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Full Length Research Paper

Evaluations of four mite predators (Acari: Phytosiidae) released for suppression of spider mite infesting protected crop of sweet pepper (*Capsicum annuum* L.)

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This study examined the efficacy of 4 mite predators such as *Neoseiulus pseudolongispinosus* (Xin, Liang and Ke), *Euseius castaneae* (Wang and Xu), *Euseius utilis* (Liang and Ke) and *Euseius finlandicus* (Oudemans) (Phytosiidae) released for the suppression of spider mite *Tetranychus urticae* (Koch) infesting sweet pepper (*Capsicum annuum* L.) in greenhouse. When the predatory mites were released on sweet pepper plants, their establishment was successful to control the population of spider mite at a lower level and results revealed significant differences in declining pest density among predators released and non released plants; in addition, non significant differences were detected in treated plants. Released predators along an untreated control treatment to manage *T. urticae* resulted decline in pest densities from 0.82 to 1.02 per leaf, compared to 1.42 observed within the plants served as control, indicating the potential of these mite predators for augmentative releases. Among all the treatments tested, mite predators recovered were higher with *N. pseudolongispinosus* and *E. utilis* in equal proportion (0.12 per leaf) followed by *E. castaneae* and *E. finlandicus* (0.11 per leaf) inside released plants in comparison to check treatment where no predators were released (0.03 per leaf). The results for allocations of the pest and predatory arthropods on the plant canopy indicated that more prey and predator mites were found on middle and bottom leaves as compared with upper leaves of plant. Observational data suggested that predation on spider mite by the existing predators fauna may have perfect potential to provide biological control of pest in greenhouse crops.

Key words: Biological control, phytosiidae, tetranychidae, sweet pepper, China.

INTRODUCTION

Sweet pepper (*Capsicum annuum* L.), an economically potential vegetable is generally gaining popularity. But its fruit size and productivity is very poor because of fluctuations in temperature and attack of pests such as mites in field conditions (Singh et al., 2004). The tetranychidae, or spider mites, are a large family of plant feeding mites. The species of the genus *Tetranychus* that occur in greenhouses produce copious amount of webbings that are used for dispersal and protection processes. The *Tetranychus* species cause severe damage expressed as leaf wilting, losses of yield quantity and quality and even plant death to diverse crops. Many spider mites have become resistant to most of the commonly used pesticides.

The *Tetranychus urticae* (Koch) known as the two-spotted spider mite has an almost cosmopolitan distribution. It is among major pests of many greenhouse crops, both vegetables and ornamentals. The mite usually colonizes the lower side of the leaves whereon it feeds, damage is also caused due to their webbings, which may cover different parts of plants and impede pest's chemical control (Gerson and Weintraub, 2006).

Chemical insecticides are widely applied for the control of *T. urticae*, but the number of pest may temporarily be depressed by the spraying of insecticides. However, their short life cycle and strong reproductive abilities cause in population densities to recover rapidly. Short generation times and excessive pesticide treatments also contribute

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to the rapid development of resistance to insecticides. Biological control is often viewed as a promising alternative or complement to pesticides in integrated pest management programs (Mcdougall and Mills, 1997). Biological control has great potential for use against *T. urticae* as based on successes of biological control and due to the abundance of potential biological control agents. Some insect predators like *Orius laevigatu* have been found to be less effective to control pests on sweet pepper as compared with the phytoseiid natural enemies such as *Iphiseius* (*Amblyseius*) *degenerans* Berlese (Rubin et al., 1996). Two phytoseiidae genera, *Neoseiulus* and *Euseius* were found as predators associated with *T. urticae* in sweet pepper (Gallardo et al., 2005). There has been little research conducted on the effects of native or established mite predators on population suppression of *T. urticae* in China. In this study, the tests were conducted to evaluate the effects of 4 mite predators as bio-control agents of *T. urticae* on sweet pepper grown in protected environment. In addition, the distribution of the predators and their *T. urticae* prey was also monitored on crop. Specific objectives were to examine and evaluate the predator's effectiveness in suppressing *T. urticae* population infesting sweet pepper and to observe the allocations of the introduced predators with respect to plant canopy.

MATERIALS AND METHODS

Experimental spot

This study was conducted in 2007 at the Bikun Greenhouse, Mentougou District, Beijing, China. The experimental plots were consisted of 0.4 ha of sweet pepper planted on 5 May, 2007 and spatially separated with a minimum of 1 m inter-plot distance to prevent cross-contamination among plots. These plots were maintained following standard commercial growing practices with the exception that no insecticide applications were made.

Mite colonies

Laboratory scale mass rearing of predator mites species was established since the last 2 years according to proper rearing requirements. Initially, predators were collected from field's survey and reared on snap bean (*Phaseolus vulgaris* L.) seedlings infested with *T. urticae* and their individual stock colonies maintained at the laboratory of Insect Natural Enemies, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China, after which these predators were removed and collected from these leaves for use in the experiment. For the efficient predator production, 25 - 30°C temperature, relative humidity 70% and photoperiod 16 L: 8 D were maintained.

Experimental technique

Four weeks old sweet pepper plants raised in greenhouse were inoculated with 15 predators per plant (shipped and mixed with a bran carrier) by shaking the contents of container gently onto the top growing portion of crop and allowed to spread and settle the populations of natural enemy over all the leaves of the plants. Prior to predators' releases, one leaf each from top, middle and bottom of each 3 plants was selected at random and acarine populations of prey and predators were recorded with the help of magnifier. Five treatments; *Neoseiulus pseudolongispinosus*, *Euseius castaneae, Euseius utilis* and *Euseius finlandicus* (adults and 3 - 5 days old instars) and non release plots were replicated 3 times. Predators releases were made into the plant canopy at the top portion of each plant and at a height about two-thirds from the base of the stem, so, that the moving ability of the predators could be noted. Further, such predator placements were used to reduce the light and wind effects on the predators. There were three buffer rows of plants left between the experimental treatments to restrict predator's movement and to aid in their establishment. Thousands of adult and young predators were released in the biocontrol area for 3 consecutive periods. The release times were; before flowering, at flowering and at the end of the flowering stages. Before and after 1 week of predators released, observations on reduction or increase of spider mite population and number of predators recovered were recorded separately from 3 randomly selected plants (1 top, middle and bottom leaves of each plant) with fine magnifier. Pest and predator mites recorded were identified using standard Departmental reference material. Populations of predators and prey mites were evaluated weekly for 7 weeks following initial release of predators. Every week, a total of 9 leaves per replicate were chosen randomly from each experimental treatment and the numbers of pest and the predators were counted. To determine the movement and allocations of the predatory and pest mites on the plant, they were observed briefly at the top, middle and lower levels of the plant canopy to ensure that they were moving and distributing properly. At the end of the crop season, an evaluation of the predator potential to effective control of spider mite was developed and its details is presented in the results section. At the end of the season, the data on observations of pest and the predators' densities were analyzed by ANOVA after transformation into mean values of each treatment using SPSS (2005).

RESULTS

Comparison of observations among treated blocks of sweet pepper with control where no predators were released indicated that the populations of predators and prey acarines remained at different levels over the same period. Overall, due to combined effects of all 4 predator species, the numbers of *T. urticae* density differed significantly in treated and non treated blocks. These results are indicative that after the release of predators the results were significantly encouraging in reducing the numbers of *T. urticae* arthropod in treated blocks of pepper. In general, all the treatments invariably reduced the pest populations, but, *N. pseudolongispinosus, E. utilis* and *E. castaneae* greatly and slightly to a lesser extent *E. finlandicus* had negative effects on spider mite population increase.

Pest mite spectrum in sweet pepper

In the second week of April prior to the release of predators (pre treatment), the number of *T. urticae* in all to be treated replicates differed non-significantly, but showed an overall level from 0 to 0.01 per leaf ($F = 0.750$; $df = 4$; $P = 0.580$) (Table 1). However, following the release of

Table 1. Mean numbers of predators and prey observed in the release versus non release treatments at the beginning (''Initial'') and at the end (''Final'') of experiments.

Treatments	Spider mite pre treatment/ leaf	Spider mite post treatment/ leaf	Predator mite recovered/ leaf
T^1 = *N. pseudolongispinosus*	0.01 ± 0.01 a	0.82 ± 0.12 a	0.12 ± 0.01 b
T^2 = *E. castaneae*	0.00 ± 0.00 a	0.94 ± 0.15 a	0.11 ± 0.02 b
T^3 = *E. utilis*	0.01 ± 0.01 a	0.87 ± 0.15 a	0.12 ± 0.02 b
T^4 = *E. finlandicus*	0.00 ± 0.00 a	1.02 ± 0.14 ab	0.11 ± 0.02 b
T^5 = Check	0.00 ± 0.00 a	1.42 ± 0.20 b	0.03 ± 0.02 a

Similar values at p > 0.05 indicate that the means were not significantly different.

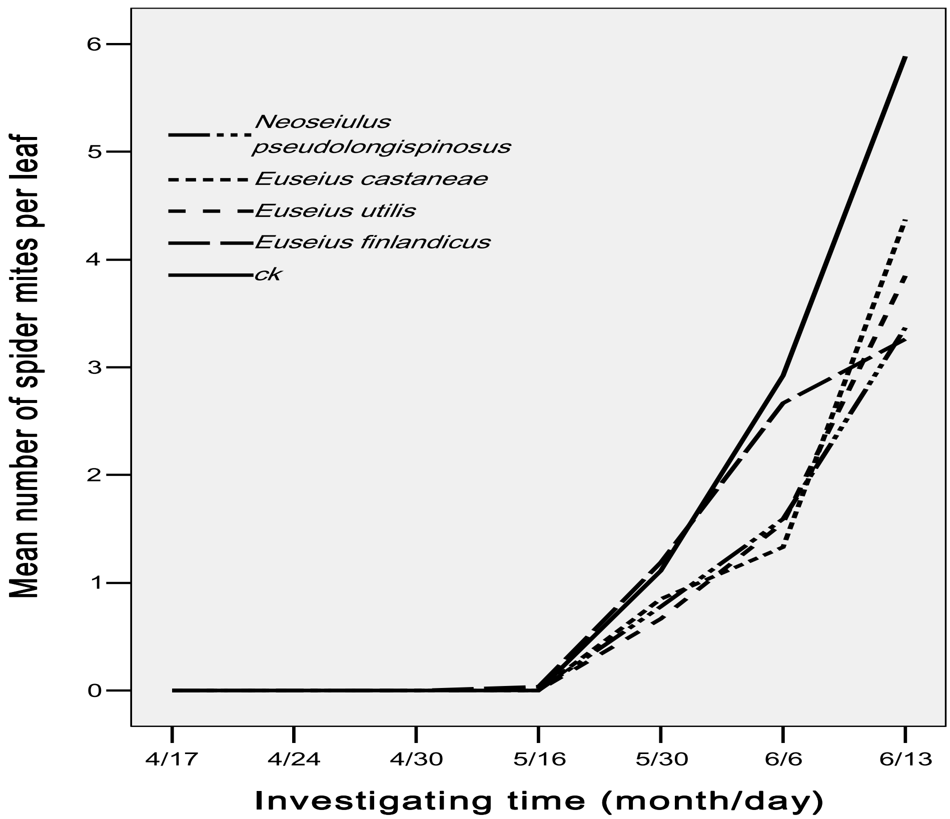

Figure 1. Population frequency of *T. urticae* found on protected crop of sweet pepper.

predators (post treatment), *T. urticae* numbers were significantly lower in the biocontrol areas than the control area (F = 2.260; df = 4; P = 0.061). The numbers of *T. urticae* in treated areas were decreased to 0.82 and 0.87 per leaf in *N. pseudolongispinosus* and *E. utilis* treated areas, respectively. In the biocontrol areas where *E. castaneae* and *E. finlandicus* were released the numbers of *T. urticae* recorded were 0.94 and 1.02/ leaf, respectively, within eight weeks. Population of *T. urticae* were not controlled in the non released area as the pest remained significantly higher (1.42 per leaf) than in the biocontrol areas. Taken as a whole, the results (Figure 1) revealed that *T. urticae* population density monitored weekly following predators released remained zero up to mid May in all treatments. During last week of May and 1st and 2nd weeks of June, *T. urticae* population was 0.78, 1.59 and 3.37 per leaf, respectively, in *N. pseudolongispinosus* treated plants. During the same weeks of May and June, population trend remained 0.85, 1.33 and 4.37 per leaf, respectively, in crop where *E. castaneae* was released. Pest's population remained 0.67, 1.56 and 3.85 per leaf, accordingly, in last week of May, and 1st and 2nd weeks of June following the release of *E. utilis.* Among dates of observation, *T. urticae* showed maximum population (2.67 and 3.26 per leaf, respectively) during 1st and 2nd weeks of June, while, minimum within 2nd and 4th weeks of May (0.04 and 1.19 per leaf, accordingly) in *E. finlandicus* treatment.

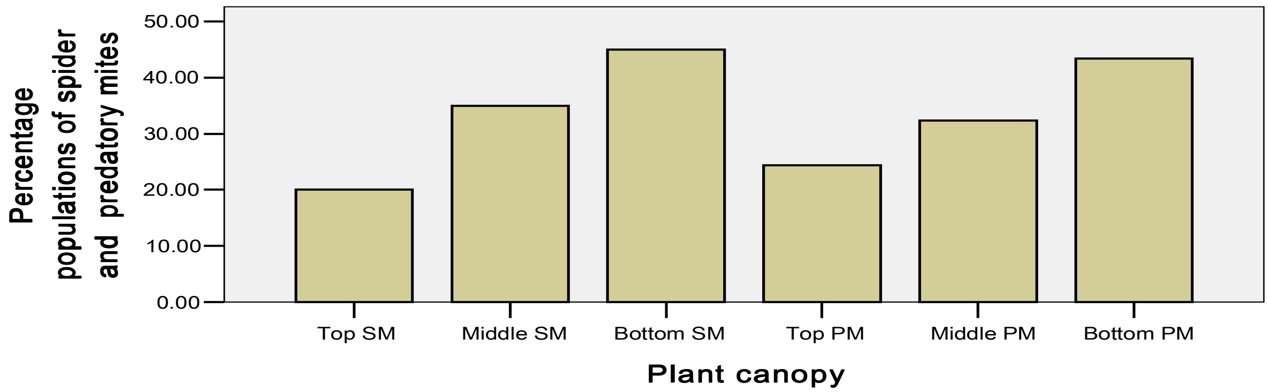

Figure 2. Relative distribution (%) of mites populations found on protected crop canopy of sweet pepper.

Comparison of pest's population across all sampling dates revealed that non released plots showed zero level of net population from April to mid May. The pest's numbers rose continuously with the further passage of time with peak at the end of May (1.11 per leaf) and in the beginning of June (2.93 - 5.89 mite per leaf).

Predator mites' spectrum in sweet pepper

A survey made in crop prior to predators released showed that natural enemies' densities were zero in all replicates. In the trial, when natural enemies were released *N. pseudolongispinosus, E. utilis* and *E. castaneae* had greater impacts in decreasing the density of *T. urticae* (0.82 - 0.94 per leaf) after their releases (Table 1) whether introduced as adult or nymphs. The mean numbers of *T. urticae* reduced were less (1.02 per leaf) after the introduction of predator *E. finlandicus*. All predators had considerably negative effects on *T. urticae* population than that of the check (1.42 per leaf). Overall, *N. pseudolongispinosus, E. utilis* and *E. castaneae* were the most efficient and consistent predators in controlling *T. urticae* than *E. finlandicus*. The results showed good establishment of the predatory mites in all the treatments from 0.11 to 0.12 mites per leaf and these levels were able to decrease the *T. urticae* population significantly as compared with the control. The results achieved on the population recovery of predators after their releases appeared to be significant (F= 3.107; df= 4; P = 0.066) in treated and non treated areas. In areas given control treatment, the number of natural enemies' recovery was the minimum (0.03 per leaf) in the eighth week. In contrast, their recovery numbers were increased in the biocontrol areas gradually (0.11 to 0.12 per leaf) (Table 1). Comparison of predator's populations across all sampling dates revealed that immediately following their releasing, both *N. pseudolongispinosus* and *E. utilis* predators had more mean populations in experimental field each with a density of 0.12 per leaf as compared with *E. castaneae* and *E. finlandicus* (0.11 per leaf). The predator's numbers rose continuously after their releases, the increase in predators matched a decrease in *T. urticae* in the treated areas.

Distribution of pest and predatory mites on pepper

Percentages of the total pest and predatory mites' population distributions on pepper plant for whole season are shown in Figure 2. The X-axis represents the top, middle and bottom of plant canopy from where the samples were taken and the Y-axis represents the number (total %) of mites recorded after their population samplings. Figure 2 showed that the percentages of the total population counts at the three sampling locations on the plants were different. Overall, the results indicated that more spider mite (SM) and predatory mites (PM) were found in the middle (35 and 32.33%) and bottom (45 and 43.33 %) leaves of plant as compared with upper leaf (20 and 24.33%), respectively.

DISCUSSION AND CONCLUSIONS

When the predatory mites were released preventively on sweet pepper plants, their establishment were successful to alleviate the population of pest mite at a lower level, whereas, thrips and whitefly were hardly found during the growing season. The predators reduced *T. urticae* density in our experiments, although the dynamics they created differed non-significantly among treatments. However, the impacts of the predators on *T. urticae* density were additive. When the predators were released at the same rates, *E. finlandicus* exerted a slightly weaker suppression of *T. urticae* population; in contrast, the predators' *N. pseudolongispinosus, E. utilis* and *E. castaneae* caused an immediate and higher mean decrease in pest population that remained constant throughout the experiments. When all natural enemies were present in treated areas, *T. urticae* population dynamics reflected its reduction due to impacts of natural enemies; but its density peaked at non treated area.

Further, in the present study, *Amblyseius pseudolongispinosus* [*Neoseiulus pseudolongispinosus*] and *E. utilis* on sweet pepper were efficient as compared with other species with respect to their performance as biological control agents of *T. urticae*. Additionally, when the predators were released at the same rates in greenhouse, the establishment of both species on sweet pepper plant was better than the establishment of *E. castaneae* and *E. finlandicus*. From the results examined, it appears that predators released at the proper stage have proven to be strong natural enemies for the control of mite pest in test crop of biocontrol area. Similar to current findings, efficiency of the predatory mites to control mite pests after their release has been reported by earlier workers. For example, predaceous mites such as *Euseius* species have been recognized as highly important predators in regulating phytophagous mites and other pests (Mcmurtry et al., 1992). Predaceous mites are often released in field to control phytophagous pests, for instance, their releases have been made on crops and *Euseius tularensis* Congdon was released to control citrus thrips (Grafton-Cardwell and Ouyang, 1995).

Observations regarding distribution of predatory mites on pepper plants after they were released showed that these were established principally in the central and beneath leaves than top leaf. It was ascertained that phytoseiid mites were primarily located minimum on the top portion of plant, feasible justification for such analysis may be that prey mite *T. urticae* may have negligible feeding on apical tissues and small leaves, therefore, naturally mite predators were least recorded on top plant canopy. Further, these predatory mites may be subjected to predation by other insect predators such as anthocorid *Orius* species which also populates in pepper plant and to avoid predation by such an arthropod, mite predators remained active in the middle and bottom canopy. Interrelated interpretations were reported by Wittmann and Leather (1997) that when *O. laevigatus* was released before, the predatory mite *N. cucumeris* was found primarily in the middle and bottom leaves as it was subjected to predation by *O. laevigatus.* Urbaneja et al., (2003) detailed that when predatory bug *O. laevigatus* was released in a sweet pepper greenhouse after *N. cucumeris* had established, *N. cucumeris* remained dominant for a month and then declined drastically, being replaced by the anthocorid. It has been clearly shown that *Orius* species congregates in sweet pepper flowers in greenhouse and field plants (Hansen et al., 2003). Weintraub et al., (2004) reported the distribution of *N. cucumeris* and found significantly more on leaves in the middle and bottom of the plants at all hours as compared with top leaves. This distribution pattern of predator is similar to the distribution trend of spider mites, *T. urticae*. Perhaps, certain biotic (pollen) and abiotic (temperature, humidity) factors from the three plant levels may be responsible for such distribution. Contrary to ours, this hypothesis, Weintraub et al. (2007) determined that though temperature and humidity varied from the top to the lower level of the plants, apparently neither these factors nor the presence of pollen outside the flowers influenced mite distribution. Nevertheless, *N. cucumeris* was found to be negatively phototropic; that caused only a temporary and non-significant change in its distribution. It is believed that light may possibly be a most important feature that caused mite predators to be more frequent on the middle and lower leaves of the plant than away from top portion. These results are consistent with Ramakers (1988) that population dynamics of predacious phytoseiid mites as potential biological control agents investigated on sweet peppers had apparent adverse effects on the predator population, where *Amblyseius cucumeris* [*Neoseiulus cucumeris*] established more easily and reached higher population densities than *A. mckenziei*, but eventually *A. mckenziei* was superseded by *N. cucumeris*. However, it is necessary to undertake further research to more clearly elucidate the effects of these biotic and abiotic factors on the movement of predatory mites and their efficacy as biological control agents. So, the findings from these studies would have direct benefits for future studies.

From the evaluation of potential of predator mites as based on effective control of *T. urticae*, it is apparent and as the numerous positive sightings indicated that the mite predators can survive and reduce *T. urticae* population during the critical months of crop production. These results suggest that there is a great scope for protection of *Capsicum* cultivation using natural enemies and there is further need to develop IPM practices for the management of insect pests to reduce the dependence on insecticides. Further, improvement in mass production and utilization of these predators is needed for their successful utilization in biocontrol programs. For implementing a successful and effective integrated pest management program for pepper production, several key developments along with the identification and integration of sustainable biocontrol practices that will maximize crop protection and yield are further needed.

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