider mite by *N. californicus* was also conducted in the potted bean plants. Four treatments were setup with a control group (no release of predator) and each treatment were replicated with five times. The effectiveness of the predatory mite, *N. californicus*, as a suppressive agent of the two spotted spider mite was evaluated at predator: prey ratio of 1:10, 2:10, 3:10, 4:10 and a control group. At ratios 4:10 *N. californicus* significantly reduced *T. urticae* population about 85.63% and 1:10 ratio shown the lowest reduction of prey populations 72.09% one week after release of predator. The average motiles number of predator were found maximum on T_4 (0.78) and minimum in T_1 (0.04). *N. californicus* significantly reduced TSSM in treatments with high predator: prey ratio. All treatments significantly reduced TSSM compared with the control groups (no releases). Results indicated that release of *N. californicus* is able to sustained control of TSSM populations.

Keywords: *Neoseiulus californicus*; *Tetranychus urticae*; Functional response; Biological control; Predator: prey ratio

CONTENTS

CONTENTS (Contd.)

CONTENTS (Contd.)

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

LIST OF FIGURES (Contd.)

CHAPTER I

INTRODUCTION

One of the most polyphagous arthropod herbivores, *Tetranychus urticae* Koch (Acari: Tetranychidae) is the two-spotted spider mite (TSSM), eating on over 1100 plant species, including over 150 species of commercial value and belonging to over 140 distinct plant families (Pavela, 2017). TSSM feeding on plants results in 40–60% losses in economic yield due to leaf curling and yellowing, reduced photosynthesis, and the injection of phytotoxic substances (Jhonson and Lyon, 1991; Thomas, 1969). Of the most commonly used methods, such as cultural, chemical and biological control, that have been employed against *T. urticae,* the use of synthetic acaricides and insecticides is the most widespread at the present time (Bethke *et al.,* 2001; Opit *et al.,* 2004;Ay *et al.,* 2005; Van Leeuwen *et al.,* 2006;Sato *et al.,* 2011).Even though pesticides work well against two spotted spider mite in the short term, they have a long-term negative impact on the environment and human health. Among these issues are the two-spotted spider mites' (İnak *et al.,* 2019; Whalon *et al.,* 2008) development of resistance to acaricides and insecticides, the detrimental effects of pesticides on human health (Hernández *et al.,* 2011a,b), the marked rise in environmental pollution, and the persistence of chemical residues in consumed fruits and vegetables (Hoai *et al.,* 2011; Türköz Bakırcı *et al.,* 2014). There is less pressure to prey on spider mites as a result of the widespread use of acaricides, which has destroyed many of their natural enemies (Wu *et al.,* 2016). Moreover, this mite species quickly acquires resistance to pesticides due to its high net reproduction rate and quick pace of development, even after only a few treatments (Uddin *et al.,* 2017). Therefore, one common method for controlling spider mite outbreaks is the augmentative release of biological control agents, such as predatory mites (Amoah *et al.,* 2016; Gigon *et al.,* 2016; Seiedy *et al.,* 2017; Uddin *et al.* (2017).

As part of augmentative biological management against mites and thrips, two of the most significant predators are *Neoseiulus californicus* (McGregor) and *Neoseiulus cucumeris* (Oudemans) (Mendel and Schausberger 2011). One of the main elements controlling the population dynamics of predator-prey systems is the functional response of a predator. The functional response is defined as the link between a consumption rate and the density of prey (Abrams and Ginzburg, 2000; Jeschke *et al.,* 2002). The following characteristics should be taken into account when measuring a predator's efficiency: a) good dispersion ability, b) distribution with respect to the prey, c) good reproductive capacity, d) voraciousness, e) high degree of specificity to the prey, f) morphological traits; additionally, g) taxonomy classifications (McMurtry,1982). A predator must also be assessed based on its ability to hunt in its environment, its functional and numerical reaction, and its spatiotemporal matching up with the target (Huffaker *et al.,* 1971). Three basic types of functional responses were identified by Holling (1959): a convex response (Type II), in which the number of consumed prey rises with prey density but begins to decrease upon approaching a maximum point (dome-shaped curve); a linear response (called Type I), in which the number of consumed prey increases linearly to a plateau; and a sigmoidal response (Type III), in which predation results in a sigmoid-shaped response with increasing prey density. The rate at which the functional reaction grows with host density, or attack rate, or searching efficiency (a), and handling time (Th), or the amount of time it takes a predator to find and kill a victim, are two crucial characteristics of the functional response (Hassell 1978). Researchers have focused mostly on kinds II and III of the three types of functional response described by Holling (1959) Fernandez-Arhex and Corley (2003); Pervez and Omkar (2006); Xiao and Fadamiro (2010). Numerous factors affect the functional response parameters and type: temperature (Gorji *et al.*, 2009), insecticides (Poletti *et al.*, 2007), prey stage (Farazmand *et al.,* 2012), experimental unit (Madadi *et al.,* 2011), plant characteristics (Cédola *et al.,* 2001; Ahn *et al.,* 2010), and nature of the adversary (Asadi *et al.,* 2012; Nikbin *et al.,* 2014). As per McMurtry *et al.* (2013), *N. californicus* is classified as a type II selective predator of tetranychid mites and has evolved to live in colonies of spider mites with heavy webbing. Despite this, the life styles of these mites are very different from each other. The potential predation rate of a predator is one of the most important factors in biological control programs (Fan and Petitt 1994). On cultivated crops, certain phytoseiid species are employed as biological control agents of herbivorous mites (McMurtry *et al.,* 2013). It has been used in field and greenhouse horticultural crops in North and South America and Europe to control spider mites.

(Swirski *et al.,* 1970; Oatman *et al.,* 1977; Picket and Gilstrap 1986a and b; Castagnoli and Simoni 1991; Raworth *et al.,* 1994; McMurtry and Croft 1997; Jolly 2000). The predator's remarkable adaptability has been demonstrated by its ability to feed on nearly every stage of the two-spotted spider mite, *T. urticae* Koch (Acari: Tetranychidae), as well as other tetranychid species, other pest mites, insects, and pollen (Swirski *et al.,* 1970; McMurtry 1977; Friese and Gilstrap 1982; Castagnoli and Liguori 1991; McMurtry and Croft 1997; Croft *et al.,* 1998). The *N. californicus* is a predator that feeds on various tetranychid species and other pest mites in addition to attacking all stages of the two spotted spider mite (McMurtry and Croft 1997, Croft *et al.,* 1998). The knowledge of efficiency and functional response would allow growers to make control decisions based on monitoring schedule of predator and pest, using pesticide only when predation cannot impose to prevent pest damage. Considering the exponential growth of the pest population of *T. urticae* and the prey *N. californicus,* the efficiency and functional response can be controlled the initial pest densities successfully. Therefore, the present study was investigated how *N. californicus* will respond to changing prey density under simplified experimental conditions

Objectives:

i) To examine the biological regulation of *T. urticae* by predatory mites and

ii) To observe the best functional response of *N. californicus* while provided different densities of egg, larva, protonymph and deutonymph of *T. urticae.*

CHAPTER II

REVIEW OF LITERATURE

Of the many mite species, the majority of polyphagous species are widespread pests in contemporary agro ecosystems across the globe, with some of them ranking among the most significant crop pests (Pokele and Sukla, 2015). According to Jeppson *et al.* (1975), the two-spotted spider mite is also referred to as the glasshouse spider mite, red spider mite, and red spider. *Tetranychus bimaculatus* (Harvey), *Tetranychus cinnabariuns* (Boisduval), *Tetranychus telarium* (Linnaes), *Tetranychus urticae* (Koch), and more than 59 synonyms are among the common names (Jeppson *et al.*, 1975; Kono and Papp, 1977; Smith and Baker, 1968). However, *Tetranychus urticae* is a legitimate name for the two-spotted spider mite, according to Boudreaux and Dosse (1963). Koch (1836) first described the spider mite, *Tetranychus urticae* Koch (Acarina: Prostigmata: Tetranychidae), as a cosmopolitan pest and member of the Tetranychidae family (Hinomoto *et al.*, 2001). Pritchrad and Baker (1995). Four phases make up the lifecycle of the Two Spotted Spider Mite (TSSM): egg, larva, protonymph, deutonymph, and adult (Krantz, 1978). The larvae is different from the other phases as they have three pair of legs. The two spots are usually prominent on the abdomen found in the protonymph, deutonymph and adult phases is the reason of their naming and the webbing power which is the symptom of high infestation help them to escape from the predators established their " SPIDER" image.

According to Shih *et al.* (1976), a spider mite population can grow by up to 40% every day. However, overexploitation of food supplies and natural enemy predation could cause a sudden decline in population. Furthermore, TSSMs are well-suited to acquire pesticide resistance due to their arrhenotokous genetic makeup. It has become harder to control outbreaks as a result of their development of resistance to several acaricides (Carbonaro *et al.,* 1986).

2.1 *Tetranychus urtice* Koch 1836

One of the worst agricultural pests, *Tetranychus urticae*, can harm a variety of crops, including fruits and vegetables. USA was where (Tuttle and Baker, 1968) initially reported it.

2.1.1 Taxonomic Position of *Tetranychus urticae*:

Kingdom: Animalia
  Phylum: Arthropoda
    Subphylum: Chelicerata
      Class: Arachnida
        Order: Trombidiformes
          Family: Tetranychidae
            Genus: *Tetranychus*
            Species: *Tetranychus urticae* (Koch, 1836)

2.1.2 Distribution

The most common spider mite, the two spotted spider mite (*Tetranychus urticae*), has a cosmopolitan distribution and has been recorded on more than 300 species of plants, including all of the tree fruit crops as well as small fruits, vegetables and ornamentals. The global status of pests on vegetables, fruit trees, fiber crops, and ornamental plants is widely documented. According to reports, the following crops are highly susceptible: beans, brinjals, cucurbits, tomatoes, soybeans, okra, cowpeas, and cucumbers (Rahman and Sapra, 1940; Bindra and Singh, 1970; Singh, 1995; Sirvi and Singh, 2014). The majority of the nations in Europe, Asia, Africa, Australia, the Pacific and Caribbean islands, North, Central, and South America are among the regions where *Tetranychus urticae* is found globally.

2.1.3 Host

Tetranychus urticae (Acari: Tetranychide) is a highly polyphagous herbivorous arthopod that consumes over 1,100 plant species from over 140 families, including some that are recognized for their ability to produce harmful compounds. Additionally, it is a significant problem in fields and greenhouse cultivations (Grbicet *et al.*, 2011). According to Khajehali *et al.* (2011), one of the most significant pests of roses economically is *Tetranychus urticae*. *Tetranychus urticae* Koch is a phytophagous and highly polyphagous herbivore that causes significant economic loss worldwide by heavily feeding on agricultural crops. According to Bolland *et al.* (1998) and Megeon and Dorkeld (2013), *T.urticae* infects roughly 88 host plants, including some palms, common beans, soybeans, cucumbers, melons, peanuts, sweet potatoes, and papaya. It has a wide range of hosts, including woody, herbaceous, and ornamental landscape plants; it has been observed to feed on over 180 different plant species (Johnson and Lyon 1988). The nutritional status of the host plant is one of the most important factors in the establishment and reproduction of agricultural pests (Motaheri *et al.*, 2014).

2.1.4 Biology

T. urtiace follows the standard warm-weather spider mite life cycle. It develops from egg to adult in 7 to 8 days at 27.5 ^{0}C to 32.5 ^{0}C, depending on the environmental conditions, with all life stages present throughout the year (Helle and Sabelis 1985). The five main stages of *T. urticae* are typically the egg, larva, protonymph, deutonymph, and adult stages. Following the feeding stages in their life cycle, they go through three brief quiescent stages: nymphocrysalis, deutocrysalis, and teleocrysalis. On the other hand, a spider mite colony can expand by as much as 40% every day (Shih *et al.,* 1976). The ideal environmental conditions for TSSM populations to quickly grow to harmful levels are temperatures of 26.5°C degrees Celsius or higher, minimal rainfall, and low to moderate humidity (Helle *et al.*, 1985).

2.1.4.1 Egg

T.urticae typically deposit their eggs on the undersides of leaves. The egg masses are attached to the leaves by fine silk webbing. *T. urticae* eggs are translucent, spherical, and have a diameter of 0.15 mm when they are first laid; as hatching approaches, they turn white (Brrust and Gotoh 2017). In mated females, the female-to-male sex ratio is 3:1. Females lay both fertilized and unfertilized eggs (Shih *et al.,* 1976; Gotoh 1997; Rote and Agrawal 2003). Usually, eggs hatch in approximately 3 days

2.1.4.2 Larva

The larva has six pairs of legs when it hatches. The larval phase lasted between one and two and a half days for males and between one and four days for females (Sandeepa *et al.,* 2019). Though moulting, it enters the nymphocrysalis stages, when they remain attached to the plant surfaces.

2.1.4.3 Nymph

After a period of quiescence, the larva grows into a slightly larger, eight-legged protonymph. The protonymph transforms into the slightly larger deutonymph upon emerging from the deutochrysalis stage. Males and females at this stage are usually distinguishable from one another despite having the same coloration (Tjosvold and Karlik 2003). Two dark patches gradually appear on the back during periods of active feeding.

2.1.4.4 Adult

The eight-legged adult emerges after it has finished feeding and reached the last stage of quiescence. Before beginning to lay eggs, the adult female may wait up to three days (Tjosvold and Karlik, 2003). The life cycle usually lasts between two weeks and ten days. The most important element is temperature, but other elements that influence development time are humidity, host plant, leaf age, and so forth. The lower bound for development is approximately 12°C, and the upper bound is approximately 40°C (Tjosvold and Karlik, 2003).

2.1.5 Season

In temperate or colder climates, there is a winter hibernating period known as the diapause, which is caused by the effects of temperature, photoperiod, and mite nutrition. Adult females change to orange in order to hibernate, and they then seek refuge in cracks and crevices, beneath leaves, or in another safe place. These mites do not feed or reproduce until favorable conditions return. Even in the winter, mites can survive if their host plants are available or the environment is sufficiently warm (Tjosvold and Karlik 2003). Additionally, this pest prefers warm, dry weather to proliferate and spread (Jeppson *et al.,* 1975). According to Nasreen *et al.* (2021), there was no mite incidence from September to November, January to March, and from the first week (6.20/3 leaves/plant) to the third week (6.95 mites/3 leaves/plant) of December.

2.1.6 Damage

Tetranychus urticae is only polyphagous, and compared to the upper leaf surface, the lower leaf surface is where they are most abundant. According to (Jeppson *et al.,* 1975), two spotted spider mites spin a thick sheath of web around the entire plant. Yield loss is the outcome, and it also impacts the photosynthetic rate (Butani and Mittal, 1992), Furthermore, a severe infestation causes the plants to die (Jeppson *et al.,* 1975). During sever infestations, they occur on both surfaces of the leaves. The stylet-like chelicera are used for piercing the epidermal cells. The mite uses its rostrum to suck up the parenchymal cells' released celluar content (Brust and Gotoh, 2018). Consequently, the leaves turn brown, though they start off as white and yellow chlorotic spots.18 to 22 plant cells can be consumed by two-spotted spider mites every minute (Rinehold *et al.,* 2015). An adult spider mite is predicted to consume roughly half of its mass every hour (Theri 2014).

T.urticae is widely distributed on bean plants in Rajshahi's city Corporation area. Bean plants are seriously harmed by this mite (Naher, 2005).

The browning and white ring of the petals on the tomato blossoms are caused by *Tetranychus urticae* feeding damage. As stated by Meck *et al.* (2013), mites will eat tomato fruit directly, usually around the cap and at the stem end.

In Egypt, *T.urticae* is primarily responsible for defoliation, leaf yellowing, and leaf burning (Abdulallah *et al.,* 2019).

Without a doubt, *T. urticae* is well-known in Morocco for its detrimental impacts on agriculture and its unlikely propensity to acquire resistance to pesticides (Grbic *et al.,* 2011).

These spider mites are a pest to hundreds of plant species in Europe and Israel (Bolland *et al.,* 1998).

For the most part, spider mites cause enormous economic damage because of their quick development and low mortality, especially in low humidity and moderate to high temperature conditions (Hazan *et al.,* 1974; Northcraft and Watson, 1987) and their resistance to numerous chemical pesticides that are commonly used, many of which destroy their natural predators (Gerson and Weintraub, 2007).

2.2 *Neoseiulus californicus* (McGregor) 1954

Predatory phytoseiid mite *Neoseiulus californicus* consumes mite pest species. *N. californicus* was originally identified as *Typhlodromus californicus* by McGregor in 1954 from lemons in California.

Common name: Predatory mite

Scientific name: *Neoseiulus californicus*

2.2.1 Taxonomic Position

Kingdom: Animalia

- **Phylum**: Arthropoda
  - **Subphylum**: Chelicerata
    - **Class**: Arachniida
      - **Order**: Mesostigma
        - **Family**: Phytoseiidae
          - **Genus**: *Neoseiulus*
            - **Species**: *Neoseiulus californicus*

2.2.2 Distribution

Natural populations of *N. californicus* can be found in parts of southern Europe, Argentina, California, Chile, Florida, Japan, South Africa, Texas, and all the way around the Mediterranean Sea's border (Rhodes and Liburd, 2019). Many agricultural cropping systems, such as those that grow strawberries, raspberries, roses, grapes, citrus, ornamentals, and vegetables, are linked to this predatory mite (Hoddle 2000; Johnson and Lyon, 1988).

2.2.3 Host

Important fruits and decorative pests like the two-spotted spider mite (*Tetranychus urticae* Koch), broad mite (*Polyphagotarsonemus* (*Stenotarsonemus*) *ltus* Banks), cyclamen mite (*Tersonemus pallaidus* L.), and other mite species are among the foods that *N. californicus* consumes (Hoddle 2000). When the two-spotted spider mite is present in low densities, *N. californicus* exhibits a preference for feeding on its larval and nymphal stages (Maleis and Ravenberg, 2004). Nonetheless, *N. californicus* can subsist on pollen alone for a few days without consuming any prey (Malais and Ravensberg, 2004).

2.2.4 Biology

Although *N. californicus* can withstand cold temperatures for brief periods of time, it prefers warm temperatures (10–30 ^{0}C).Wide variations in humidity have no effect on them. Depending on the temperature, the development period from egg to adult can take anywhere from 4 to 12 days (Rhodes and Liburd, 2005).

2.2.4.1 Egg

A female *N. californicus* can lay up to four eggs in a single day, on average. The single oval eggs are 0.04 mm long and have a light yellow hue. Depending on the temperature, the incubation period lasts between 1.5-4 days (Rhodes and Liburd, 2005).

2.2.4.2 Larva

The tiny, translucent, six-legged larva that emerges from the eggs is primarily not a feeding stage. About 0.5 to 1 day is how long it lasts (Rhodes and Liburd, 2005).

2.2.4.3 Nymph

Protonymphs and deutonymphs, the two nymphal stages, are active feeders. Every step takes one to three days. Except for their diminutive size, they are exactly like adults (Rhodes and Liburd, 2005).

2.2.4.4 Adult

Compared to males, adult females are larger. They have a translucent body, an oblong shape, and a length of about 0.1 mm. They can be transparent, pale orange, pink, or peach in color. Twenty days make up the adult stage (Rhodes and Liburd, 2005).

2.2.5 Season

Their ability to adjust to challenging environments is a crucial criterion for evaluating the quality of natural adversities (Shipp and Van, 1997; Singh, 2018). Extreme summer heat and food scarcity have a major impact on predatory mite populations (Li *et al.,* 2016; Huang *et al.,* 2019). Predatory mites' ability to withstand hunger in hot temperatures is strongly associated with both their survival and population stability (Montserrat *et al.,* 2013; Zhang *et al.,* 2018). According to (Gotoh *et al.,* 2004), *N. californicus* was able to lay eggs at 37.5°C, but the eggs were unable to hatch at that temperature. However, females could not lay eggs when the temperature rose above 40°C. According to (Fraulo and Liburd, 2008), 40–80% relative humidity was ideal for *N. californicus* survival.

2.2.6 *Neoseiulus californicus* as a predator

Worldwide, predaceous phytoseiid mites are important biological pest control agents for a variety of crops (Helle and Sabelis, 1985). The number of spider mites in some agroecosystems may be lowered by these natural enemies to levels that are not detrimental to the economy (Nyrop *et al.,* 1998). It has been demonstrated that the phytoseiidae family of predatory mites effectively controls *T.urticae* populations. Numerous predator species are members of the phytoseiidae family, such as *Neoseiulus fallacis* (Garman), *Neoseiulus californicus* (McGregor), and *Phytoseiulus persimillis* Athias-Henriot. In many nations, they are employed through argumentative techniques (Hussey and Scopes, 1985; Waite, 1988; Raworth 1990; Zalom *et al.,* 1990).

The mite predators *Neoseiulus californicus* (McGregor) and *Neoseiulus cucumeris* (Oudemans) are more flexible than generalist feeders (TSSM and broad mites), and they can utilize alternative food sources like pollen (McMurthy and Croft, 1997; Weintraub *et al.*, 2003). As a result, they can be employed in the early stages of mite outbreaks.

Numerous research works have looked into the field uses of *N. californicus*. For instance (Abad-Moyano *et al.*, 2010) discovered that *Tetranychus urticae* can be successfully controlled by repeatedly releasing *N. californicus* into citrus orchards. *N. californicus* significantly decreased avocado damage in Spain, according to Monsterrat *et al.* (2013). In Florida, *T.urticae* on strawberris is effectively controlled by applying acaricides and *N. californicus* together, as confirmed by (Liu *et al.,* 2016). *N. californicus* is resistant to pesticides and can withstand extremes in temperature and humidity. As a result, it has been used to control *Tetranychus* urticae Koch, *Panonuchus ulmi* Koch, *Raoiella indica* Hirst, and *Polyphagotarsonemus latus* Banks (Monterio *et al.*, 2008; Kishimoto *et al.*, 2007; Katayama *et al.*, 2006).

2.2.7 Functional Response of *Neoseiulus californicus*

Natural enemies' functional response may be impacted directly by host-plant structures that impede their ability to move about, or indirectly by these structures' ability to provide refuge from predators and modify the suitability or palatability of prey (Jalali and Ziaaddini, 2017, Madadi *et al.*, 2007, Paspati *et al.*, 2021). Only a small amount of information is known about the indirect transgenerational effects of host plants—which are defined as any effects on the phenotype of the offspring caused by experiences of their mothers—on the functional response of predators. Direct effects of host plants on the functional response of predators are more frequently studied (Bonduriansky and Day, 2009). Typically, predatory mites are raised on specific host plants and then released as biocontrol agents in a variety of cropping systems. As of right now, nothing is known about how transgenerational factors involving prey or predators may impact predators' functional response and, ultimately, their effectiveness as biocontrol agents. (Williams *et al.,* 2004; Rahmanietal, 2009) Phytoseiid mites are well-known biocontrol agents of pests, such as spider mites and thrips. Studies conducted on *N. californicus* in the past (Castagnoli and Simoni, 1999; Cedola *et al.,* 2001; Gotoh *et al.,* 2004; Ahn *et al.,* 2010; Xiao and Fadamiro, 2010) have reported a type II functional response. Compared to Castagnoli *et al.* (2001), who discovered that a mated female *N. californicus* consumed 9.04 and 11.41 eggs after 1 and 5 days of exposure, respectively, there was more predation by *N. californicus* on two spotted spider mites. In addition, there was more nymphal predation than in the Castagnoli *et al.* (2001) report that found that on the first and fifth days of exposure, the average consumption was 6.97 and 6.48 protonymphs (at <25ºC), respectively.

CHAPTER III

MATERIALS AND METHODS

The experiment was conducted in the laboratory of the Department of Entomology, Hajee Mohammad Danesh Science and Technology University (HSTU) Dinajpur from May to November 2023.

3.1 Culture of host plant *Lablab purpureus* for *Tetranychus urticae* rearing

Country bean plant, *Lablab purpureus* was cultured as host plants of two spotted spider mite, *Tetranychus urticae* within the pot and the experimental field of Entomology department of Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur. Bean seeds were sown twice in a week from May 24, 2023 for constant and continuous offer of individual TSSM's species to maintain the *Neoseiulus californicus* colonies and experimental purposes. Proper intercultural practices were maintained to grow the host plants as per necessary. Plants were watered regularly. No pesticides were used for the assembly of host plants.

3.2 Mass culture of two-spotted spider mite

To initiate the mass culture, TSSM were collected from some infested vegetable gardens of the university field. After getting the bean seedlings, two spotted spider mites were inoculated from the field and maintained on bean seedlings outside of the laboratory. The mass culture was maintained all stages of two-spotted spider mite.

3.3 Predator source and rearing

Live specimens of the predatory mite, *Neoseiulus californicus* were collected from country bean (*Lablab purpureas;* Family: Legumionsae) cultivated in nearby open fields located at Hajee Mohammad Danesh Science and Technology University campus, Dinajpur. Stock cultures of all studied species were maintained on country bean leaf arenas placed upside down on water saturated foam pads of 2.5cm thickness and placed inside round plastic box (17.5cm D and 10.3 cm H). The water level inside the box was maintained at an optimum level to keep the leaves fresh and prevent the escape of mites from the leaves.

Separate culture sets were maintained for each mite species under laboratory conditions of 25 ± 2 ^{0}C and 70 ± 5% Relative Humidity (RH) and photoperiod of 12 L:12 D h. Bean leaves with *T. urticae* were added to the petridish arena daily to obtain sufficient predators for the experiment.

3.4 Functional response of *N. Californicus* on different densities of *T. urticae*

Mature fresh uninfested bean leaves were collected from potted bean plants. The leaves were cut into disc (3cm diameter) and placed into the Petridish. A clean petridish was used to starve an adult female N. californicus for four hours after she was removed from the stock culture. Following that, an adult female *N. californicus* was provided five densities of *T. urticae* eggs, larva, protonymph, and deutonymph individually at 10, 15, 20, 25, and 30. A total of required number of eggs, larva, protonymph and deutonymph were retained in the experimental arena and the rest were removed without damaging the webbings over the eggs and surroundings. *N. californicus* were gently transferred to each experimental area using fine camel hairbrush. Each treatment replicated with five times. Predation efficiency was assessed by placing a single female adult *N. californicus* in an experimental arena. To avoid drought the leaf discs were placed in water saturated cotton. The experiment duration was as fixed as 24 hr. and observations were made carefully by counting the number of shriveled carcasses of the life stages of prey mite and the number of intact eggs left on the leaf discs two parameters were widely used to describe the functional response of predator feeding on a single species of prey: these are predator’s “rate of attack” or “searching rate” (α) and it’s handling time (Th). These are the "rate of attack" (a) and "handling time" (Th) of the predator (Hassell *et al.,* 1976). Each predator-prey combination will have a different value for a and Th as the predator moves through a succession of size classes. The steepness of an increase in predation with the satiation threshold is estimated by the coefficient of searching efficiency (Hassell, 1981).

Mite infested bean leaf

Host plants

Plate 1. Cultivated host plants of country bean, *Lablab perpureus*

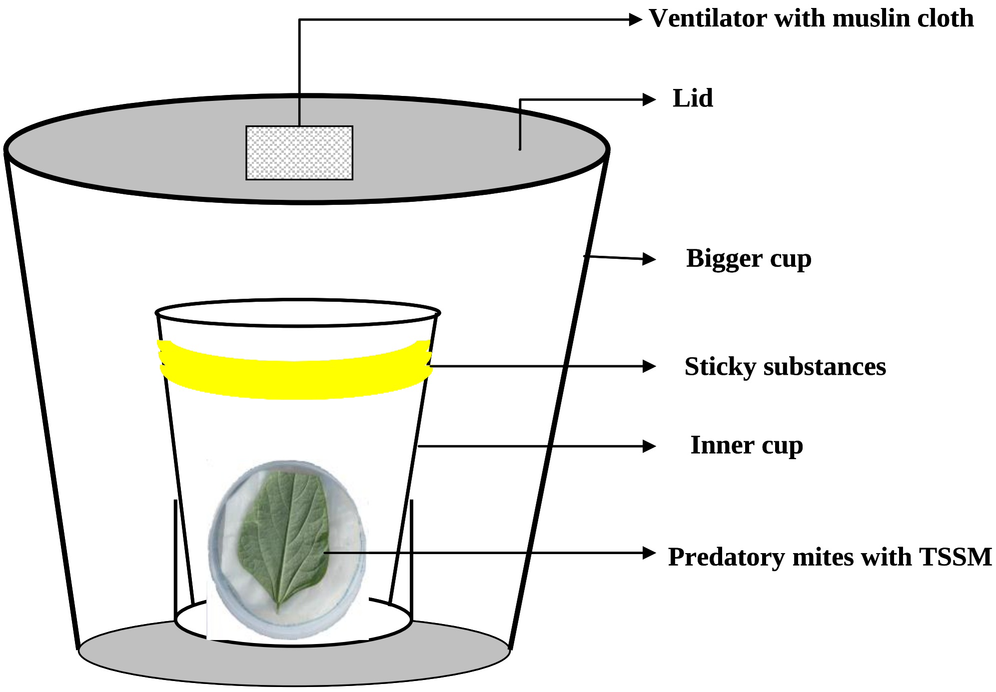

Plate 2. Rearing apparatus of the predatory mite

3.5 Statistical Analysis for functional response of *Neoseiulus californicus*

The relationships between consumption rate (prey consumed / prey offered × 100) by adult males, females and different nymphal instars in relation to prey density were analyzed using linear regression procedures (R studio 3.1.3). Holling curvilinear type II equation (Holling, 1959) was used for calculation of functional response. In this model, the number of prey consumed (Na) is a function of prey density (n) as follows:

$$Na = (aTN)/(1 + aThN)$$

Where, a is the rate of attack (discovery) of the prey, T is the total time available (1 d or 24 h in this experiment), and Th is the handling time for one prey.

The parameter of Holling curvilinear type II equation was the handling time (Th) and the attack constant (a) were estimated using Holling's disc equation (1959) modified by reciprocal linear transformation (Livdahl and Stiven, 1983). The modified equation is as follows:

$$\frac{1}{Na} = \frac{1}{a} . \frac{1}{NT} + \frac{Th}{T}$$

Where $\frac{1}{Na}$ represents y, $\frac{1}{a}$ represents α, $\frac{1}{NT}$ represents x and $\frac{Th}{T}$ represents ß. The linear regression form becomes y = α x + ß. The maximum number of consumed prey per predator (asymptote), H$a_{\max} = \frac{T}{Th}$ was found.

Plate 3. Mass culture of *N. californicus* inside the plastic box in protected conditions

Plate 4: Stereo microscope (model: BST 606) for observing and counting all stages of mite

Plate 5: Camel hair brush for picking mite

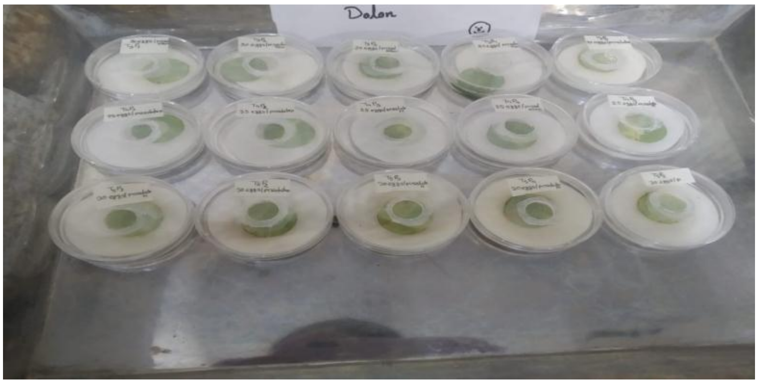

Plate 6. Experimental set up for determination of functional response

3.6 Biological control of two spotted spider mite by predatory mite

The experiments were conducted from October to November 2023 in the faculty of Agriculture building at Hajee Mohammad Danesh Science and Technology University, Dinajpur. The host plants were planted in Mid–October and watered regularly. Potted bean plants with eight to ten compound leaves infested with 10 adult females of TSSM. Females were released to the middle of the plants and allowed to oviposit for 7 days for the establishment of TSSM colonies. Before released of predatory mites, a leaflet was collected from the middle of each plant. The number of two spotted spider mite motile and eggs on each leaflet was counted, and the mean number per leaflet was calculated. There were five treatments and each treatments were replicated with five times. Treatments included 1) 10 TSSM and 1 predator 2) 10 TSSM and 2 predator 3) 10 TSSM and 3 predator 4) 10 TSSM and 3 predator 5) Untreated (control) without predatory mite. After a week the population of predators and two spotted spider mites were sampled by taking one leaflets from the middle of each plants (One leaflet from each individual treatment) and counting the number of TSSM as well as predatory mites (motile and eggs). The terms motiles refers to all life stages except eggs. Samples were recorded for 2 weeks.

3.7 Statistical analysis for biological control of *T. urticae*

The data for the number of TSSM motiles and eggs were found on the leaf of the plant were analyzed by using Least Significant Differences (LSD) test was performed to separate means among the treatments ($P<0.005$). Differences in TSSM motiles and eggs observed on the leaves were analyzed using ANOVA to separate means. All statistical analyses were performed using SPSS.

Plate 7. Experimental setup of biological control of TSSM by predatory mite

CHAPTER IV

RESULTS AND DISCUSSION

The present study investigated the functional responses of *Neoseiulus californicus* to different densities of egg, larva, protonymph, deutonymph of two-spotted spider mite (*Tetranychus urticae*) and biological control of two spotted spider mite by *Neoseiulus californicus.*

4.1 Functional response of *N. californicus*

The attack rates, handling time, maximum prey consumption and regression coefficient (r^2) of *N. californicus* while provided to various densities of *T. urticae* are presented in Table.1. The widely used functional response parameters exercised the rate of attacked and handling times of predators to prey. Among the different densities of egg, larva, protonymph and deutonymph of TSSM the highest rate of attack of *N. californicus* female shown in protonymph (0.626 d^{-1}) while predatory female exhibited the lowest rate of attack on deutonymph (0.504 d^{-1}).

Handling time was same (0.0052 min) in case of egg and larval periods and increased as two spotted spider mite progressed to the next stage and it was maximum (0.0283 min) in deutonymph stage. The predicted maximum consumption on egg, larva, protonymph and deutonymphs were 192.30, 192.30, 50.761 and 35.33, respectively for each female predatory mite in 1 day or 24 hour (Table 1). But the lowest consumption was shown in deutonymph (35.33) for 1 day or 24 hour. It is indicated that various prey stages of a predator have different abilities in respond to increasing prey densities.

Prey consumption rates of adults female of *N. californicus* was observed by providing various densities of prey *T. urticae* were estimated as attack rate and handling time (Table 1).

Predation efficiency, *N. californicus* was estimated by offering 5 densities of egg, larva, protonymph and deutonymph to *T. urticae* to judge the form of its efficient response. Figure (1a-1d) showed the curve of the functional response of *N. californicus* female on different life stages of two spotted spider mite. The functional response curve was established following Holling's type II equation (1959). The handling times of *N. californicus* feeding on *T. urticae* in this study were longer with the exception of *T. urticae* larvae. Additionally, the daily consumption by the *Neoseiulus* species at varying densities of eggs and larvae indicated that this predator preferred to consumed more eggs overall. The functional response of predatory female was determined by the consumption of prey egg in different densities. The graph titled Figure 1(a): Functional response of predatory *N. californicus* on eggs of *T. urticae* in Petri dishes illustrate the relationship between prey density and the number of eggs consumed by the predatory. The consumption of prey by this predatory varied with minimum 5.59 in the lowest predatory densities (10) and maximum 15.26 in the highest prey densities (30) which was second highest than all other prey densities. Initially, as prey density increases, the number of eggs consumed rises sharply. However, the curve eventually levels off, indicating a saturation point where predators cannot consume more prey, regardless of increased availability. The R-squared (R^2) co efficient of determination value of 0.92 indicates a strong correlation between prey density and egg consumption. Approximately 92% of the variance in egg consumption can be explained by prey density.

Table 1. Type II functional response parameters of *N. californicus* feed on different densities of *T. urticae.*

Life stages	a	Th **(min)**	T/Th	r^2
Egg	0.588	0.0052	192.30	0.8547
Larva	0.593	0.0052	192.30	0.9536
Protonymph	0.626	0.0197	50.761	0.6922
Deutonymph	0.504	0.0283	35.33	0.6604

a = Rate of attack, Th = Handling time, $\frac{T}{Th}$ = Prediction of maximum prey consumption, r^2 = Regression coefficient

When the two-spotted spider mite larval stage reached its fifth density of prey consumption, the predatory mite's functional response was ascertained. Pray consumption rate rose as prey densities increased, according to the result displayed in Figure (1b). The female predator consumed varying densities of larva; at the lowest (10), they consumed 5.64, and at the highest (30), they consumed 15.40. For the model employed in this analysis, a perfect fit is indicated by the coefficient of determination value labeled $R^2=1$ on the graph. Indicating the strong correlation between prey density and predation rate, this shows that there is no variation that the model cannot account for.

In summary, this graph provides valuable insights into predator-prey dynamics and can inform ecological studies and pest management research. The functional response of predatory female was determined by the consumption of prey protonymph in five densities. The result presented in Figure (1c) indicated that the consumption by the female predatory mite increased with increasing densities of protonymph. The consumption of prey by this predator varied with minimum 5.23 in the lowest predatory densities (10) and maximum 11.80 in the highest prey densities (30) which is lowest than egg and larval stage of prey. The graph represents a Type II functional response, as described by Holling's (1959) equation. This type of response typically shows a decelerating intake rate, which means that the consumption rate increases with prey density but at a diminishing rate. At low prey densities (1), the average prey consumed is 5.23.As prey density increases to 5, the number of prey consumed rises to 11.80, indicating that *N. californicus* is more efficient at higher prey densities but shows signs of satiation.

Adult female predator showed the functional response by consuming the prey in different densities. The result presented in Figure (1d) indicated that the prey consumption by the female predator mite increased with increasing densities of deutonymph. The consumption of prey by this predator varied with minimum 3.93 at the lowest predator densities (10) while maximum 8.17 at the highest prey densities (30) which is the lowest than egg, larva, protonymph of *T. urticae.* The co efficient of determination value of 1 indicates a perfect fit of the model to the data, implying that 100% of the variance in prey consumption is explained by prey density. The data demonstrates that *N. californicus* exhibits a Type II functional response when preying on *T. urticae* deutonymphs. The predator's consumption rate increases with prey density but eventually plateaus, reflecting a typical decelerating intake rate as described by Holling's equation. The perfect R^2 value and low standard errors underline the robustness and accuracy of the experimental results. This study underscores the potential of *N. californicus* as an effective bio control agent for managing *T. urticae* populations.

The results of this study support those of Zhang and Croft (1995), who found that when predator numbers rose, there was a discernible decrease in the quantity of prey consumed by female predators. Compared to egg and larva to deutonymph, the handling time of *N. californicus* feeding on *T. urticae* in this study was longer, and the daily consumption by the *Neoseiulus* species at varying densities of eggs and larvae demonstrated that *N. californicus* consumed more eggs overall. When Cuellar *et al.* (2001) examined how six phytoseiid species fared on *Mononychellus tanajoa* (Bondar) eggs, they discovered that *N. californicus* could only eat up to 40 eggs at a starting prey density of 200. Employing a density of 80 eggs per 5 cm^2, Castagnoli and Simoni (1999) documented a maximum daily predation of 17 two spotted spider mite eggs by one strain of *N. californicus* raised in a laboratory on two spotted spider mites collected in strawberry fields. In comparison, strains raised on American house dust mite, *Dermatophagoides farinae* Hughes, and *Quercus* spp. pollen consumed roughly 12 and 14 eggs, respectively.

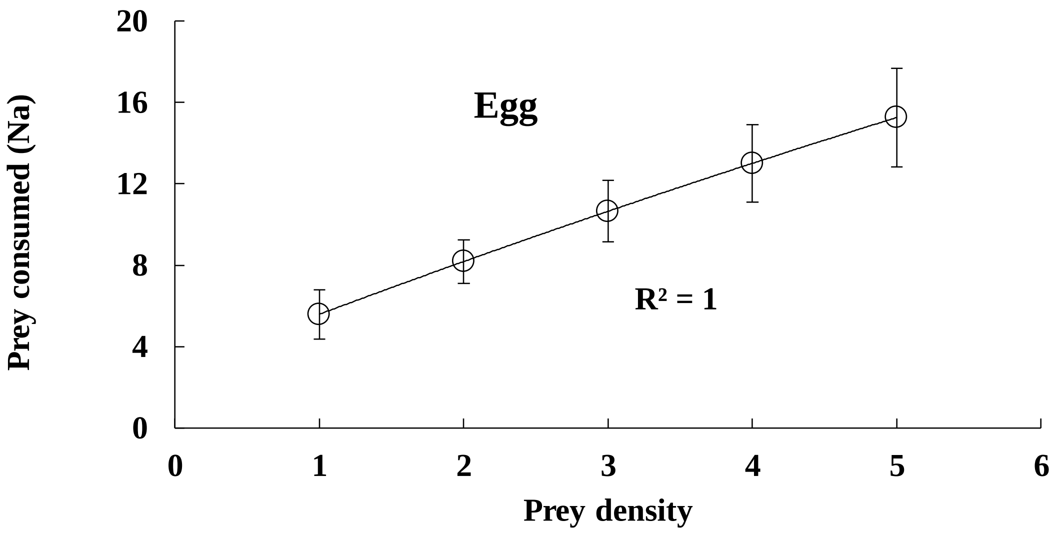

Figure 1(a). Functional response of predatory *N. californicus* on egg of *T. urticae* in petridish. Points show average number of eggs eaten or killed by *N. californicus* at each densities of prey availability as Hollings equation of type II functional response. Bars show standard errors.

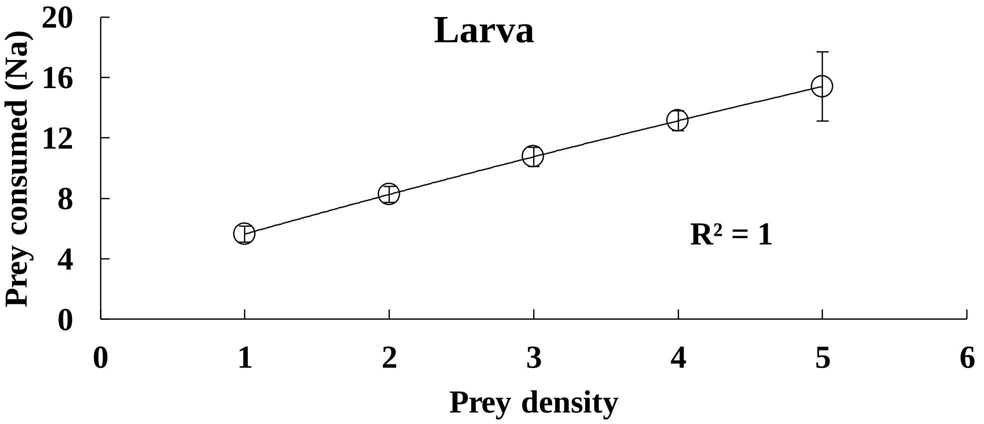

Figure 1(b). Functional response of predatory *N. californicus* on larva of *T. urticae* in petridish. Points show average number of larva eaten or killed by *N. californicus* at each densities of prey availability as Hollings equation of type II functional response. Bars show standard errors.

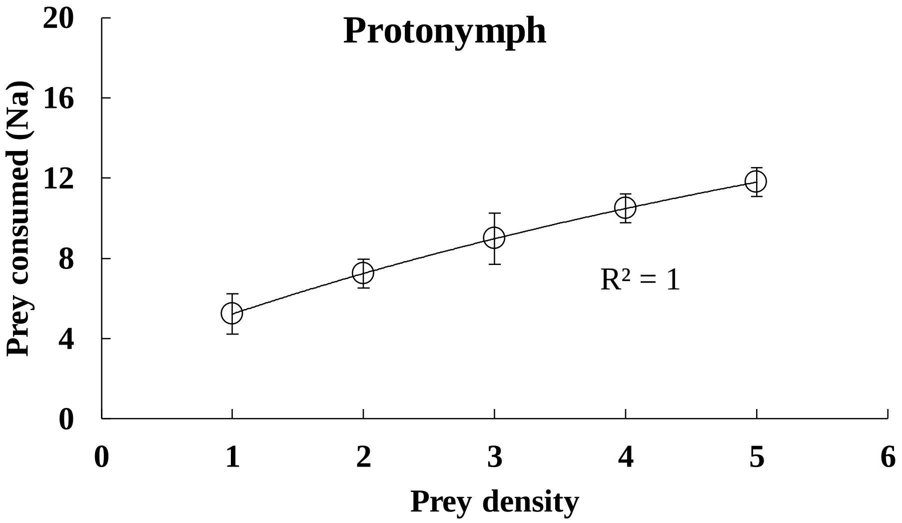

Figure 1(c). Functional response of predatory *N. californicus* on protonymph of *T. urticae* in petridish. Points show average number of protonymph eaten or killed by *N. californicus* at each densities of prey availability as Hollings equation of type II functional response. Bars show standard errors.

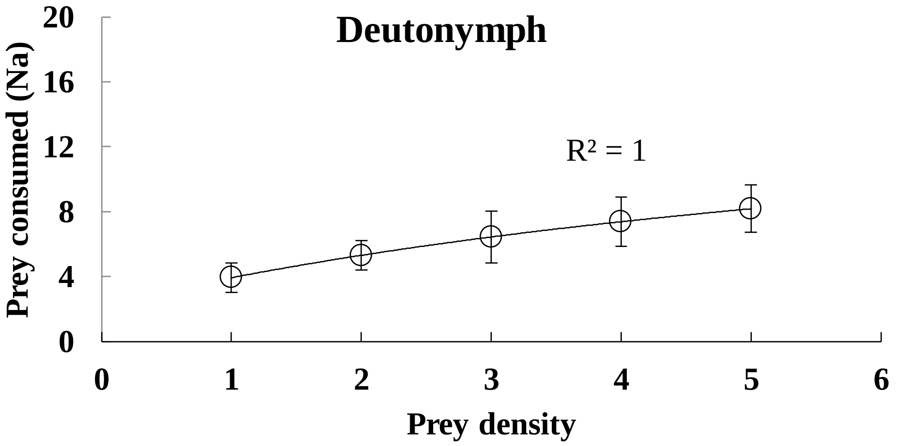

Figure 1(d). Functional response of predatory *N. californicus* on deutonymph of *T. urticae* in Petridis. Points show average number of deutonymph eaten or killed by *N. californicus* at each densities of prey availability as Hollings equation of type II functional response. Bars show standard errors.

We can conclude that Type II Holling was the functional response of *N. californicus* on two spotted spider mite. According to Gotoh *et al.* (2004), *N. californicus* females feeding on two spotted spider mite eggs at 25°C exhibited a Type II functional response, with a daily egg consumption rate of 13.4, a rate of successful search (a) of 1.3000, and handling time of 0.0290.

4.2 Biological Control of *T. urticae* by *N. californicus*

Neoseiulus californicus, formerly known as *Amblyseius californicus*, is a widely researched and utilized biological control method in integrated pest management (IPM) for the two-spotted spider mite (*Tetranychus urticae*). *Neoseiulus californicus* can significantly reduce spider mite populations. Biological control aims to achieve a number of sustainable and successful pest management objectives, especially when applying *Neoseiulus californicus* to control populations of two-spotted spider mites.

During the release of TSSM populations were low. Therefore, data were not analyzed. Two spotted spider mite numbers began to increase day by day. Data were collected after 7 days of releasing TSSM. The release of *N. californicus* resulted in significantly fewer TSSM motiles and eggs on each treatments than no releases of *N. californicus*.

The average number of *Tetranychus urticae* motiles per treatment from field samples taken prior to the release of predators is shown in **Table 2**. The information contains the standard deviation (SD), standard error (SE), and average (Avg) for each of the five treatments labeled T_1 through T_4 as well as the control group. The standard deviation and standard error of each value are included to show the accuracy and variability of the estimations. In Treatment 1 (T_1), the average number of TSSM motiles was 17.83, with a standard error (SE) of 8.67 and a standard deviation (SD) of 19.39. This suggests that there is a great deal of variation in this treatment group. TSSM motiles averaged 13.08 in

Treatment 2 (T_2), with a 7.25 SD and a 10.67 SE. The SE is higher even though the average is lower than T_1, indicating less accurate (T_3) estimates in this treatment. In comparison to T_1 and T_2, the mean for Treatment 3 (T_3) was 10.73, with a significantly lower SD of 4.76 and a SE of 2.13, indicating more consistent counts of TSSM motiles. The results of Treatment 4 (T_4) revealed an average of 11.22 TSSM motiles, a 6.02 SD, and a 2.69 SE. While not as high as in T_3, this variability is still lower than in T_1 and T_2. The control group had the lowest mean (SD = 6.90, SE = 3.08) number of TSSM motiles (9.22). To sum up, group T_1 displayed the highest mean number of TSSM motiles along with the highest variability, whereas group T_2 displayed the lowest mean and most moderate variability. The means of treatments T_2, T_3, and T_4 were in the middle range; treatment T_3 had the lowest SE, indicating the least variability and most accurate estimates.

After predators were released, field samples are used to calculate the mean number of *Tetranychus urticae* motiles per treatment, as shown in **Table 3.** The aforementioned metrics comprise the mean (Avg), standard deviation (SD), standard error (SE), and the percentage change (or reduction) in TSSM motiles (%). Following the release of the predator, Treatment 1 had an average of 5.53 TSSM motiles. With a standard deviation (SD) of 3.93, this treatment group's variability was found to be moderate. The accuracy of the mean estimate was demonstrated by the 1.89 standard error (SE). The percentage reduction was 83.15%, with a standard deviation of 2.60%. This indicates a substantial decrease in TSSM motiles, suggesting a very effective treatment. Treatment 4 (T_4) showed an average of 1.53 TSSM motiles. The SD was 2.30, reflecting moderate variability. The SE was 1.03, indicating a precise mean estimate. The percentage reduction was 85.63%, with a standard deviation of 2.34%, the highest reduction among the treatments, showing the most effective control of TSSM motiles.

Table 2. Mean number of TSSM motiles per treatment counted from the field samples before released predator.

Treatments	Average	SD	SE
T_1	17.83 ± 11.48	19.39 ± 13.08	8.67 ± 5.85
T_2	13.08 ± 5.91	7.25 ± 4.13	10.67 ± 9.26
T_3	10.73 ± 3.93	4.76 ± 1.46	2.13 ± 0.65
T_4	11.22 ± 4.96	6.02 ± 3.08	2.69 ± 1.38
Control	9.22 ± 4.87	6.90 ± 3.61	3.08 ± 1.61

Table 3. Mean number of TSSM motiles per treatment counted from the field samples after releasing predator.

	Average	SD	SE	Percent
T_1	5.53 ± 3.88	3.93 ± 2.40	1.89 ± 1.02	72.09 ± 3.45a
T_2	3.50 ± 1.86	4.11± 2.22	1.84 ± 0.99	75.55 ± 3.09a
T_3	1.76 ± 0.75	1.81 ± 0.50	1.12 ± 0.64	83.15 ± 2.60a
T_4	1.53 ± 0.69	2.30 ± 1.08	1.03 ± 0.48	85.63 ± 2.34a
Control	8.82 ± 3.19	5.23 ± 1.78	2.36 ± 0.79	-28.12 ± 33.68b

Means with the same letter are not significantly different ($P<0.005$) using LSD test.

The control group had a mean of 8.82 TSSM motiles, higher than any of the treatment groups. The SD was 5.23, indicating high variability. The SE was 2.36, reflecting less precise estimates. Interestingly, the percentage change was -28.12%, with a standard deviation of 33.68%, indicating an increase in TSSM motiles rather than a reduction, contrasting with the treatment groups. This data showed that without predator release, the TSSM population increased. In summary, all treatments (T_1 to T_4) showed significant reductions in TSSM motiles after predator release, with T_4 being the most effective, achieving an 85.63% reduction. In contrast, the control group experienced an increase in TSSM motiles. The data underscores the effectiveness of predator release in controlling TSSM populations in the treated groups compared to the control.

The **Table 4** presents data on the total number of motiles and eggs of the predatory mite *Neoseiulus californicus* across different treatments. The mean number of motiles and eggs for Treatment 1 (T_1) was 0.40. The standard deviation (SD) was 0.42, indicating moderate variability within this treatment group. The standard error (SE) was 0.19, reflecting the precision of the mean estimate. In summary, the treatments T_1, T_2, T_3, and T_4 showed varying levels of *N. californicus* motiles and eggs, with means ranging from 0.40 to 0.78. The control group had no motiles or eggs, indicating the absence of predatory mites. While there was some variability within treatments, the differences among the treatments were not statistically significant.

When compared to the untreated control, the control group, denoted by "b," differed significantly from all treatments, indicating that the treatments were successful in introducing *N. californicus*.

Table 4. Shown that the total number of motiles and eggs of the predatory mite *N. californicus.* The number of predator on each treatment were not significantly different.

Treatments	Average	SD	SE
T_1	0.40±0.19ab	0.42±0.17ab	0.19±0.08ab
T_2	0.77±0.20a	0.82±0.14a	0.37±0.06a
T_3	0.78±0.23a	0.80±0.18a	0.36±0.08a
T_4	0.78±0.20a	0.80±0.15a	0.36±0.07a
Control	0.00±0.00b	0.00±0.00b	0.00±0.00b

Means with the same letter are not significantly different ($P<0.005$) using LSD test.

The data show the average measurements of an unidentified variable before and after the introduction of predators for four treatments (T_1, T_2, T_3, T_4) and a control group in the graph shown in **Figure 2(a)**. The average values for T_1, T_2, T_3, and T_4 were 17.82, 13.08, 10.73, and 11.22, respectively, prior to the release of the predator, whereas the control group had an average of 9.22. These values dramatically dropped to 5.53, 3.5, 1.76, and 1.53 for T_1 through T_4 after the release, with the control showing a minor decline to 8.82. Predators were not present in the control group; in T_1, their presence was measured at about 0.4, and it increased slightly about 0.78 in T_2, T_3, and T_4. In contrast to the control condition, which showed less noticeable changes.

The graph in **Figure 2(b)** shows the measurements' standard deviation (SD) for four treatments (T_1, T_2, T_3 and T_4) as well as a control group, both before and after the predators were released. At the beginning, the control group's SD was 6.9, while the SDs for T_1, T_2, T_3, and T_4 were 19.39, 7.25, 4.76, and 6.02, respectively. Following the release, the SDs for T_1, T_2, and T_3 significantly decreased to 3.93, 4.11, 1.81, and 2.29, respectively, while the SD for the control group only slightly decreased to 5.23. Furthermore, the predator counts in the control group were zero, despite being measured as roughly 0.42 in T_1 and roughly 0.8 in T_2, T_3, and T_4. While the control group showed less change, indicating the absence of such influence.

The standard error (SE) of measurements for four treatments (T_1, T_2, T_3 and T_4) and a control group are shown in **Figure 2(c)** as a graph that compares values before and after the release of predators. At the beginning, the control was 3.08 and the SE for T_1, T_2, T_3, and T_4 were 8.67, 10.67, 2.13, and 2.69, respectively. Following the introduction of the predator, the SE values for T_1, T_2, T_3, and T_4 significantly dropped to 1.89, 1.84, and 1.12, respectively, while the SE for the control group dropped to 2.36. Predators made up only 0.19 of the population in T_1, roughly 0.36 in T_2, T_3, and T_4, and none in the control group. The control group's comparatively small change suggests the absence of this effect, whereas the reduction in SE across all treatments shows a significant improvement in the precision of the measured variable following predator release, highlighting the homogenizing effect of predators.

The findings on the reduction of *Tetranychus urticae* (TSSM) populations after the release of predatory mites, *Neoseiulus californicus,* are consistent with those reported in other studies. Opit *et al.* (2005) and Xia *et al.* (2019) observed that releasing *N. californicus* significantly reduced TSSM densities by over 70%, aligning with your documented reductions of 72.09% to 85.63%. *Neoseiulus californicus* has a slower metabolism and a lower searching efficiency, but it can withstand starvation and has a high rate of spatial coincidence with TSSM (Greco *et al.,* 2004) in comparison to other phytoseiids. Additionally, the variability in TSSM counts before predator release in your treatments, particularly the higher variability in T_1, corresponds with Kergunteuil *et al.* (2016), who found that initial pest densities often vary due to environmental factors and initial infestation levels. Moreover, the increase in TSSM populations in your control group, which lacked biological control agents, mirrors findings by Pratt and Croft (2000), where unchecked TSSM populations were observed to increase rapidly.

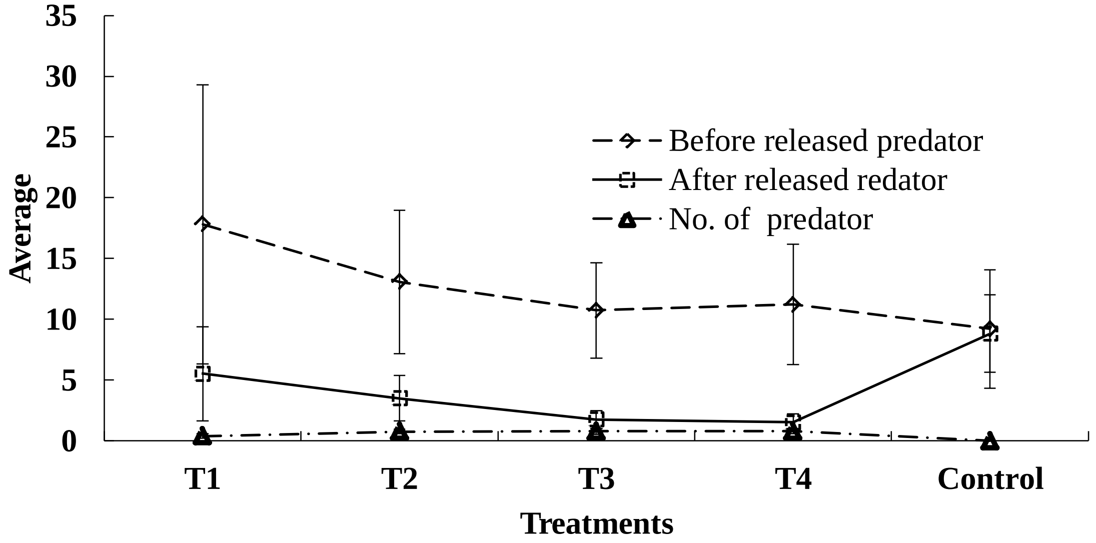

Fig. 2(a). The graph illustrates the Average (Avg) of some measurements before and after the release of predators in different treatments.

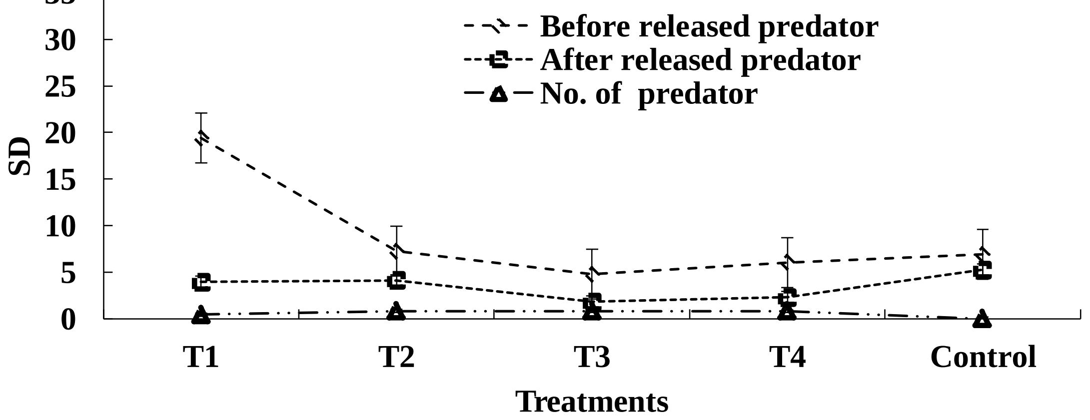

Fig. 2(b). The graph illustrates the standard deviation (SD) of some measurements before and after the release of predators in different treatments.

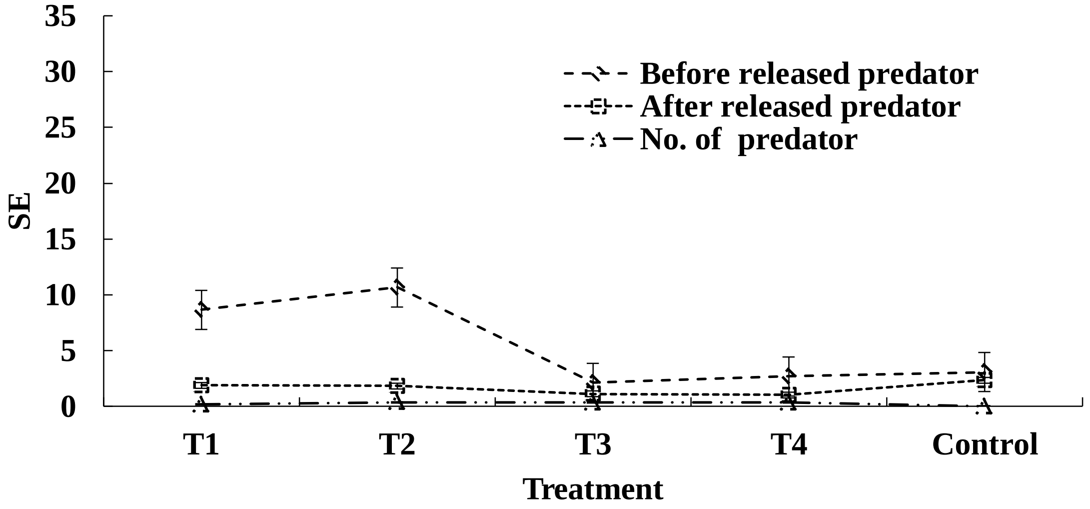

Fig. 2(c). The graph illustrates the standard error (SE) of some measurements before and after the release of predators in different treatments.

CHAPTER V

SUMMARY AND CONCLUSION

Two spotted spider mite (TSSM) is highly destructive pest that causes significant damage to agricultural crop. A well-known beneficial predator for TSSM population suppression is the predatory mite species *Neoseiulus californicus*. A detailed research work was carried out in the laboratory of the Department of Entomology, Hajee Mohammad Danseh Science and Technology University, Dianjpur to study the functional response of *Neoseiuls californicus* on two spotted spider mite and biological control of *Tetranychus urticae* by predatory mite during May 2023 to December 2023. All experiments were carried out under laboratory conditions at 25 ± 2 ^{0}C and 70 ± 5% RH.

The predatory female *N. californicus* showed the maximum rate of attack in TSSM's protonymph (0.626 d^{-1}) but minimum on deutonymph (0.504 d^{-1}). The adult female had highest handling time in deutonymph (0.0283 min) while the lowest on egg and larva (0.0052 min). The predicted maximum consumption on egg, larva 192.30 and minimum consumption on deutonymph were 35.33 for each female predatory mite in 1 day or 24 hour. On biological control of two spotted spider mite by *N. californicus* demonstrated that *N. californicus* released at ratios between 4:10 and 1:10 leads to significant reduction caused by *T. urticae.* After releasing predatory female maximum reduction of two spotted spider mite found on the T_4 that is 85.63% and lowest in T_1 72.09% and ratio of TSSM and predatory female was 4:10 and 1:10 respectively. The control group that had no predator showed the increased number of two spotted spider mite populations rather than reduction. The average number of predatory motiles maximum in T_4 (0.78) but minimum in T_1 (0.04).The findings of the present study indicated that *N.californicus* might play a significant role in two spotted spider mite management. The functional response of *N. californicus* on different densities of egg, larva, protonymph and deutonymph of two spotted spider mites indicates that handling time, prey consumption rate, rate of attack are different in different prey stages. *N. californicus* is able to maintain populations of TSSM below damaging levels. However, before releasing *N. californicus* as a biological control agent in agro-ecosystem, further researches on its functional response and susceptibility to insecticides in the field level need to be investigated as insecticides affect their biology and functional response.

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