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EVALUATION OF UTILIZING INTEGRATED MANAGEMENT AS A SUBSTITUTED FOR THE BIOLOGICAL CONTROL TO CONTROL THE TWO SPOTTED SPIDER MITE, *TETRANYCHUS URTICAE* IN THE OPEN FIELDS IN EGYPT

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ABSTRACT

Neoseiulus californicus is considered a promising agent for successful controlling the two spotted spider mite, *Tetranychus urticae* Koch. Vertimec, in selective doses, used to reduce the number of the two-spotted spider mite population withoutaffecting the survival of the predator. An open area was selected in El-Mahala district, El-Gharbia governorate to evaluate the utilizing of Vertimec followed by releasing *N. californicus* to control the population number of the two-spotted spider mite during unsuitable weather conditions for *N. californicus*, and activating the role of *N. californicus* to suppress the population number of *T. urticae*. An open area divided into two equal areas. The first area was allocated without Vertimec treatment and the second area was left for Vertimec treatment. The two areas are cultivated watermelon variety Sikata. The results obtained from releasing the predatory mite, *N. californicus* did not provide an advantage to prevent the two-spotted spider mite, *T. urticae* from exceeding the economic threshold level and subsequently deteriorated watermelon plant. In addition, the results obtained from spraying Vertimic pesticide before releasing the predatory mite *N. californicus* and duplicate the release of *N. californicus* was better of that obtained by releasing the predatory mite without using Vertimic in terms of suppressing the two-spotted spider mite, *T. urticae*.

Keywords*:* Integrated management, biological control, *Neoseiulus californicus, Tetranychus urticae*, Vertimec.

Introduction

Two spotted spider mites, *Tetranychus urticae* (Koch), is considered one of the most serious pests that attack more than 900 plant species and causing serious damage to the plant. The signs of infestation is tiny pale spots or scars where the green epidermalcells, which contains chlorophyll and mesophyll cells within the leaf tissue, thereby reducing the photosynthetic capacities of, infested leaves. This deformation of leaflet causes the plant's inability to regulate exchanges of gases, metabolic and decreasing of absorbs water and mineral nutrients, which eventually results leaflets drop, reduced growth rates, flowering and yield. (Sances *et al*., 1982, Bolland *et al*., 1998; Lette *et al*., 2003).

In Egypt, either insecticide or many varieties of acaricides with differing chemical structures and modes of action are considered a primary role in controlling populations of the two spotted spider mites that attacks vegetables, fruits, agricultural crops, and a broad range of ornamental plants. Carbamates organophosphates, pyrethroids, amidine and specific acaricides such as mitochondrial electron transport inhibitor avermectins, and milbemycins are not considered labelledfor use against the two spider mites and if not properly applied the development of resistance can be accelerated.This may rely on primarily because of a seriously increasing problem of pest resistance to pesticides (Thind and Ford 2007, Van Leeuwen *et al*., 2009, Van Leeuwen *et al*., 2010). High fecundity, Arrhenotokous, propensity for inbreeding and short life cycle, are major phenomenon belonging to resistance of the two-spotted spider mite.

Vertimec is a leading acaricide/insecticide in numerous countries on a wide variety of crops. Vertimec has an active matter Abamectin, which is a bacterium derived pesticide. It is belonging to family of macrocyclic lactones either produced directly by the soil organism Streptomyces avermitilis or generated through semisynthetic modifications (Fisher and Mrozik, 1989, Burg and Stapley 1989). Abamectin has the same mode of action of other insecticides, avermectins are nerve poisons. They stimulate the gamma-aminobutyric acid (GABA) system, a chemical "transmitter" produced at nerve endings, which inhibits both nerve to nerve and nerve to muscle communication, which becomes paralyzed, stops feeding, and dies. Although Abamectin and oil at 3 ppm is considered highly toxic to *T. urticae* after 33 days, either females and larvae of the predators belonged to family phytosiidae has ability to survive well on 48-to 96-hour-old residues. Therefore, Abamectin, in selective doses, can be used to reduce the number of red spider mite population without affecting the survival of the predator depends on learning to use it at rates that will allow the retention of sufficient prey so that surviving predators can persist (Hoy & Cave, 1985)

Neoseiulus californicus (McGregor) is economically important predators belongs to family Phytoseiidae that has characteristics of both type II specialist predatory mites and type III generalist predatory mites. *N. californicus* is considered a promising agent for successful controlling the two spotted spider mite, *Tetranychus urticae* Koch through pest/predator ratio was 5/1 (at initial pest densities from 5 to 15 females/leaflet) (Greco et. al. 2005). *N. californicus* reproduced exclusively when fed on *T. urticae* at temperatures of 15–35°C Gotoh *et al*. (2004). *N. californicus* has a potential to effectively reduce the population number of the two spotted spider mites below economic thresholds level under greenhouse (Greco *et al*., 2005; Rhodes and Liburd, 2006). On the other hand, *N. californicus* has ability to resist pesticides (Castagnoli *et al*., 2005).

The main objective of this manuscript is to evaluate of utilizing Vertimec to suppress the population number of the two-spotted spider mite during unsuitable weather conditions for *N. californicus*, andto activate the role of *N. californicus* to control the population number of *T. urticae*.

Materials and Methods

Plants

An open area 8400 m^2 divided into two equal area, each has about 4200 m^2. The first area was allocated without Vertimec treatment and the second area was left for Vertimec treatment. The two areas are separated by a two-meter-wide watermelon area located in the center of the two test areas as a barrier. each field divided into 30 equal plots. Each plot divided into two beds. Each plot planted with sixteen seeds of watermelon using double row per bed. The bed designed as slightly flat and hollow surface at the top of the hills. Three or four small pits were dig into the soil with 2.5 cm deep using fingers. One to four seeds of watermelon, variety Sikata, treated with 0.1% Vitvax were placed in each hole. Seeds covered with thin layer of soil and thin layer of sulfur, to protect seeds against birds. Then each hole was gently pressed properly to prevent moisture from rapid evaporating around the seeds. Three treatments areas were applied for releasing the predatory mite, *N. californicus*. Each treatment included nine replicates plots, when plots were established in a randomized complete block design. Three plots were left without releasing as control, restricted in the field corner and separated with buffer watermelon lines.

Releasing

The time of releasing the predatory mite, *N. californicus* were as follows:

1. untreated Vertimec area

Ten days after emergence of the watermelon first leaflets to release the predatory mite, *N. californicus*.

2. Treated Vertimec area

Seven days after emergence of the watermelon first leaflets, the area was treated using Vertimec at rate 0.5%. 20 ml of VERTIMEC, added to the partly filled spray tank 20 L, and then the water was added to complete the solution to reach 20 L. Three days were left after spraying Vertimec before releasing the predatory mite, *N. californicus*.

The predator individuals were released in each predator's area twice at two weeks intervals, at the rate of 6 and 9 individuals/2 plants. Each predator individuals were collected in gelatin capsules number 3 (0.5 – 1.5 cm) by using a special vacuum pump. Each predator individuals were released in its own field area by opening the gelatin capsules and pasting (by stick glue) the separated capsule parts on the watermelon leaflets. Randomized samples of 30 leaflets/replicate were taken just before every release and then biweekly, where the first sample was considered as pre-count and the second one as first post-count and so on with the subsequent samples.

Laboratory examination

The collected leaflets were put in plastic bags on cooled icebox. Then the icebox transferred to the laboratory to examine using stereomicroscope. Eggs and postembryonic stages of *N. californicus* and only postembryonic of *T. urticae* were counted with aid of a stereo-microscope.

Statistical analysis

The statically equation of Henderson and Tilton (1955) was applied to calculate the reduction in the two spotted spider mite populations.

Results

The suppression number of *T. urticae* outcome of releasing *N. californicus* on unsprayed watermelon plants by Vertimec is presented in table 1. The infestation of the two-spotted spider mite, *T. urticae* was generally high in the pre-count, just before the predator release, on April 28, 2019 reached to 84% and 91% in the allocated area for releasing predators at level 9 and 6 predator / pit, respectively. While the number of *T. urticae* ranged between **81** and **62** moving stages / replicate. These values were 93% and 94 moving stages / replicate in the non-release plots. The first and second post-counts shows frequent increase of the mean numbers of *T. urticae* to each **297** and **227** moving stages / replicate in the first post-count when the predator was released at the levels of 9 and 6 predators/pit, respectively. These values were 1635 and 1720 moving stage / replicate in the second post-count. On the other hand, the number of *T. urticae* showed greatly increase in the no release plots to reach **484** and 3022 moving stage / replicate in the first and second post-counts, respectively. Then the number of *T. urticae* increased to reach 2105 and 221**5** moving stage / replicate in the third post-count.

Unfortunately, at the fourth post-count the huge population number of *T. urticae* caused a significant deterioration in watermelon leaves, which resulted in the difficulty counting the mite. On the other hand, numbers of *T. urticae* showed greatly increase in the no release plots to reach **5024** moving stage/replicate at the third post-count.

Reduction of red spider mite population at the first post-count reached **59.1** and **65.9**% at the releasing level 9 and 6 predators/ pit, respectively. These values increased gradually after each release to reach **72.1** and **67.9**%, at the fourth post-count, on the previously plots respectively. Few numbers of the predatory mite, *N. californicus* were observed in deferent releasing plots at the first and second post-counts. The numbers of *N. californicus* / replicate at the first post-count were 15 and 3 at the releasing levels 9 and 6 predators/ pit, respectively. Were direct proportions, were observed between the number of predatory mite and the releasing levels. The number of predatory mite, *N. californicus* greatly increased at the third post-count to reach 180 and 39predators / replicate in the releasing plots 9 and 6 predators/ pit, respectively.

Table 1 : Suppression number of *T. urticae* outcome of releasing *N. californicus* on unsprayed watermelon plants by Vertimec

Sampling date	treatments[1]	Mean no. of *T. urticae*/ replicate	Reduction	infested leaflets%	No. of *N. californicus* / replicate		
					Eggs	M. S.	Total
4/28/2019 *	A (9 predators/2plants)	81		84	0	0	0
	B (6 predators/2plants)	62		91	0	0	0
	No release	94		93			
5/12/2019 **	A (9 predators/2plants)	297	59.1	100	6	9	15
	B (6 predators/2plants)	227	65.9	100	0	3	3
	No release	484		100	0	0	0
5/26/2019 ***	A (9 predators/2plants)	1,635	63.9	100	25	54	79
	B (6 predators/2plants)	1,720	58.6	100	6	11	17
	No release	3,022					
6/9/2019 ****	A (9 predators/2plants)	2,105	86.8	100	108	72	180
	B (6 predators/2plants)	2,215	84.9	100	31	8	39
	No release	10,661		100			
9/23/2019 *****							

1 (rate of predator release)

* Pre-count, time of first release

** First post-count, time of second release

*** Second post-count.

**** Watermelon leaves were completely affected by the huge numbers infested with the two-spotted spider mites. This causes the plant damage and the difficulty of taking the mite count.

Data of suppression number of *T. urticae* outcome of releasing *N. californicus* on sprayed watermelon plants by Vertimec is presented in table 2. The percentage of infested leaflets at the pre-count the time of first release were 86 and76% and the main number of *T. urticae* / replicate were 12 and 11 moving stage/replicate at plots of release levels of 9 and 6 predator / 2 plants, respectively. These values were 42% and 8 moving stage/ replicate at the no releasing plots. The main number of *T. urticae* / replicate increased in the first post-count to reach 15.4and 21.8 moving stage / replicate at the releasing rate 9 and 6 predator / 2 plants, respectively. The infested leaflets were also increased in the releasing plot to reach 76 and 78% at the same releasing levels, respectively. The population of *T. urticae* / replicate continued to increase at the next count to reach 33.0and 46.7at the fourth inspection at the releasing rates 9 and 6 predator / 2 plants respectively. While they were 1672.3 moving stage / replicate and 100% infestation of leaflets on the no releasing plots. The reduction of *T. urticae* were 63.6 and 43.8% at first post-count on May, 12, 2019 and increased at the fourth-count to reach 98.7 and 98.0% at the releasing levels 9 and 6 predators / 2 plants, respectively.

Few numbers of predators were observed after the first release in plots with the releasing level 9 predators / 2 plants. The predator increased rapidly after the second release in all releasing plots with maximum population on Jun 23, 2019 outcome of abundant of prey individuals in these plots to reach 171 and 103 predators/ replicates on the releasing levels 9 and 6, respectively.

Table 2 : Suppression number of *T. urticae* outcome of releasing *N. californicus* on sprayed watermelon plants by Vertimec

Sampling date	treatments1	Mean no. of T. urticae/ replicate	Reduction	infested leaflets%	No. of N. californicus / replicate		
					Eggs	M. S.	Total
4/28/2019 *	A (9 predators/2plants)	12		86	0	0	0
	B (6 predators/2plants)	11		76	0	0	0
	No release	8		42	0	0	0
5/12/2019 **	A (9 predators/2plants)	15.4	63.6	76	3	2	5
	B (6 predators/2plants)	21.8	43.8	78	2	4	6
	No release	28. 2		63	0	0	0
5/26/2019 ***	A (9 predators/2plants)	19.9	94.5	66	7	4	11
	B (6 predators/2plants)	28.2	91.5	73	4	3	7
	No release	240.3		86			
6/9/2019 ****	A (9 predators/2plants)	25.6	98.2	56	23	11	34
	B (6 predators/2plants)	36.3	97.2	69	12	8	20
	No release	926.7		92			
6/22/2019 *****	A (9 predators/2plants)	33.0	98.7	53	116	55	171
	B (6 predators/2plants)	46.7	98.0	67	61	42	103
	No release	1672.3		100			

1 (rate of predator release)

* Pre-count, time of first release

** First post-count, time of second release

*** Second post-count.

**** Third post-count

***** Fourth post-count.

Both average temperatures and relative humidity for a quarter of the day are presented in Figure 1. Recorded average temperatures were lowest in the early hours of morning from 12 am to 6 am and gradually increasing to reach the greatest heat in the early afternoon from 12 pm to 6 pm then gradually decreasing. On contrary, relative humidity is highest in the early hours of the morning from 12 am to 6 am, then decreases gradually from 6 am to 6 pm and then gradually increasing.

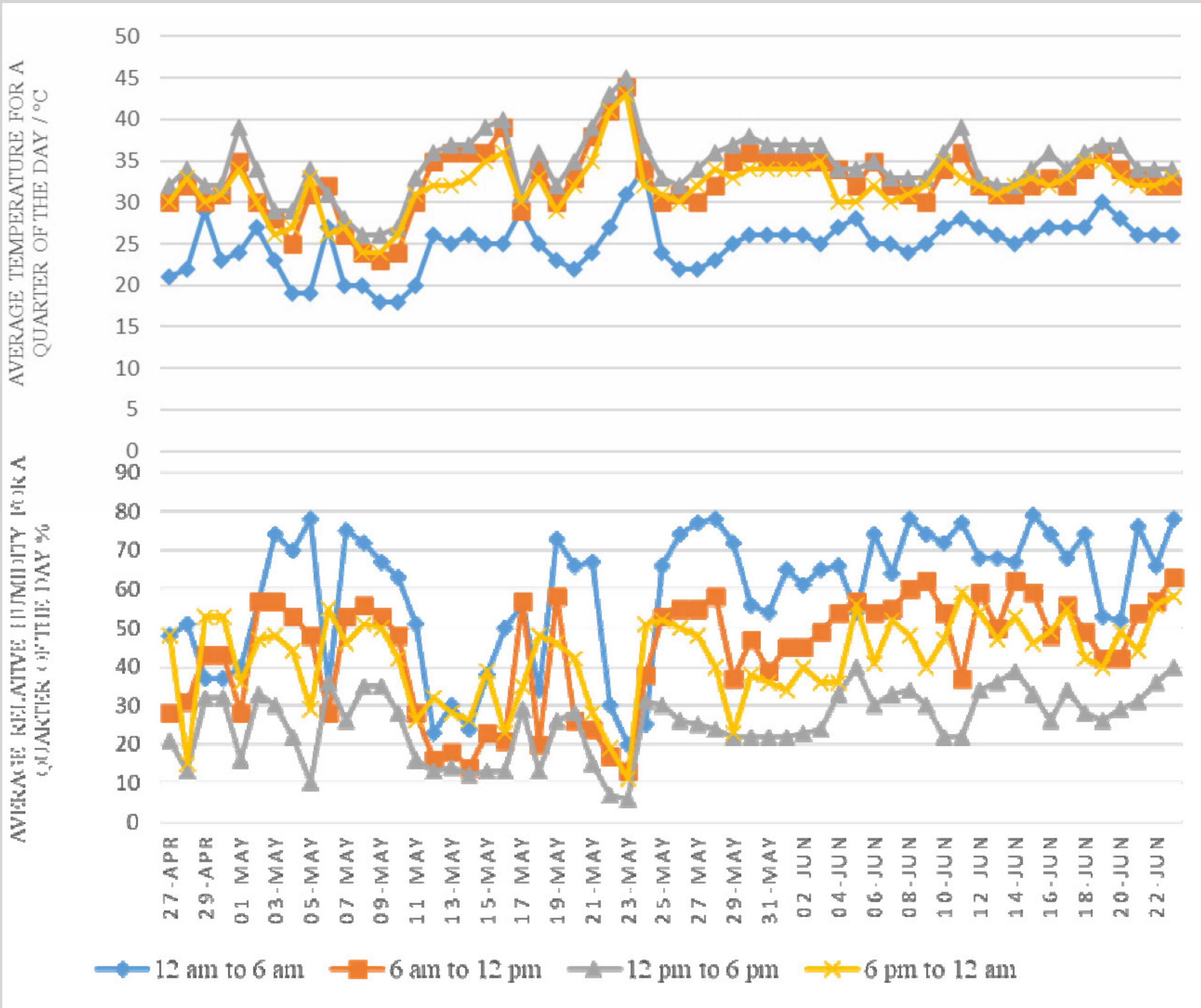

Fig. 1 : Average both temperatures and relative humidity for a quarter of the day

Discussion

Although the current study is followed the recommended prey/predator ratios which is 10:1 (Croft and Hoyt 1983; Wilson *et al.*, 1984; Gonzalez Zamora *et al.*, 1991; Strong and Croft 1995), the results obtained from releasing the predatory mite, *N. californicus* did not provide an advantage to prevent the two-spotted spider mite*, T. urticae* from exceeding the economic threshold level and subsequently deteriorated watermelon plant. In addition, the results obtained from spraying Vertimic pesticide before releasing the predatory mite *N. californicus* was better of that obtained by releasing the predatory mite without using Vertimicin terms of suppressing the two-spotted spider mite, *T. urticae*. In the open field, the prey/predator ratio cannot be determined as general methods to control the two-spotted spider mite while ignoring other ecological factors that affect the predator's capability to suppress the *T. urticae*. In Egypt, during implementing the current experiment from 27th of April to 23rd of June, 2019, the minimum and maximum temperature were 18 and 45°C, respectively, with an average temperature ranged between 25 and 35°C. Actually, that temperature is ideal for *T. urticae* by accelerating the population number through decreasing the developmental time from egg to adult to reach 8.3 days (Sabelis, 1981). It is worth to mention that the total number of eggs laid by *T. urticae* female per day was affected by the temperature. For instance, the temperature that ranged between 25 to 35°C affects the fecundity at a lower rate, in which the female of the two-spotted spider mite has the ability to lay 12 eggs per day Sabelis 1981.In contrary, both developmental time and number of daily eggs laid of *N. californicus* decreased by increasing the temperature from 25 to 35°C to reach 3.8 days and 2 eggs/day, respectively (Gotoh *et al.*, 2004, Canlas *et al.*, 2006, Kim *et al.*, 2009).

On the other hand, during implementing the current experiment the minimum and maximum relative humidity were 6 and 79%, respectively, with an average relative humidity ranged between 30 to 60%. In facts, the variation of relative humidity affects the developmental time of the predator. For instance, at relative humidity which ranged between 40 to 70% increase the developmental time (Begljarow 1967, Stenseth 1979). In addition, the developmental time is almost stopped at relative humidifies below 35% (Begljarow 1967, Stenseth 1979). On the other hand, the mortality rates increased for all predator stages by decreasing the relative humidifies. For instance, at temperature 21°C and relative humidity 40%, the mortality of predator eggs reached 10% (Stenseth 1979).

It could be concluded that, at open field, sometimes both high temperature and low relative humidity did not suitable for *N. californicus* to play it is role to suppress the population number of the two-spotted spider mite. If we considered that Egypt's agriculture focuses mainly on open agriculture area where temperature and humidity are difficult to control unlike greenhouses. In fact, The predator cannot adapt on these heat and relative humidity conditions in Egypt to control the rapid increasing numbers of its prey, which causes the deterioration of the plants. Not to mention the fact that high temperature and low relative humidity affects the developmental time of the predatory mite, *N. californicus*, which represents half of the developmental time of the two-spotted spider mite, *T. urticae* and meanwhile decreased the consumption rate and increased the mortality of *N. californicus*. Therefore, it should be using acaricide to compensate the low capability of *N. californicus* to control *T. urticae.* Although utilizing of acaricides has positive effects in terms of rapid suppressing the two-spotted spider mite, the extensive utilize of acaricide has negative effects on natural enemies, environments and human health (Lette *et al*., 2003). Vertimec is recommended to use carefully with specific dosage during Integrated Pest Management (IPM) programs (Abd-Elhady and Heikal 2011).Therefore, it is recommended to release the predator after two weeks intervals of spraying the Vertimec in order to ensure that the residual effect of the pesticide is lost. On the other hand, the double release of N. californicus with two weeks intervals is suitable in terms of suppressing the number of the two-spotted spider mite, *T. urticae*.

Conclusion

The results obtained from spraying Vertimic pesticide before releasing the predatory mite *N. californicus* and duplicate the release of *N. californicus* was better of that obtained by releasing the predatory mite without using Vertimic in terms of suppressing the two-spotted spider mite, *T. urticae*. In addition, It is recommended to release the predator after two weeks intervals of spraying the Vertimec in order to ensure that the residual effect of the pesticide is lost. On the other hand, the double release of *N. californicus* with two weeks intervals is suitable in terms of suppressing the number of the two-spotted spider mite, *T. urticae*.

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Competing interests

The authors do not have competing interests.

Availability of data and material

All data obtained or analyzed during this study are presented in this manuscript

Author Contributions

AAE, AMA and KAA

The final manuscript was approved by AAE, AMA and KAA. selecting the open field, cultivating with watermelon, collecting the predator individuals in the gelatin capsules and releasing are designed by AAE, AMA and KAA.