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## Evaluation of different bio-pesticides against red spider mite, *Tetranychus urticae* Koch in marigold

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### Abstract

The efficacy of different bio-pesticides was evaluated against red spider mite, *Tetranychus urticae* in marigold viz. Neem seed kernel extract 5% (NSKE), aqueous tobacco extract (2%), neem oil (0.5%), azadirachtin 10,000 ppm (0.002%), *Metarhizium anisopliae* NBAIR Ma4 1% WP ( $2 \times 10^8$  cfu/g), *Beauveria bassiana* NBAIR Bb5a 1% WP ( $2 \times 10^8$  cfu/g) and *Lecanicillium lecanii* ICAR NBAIR V18 1% WP ( $2 \times 10^8$  cfu/g) compared with control at Biological Control Research Farm, ICAR Unit-9, Anand Agricultural University, Anand (Gujarat). The results showed that neem oil (0.5%) and azadirachtin 10,000 ppm (0.002%) were the most effective treatments that reduced the mite population and demonstrated remarkable efficacy in managing mite infestations. In contrast, *Metarhizium anisopliae* and *Beauveria bassiana* were the least effective treatments, resulting in the highest mite population. While the remaining bio-pesticides exhibited moderate efficacy. These findings suggest that Neem oil and azadirachtin can be used as effective bio-pesticides for managing red spider mite infestations in marigold.

**Keywords:** *Tetranychus urticae*, bio-pesticides, efficacy, marigold, mite

### Introduction

Marigold belongs to the genus *Tagetes* is a flowering plant in the *Asteraceae* family. The name "marigold" is derived from "Mary's Gold," associated with the Virgin Mary in Christian stories [1]. There are approximately 33 species of *Tagetes* with five introduced to Indian gardens i.e., *Tagetes erecta* L., *Tagetes minuta* L., *Tagetes patula* L., *Tagetes lucida* Cav. and *Tagetes tenuifolia* Cav. [2]. The area under marigold cultivation in India is about 87.07 thousand ha with a production of 952.60 thousand MT, Gujarat contributes 92.84 thousand tonnes to the National production with a cultivation area of 10.09 thousand hectares [3]. Currently, the predominant obstacle impeding the attainment of high productivity and quality flower production in marigold is the prevalence of a variety of pests including aphid (*Lipaphis erysimi* Kalténbach), thrips (*Thrips tabaci* Lindeman), red spider mite (*Tetranychus urticae* Koch), bud borer (*Helicoverpa armigera* (Hübner)), leaf miner (*Liriomyza trifolii* Burgess), mealy bug (*Phenacoccus solenopsis* Tinsley), hadda beetle (*Henosepilachna vigintioctopunctata* Fabricius), tarnished plant bug (*Lygus lineolaris* (Palisot de Beauvois)), green stink bug (*Nezara viridula* Linnaeus) and grasshopper (*Bycotophus longiceps* Dirsh) have been identified as detrimental to marigold crops [4]. Among the different insect-pests infesting marigold, red spider mite or two-spotted spider mite is a sucking pest belonging to the sub-class Acarina within the Arachnida class of the phylum Arthropoda. Among the Arachnida, the Acari is the only subclass that feeds on plants. Mites exhibit diverse feeding behaviors, leading to their classification as phytophagous, predatory, parasitic, or stored mites. Globally, there are approximately 7,000 recognized species of phytophagous mites distributed across five families viz., Tetranychidae, Eriophyidae, Tarsonemidae, Tenuipalpidae and Tuckerellidae [5]. The red spider mite, *T. urticae* belongs to the Tetranychidae family, a substantial group of spider mites that encompasses close to 70 genera and approximately 1,200 species [6]. Persistent mite feeding initiates the formation of spots on leaves, marked by the emergence of whitish streaks that transform into pale yellowish patches. Upon consolidation, these patches give rise to distinct chlorotic patterns, commonly recognized as the 'bronzing' effect [7]. Plants infested by protonymphs, deutonymphs, and adult spider mites become enveloped in protective webbing. This intricate web serves as a shield

against natural predators and hinders the efficacy of pesticide application [8].

Management of *T. urticae* with indiscriminate use of chemicals leads to the development of resistance, outbreak of secondary pests, destruction of natural enemies, pesticide residues in food, hazards to human and animal life, and environmental pollution. Therefore, employing biological control methods appears to be an effective strategy for managing phytophagous mites. The bio-pesticides have also proven successful in the management of mites. As a result, there is a requirement to investigate the effectiveness of various bio-pesticides for the efficient and cost-effective management of mites in marigold.

### Materials and Methods

A field experiment was carried out to evaluate different bio-pesticides against red spider mites in marigold at Biological Control Research Farm, ICAR Unit-9, AAU, Anand, Gujarat during the *kharif-rabi* season of 2023-24. A Randomized Block Design (RBD) was employed to evaluate the efficacy of seven bio-pesticides with three replications. For the study, the marigold variety Orange Bunch was transplanted during the last week of September with a spacing of 30 x 30 cm having a plot size of 1.5 x 3.0 m. All the recommended agronomical practices were followed for raising the crop. The treatments included NSKE (5%), aqueous tobacco extract (2%), neem oil (0.5%), azadirachtin 10,000 ppm (0.002%), *Metarhizium anisopliae* NBAIR Ma4 1% WP ( $2 \times 10^8$  cfu/g), *Beauveria bassiana* NBAIR Bb5a 1% WP ( $2 \times 10^8$  cfu/g) and *Lecanicillium lecanii* ICAR NBAIR V18 1% WP ( $2 \times 10^8$  cfu/g) compared with control. The first spray of respective bio-pesticides was applied at the appearance of mite and the subsequent two sprays were done after 10 days of the first spray by using a knapsack sprayer. To ascertain the field efficacy of various bio-pesticides against *T. urticae*, observations on the mite population were recorded by randomly selecting five plants from each net plot. From each plant, three leaves one each from the top, middle, and bottom canopies were sampled and the mite population which included mobile stage was recorded one day before spraying (pre-treatment) and 3, 7, and 10 days after spraying using a stereo zoom microscope. The mite population was recorded in one cm<sup>2</sup> area per leaf. Considering the activity of mites, three sprays were done during the crop period. The data was statistically analyzed using square root transformation and subjected to ANOVA.

### Results and Discussion

Before the application of any treatments, the mite population was notably high and uniformly distributed throughout all the plots according to the data presented in Table 1, and there was no discernible difference between any of the treatments before spraying. There were 7.85 to 10.99 mites per cm<sup>2</sup> of leaf.

The pooled data computed for the first spray revealed that all the treatments were significantly superior to the control (15.10 mites/cm<sup>2</sup> leaf). The treatment of neem oil 0.5 per cent recorded the lower mite population (4.98 mites/cm<sup>2</sup> leaf) and it was at par with azadirachtin 10000 ppm and NSKE 5% with 5.21 and 5.80 mites/cm<sup>2</sup> leaf, respectively. Thus, all these treatments were found to be more effective than the rest of the treatments in controlling mite population. It was also discovered that the treatments using *L. lecanii* 1 per cent (7.85 mites/cm<sup>2</sup> leaf) and aqueous tobacco extract 2 per cent (7.74 mites/cm<sup>2</sup> leaf), were moderately successful in reducing the mite population. Among all the treatments *B. bassiana* 1 per cent had a higher mite population (11.13 mites/cm<sup>2</sup> leaf) and was found to be the least

efficient against mites. This treatment was also found statistically at par with *M. anisopliae* 1 per cent, which recorded 10.86 mites per cm<sup>2</sup> leaf and was superior to control plots.

The second spray (Table 2) exhibited that the reduction in mite population was maximum in plots treated with neem oil 0.5 per cent (4.52 mites/cm<sup>2</sup> leaf). However, this treatment was statistically comparable with the treatment of azadirachtin 10000 ppm (4.98 mites/cm<sup>2</sup> leaf) and NSKE 5 per cent (5.16 mites/cm<sup>2</sup> leaf) which revealed the supremacy of these treatments in comparison to the remaining treatments. Conversely, the treatment of aqueous tobacco extract 2 per cent (11.26 mites/cm<sup>2</sup> leaf) proved to be least effective against mites and it was at par with *M. anisopliae* 1 per cent (10.59 mites/cm<sup>2</sup> leaf). However, these biopesticides exhibited a significantly lower incidence of mites than the untreated control (15.58 mites/cm<sup>2</sup> leaf).

The data pooled over periods for the third spray presented in Table 3 indicated that there was a significant difference among the various biopesticide treatments. The treatment of neem oil 0.5 per cent was found as the most effective treatment and recorded a lower mite population (3.62 mites/cm<sup>2</sup> leaf). The treatment with azadirachtin 10000 ppm (3.78 mites/cm<sup>2</sup> leaf) was registered as the second-best treatment and this was followed by NSKE 5 per cent (4.25 mites/cm<sup>2</sup> leaf). All these three treatments were statistically comparable with each other and demonstrated their superiority over the other treatments. While, treatments of *L. lecanii* 1 per cent and aqueous tobacco extract 2 per cent recorded 6.74 mites per cm<sup>2</sup> leaf and 7.01 mites per cm<sup>2</sup> leaf area, respectively. These treatments moderately reduced the population of red spider mite. Whereas, treatment of *B. bassiana* 1 per cent (10.72 mites/cm<sup>2</sup> leaf) and *M. anisopliae* 1 per cent (10.19 mites/cm<sup>2</sup> leaf) proved to be least effective in controlling the mite population but was superior to control which recorded 15.10 mites per cm<sup>2</sup> leaf.

The data on pooled over periods and sprays presented in Table 4 recorded maximum reduction in mite population was found in a plot treated with neem oil 0.5 per cent (4.38 mites/cm<sup>2</sup> leaf) and this was followed by the treatment using azadirachtin 10000 ppm (4.65 mites/cm<sup>2</sup> leaf). These two treatments were statistically at par with each other. The treatment of NSKE 5 per cent was registered as the third-best treatment by recording 5.07 mites per cm<sup>2</sup> leaf. While *L. lecanii* 1 per cent (7.34 mites/cm<sup>2</sup> leaf) and aqueous tobacco extract 2 per cent (8.56 mites/cm<sup>2</sup> leaf) were proved to have a moderate effect in reducing mite population. Whereas, treatment of *M. anisopliae* 1 per cent (10.52 mites/cm<sup>2</sup> leaf) proved least effective against mite and it was at par with *B. bassiana* 1 per cent (9.68 mites/cm<sup>2</sup> leaf).

Neem oil effectively reduced *T. urticae* population in ashwagandha by 35.33% after one day of treatment [9]. Neem extract showed higher mortality rates against red spider mites recorded at 61.95, 64.63 and 63.29% after the first, second, and on average spray, respectively in okra [10]. Neem oil 3 per cent with a mean reduction of 68.8% in egg counts and 74.7% in *T. urticae* population under laboratory studies [11]. Neem oil 5 per cent demonstrated maximum *T. urticae* mortality of 80.72% and a maximum reduction in egg count of 74.41% under *in-vitro* conditions [12]. Azadirachtin 1% and mahua oil + neem oil 3% were found effective and achieved mean reduction percentages of 84.05 and 68.40, respectively against red spider mite, *T. urticae* in okra [13]. Neem oil (0.5%) recorded higher efficacy followed by NSKE (5%) against mite, *T. urticae* infesting brinjal [14]. These findings follow the result of the present study which demonstrated the effectiveness of neem oil in suppressing the population of red spider mites.

**Table 1:** Evaluation of different biopesticides against red spider mite, *T. urticae* infesting marigold (First spray)

Sr. No.	Treatments	Before spray	No. of mites/cm <sup>2</sup> leaf area at indicated days after spray			Pooled
			3	7	10	
1	Neem Seed Kernel Extract 5%	2.99 (8.44)	2.56 <sup>ab</sup> (6.05)	2.43 <sup>abc</sup> (5.40)	2.55 <sup>ab</sup> (6.00)	2.51 <sup>a</sup> (5.80)
2	Aqueous tobacco extract 2%	3.16 (9.49)	2.86 <sup>b</sup> (7.68)	2.81 <sup>bc</sup> (7.40)	2.93 <sup>bc</sup> (8.08)	2.87 <sup>b</sup> (7.74)
3	Neem oil 0.5%	2.89 (7.85)	2.33 <sup>a</sup> (4.93)	2.32 <sup>ab</sup> (4.88)	2.39 <sup>a</sup> (5.21)	2.34 <sup>a</sup> (4.98)
4	Azadirachtin 10000 ppm 0.002%	2.93 (8.08)	2.43 <sup>ab</sup> (5.40)	2.28 <sup>a</sup> (4.70)	2.46 <sup>ab</sup> (5.55)	2.39 <sup>a</sup> (5.21)
5	<i>Beauveria bassiana</i> - NBAIR Bb5a - 1% WP	3.33 (10.59)	3.40 <sup>c</sup> (11.06)	3.38 <sup>d</sup> (10.92)	3.46 <sup>d</sup> (11.47)	3.41 <sup>c</sup> (11.13)
6	<i>Metarhizium anisopliae</i> - NBAIR Ma4 - 1% WP	3.39 (10.99)	3.38 <sup>c</sup> (10.92)	3.37 <sup>d</sup> (10.86)	3.36 <sup>cd</sup> (10.79)	3.37 <sup>c</sup> (10.86)
7	<i>Lecanicillium lecanii</i> 1% WP (ICAR-NBAIR V18)	3.17 (9.55)	2.90 <sup>bc</sup> (7.91)	2.85 <sup>c</sup> (7.62)	2.92 <sup>bc</sup> (8.03)	2.89 <sup>b</sup> (7.85)
8	Untreated Control	3.37 (10.86)	3.92 <sup>d</sup> (14.87)	3.92 <sup>c</sup> (14.87)	4.02 <sup>e</sup> (15.66)	3.95 <sup>d</sup> (15.10)
	S.Em. ± Treatment (T)	0.21	0.16	0.16	0.16	0.08
	Period (P)	-	-	-	-	0.06
	P × T	-	-	-	-	0.16
	F test (T)	NS	Sig.	Sig.	Sig.	Sig.
	C.V. (%)	11.61	9.20	9.58	9.09	9.29

Note: 1) Figures in parentheses are retransformed values and those outside are  $\sqrt{x+0.5}$  transformed values

2) Treatment means with the letter(s) in common are not significant by Duncan's New Multiple Range Test (DNMRT) at 5% level of significance, NS = Non-Significant, Sig.=Significant

3) Significant interactions and its parameters: P

**Table 2:** Evaluation of different biopesticides against red spider mite, *T. urticae* infesting marigold (Second spray)

Sr. No.	Treatments	No. of mites/cm <sup>2</sup> leaf area at indicated days after spray			Pooled
		3	7	10	
1	Neem Seed Kernel Extract 5%	2.33 <sup>ab</sup> (4.93)	2.32 <sup>ab</sup> (4.88)	2.48 <sup>ab</sup> (5.65)	2.38 <sup>a</sup> (5.16)
2	Aqueous tobacco extract 2%	3.44 <sup>c</sup> (11.33)	3.41 <sup>c</sup> (11.13)	3.44 <sup>d</sup> (11.33)	3.43 <sup>c</sup> (11.26)
3	Neem oil 0.5%	2.24 <sup>a</sup> (4.52)	2.21 <sup>a</sup> (4.38)	2.27 <sup>a</sup> (4.65)	2.24 <sup>a</sup> (4.52)
4	Azadirachtin 10000 ppm 0.002%	2.36 <sup>ab</sup> (5.07)	2.28 <sup>ab</sup> (4.70)	2.38 <sup>ab</sup> (5.16)	2.34 <sup>a</sup> (4.98)
5	<i>Beauveria bassiana</i> - NBAIR Bb5a - 1% WP	2.82 <sup>b</sup> (7.45)	2.78 <sup>b</sup> (7.23)	2.85 <sup>bc</sup> (7.62)	2.82 <sup>b</sup> (7.45)
6	<i>Metarhizium anisopliae</i> - NBAIR Ma4 - 1% WP	3.35 <sup>c</sup> (10.72)	3.32 <sup>c</sup> (10.52)	3.34 <sup>cd</sup> (10.66)	3.33 <sup>c</sup> (10.59)
7	<i>Lecanicillium lecanii</i> 1% WP (ICAR-NBAIR V18)	2.80 <sup>b</sup> (7.34)	2.79 <sup>b</sup> (7.28)	2.84 <sup>bc</sup> (7.57)	2.81 <sup>b</sup> (7.40)
8	Untreated Control	3.98 <sup>d</sup> (15.34)	4.00 <sup>d</sup> (15.50)	4.05 <sup>e</sup> (15.90)	4.01 <sup>d</sup> (15.58)
	S.Em. ± Treatment (T)	0.17	0.17	0.17	0.09
	Period (P)	-	-	-	0.06
	P × T	-	-	-	0.17
	F test (T)	Sig.	Sig.	Sig.	Sig.
	C.V. (%)	9.82	10.01	10.21	10.02

Note: 1) Figures in parentheses are retransformed values and those outside are  $\sqrt{x+0.5}$  transformed values

2) Treatment means with the letter(s) in common are not significant by DNMRT at 5% level of significance, Sig.=Significant

3) Significant interactions and its parameters: P

**Table 3:** Evaluation of different biopesticides against red spider mite, *T. urticae* infesting marigold (Third spray)

Sr. No.	Treatments	No. of mites/cm <sup>2</sup> leaf area at indicated days after spray			Pooled
		3	7	10	
1	Neem Seed Kernel Extract 5%	2.23 <sup>abc</sup> (4.47)	2.07 <sup>a</sup> (3.78)	2.23 <sup>ab</sup> (4.47)	2.18 <sup>a</sup> (4.25)
2	Aqueous tobacco extract 2%	2.75 <sup>cd</sup> (7.06)	2.69 <sup>b</sup> (6.74)	2.79 <sup>cd</sup> (7.28)	2.74 <sup>b</sup> (7.01)
3	Neem oil 0.5%	2.09 <sup>a</sup> (3.87)	1.87 <sup>a</sup> (3.00)	2.13 <sup>a</sup> (4.04)	2.03 <sup>a</sup> (3.62)
4	Azadirachtin 10000 ppm 0.002%	2.13 <sup>ab</sup> (4.04)	1.99 <sup>a</sup> (3.46)	2.10 <sup>a</sup> (3.91)	2.07 <sup>a</sup> (3.78)
5	<i>Beauveria bassiana</i> - NBAIR Bb5a - 1% WP	3.37 <sup>e</sup> (10.86)	3.32 <sup>c</sup> (10.52)	3.36 <sup>e</sup> (10.79)	3.35 <sup>c</sup> (10.72)
6	<i>Metarhizium anisopliae</i> - NBAIR Ma4 - 1% WP	3.29 <sup>de</sup> (10.32)	3.25 <sup>c</sup> (10.06)	3.28 <sup>de</sup> (10.26)	3.27 <sup>c</sup> (10.19)
7	<i>Lecanicillium lecanii</i> 1% WP (ICAR-NBAIR V18)	2.68 <sup>bc</sup> (6.68)	2.67 <sup>b</sup> (6.63)	2.72 <sup>bc</sup> (6.90)	2.69 <sup>b</sup> (6.74)
8	Untreated Control	3.9.8 <sup>f</sup> (15.34)	3.94 <sup>d</sup> (15.02)	3.93 <sup>f</sup> (14.94)	3.95 <sup>d</sup> (15.10)
	S.Em. ± Treatment (T)	0.18	0.18	0.17	0.09
	Period (P)	-	-	-	0.06
	P × T	-	-	-	0.18
	F test (T)	Sig.	Sig.	Sig.	Sig.
	C.V. (%)	11.04	11.36	10.48	10.96

Note: 1) Figures in parentheses are retransformed values and those outside are  $\sqrt{x+0.5}$  transformed values

2) Treatment means with the letter(s) in common are not significant by DNMRT at 5% level of significance, Sig.=Significant

3) Significant interactions and its parameters: P

**Table 4:** Evaluation of different biopesticides against red spider mite, *T. urticae* infesting marigold (Pooled over periods over sprays)

Sr. No.	Treatments	No. of mites/cm <sup>2</sup> leaf area at			Pooled over periods over sprays
		1 <sup>st</sup> Spray	2 <sup>nd</sup> Spray	3 <sup>rd</sup> Spray	
1	Neem Seed Kernel Extract 5%	2.51 <sup>a</sup> (5.80)	2.38 <sup>a</sup> (5.16)	2.18 <sup>a</sup> (4.25)	2.36 <sup>b</sup> (5.07)
2	Aqueous tobacco extract 2%	2.87 <sup>b</sup> (7.74)	3.43 <sup>c</sup> (11.26)	2.74 <sup>b</sup> (7.01)	3.01 <sup>d</sup> (8.56)
3	Neem oil 0.5%	2.34 <sup>a</sup> (4.98)	2.24 <sup>a</sup> (4.52)	2.03 <sup>a</sup> (3.62)	2.21 <sup>a</sup> (4.38)
4	Azadirachtin 10000 ppm 0.002%	2.39 <sup>a</sup> (5.21)	2.34 <sup>a</sup> (4.98)	2.07 <sup>a</sup> (3.78)	2.27 <sup>ab</sup> (4.65)
5	<i>Beauveria bassiana</i> - NBAIR Bb5a - 1% WP	3.41 <sup>c</sup> (11.13)	2.82 <sup>b</sup> (7.45)	3.35 <sup>c</sup> (10.72)	3.19 <sup>e</sup> (9.68)
6	<i>Metarhizium anisopliae</i> - NBAIR Ma4 - 1% WP	3.37 <sup>c</sup> (10.86)	3.33 <sup>c</sup> (10.59)	3.27 <sup>c</sup> (10.19)	3.32 <sup>e</sup> (10.52)
7	<i>Lecanicillium lecanii</i> 1% WP (ICAR-NBAIR V18)	2.89 <sup>b</sup> (7.85)	2.81 <sup>b</sup> (7.40)	2.69 <sup>b</sup> (6.74)	2.80 <sup>c</sup> (7.34)
8	Untreated Control	3.95 <sup>d</sup> (15.10)	4.01 <sup>d</sup> (15.58)	3.95 <sup>d</sup> (15.10)	3.97 <sup>f</sup> (15.26)
	S.Em. ± Treatment (T)	0.08	0.09	0.09	0.05
	Period (P)	0.06	0.06	0.06	0.03
	Spray (S)	-	-	-	0.03
	T×P	0.16	0.17	0.18	0.09
	T×S	-	-	-	0.09
	S×P	-	-	-	0.06
	T×S×P	-	-	-	0.16
	C.D. at 5% T	0.23	0.24	0.25	0.15
	P	0.17	0.17	0.17	NS
	S	-	-	-	0.09
	T×P	NS	NS	NS	NS
	T×S	-	-	-	0.26
	S×P	-	-	-	NS
	T×S×P	-	-	-	NS
	C.V. (%)	9.29	10.02	10.96	9.53

**Note:** 1) Figures in parentheses are retransformed values and those outside are  $\sqrt{x+0.5}$  transformed values

2) Treatment means with the letter(s) in common are not significant by DNMR at 5% level of significance, NS = Non-Significant

3) Significant parameters and its interaction: S, T×S

## Conclusion

Among the seven bio-pesticides evaluated against red spider mite, *T. urticae* in marigold, neem oil 0.5% and azadirachtin 10000 ppm emerged as the most effective treatments that demonstrated exceptional potential in mitigating mite infestation. Inversely, *M. anisopliae* 1% and *B. bassiana* 1% exhibited the least effectiveness, with a higher mite population. The treatments of NSKE 5%, *L. lecanii* 1%, and aqueous tobacco extract 2% displayed moderate efficacy.

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