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Evaluation of prey stage preference of the predatory mite *Neoseiulus longispinosus* (Evans) on the spider mite pest *Tetranychus neocaledonicus* (André) (Acari: Phytoseiidae, Tetranychidae)

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Original research

ABSTRACT

Tetranychus neocaledonicus, the commonly called vegetable spider mite is a highly polyphagous pest, infesting over 500 species of economic plants. The species has been categorized as a major pest, inducing considerable feeding damage on vegetables, fruit crops, medicinal plants, ornamentals, plantation crops etc. The present study was concentrated to assess the predatory potential of a phytoseiid predator, *Neoseiulus longispinosus* on the different life stages (egg, larva, nymphs and adults) of *T. neocaledonicus* under laboratory conditions. Separate experimental set ups were prepared with leaf arena of mulberry plants and known numbers of different life stages of the pest mite were maintained on individual leaf arena. For evaluating the predatory potential, adult females of *N. longispinosus* were introduced on to the individual leaf discs in the arenas and observations were made under a stereomicroscope to record the response of the predator to the individual life stage of the pest mite. The number of prey stages consumed by the predator on individual leaf arena was recorded for a time interval of 24 hrs and the rate of consumption on individual prey instar was calculated. Except at the time of oviposition, the adult predator was found to make active prey searching movement randomly, in and around the prey patches. By sensing the prey through successive contacts with the first pair of legs and pedipalps, the predator initiated feeding actively, and sucked out the entire fluid content with its mouth parts. The adult predator showed a specific preference to feed on the egg stage of the prey mite while the predator nymph exhibited preference to the nymphal stages of the prey. A decreasing sequence in the order egg > larva > nymphs > adult was recorded in the feeding preference of the adult predator towards life stages of the prey mite and the per cent consumption on egg, larva, nymphs and adult female stages was 48.4, 29.9, 26.6 and 9.3 %, respectively. However, the feeding preference of the nymphal stage of the predator showed a slight deviation in the order nymph > larva > adult > egg and the mean rates of consumption were 31.3, 22, 11.2 and 10.3 %, respectively. Data gathered on consumption rates of the predator upon statistical analysis revealed a significant variation towards different stages of *T. neocaledonicus*, thereby establishing a specific preference to prey eggs by the predator.

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Keywords stage wise preference; leaf disc; *Neoseiulus longispinosus*; spider mite

Introduction

Tetranychid mites constitute one of the most severe agricultural pests recognized globally and the accomplishment of pest status by these mites depends on a combination of factors such as high biotic potential, fecundity, short generation time, wide geographic distribution, highly polyphagous nature, etc. (Helle and Sabelis, 1985; Seeman and Beard, 2011). Among the common tropical species of spider mites, *Tetranychus neocaledonicus* André, commonly called vegetable mite inflicts heavy damage to a variety of crops and has been ranked as a major pest (Chillar *et al.*, 2007). Prevalence of high temperature and low humidity favours incidence and multiplication of *T. neocaledonicus* in peak levels. Active feeding activity of all life stages of the mite on plant sap leads to the disappearance of chloroplasts, thereby leading to the development of white chlorotic spots on the leaf surface (Roy *et al.*, 2011). Severe infestation would induce immense webbing over the leaves, which in turn would lead to a decrease in the rate of photosynthesis and substantial reduction in yield (Antonio *et al.*, 2017).

Chemical means of pest control was widely practiced by the farmers and agriculturists based on its ease of application and fast eradication of pests. However, application of chemical pesticides always has been proved to induce varying backlash phenomena, ultimately leading to severe environmental degradation (Roy *et al.*, 2011). As a better and safe substitute to overcome the hazards of synthetic pesticides, biological control through inoculative release of natural enemies has been practiced in various countries (Helle and Sabelis, 1985). Among the natural enemies of pest mites, especially the spider mites, predatory mites of the family Phytoseiidae have been proved to be highly promising against phytophagous mites like spider mites (McMurtry and Croft, 1997; Puchalska and Kozak, 2016), tarsonemids (Svensson, 2009) and eriophyids (Carrillo and Pena, 2011). The species *Neoseiulus longispinosus* (Evans) has been included under the category of type II predators based on its exclusive preference to spider mites (McMurtry *et al.*, 2013). Based on the feeding specificity of *N. longispinosus* on spider mites, detailed studies have been undertaken to elucidate its potential in controlling bamboo mite populations such as *Aponychus corpuzae* Rimando and *Schizotetranychus nanjingensis* Ma and Yuan in China (Zang *et al.*, 1998; 1999). Similarly Song *et al.* 2016 studied the prey consumption and functional response of *N. longispinosus* on *Tetranychus urticae* Koch and *Tetranychus kanzawai* Kishida. There are studies showing the close association of *N. longispinosus* and *Tetranychus macfarlanei* Baker and Pritchard in the Okra plants of all growing seasons suggesting that *N. longispinosus* is an efficient natural predator of *T. macfarlanei* (Rajgopal and Srinivasa, 2017). Likewise close association of *N. longispinosus* and *T. neocaledonicus* have reported in Okra plants (Rachana *et al.*, 2009).

In the present study, the predatory potential of the species on the different life stages of a selected spider mite pest, *T. neocaledonicus*, was evaluated by making observations on the stage-wise preference of the predator in order to find out the most potential stage of the predator to be considered for pest suppression in field. The study was primarily based on field survey results obtained from different localities in two districts of northern Kerala, which revealed the close association of *N. longispinosus* with natural populations of *T. neocaledonicus* on the vegetable and fruit crops.

Material and Methods

Raising of stock cultures of *T. neocaledonicus* and *N. longispinosus*

Sufficient populations of prey cum predatory mites required for the study were raised by collecting live specimens from leaves of the host plant, *Carica papaya*, a common fruit crop cultivated in various localities of Calicut University Campus. Both the pest and the predatory species were cultured on mulberry leaves placed over wet sponge (2.5 cm thick) kept in plastic trays (30 x 26 x 6.5 cm). Pure cultures of *T. neocaledonicus* were raised by introducing a single adult female on to the mulberry leaves kept in plastic trays. The mulberry leaves were

maintained afresh till the completion of feeding experiments by keeping on a wet piece of sponge, soaked in water, up to 3/4 of its height, in a tray.

Pure cultures of the predatory mite, *N. longispinosus* were raised by introducing a single adult female on to the leaf disc harbouring all life stages of *T. urticae* and maintaining its successive generations to prevent cross contamination. Ample numbers of all stages of the prey mite were offered to raise the predatory mite population. Both the prey and predatory mite cultures were carefully observed regularly to check over population and culture contamination. Over populated leaves were cut and placed over fresh mulberry leaves and the whole set up was maintained at 27 ± 2 °C and 75 ± 10 % RH till the completion of feeding experiments.

Conduct of feeding experiments

The experimental arena consisted of mulberry leaf discs of 2cm diameter placed over water saturated cotton pads kept in a Petri dish. Feeding experiments were initiated by setting four culture sets containing leaf discs with fixed number of different stages of the prey mite, *T. neocaledonicus*. Four different sets of experiments were carried out and each experiment was replicated 15 times. In the first set of experiments, a single adult female predator was released on to each of the leaf disc kept in the culture trays. The second array of experiment was conducted by introducing a single nymph of the predator on to each of the four culture sets, as done in the previous experiment. Observations were made separately for each culture set by counting the no. of prey stages consumed by the adult/nymph of the predator in a period of 24 hrs, after initiation of feeding experiment. Each experiment replicated 15 times. Based on the data gained on each experimental set up, the percentage of consumption was calculated following the formula, $(N_e / N_o) \times 100$. Where N_e = No. of prey stage consumed; N_o = Total no. of prey stage given. Data obtained on the above parameters were subjected to ANOVA by SPSS.

Results

Results of field sampling revealed the close association of the phytoseiid predator, *N. longispinosus* on the leaves of the fruit crop, *C. papaya*, along with the pest mite, *T. neocaledonicus* in all the localities screened. In the laboratory, the predator was found to wander actively on the leaf arena and showed a random prey searching behaviour as observed during the present study. The female predator often appeared passive and inactive, assuming a resting posture, especially prior to the initiation of oviposition. The predatory behaviour was found initiated by sensing the prey by the predator with its long legs and pedipalps. When the egg stage of the prey mite was encountered, the predator was found to capture it and slowly pierced the egg shell with its chelicerae and initiated to suck out the inner contents. As the feeding process progressed, the pale yellow colour of the prey egg got diminished and finally disappeared, as the predator sucked out the entire content. The shrunken egg shell was found left behind on the leaf arena after completely devouring the inner contents.

The predator exhibited swift movements when it encountered and come in contact with the mobile stages of the prey mite, unlike that of the egg stage. The prey was found held by the predator with its chelicerae. Initially, the predator captured the prey by holding its legs or the mouth parts and subsequently an incision was made on the body of the prey with the help of its chelicerae. During most of the feeding time starting from the initiation, the predator was found catching the prey, either its leg or the pedipalp and making an incision there by sucking out the internal content through the appendage. Since the legs are transparent, the flow of internal contents from the main body to the point of incision on the leg was clearly visible. Feeding through a single leg may last 10-15 seconds.

The time taken to suck out the entire contents of a single active prey stage by the predator was 2-3 minutes. Quite often, the predator left out the prey after partial feeding and then resumed the feeding activity within a few moments. Concomitant with the progressive feeding

Table 1 Rate of consumption (Mean \pm SEM) by *Neoseiulus longispinosus* on different life stages of *Tetranychus neocaledonicus*.

Predator life stage	Prey life stage	N_e / N_o
Adult	Egg	0.48 ± 0.02
	Larva	0.29 ± 0.03
	Nymph	0.26 ± 0.03
	Adult	0.09 ± 0.03
Nymph	Egg	0.10 ± 0.00
	Larva	0.22 ± 0.02
	Nymph	0.31 ± 0.03
	Adult	0.11 ± 0.01

activity, the prey body appeared shrunken, and the legs of the predator become clogged with the red coloured internal contents of the prey mite.

Data gathered through the feeding experiments performed during the study disclosed significant variations between the adult and nymphal stages of *N. longispinosus*, in the rate of predation on the different life stages of *T. neocaledonicus* ($P < 0.05$). Results of feeding experiments showing the number of prey stages consumed by the adult and nymphal stages of the predator during the experimental hours are presented in Table 1. As indicated in the table, the order of preference of the adult predator towards the prey stage was found to be Egg > Larva > Nymph > Adult. The adult females of *N. longispinosus* exhibited high preference to the egg stage of *T. neocaledonicus*, with the mean consumption rate being 48.4%. Larval stage of the pest mite was recognized as the next preferred stage (29.9%), followed by the nymphal (26.6%) stage. The predator displayed a very low preference to the adult prey mite and its consumption rate was recorded as 9.3% (Figure 1).

Observations made on the predatory potential of the nymphal stage of the predator enabled

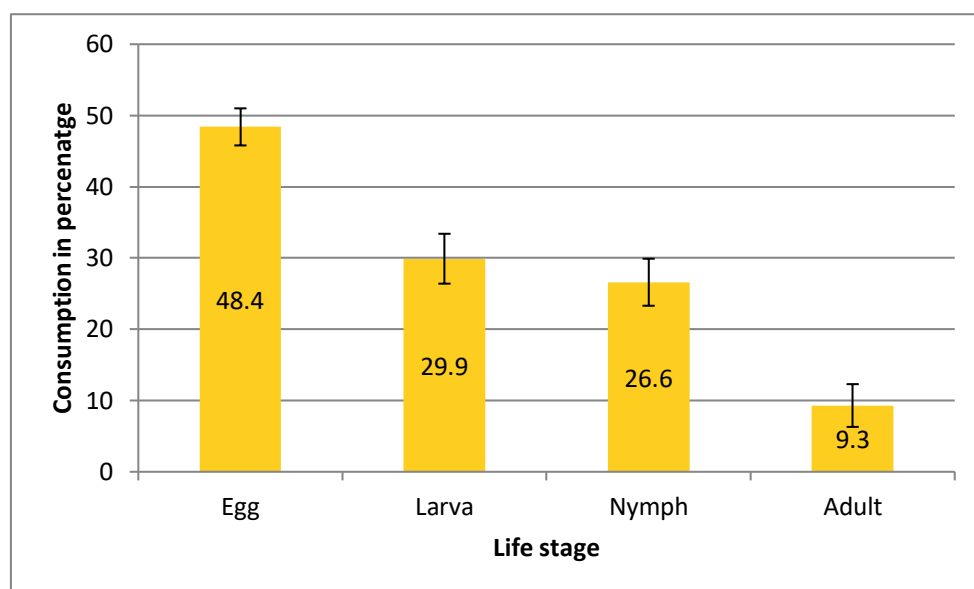


Figure 1 Mean consumption rate (%) of adults of *Neoseiulus longispinosus* on different life stages of *Tetranychus neocaledonicus*.

to assign a greater predatory efficiency to the nymphal stages of *N. longispinosus* and which consumed a significantly higher number of nymphal stages of the pest mite. The order of preference could be represented as nymph > larva > adult > Egg with respective mean consumption rates of 31.3%, 22%, 11.2% and 10.3 % (Figure 2).

Discussion

Phytoseiid mites in general are known to show preference to feed on certain types of food items rather than being indiscriminate feeders (Dicke *et al.*, 1988). In the present study, attempts were made to evaluate the instar-wise variation in the predatory potential between the adult and nymphal stages of the phytoseiid mite, *N. longispinosus* on a selected species of pest mite, *T. neocaledonicus* at constant pest densities and under controlled temperature cum humidity conditions. The species has been included under the Type-II category among the phytoseiid predators based on its exclusive preference to spider mites (McMurtry *et al.*, 2013). Type II predators exhibit a selective feeding trend with a wide prey range comprised of tetranychid mites and their reproductive success is relatively very poor on other food items (McMurtry and Croft, 1997; Croft *et al.*, 1998). Based on its promising candidature to serve as efficient bio-control agent of spider mites, the species was successfully mass reared and released in bamboo forests of China to control populations of bamboo spider mite pests like *S. nanjingensis* (Zhang *et al.* 1999) and *A. corpuzae* (Zhang *et al.* 1998, 1999, 2000). Results of feeding studies carried out during the present work enabled to identify the egg stage of the prey mite, *T. neocaledonicus* as the most preferred food item to the adult females of the species rather than the mobile prey stages like the larva and nymphs. The order of feeding preference of the adult female predator was in the sequence of egg > larva > nymph > adult. A similar feeding preference to prey mite eggs was recorded in several phytoseiids studied earlier (Badii *et al.*, 2004; Ganjisaffar and Perring, 2015). Preference to the egg stage shown by the adult females of *N. longispinosus* is to be considered advantageous in terms of its successful utilization as a bio-control agent, based on its ability to exert sufficient suppression of the pest in the egg stage itself, without giving further chance to hatch into the larval and nymphal stages and thereby

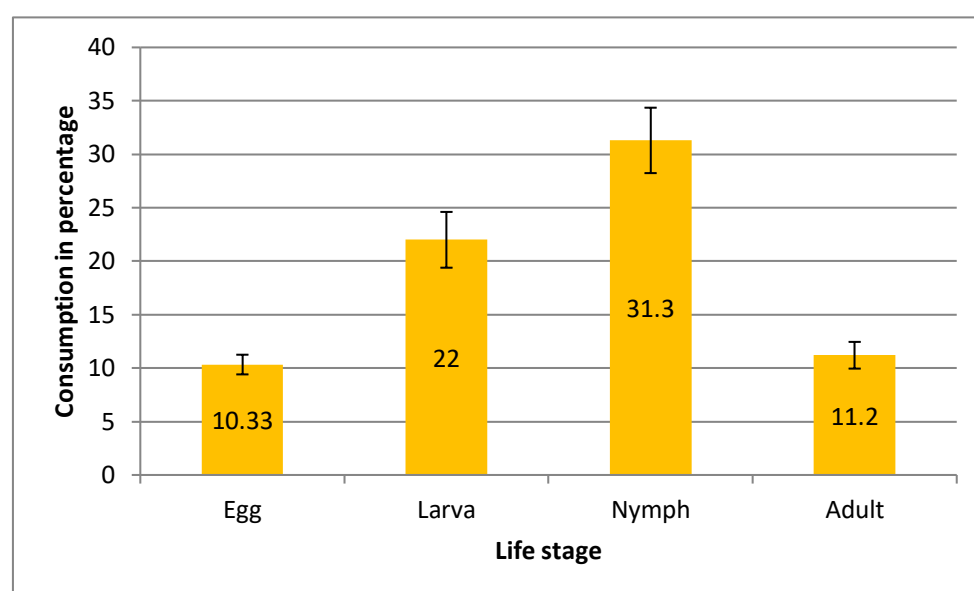


Figure 2 Mean consumption rate (%) of nymphs of *Neoseiulus longispinosus* on different life stages of *Tetranychus neocaledonicus*.

building up its population to a great extent. However, variations could be observed between the adults and nymphal stages while feeding on the life stages of the same species of prey mite. The adults of the predator showed more preference to the eggs of the prey mite followed by its larva and nymphs, while the predator nymph showed a reverse trend, showing more affinity to prey nymphs followed by its larva, adult and eggs. The order of the preference of the predator nymph could be recorded in the sequence: nymph > larva > adult > egg. This observation seems to be in agreement with the earlier finding made on the species on another prey species, *T. urticae* (Blackwood *et al.*, 2001). Thus the study provides a clear implication to confirm the egg stage preference by the adults of *N. longispinosus* among the various life stages of spider mites. Despite this, the present result contradicts the reports (Rahman *et al.* 2012) revealing the greater preference to the larval and nymphal stages of another species of spider mite, *O. coffeae* Neitner by *N. longispinosus* irrespective of its life stage variation. This may be due to the thickness of *O. coffeae*'s egg-shell (Rahman *et al.*, 2013). A similar trend was reported in the Japanese strain of *N. californicus* towards the larval stages of *T. urticae* (Canlas *et al.*, 2006). Contradictory to the present finding, a high preference of the adults of *N. longispinosus* to the larvae of *T. urticae* was also recorded earlier thereby establishing feeding variation with respect to prey mite variation (Ibrahim and Palacio, 1994). Prey-stage wise variations in the feeding preference displayed by phytoseiids were reported to be influenced by several factors like searching behaviour, hunger level, prey protection mechanism and nutritional value of the prey stage (Blackwood *et al.*, 2001; Lemos *et al.*, 2010) and any alterations in the above factors could result in variations in feeding specificity also.

Several species of phytoseiids are known to display feeding variation with respect to variations in prey life stages as observed in *Euseius hibisci* (Chant) on the prey *T. urticae* (Badiet *et al.*, 2004), *Galendromus flumenis* (Chant) on *O. pratensis* Banks (Ganjisaffar and Perring, 2015), both of which showed preference to the egg stages of the prey.

Despite the above records, another phytoseiid predator, *Kampimodromus aberrans* (Oudemans) consumed significantly higher number of pest mite larvae rather than its eggs. (Kasap and Atlihan, 2011).

Quite often, prey consumption was found to be inversely related to the prey stage (Badii *et al.*, 2004). Food preference may also be changed according to the species of prey available in the field as evidenced in *N. californicus* (McGregor) which consumed more number of immature stages (larvae, protonymph and deutonymph) of *T. urticae* when presented along with the first instar larvae of Onion thrips, *Thrips tabaci* Lindeman. At the same time the prey preference was found switched over to the first instar larvae of *T. tabaci* when supplied in combination with adult females of *T. urticae* (Rahmani *et al.* 2016). The significantly higher preference shown by phytoseiids to the egg stage over the other stages of pest mites could be explained based on several factors such as, innate tendency of the predator to feed on smaller prey, immobile and defenceless features of the egg, consumption of low biomass eggs in large numbers to meet the same energy requirement, and easy penetration of predator chelicerae in to the egg chorion. Results of feeding experiments carried out on *Typhlodromus (Anthoseius) divergentis* (Chaudhri, Akbar and Rasool) against the life stages of *T. urticae* indicated the influence of individual prey size in the food preference of the predator. The small sized prey stage was always found preferred by the predator when offered a choice between egg-immature, egg-adult and immature-adult (Naeem *et al.* 2017). Being the stage with longest duration up to 3 days, eggs of the prey mite might be available to the predator for a long period of time when compared to the other life stages and that also would be considered as a probable reason for their greater preference by the phytoseiid predators (Perring *et al.* 1984). Life style of phytoseiids also could be related to the prey stage preference with the specialists like *N. longispinosus*, *Phytoseiulus macropilis* (Banks) and *P. persimilis* Athias-Henriot, and one generalist, *N. fallacis*, exhibiting a significant preference to the eggs of *T. urticae* while generalist predators like *K. aberrans*, *N. barkeri* (Hughes), *N. californicus*, *N. cucumeris*, *Typhlodromus (Typhlodromus) pyri* Scheuten and one specialist, like *Galendromus (Galendromus) occidentalis* (Nesbitt) did not show any preference (Blackwood *et al.* 2004). However, results of the same study also showed that often

generalists like *Amblyseius andersoni* Chant, *E. finlandicus* (Oudemans) and *E. hibisci* (Chant) showed preference to the larvae of *T. urticae* rather than the eggs.

Despite the variations in the feeding preference in accordance with the prey stage, all the life instars of the predator, *N. longispinosus* were found to exhibit significant rates of consumption on the eggs and immature stages of the pest mite, *T. neocaledonicus*. Being a highly polyphagous species with high biotic potential, fecundity, rapid dispersal etc. it is high time to launch control measures against *T. neocaledonicus* so as to prevent its rapid spread to more number of host plants. In this context, the present study provided encouraging results by recording significantly higher rates of predation in all life stages of the predator on all life stages of the pest mite, thereby suggesting *N. longispinosus* as a potential candidate in pest management programs. In conclusion, our study helped to reaffirm the need for the release of the predator in to the field during the early period of infestation when the females initiated the process of oviposition to ensure a better control of *T. neocaledonicus* population.

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