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Effect of Biocide Compounds Biofly and Metarril on Three Phytophagous Mites, *Tetranychus urticae* Koch; *Brevipalpus californicus* (Banks); *Panonychus ulmi* (Koch) on Apple Trees Under Field Conditions

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ABSTRACT

Utilizing of bio-compound Biofly (*Beauveria bassiana* (Bals. - Criv.)) and Metarril (*Metarlizium anisopliae* (Metchnikoff)) against three phytophagous mites *Tetranychus urticae* Koch; *Brevipalpus californicus* (Banks); *Panonychus ulmi* (Koch) on apple trees under field conditions were conducted in Qalubia governorate. The obtained results showed that, the efficacy of tested compounds were varied according mites and fungus species namely, in case of *T. urticae* the reduction percentage reached to 95.1 % and 84.55% after applying with Biofly (*Beauveria bassiana*) and Metarril (*Metarlizium anisopliae*), in 10th 3rd Sept., resp. while, the strongest reduction recorded 74.75% on 24th Sept. and 96.9% on 10th Sept., in case of *B. californicus*. after sprayed with Biofly (*Beauveria bassiana*) and Metarril (*Metarlizium anisopliae*) respectively. On the other hand, the population of *P. ulmi* was affected with the mentioned biocides whereas reduction percentages were 77. 7 % on 24thSept., and 86.54% on 3rd Sept.,After sprayed with Biofly (*Beauveria bassiana*) and Metarril (*Metarlizium anisopliae*) respectively.

INTRODUCTION

The Phytophagous mites are universal pests, causes great damages to fruit trees over world. The negative effects of these pests on fruit trees are regarded to their short biological cycle, ability to cause injuries and their highly fertility rate (Araujo *et.al.*, 2020). Some species from family Tetranychidae Donnadieu: (*Tetranychus urticae* Koch; *Eutetranychus orientalis* (Klein); *Panonychus ulmi* (Koch) and *P. citri* McGregor); Tenuipalpidae Berlese (*Brevipalpus californicus* (Banks) and *B. phoenicis* (Geijskes)) and Eriophyidae Nalepa (*Phyllocoptruta oleivora* (Ashmead)) are the important pests on fruit trees which causes serious damages (Author). The mite's population and losses they cause can be controlling by chemical method, but it is not sustainable for agriculture as the wide spread use of acaricides which cause ecological problems such as destroying non-target useful organisms, harmful for human health because their residues on food and developing resistance to chemicals by mites (Kumral, *et al.* 2020). Moreover, many studies have been

conducted on the alternatives to the chemicals mites control strategies to get both environmentally- and human-friendly as well as to avoid the resistance (Ashrafju, *et. al*. (2014); Numa, *et. al*. (2015**)**; Yesilayer, (2015); Numa Vergel, *et. al*. (2016**)**; Basaid, *et al.* 2020). So, the presented study aims to evaluate the Biocide compounds Biofly, *Beauveria bassinet* (Bals. -Criv.) and Metarril, *Metarlizium anisopliae* (Metchnikoff) on three phytophagous mites *Tetranychus. urticae* Koch; *Brevipalpus californicus* (Banks); *Panonychus ulmi* (Koch) on apple trees under field conditions.

MATERIALS AND METHODS

The experiments were carried out in Qalubia governorate in 2021 on apple trees which were found heavily infested with three phytophagous mites (*Tetranychus urticae* (Koch); *Brevipalpus californicus* (Banks); *Panonychus ulmi* (Koch)). The experimental design was randomized complete blooks each treatment was replicated, each replicate included forty apple trees. Four applications were achieved by each commercial *Beauveria bassiana (*3×10^7Conidia/cm³) and *Metarlizium anisopliae* (1×10^8Conidia/ml) 1product. The length among first, second and third application were a week interval. The fourth application was done after 21 days from the third application. A 600-liter motor was used to spray the trees with the pesticide, and then each samples containing 40 leaves were examined for each treatment. Count of the phytophagous mites started just before treatment in a sample as pre-account followed by weekly samples after treatment from 20Augst to 7 October, the percentages of reduction in the phytophagous mite's population due to treatments were assessed according to Hendrson and Titlon equation (1955).

RESULTS

A-Effect of Biocide Compounds Biofly, *Beauveria bassiana* (Bals. -Criv.) and Metarril, *Metarlizium anisopliae* (Metchnikoff) against Some Phytophagous Mites Under Field Conditions.

1-Effect of Biocide Compound Biofly, *Beauveria bassiana* (Bals. -Criv.) against Some Phytophagous Mites Under Field Conditions.

a). Effect of Biocide compound Biofly, *Beauveria bassiana* (Bals. -Criv.) against two spotted spider mite, *Tetranychus urticae* (Koch).

Table (1) and Figure (1); showed that the population size of *T. urticae* had been affected by the releasing of the parasite fungi *B. bassiana* with a relative difference in the percentage of reduction, which released on (20^{th} Aug) 2021 by rate 100 cc 3×10^7 Conidia/cm³ during the three months of the treatment (Augst, September and October) where the pre- count of the pest was recording 450 individuals in the control group, on the other hand it reached 522 in the treatment group per 12 fruits and 40 leaves on 20^{th} Aug., 2021. The greatest reduction percentage was about a twenty day after the start of the experiment on (10^{th} Sep.),2021 it had reached 95.1 %, that may agree with **(Fargues *et al.*, 2018)** who confirmed that the optimal temperatures of the *B. bassiana* are generally between 25 and 28 °C. This result also was confirmed by the World Meteorological Organization that the average temperature in Egypt during the months of September and October arrange (23.3-26) degrees Celsius respectively.The next higher reduction ratio recorded on (17^{th} Sept.,) in was about 77.55% after one week from the over the top on the reduction.

In general, the rest of the details of the reduction percentages on both (27^{th} August, 3^{rd} September, 24^{th} September, 31^{st} September and 7^{th} October) had been reaching (50.73%, 41.22%, 47.92%, 63.02% and 61.19%) respectively.

Table 1: Effect of Biocide compound Biofly, *Beauveria bassiana* against *T.urticae* under field conditions**.**

Treatment Date	Rate of application /100 liters of water	Pre-Count		Number of mites after treatment/12 fruits+40 leaves		Reduction %
		Control	Treatment	Control	Treatment	
20 -August 2021	100 cc 3×10^7 Conidia/cm³	450	522			
27- August	100 cc 3×10^7 Conidia/cm³			350	200	50.73
3- September	100 cc 3×10^7 Conidia/cm³			220	150	41.22
10- September				880	50	95.10
17- September				4800	1250	77.55
24 -September				9600	5800	47.92
31- September	100 cc 3×10^7 Conidia/cm³			9850	4225	63.02
7- October				10138	4564	61.19

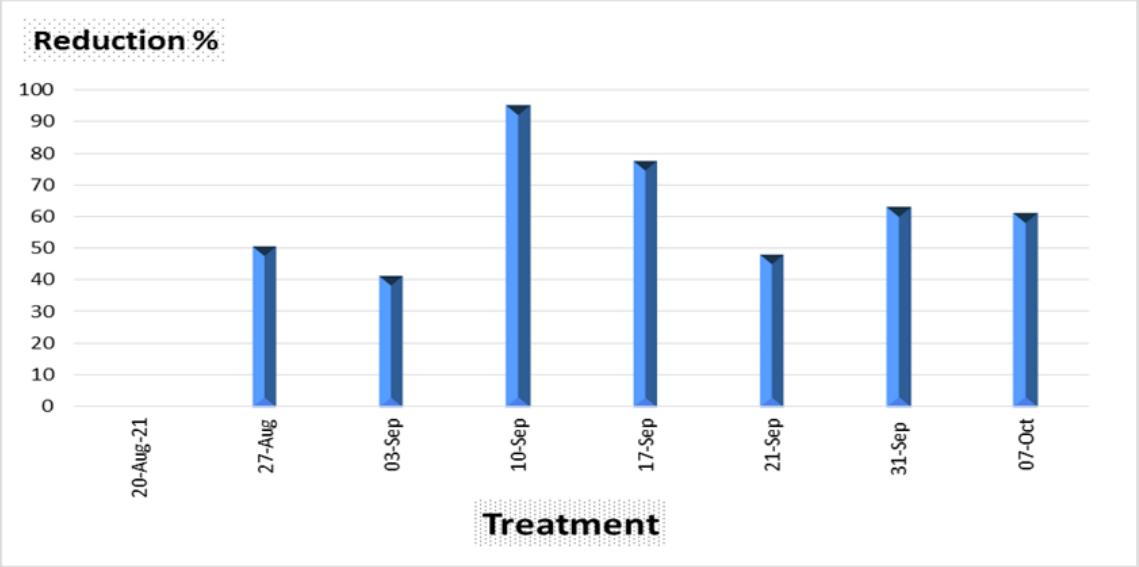

Fig. 1: Effect of Biocide compound Biofly, *Beauveria bassiana* against *T. urticae* under field conditions

b). Effect of Biocide compound Biofly, *Beauveria bassiana* (Bals. -Criv.) against flat mite, *B. californicus* under field conditions:

Data in Table (2) and Figure (2); showed that the results of *B. bassiana* which released on the host *B. californicus* on (20th Aug) by rate 100 cc 3×10^7 Conidia/cm³; were not different from the previous host *T. urticae* as the reduction rates ranged roughly during the three months of experimentation. Approximately, the highest reduction rate on this host was close in time to that of the previous host. In this case the strongest reduction recorded 74.45% on (24th Sep.,) its difference may be due to the mode of action of the parasite on the mite *B. californicus*. On the other hand, the lowest reduction percentage was recorded 37.57 % on 27th August. While the rest of the reduction rates were recorded (56.42%, 57.85%, 62.64%, 46.75% and 40.94%) on (3rd September, 10th September, 17th September, 31st September, and 7th October) respectively.

Table 2: Effect of Biocide compound Biofly, *B. bassiana* against flat mite *B.californicus* under field conditions.

Treatment Date	Rate of application /100 liters of water	Pre-Count		Number of mites after treatment/12 fruits+40 leaves		Reduction %
		Control	Treatment	Control	Treatment	
20 -August 2021	100 cc 3×10^7 Conidia/cm³	450	522			
27 -August	100 cc 3×10^7 Conidia/cm³			390	150	37.57
3- September	100 cc 3×10^7 Conidia/cm³			250	120	56.42
10- September				450	220	57.85
17 -September				1500	650	62.64
24 -September				4050	1200	74.45
31- September	100 cc 3×10^7 Conidia/cm³			10200	6300	46.75
7- October				9050	6200	40.94

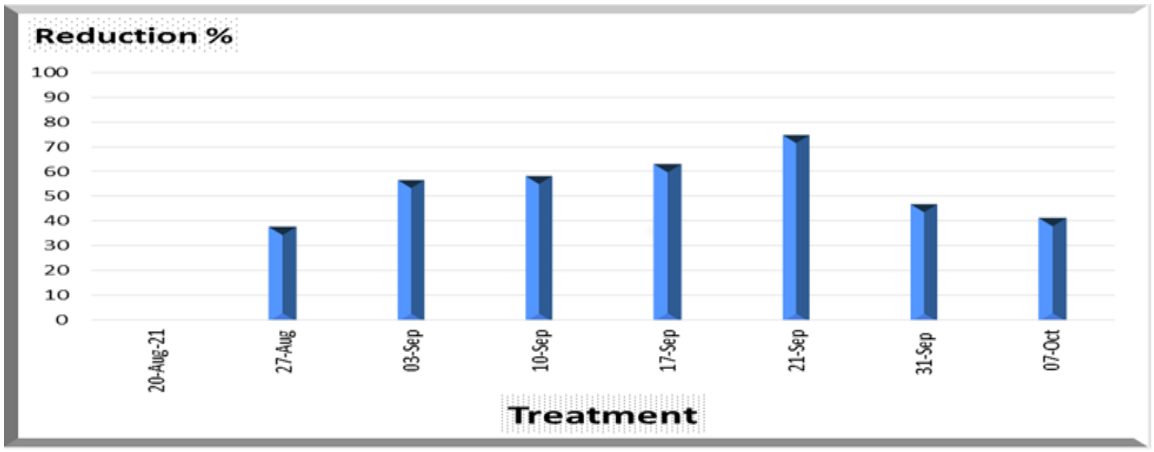

Fig (2): Effect of Biocide compound Biofly, *B. bassiana* against B. *californicus* under field conditions.

c- Effect of Biocide Compound Biofly, *B. bassiana* against *P. ulmi* Under Field Conditions:

Information that tabulated in Table (3) and Figure (3); showed Examination result after dealing with the pest *P. ulmi* with the parasite *B. bassiana* which released on (20th Aug) by rate 100 cc 3×10^7 Conidia/cm³., where the data was as follow.

The top of the reduction occurred on (24th Sept.,) 77.7 %, it was same the date of the highest reduction of the mite *B.californicus.* While in this treatment there is a close result on (3rd Sept.) that reached 75.65% reduction percentage. On the other hand, the lowest reduction was recorded on (27th Aug.,) But the total details of this treatment during the three months were as follows (42.52%, 75.65%, 49.73 %, 49.18 %, 77.7 %, 43,62 %, and 45.1 %) reduction percentages on the days of the examinations (27th August, 3rd September, 10th September, 17th September, 24th September, 31th September and 7th October) respectively.

Table 3: Effect of Biocide compound Biofly, *B. bassiana* against *P.ulmi* under field conditions**.**

Treatment Date	Rate of application /100 liters of water	Pre-Count		Number of mites after treatment/12 fruits+40 leaves		Reduction %
		Control	Treatment	Control	Treatment	
20 -August 2021	100 cc 3×10^7 Conidia/cm³	450	522			
27- August	100 cc 3×10^7 Conidia/cm³			8400	5600	42.52
3- September	100 cc 3×10^7 Conidia/cm³			354	100	75.65
10- September				600	350	49.73
17 -September				950	560	49.18
24 -September				5800	1500	77.7
31- September	100 cc 3×10^7 Conidia/cm³			25000	16350	43.62
7 -October				9900	8200	45.1

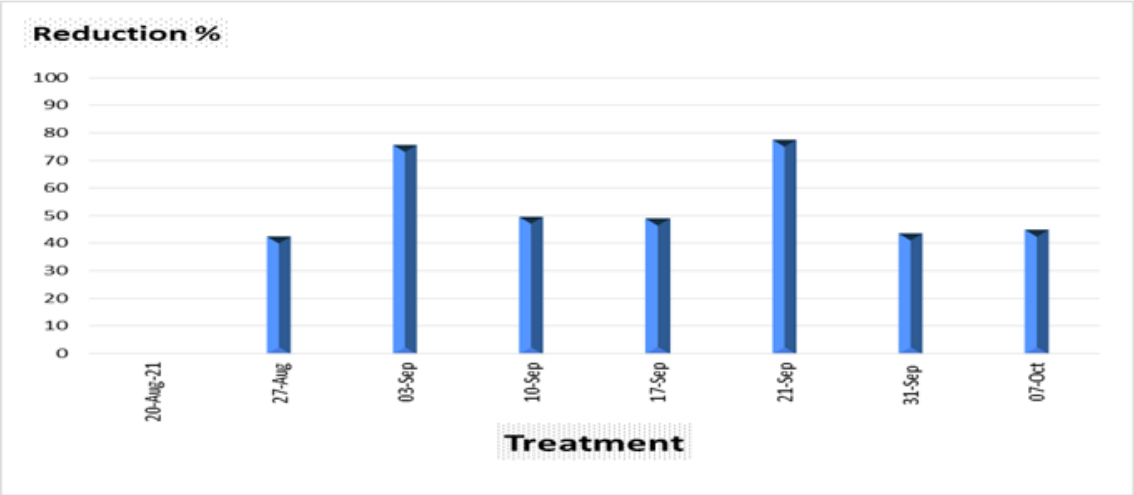

Fig. (3): Effect of Biocide compound Biofly, *B. bassiana* on *P.ulmi*) under field conditions

2-Effect of Biocide Compound Metarril, *Metarlizium anisopliae* (Metchnikoff) against Some Phytophagous Mites Under Field Conditions.

a). Effect of Biocide compound Metarril, *Metarlizium anisopliae* (Metchnikoff) against Two Spotted Spider Mite, *T. urticae*:

Results which inserted in Table (4) and illustrated in Figure (4), Showed the effect of the biocide compound biofly, *M. anisopliae* on *T. urticae* started from (20th Aug.) 2021. Where the initial number pre-count of the *T.urticae* were 460 individuals for control and 380 for the treatment per 12 fruits and 40 leaves; recorded as follow results. That parasite caused a decrease in the density of the mites at a rate 84.55% reduction percentage on (3rd Sept.), whereas it was the highest percentage in this experiment on the other hand the lowest reduction 40.68 % was recorded on (31st Sept.,). While the rest of the reduction rates were recorded as follows: (68.14%, 65.03 %, 56.76%, 60.92 %, and 53.44%) on (27th August, 10th September, 17th September, 24th September and 7th October) respectively. These results proved what (Bugeme *et al.*, 2014) reported where they mentioned that the parasite *M. anisopliae* can be as an alternative to acaricides.

Table 4: Effect of Biocide compound Metarril, *M. anisopliae* on two spotted spider mite *T. urticae* (Koch) under field conditions.

Treatment Date	Rate of application /100 liters of water	Pre-Count		Number of mites after treatment/12 fruits+40 leaves		Reduction %
		Control	Treatment	Control	Treatment	
20- August 2021	100 cc 1×10^8Conidia/ml	460	380			
27- August	100 cc 1×10^8Conidia/ml			380	100	68.14
3- September	100 cc 1×10^8Conidia/ml			980	125	84.55
10- September				4500	1300	65.03
17- September				9800	3500	56.76
24- September				16700	22200	60.92
31- September	100 cc 1×10^8Conidia/ml			12160	5940	40.86
7- October				390	150	53.44

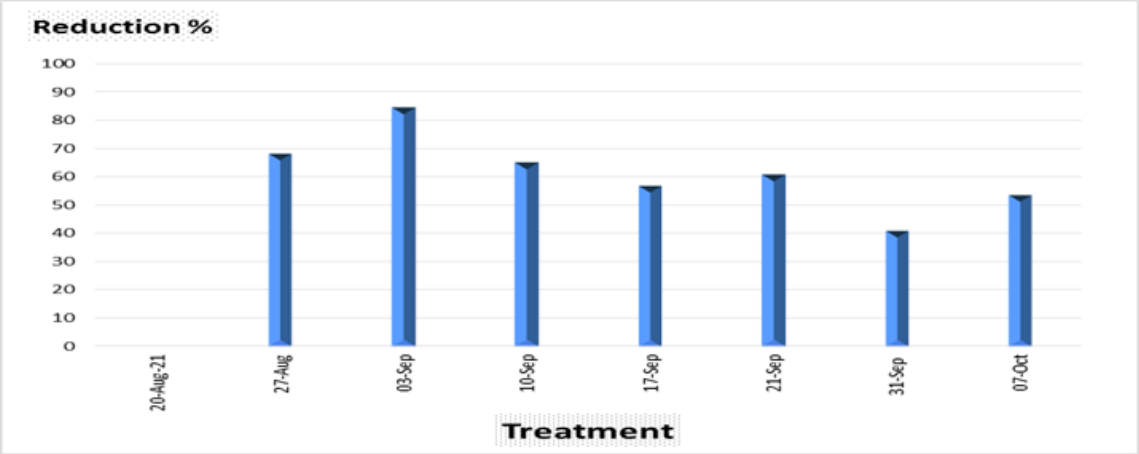

Fig. 4: Effect of Biocide compound Metarril, *M. anisopliae* on *T. urticae* under field conditions.

b). Effect of Biocide compound Metarril, *M. anisopliae* against Flat mite *B. californicus* under field conditions

Results in Table (5): and Figure (5), Where the release of Biocide compound *M. anisopliae* in the field was tested on the mite *B. californicus* Confirm The pathogenic fungus had a very high lethal effect on the mites under study During the experiment that took place in the 2021 season, which released on (20 Aug) by rate 100 cc 1×10^8 Conidia/ml during time from August to October of the same year, the percentage of reduction reached its peak during the month of September specifically in the examination that took place on September 10th 96.91%, where the control number of the mite recorded 981 individuals while the density of the mite in the treatment was 25 individuals, on the other side had been noted that the lowest reduction on 31St September 40.56%. other than, the reduction percentages were (60.04%, 70.35%, 68,69%, 41,14% and 70.65%) on both (27th August, 3rd September, 17th September,24th September and 7th October) respectively.

Table 5: Effect of Biocide compound Metarril, *M. anisopliae* on flat mite *B.californicus* under field conditions.

Treatment Date	Rate of application /100 litres of water	Pre-Count		Number of mites after treatment/12 fruits+40 leaves		Reduction %
		Control	Treatment	Control	Treatment	
20 August 2021	100 cc 1×10^8Conidia/ml	460	380			
27 August	100 cc 1×10^8Conidia/ml			380	124	60.04
3 September	100 cc 1×10^8Conidia/ml			392	96	70.35
10 September				981	25	96,91
17 Septembe				5800	1500	68,69
24 September				10168	4944	41.14
31 September	100 cc 1×10^8Conidia/ml			16700	8200	40,56
7 October				14850	3600	70.65

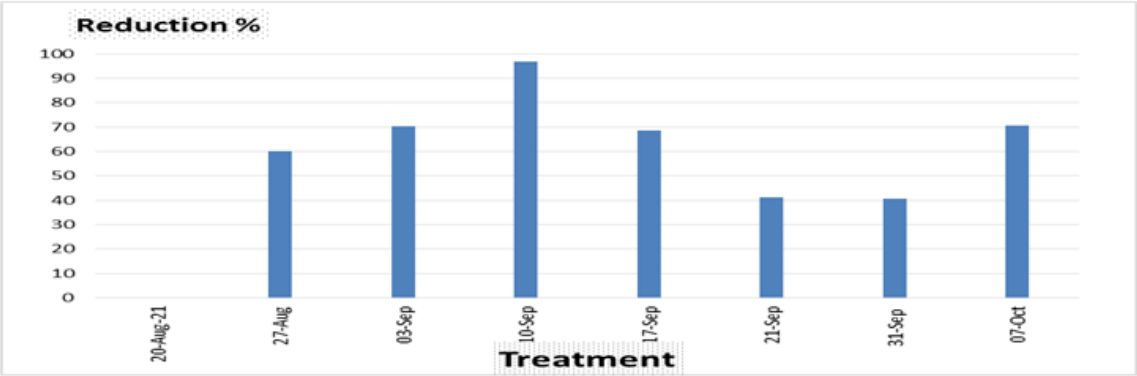

Fig. 5: Effect of Biocide compound Metarril, *Metarlizium anisopliae* against flat mite *B. californicus* under field conditions.

c). Effect of Biocide compound Metarril, *Metarlizium anisopliae* on *Panonychus ulmi* (Koch) under field conditions:

Data in Table (6) and Fig (6); showed that the effect of the biocide compound biofly *M. anisopliae* when it was tasted on P. ulmi by rate100 cc 1×108 Conidia/ml during time from August to October; that The obvious positive effect is in the percentage reduction occurred on 3rd September, when the control number of the individuals of the pest was 1080, and the treatment number recorded 120, meanwhile the reduction percentage reached 86.54%, that is considered an indicator that the strongest effect comes after about two weak of treatment, which started on 20th August in the same season. While after just two weeks that compound recorded the lowest reduction 40.68 %after the peak of reduction. While the percentages of reduction taken from the experiment were (49.32%, 75.12%, 46.19% 45.5 %. and 46.19) on (27th Augst, 10th September, 24th September, 31st September, 7th October) respectively.

Table 6: Effect of Biocide compound Metarril, *M. anisopliae* on *P. ulmi* under field conditions.

Treatment Date	Rate of application /100 litres of water	Pre-Count		Number of mites after treatment/12 fruits+40 leaves		Reduction %
		Control	Treatment	Control	Treatment	
20- August 2021	100 cc 1×10⁸Conidia/ml	460	380			
27- August	100 cc 1×10⁸Conidia/ml			430	180	49.32
3- September	100 cc 1×10⁸Conidia/ml			1080	120	86.54
10- September				5500	1130	75.12
17- September				10000	4900	40.68
24- September				18000	8000	46.19
31- September	100 cc 1×10⁸Conidia/ml			10138	4564	45.5
7- October				450	200	46.19

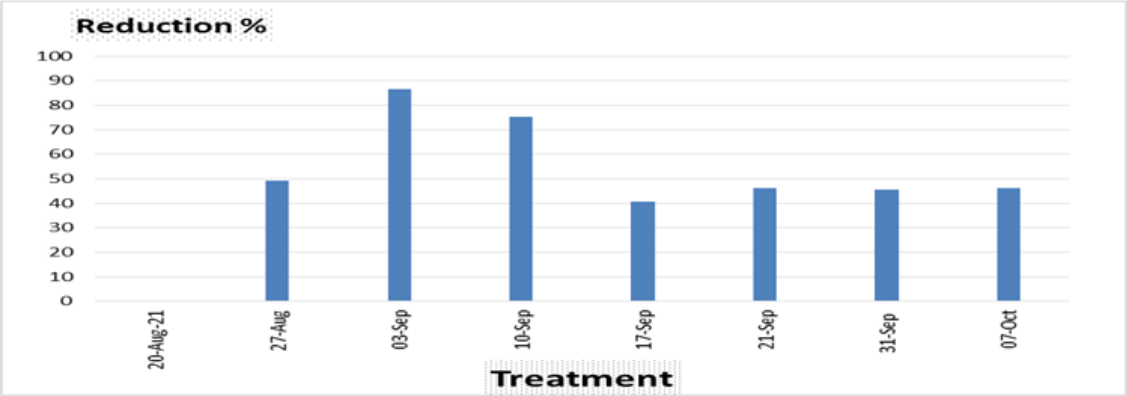

Fig. 6: Effect of Biocide compound Metarril, *M. nisopliae* on *P. ulmi* under field conditions.

DISCUSSION

The present study aimed to evaluate two biocide compounds Biofly (*B.bassiana)* and Metarril (*M. anisopliae)* by concentrates 3 x10 ^{7} conidia/ cm^{3} and 1x10^{8} conidia/ml resp. against three phytophagous mites *(T. urticae*; *P. ulmi* and *B. californicus*) on apple trees in the field. Four applications were conducted, three of them were subsequently by 7 days intervals while the last application was occurred before the end of experiment by one week .After 7 days of the first application at the 20th of August , 2021 the population reduction of *T. urticae*; *P. ulmi* and *B. californicus* were 50.73%; 42.52 % & 37.57% in case of *B. bassiana* resp. While in case of *M. anisopliae* the population reduction of *T. urticae*; *P. ulmi* and *B. californicus* were 68.14%; 49.32% & 60.04% resp. Highly decreased of the mites population after second and third applications . On the other hand, the mites population sharply decreased during the period from the third application (3rd of sept.) to 24th sept.which caused final application conducting at 31th Sept. The reduction of the mites population after last application were 61.19%; 45.1% & 40.94% in case of *B. bassiana* resp. while in case of *M. anisopliae* the population reduction of *T. urticae*; *P. ulmi and B. californicus* were 53.44%; 46.19% & 70.65% resp.the results of this study agreement with Halawa (1998) who evaluated Biofly(*Beauveria bassiana)* to control citrus rust mite, *Phyllocoptruta oleivora* by using four application at concentrate 3 x10 ^{7} conidia/ cm^{3} . His results were recorded average reduction of *P. oleivora* reached to 72.2%. Moreover, the fungi, *B. bassiana* was used on Cucumber to control two spotted spider

mite, *T. urticae*. The biocide compound which contained spores of *B. bassiana* was sprayed four time and population reduction reached to 80.86% (EL-Adawy *et al.*, 1995). Therefore, the mentioned biocides compound can be inserted as a tandem control strategy of mites as a safe method, especially many authors such as Hassan, *et. al.* (2017) who studied the side effects of Biofly(*B. bassiana)* and Metarril (*M. anisopliae)* against the adult of the two predator mites *Phytoseiulus persimilis* and *Neoseiulus californicus*. His results indicated that, the mentioned biocompound were harmless against *P. persimilis* and slightly harmful against *N. californicus*.

Declarations

Ethical Approval: Not applicable.

Competing Interests: The authors declare that they have no competing interests.

Authors' Contributions: MA, NG, SE and AT did the conceptualization. MA, NG, SE and AT contributed in the formal analysis. MA, NG and SE took part in the investigation. MA wrote the original draft. NG and AT did the writing review and approved the final manuscript. All authors read and approved the final manuscript.

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Availability of Data and Materials: All datasets analyzed and described during the present study are available.

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