

## Research Article

# *In vitro* efficacy of *Beauveria bassiana* (Balsamo) Vuillemin against *Tetranychus urticae* Koch on tuberose

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**ABSTRACT:** Bio efficacy of virulent isolate of entomopathogenic fungi, *Beauveria bassiana* (MK918495) was evaluated against two spotted spider mite, *Tetranychus urticae* on tuberose under pot culture condition at Insectary, Department of Agricultural Entomology, TNAU, Coimbatore. For effective working of any insect pathogen, it is mandatory to develop an effective formulation. Keeping this point in view, in the present study, compatibility of the fungal isolate with two different oils, viz., ground nut and sunflower were tested at different concentrations, viz., 1, 3, 5 and 10%. The results revealed that the groundnut oil was compatible with *B. bassiana* at all concentrations with respect to colony growth and spore yield compared to sunflower oil which was compatible only at one percent. Based on the results, oil in water and oil based formulations were prepared. At higher concentration of 10%, groundnut oil recorded the radial colony growth of 36.90 mm diameter and spore yield of  $1.2 \times 10^8$  spores mL<sup>-1</sup>. Among the seven formulations (A-G) tested, formulation D which contained surfactant mixture having HLB value of 10.00 (53.33% Tween 80 and 46.67% Span 80) formed a better suspension with less flocculation layer and dispersion of conidia in spray fluid and was found to be better as it formed a good emulsion with water by mere shaking. Results of pot culture studies revealed that groundnut oil based fungal formulation was effective with a cumulative mean mite population reduction of 64.98% followed by crude fungal spore suspension (62.16%) and both were statistically on par with each other. Standard chemical check, Fenazaquin 10% EC @ 1.5 mL L<sup>-1</sup> recorded significantly highest population reduction of mites (77.20%).

**KEY WORDS:** *Beauveria bassiana*, formulations, pot culture, *Tetranychus urticae*, tuberose

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## INTRODUCTION

Tuberose is an important flower crop grown in tropical regions for their good economic returns throughout the year. In Tamil Nadu, the commercial tuberose growing areas are Dindigul, Coimbatore, Erode, Madurai, Trichy and Ariyalur districts. The damage caused by arthropod pests resulted in significant yield reduction in tuberose. Due to fluctuations in the environmental conditions, red spider mite is emerging as a major pest of tuberose resulting in severe yield reduction (Safeena *et al.*, 2015).

Two spotted spider mite, *Tetranychus urticae* (Acari:Tetranychidae) is an extremely polyphagous pest that has been reported on more than 900 host species and is a serious pest of at least 150 economically important agricultural and ornamental plants (Jeppson *et al.*, 1975).

In order to reduce the damage and yield loss caused by mite pests, a large quantity of acaricides has been used which in turn lead to higher level of pesticide residues that may pose severe problem to consumers (Sardana *et al.*, 2005). Due to the short lifespan, faster multiplication rate and indiscriminate application of chemical acaricides, *T. urticae* developed resistance to many of the acaricides (Croft *et al.*, 1987).

Alternate strategies are the application of predatory mites (Simmonds, 1971). However, insufficient control by predatory mite was reported by Chandler *et al.* (2005) which lead to search for other eco-friendly methods and in particular, the entomopathogens which are considered safe to human and other non-target arthropods.

Entomopathogens are gaining increasing importance mainly due to their compatibility with other management strategies under integrated pest management, specific host range and safety to non-target organisms (Saik *et al.*, 1990) and among them, the best one to control sap feeding pest such as *T. urticae* are the entomopathogenic fungi which are capable of infecting mites directly through the integument without necessarily the pathogen being ingested by the insect.

Over 700 different fungal species from at least 90 genera including *Beauveria*, *Metarhizium*, *Isaria*, *Lecanicillium*, *Hirsutella* and *Entomophthorales* are known to be pathogenic to insects (Khachatourians and Qazi, 2008). In the present investigation, oil formulation of *B. bassiana* MK918495 with an  $LC_{50}$  value of  $1.0 \times 10^6$  spores  $mL^{-1}$  was evaluated against two spotted spider mite, *T. urticae* under pot culture conditions at Department of Agricultural Entomology, Tamil Nadu Agricultural University.

## MATERIALS AND METHODS

### Mass production of fungal isolates

The isolate of *Beauveria bassiana* (MK918495) (Athisintha *et al.*, 2019) was mass multiplied in Potato Dextrose Broth (PDB) for carrying out pot culture studies. 250 mL of PDB was poured onto round bottom flask of 500 mL capacity and autoclaved at 121°C for 20 minutes. After cooling, one mL of spore suspension was inoculated into each flask separately and kept in orbital shaker for three days and incubated at room temperature for 10 days. After sporulation, fungal isolates were ground in a mixer and filtered through double layered muslin cloth. The suspension was shaken thoroughly with a drop of Tween 80 in order to disperse the spores in solution. The conidial suspension was vortexed for five minutes to produce a homogenous conidial suspension.

## DEVELOPMENT AND STANDARDIZATION OF OIL BASED FORMULATIONS

### Compatibility of *Beauveria bassiana* (MK918495) with different oils

The compatibility of selected *B. bassiana* isolate (MK918495) with different oils was investigated by following poison food technique (Olmert and Kenneth, 1974). The principle involved in this technique is to poison the nutrient medium with a toxicant and then allowing the test fungus to grow on it.

Sterilized PDA media were amended with groundnut and sunflower oil at 1, 3, 5 and 10% concentrations. Amended media were poured into 9 cm diameter Petri dish and allowed for solidification. Two week old *B. bassiana* (MK918495) grown on PDA medium was cut into 6mm disc by using sterile cork borer and the discs were kept in an inverted position

onto the centre of well solidified oil amended media plates. Control plate was maintained without amending oil. The plates were kept for incubation at  $25 \pm 2^\circ C$  for fifteen days. Each treatment was replicated thrice. Colony diameter was recorded on seven, ten and fourteen days after inoculation using a ruler (average of two measurements made at right angles), whereas spore count was made from fully sporulated plates on fifteenth day (Visalakshy *et al.*, 2006).

Observations on vegetative growth and sporulation were transferred relative to the control (100) and the product's toxicity (T) values were calculated by following the formula adopted by Alves *et al.* (2002).

$$T = \frac{20 (VG) + 80 (SP)}{100}$$

T – Toxicity value

VG – Vegetative growth; SP – Sporulation

Toxicity range based on T value

T value	Toxicity/ Compatibility
0–30	Very toxic
31–45	Toxic
46–60	Moderately toxic
>60	Compatible

Based on the results of compatibility study, groundnut oil was selected for carrying out the formulation of *B. bassiana* (MK918495).

### Oil in water formulation (o/w)

Oil in water formulation was developed by mixing oil phase with water phase.

Oil phase is constituted by surfactant and groundnut oil each at 1%. Different surfactants viz., Tween 80 and Span 80 alone and in combination were used to formulate oil phase. Surfactant mixture were obtained by mixing Tween 80 and Span 80 in different proportions. Surfactant proportions were fixed based on specific Hydrophilic-Lipophilic Balance (HLB value) according to Griffin (1954). Oil phase was prepared by adding one mL of the different proportion of surfactant mixture to the groundnut oil (1%) and kept in a magnetic stirrer for fifteen minutes. 10 mL of oil phase from different proportion was then fortified in 90 mL of *B. bassiana* (MK918495) spore suspension ( $10^9$  spores  $mL^{-1}$ ) and kept in a magnetic stirrer for one hour and left to stand for 2 h. The dispersion of the conidia in the spray mixture was observed every 24 hours for three days. HLB value was arrived using the formula,

$$HLB_{oil} = \frac{W_A \times HLB_A + W_B \times HLB_B}{W_A + W_B}$$

Where  $W_A$  = Amount of first surfactant (Tween 80) required.

$W_B$  = Amount of second surfactant (Span 80) required.

$HLB_A$  = HLB value of first surfactant.

$HLB_B$  = HLB value of second surfactant.

$$\%A = \frac{100 \times (x - HLB_B)}{HLB_A - HLB_B}$$

% B = 100- % A

Where % A = Amount of first surfactant required.

% B = Amount of second surfactant required.

### Oil based formulation

1 g of pure conidia ( $10^9$  spores  $mL^{-1}$ ) was dissolved in 10 mL of glycerol and to this mixture, 10 mL of surfactants mixture (Tween 80, Span 80 and combinations of Tween 80 and Span 80) was added followed by adding 80 mL of groundnut oil.

100 mL formulation = 1 g conidia + 10 mL glycerol + 10 mL surfactant mixture + 80 mL of groundnut oil.

The formulations were kept undisturbed for 24 h at room temperature (25 to 30°C). The dispersion of the conidia was observed by adding one mL of the formulation to 99 mL of water.

### Evaluation of *B. bassiana* against *T. urticae* under pot culture experiments

Based on the results of the above experiments, *B. bassiana* (MK918495) and its oil formulation D were tested in comparison with chemical standard, fenazaquin 10% EC under pot culture conditions against *T. urticae*.

The experiment was conducted on potted tuberose plants at the Insectary Unit of Department of Agricultural Entomology, TNAU, Coimbatore in a completely randomized design with five treatments and three replications. Mite population was artificially inoculated on potted tuberose plants and a fortnight after inoculation, the following treatments were imposed and spraying was carried out during evening hours using a hand sprayer. The treatment details are as follows:

Two rounds of treatments were imposed at fortnightly intervals with the help of a hand sprayer. The pre and post

Treatments	Details
T1	Groundnut oil based formulation without fungus
T2	Groundnut oil based fungal formulation @ $10^8$ spores $mL^{-1}$
T3	Crude fungal suspension @ $10^8$ spores $mL^{-1}$
T4	Fenazaquin 10 % EC @ 1.5 mL $L^{-1}$
T5	Control (Water spray)

treatment observations on live mite population were assessed on 0, 3, 5, 7, 10 and 14 days after application. Three leaves one each from top, middle and bottom region of each plant were assessed for the live mites on the undersurface in an area of 5 x 2 cm (length x breadth). The population was assessed using 10X hand lens. The observations of two consecutive sprays were pooled and cumulative percent reduction of mite population over control was worked out. Statistical analysis were computed using SPSS version 21.0 software for windows.

## RESULTS AND DISCUSSION

*Tetranychus urticae* became a significant pest of crops because of its capability to develop resistance rapidly against chemical pesticides (Chandler *et al.*, 2005). Therefore, resistance development due to the indiscriminate use of the acaricides and the concerns about public health and environmental issues has necessitated the researchers to unveil the alternative control strategies. Pathogenicity of entomopathogenic fungi viz., *Beauveria bassiana*, *Hirsutella thompsonii* and *Lecanicillium lecanii* against *T. urticae* were reported (Alves *et al.*, 2002). Among the entomopathogenic fungi, the white muscardine fungus, *B. bassiana*, the green muscardine fungus, *Metarhizium anisopliae* and the white halo fungus, *L. lecanii* are in the forefront (Butt *et al.*, 2001; Chavan *et al.*, 2009).

The successful use of entomopathogenic fungi is mainly dependent on their infectivity and persistence in the environment (Moore and Prior, 1993). Use of oil as an active ingredient or carrier material in the formulation had a clear advantage since oil enhances the virulence and persistence nature of the entomopathogenic fungi against insects compared to other formulations (Prior *et al.*, 1988; Bateman *et al.*, 1993).

Key factors which played a major role in the success of any formulation of entomopathogenic fungi included the

proper selection of suitable carrier and additive materials which could enhance the virulence and viability of the spores during the storage period. Since, long term storage without loss in virulence is one of the essential attribute of a biocontrol agent, the present study was carried out to develop oil based formulation of *B. bassiana* (MK918495).

Compatibility of selected *B. bassiana* isolate (MK918495) with two different oils, viz., groundnut and sunflower oil were tested under *in vitro* condition (Figure 1). Experimental results showed significant variation in compatibility of *B. bassiana* (MK918495) with these two oils at different concentration (Table 1). Based on the T-value, groundnut oil was classified as compatible at all concentrations with respect to colony growth and spore yield. The T value for groundnut oil was in the range of 88.10, 79.64, 67.55 and 63.05 at 1, 3, 5 and 10% concentrations respectively and it was found to be compatible (D) with *B. bassiana* MK918495. In contrast, sunflower oil was found to be compatible with *B. bassiana* (MK918495) only at 1% concentration with T value of 60.06 whereas with other concentrations, viz., 3, 5 and 10%, it recorded T value of 55.71, 47.61, 43.47 showing toxicity range from toxic (B) to moderately toxic (C).

With respect to colony growth and spore yield, a decreased trend was observed with increase in concentration of oils. Groundnut oil recorded the highest colony growth of 41.60 mm diameter and spore yield of  $1.8 \times 10^8$  spores  $\text{mL}^{-1}$  at 1% concentration after 15 days of inoculation. At 10% concentration, it recorded the colony growth of 36.90 mm diameter and spore yield of  $1.2 \times 10^8$  spores  $\text{mL}^{-1}$ . Sunflower oil recorded lesser colony growth and spore yield ranging from 31.80 to 27.70 mm diameter and  $1.2 \times 10^8$  spores  $\text{mL}^{-1}$  to  $0.2 \times 10^8$  spores  $\text{mL}^{-1}$  respectively at different concentrations compared to groundnut oil. PDA media without oil recorded colony growth of 42.60 mm diameter and  $2.1 \times 10^8$  spores  $\text{mL}^{-1}$ . Since the media amended with groundnut oil at different concentration and the media without oil were found to be statistically on par, groundnut oil at 1% was used for development of oil in water formulation and groundnut oil at 8% was used for oil based formulations. Compatibility studies conducted revealed that groundnut oil was more compatible with *B. bassiana* (MK918495) over sunflower oil when tested at different concentrations (Figure 4). Colony growth and spore yield were found to decrease with increase in concentration of oil. Gatarayihya *et al.*, (2010) attributed that spore yield and mycelial growth were considered as factors for testing the compatibility of oils with entomopathogenic fungus. The same was proved in present study wherein groundnut oil recorded the maximum colony growth and spore yield indicating its compatibility with *B. bassiana* (MK918495).

Based on the results of studies on compatibility, groundnut oil at 1% was selected and used for developing oil in water formulation. The different surfactants, viz., Tween 80, Span 80 and their combinations were tested in order to get a good emulsion. Wide number of surfactants is available in market and suitable one need to be selected for which HLB systems are useful.

Among the tested combinations of surfactants with groundnut oil and fungal spore, the final product after a period of two hours showed sedimentation (Figure 2) which indicated the formation of poor emulsion (Table 3). Since the aqueous formulations were stable only for a shorter period of time, steps were taken to develop oil based formulation. The necessity for developing formulation other than aqueous was indicated by Kaaya and Hedimbi (2012). They reported that oil formulated conidia were found to provide thermotolerance to the conidia and enhanced its effectiveness even under low humidity.

Accordingly in the present investigation, efforts were made for developing oil based formulation. The success of oil-based formulation mainly depended on the effective combination of emulsifier, dispersion and suspension particles at right proportion. Key factors contributing for a good formulation includes purity and compatibility of the oil, effective surfactant, Hydrophilic-Lipophilic Balance (HLB) and suitable dispersing agent.

Oil based formulation was prepared by dissolving 1 g of pure conidia ( $10^9$  spores  $\text{mL}^{-1}$ ) in 10 mL of glycerol followed by addition of 10 mL of various surfactants, viz., Tween 80, Span 80 and their combinations (Table 2) and 80 mL groundnut oil. Different combinations were observed for sedimentation after 24 hours. Gillespie and Crawford (1986) reported that germination and viability of *B. bassiana*, *M. anisopliae* and *Paecilomyces farinosus* were enhanced by the addition of glycerol under field conditions. In the present study, glycerol was used for the development of oil based formulation along with surfactant and oil. This might have contributed for the better efficacy of oil based fungal formulation against *T. urticae* under pot culture conditions.

Among various combinations, formulation D which contained surfactant mixture having HLB value of 10.00 (53.33% Tween 80 and 46.67% Span 80) formed a better suspension with less sedimentation (Figure 3) compared to other treatments containing different proportion of surfactant mixture (Table 4). Dispersion of conidia in spray fluid was checked by adding one mL of the formulation to 99 mL of water and it was found to be better for formulation D which formed a good emulsion with water.



HLB concept states that the surfactants having low HLB tend to be oil soluble and the one with high values tend to be water soluble. However, this does not always seem to be the same in all situations. In our study, surfactant mixture having high HLB value (10.00) exhibited high solubility which corroborates with the findings of Gadhave (2014) who reported that surfactants with same HLB value may exhibit different solubility characteristics.

Further, formulation D was evaluated for their efficacy under pot culture against *T. urticae* on tuberose in comparison with crude fungal spore suspension and standard chemical check, fenazaquin 10% EC. The plants sprayed with groundnut oil based formulation without fungus did not show any symptoms of phytotoxicity thus ensuring that groundnut oil can be used as an ingredient for developing formulations of entomopathogenic fungi.

Pre-treatment count of *T. urticae* was in the range of 8.84 to 11.52 numbers per plant and was statistically non-significant. After first round of spraying, the standard chemical check, fenazaquin 10% EC (1 mL L<sup>-1</sup>) recorded significantly lowest mite population of 7.12, 4.32, 3.20, 2.62 and 4.72 numbers per plant at 3, 5, 7, 10 and 14 days after treatment with highest population reduction of 69.74% followed by groundnut oil based formulation of *B. bassiana* MK918495 (55.63%) and crude fungal suspension (52.42%). At 14 days after application, fenazaquin recorded significant lowest population of 4.72 numbers per plant followed by groundnut oil based fungal formulation (8.26 numbers/plant) and crude fungal suspension (7.60 numbers/plant) and both were on par with each other. The population of mites were significantly higher in groundnut based oil formulation without fungus (16.25 numbers/plant).

After second round of spraying, fenazaquin recorded significantly lowest population (3.21, 2.63, 2.41, 1.97 and 6.21 numbers/plant at 3, 5, 7, 10 and 14 days after spraying) and highest percent reduction in population (80.16%) followed by groundnut oil based fungal formulation (7.92, 6.29, 4.62, 4.10 and 7.23 numbers/plant) with 74.33% reduction and crude fungal suspension (7.12, 6.23, 5.62, 3.26 and 5.32 numbers/plant) with population reduction of 71.90%. At 14 days after application, fenazaquin recorded significantly lowest population of 6.25 numbers per plant followed by groundnut oil based fungal formulation (7.23 numbers/plant) and crude fungal suspension (5.32 numbers/plant).

At the end of second round of application, groundnut oil based formulation of *B. bassiana* (MK918495) was found to be effective against *T. urticae* with a cumulative mean mite

population of reduction of 64.98% reduction followed by the crude fungal spore suspension (62.16%) and both were statistically on par with each other. However, the standard chemical check recorded the significantly highest population reduction (77.20%). It was observed that the population was reduced significantly upto ten days after each round of spraying and increased thereafter.

The results showed that both the groundnut oil based fungal formulation and the crude fungal suspension were statistically on par with each other and recorded the cumulative mean mite population reduction of 61.18 and 58.00% respectively (Figure 5). Manivannan *et al.*, (2018) reported that *B. bassiana* recorded 48.33% mortality of cotton leafhopper under pot culture experiment. Prithiva *et al.* (2017) reported that oil formulation of *B. bassiana* recorded 45.86% reduction in population of *Bemisia tabaci* under microplot conditions. The better efficacy of formulation over crude suspension was reported by several authors (Agarwal *et al.*, 2012; Alves *et al.*, 2002; Batta, 2003). However, no significant difference was observed in the present investigation. This might be due to spraying of freshly prepared conidial suspension and existence of favorable environment for establishment of EPF. The favorable environment might have contributed for enhanced adhesion, germination and penetration of conidia. However, oil based formulation might prove better than crude suspension under long term storage conditions.

Formulating the entomofungal pathogens in oil enhanced their effectiveness by means of increasing adhesion, spreading the inoculums and probably interfering with the defensive nature of the cuticle thereby facilitating the easy penetration of the germinated spores (Prior *et al.*, 1988) and the same was proved by many authors (Chavan *et al.*, 2009; Gatarayiha *et al.*, 2010; Luz and Batagin, 2005).

Eco-friendly management of mite pest is the need of the hour and the present study supports this concept. *B. bassiana* (MK918495) was found effective against two spotted spider mite, *T. urticae* under *in vitro* condition. Groundnut oil based formulation containing 10<sup>8</sup> spores mL<sup>-1</sup> was found promising under pot culture conditions against *T. urticae* on tuberose. In this study, with two sprays mite population was brought under ETL. Hence further spraying was not taken up. As the crop is perennial, need based application of beauveria can be carried out when mite infestation is observed. Testing the shelf life and stability of the developed oil formulation will add value to the formulation. Further research on temperature and UV tolerance may pave way for developing a commercial formulation with good persistence in the environment.

**Table 1. Compatibility of *Beauveria bassiana* (MK918495) with oil**

Treatments	Concentration of oil (%)											
	1			3			5			10		
	VG	SP	T *	VG	SP	T*	VG	SP	T*	VG	SP	T*
Groundnut oil	41.60 (97.65)	1.80 (85.71)	88.10 (D)	39.80 (93.42)	1.60 (76.19)	79.64 (D)	38.40 (90.14)	1.40 (71.36)	67.55 (D)	36.90 (86.61)	1.20 (57.14)	63.05 (D)
Sunflower oil	31.80 (74.64)	1.20 (57.14)	60.06 (D)	29.40 (69.01)	1.10 (52.38)	55.71 (C)	28.40 (66.67)	0.90 (42.85)	47.61 (C)	27.70 (65.00)	0.80 (38.09)	43.47 (B)
Control	42.60 (100.00)	2.10 (100.00)		42.60 (100.00)	2.10 (100.00)		42.60 (100.00)	2.10 (100.00)		42.60 (100.00)	2.10 (100.00)	

VG – Vegetative growth (mm), SP – Sporulation ( $\times 10^8$  spores mL<sup>-1</sup>)

Figures in parentheses are values relative to control (100%)

\*Classification based on formula:  $T = (20VG + 80SP)/100$ 

A- Very Toxic (T value 0-30), B – Toxic (T value 31- 45), C – Moderately toxic (T value 46-60), D – Compatible (T value &gt;60)

**Table 2. Selection of surfactant combination based on Hydrophilic–Lipophilic Balance (HLB) value for a good emulsion (Oil in water formulation)**

Formulation	HLB value	Tween 80 (%)	Span 80 (%)	Surfactant combination (mL)	Groundnut oil (mL)	Spore suspension (mL)	Description*
A	4.3	-	100	10	10	80	Negligible
B	8	34.57	65.43	10	10	80	Negligible
C	9	43.92	56.08	10	10	80	Negligible
D	10	53.33	46.67	10	10	80	Negligible
E	12	71.96	28.04	10	10	80	Poor
F	14	90.65	9.35	10	10	80	Poor
G	15	100	-	10	10	80	Poor

Quantity of formulation = 100 mL

\*Based on sedimentation

**Table 3. Selection of surfactant combination based on Hydrophilic–Lipophilic Balance (HLB) value for a good emulsion (Oil formulation)**

Formulation	HLB value	Tween 80 (%)	Span 80 (%)	Surfactant combination (mL)	Groundnut oil (mL)	Glycerol (mL)	Description*
A	4.3	-	100	10	80	10	Negligible
B	8	34.57	65.43	10	80	10	Negligible
C	9	43.92	56.08	10	80	10	Negligible
D	10	53.33	46.67	10	80	10	Good suspension
E	12	71.96	28.04	10	80	10	Poor
F	14	90.65	9.35	10	80	10	Poor
G	15	100	-	10	80	10	Poor

Quantity of formulation = 100 mL

Dose of fungus = 1g of pure conidia for 100 mL of formulation ( $10^9$  spores mL<sup>-1</sup>)

\*Based on sedimentation

**Table 4. Pot culture studies on the efficacy of *Beauveria bassiana* (MK918495) against *Tetranychus urticae* on tuberose (First spray)**

Treatments	PTC (No. of mites / plant)	Number of mites / plant at different days after spraying					Mean	Reduction over control (%)
		3	5	7	10	14		
Groundnut oil based formulation without fungus	11.52 (3.84)	12.23 <sup>b</sup> (3.57)	13.02 <sup>c</sup> (3.68)	13.95 <sup>c</sup> (3.80)	14.26 <sup>c</sup> (3.84)	16.25 <sup>c</sup> (4.09)	13.94	4.03
Groundnut oil based fungal formulation	8.84 (3.06)	7.91 <sup>a</sup> (2.77)	6.92 <sup>b</sup> (2.72)	6.23 <sup>b</sup> (2.59)	5.96 <sup>b</sup> (2.54)	8.26 <sup>b</sup> (2.76)	6.45	55.63
Crude fungal suspension	9.83 (3.21)	7.62 <sup>a</sup> (2.85)	7.12 <sup>b</sup> (2.75)	6.21 <sup>b</sup> (2.59)	3.68 <sup>b</sup> (2.03)	7.60 <sup>b</sup> (2.85)	6.91	52.42
Fenazaquin 10% EC (1.5 mL L <sup>-1</sup> )	11.52 (3.46)	7.12 <sup>a</sup> (2.76)	4.32 <sup>a</sup> (2.19)	3.20 <sup>a</sup> (1.89)	2.62 <sup>a</sup> (1.73)	4.72 <sup>a</sup> (2.20)	4.40	69.74
Control	11.12 (3.39)	13.21 <sup>b</sup> (3.69)	13.61 <sup>c</sup> (3.75)	14.01 <sup>c</sup> (3.80)	15.67 <sup>c</sup> (3.94)	16.14 <sup>c</sup> (5.03)	14.53	
SE(d)	-	0.13	0.14	0.20	0.22	0.30	-	-
CD (0.05)	NS	0.29	0.31	0.43	0.48	0.65	-	-

Dose of fungus -  $10^8$  spores mL<sup>-1</sup>PTC- Pretreatment count; Figures in the parentheses are  $\sqrt{x + 0.5}$  transformed values

In a column, means followed by a common letter (s) are not significantly different at P=0.05 by LSD

NS – Non - significant

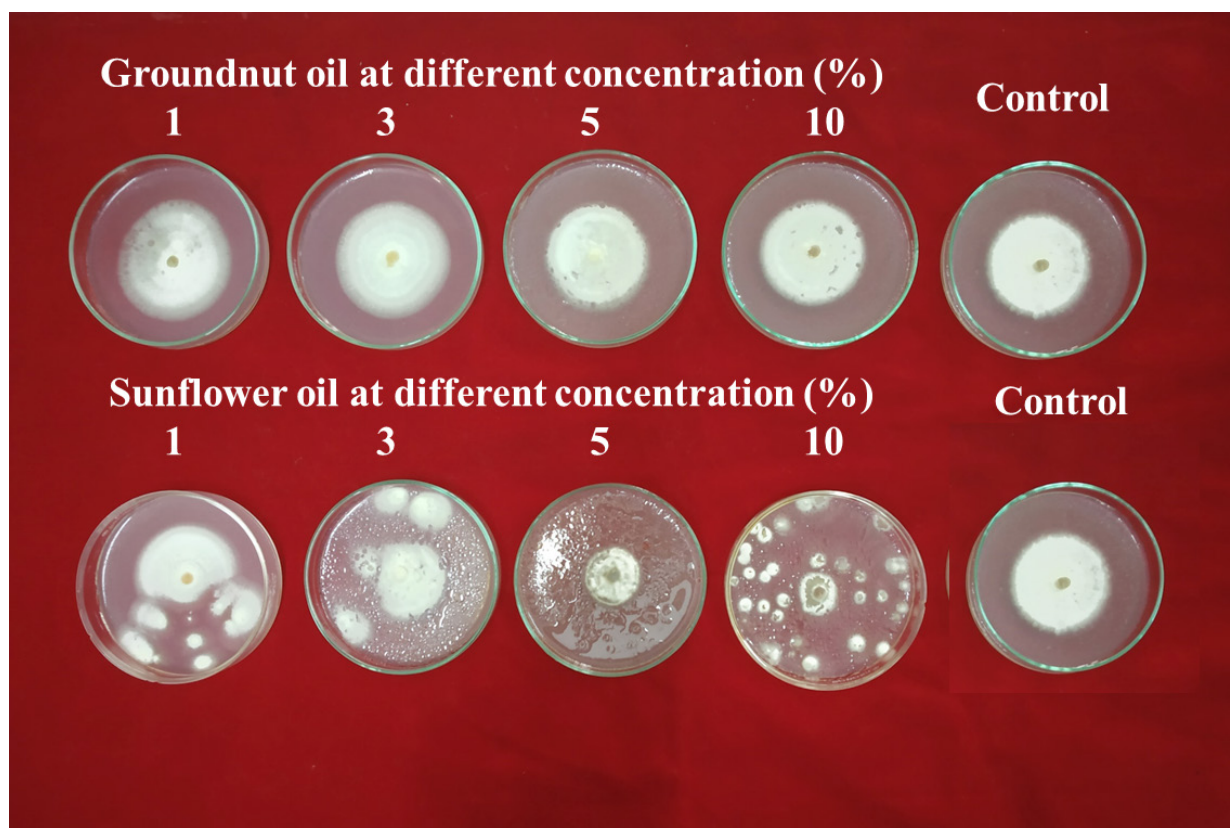
**Table 5.** Pot culture studies on the efficacy of *Beauveria bassiana* (MK918495) against *Tetranychus urticae* on tuberose (Second spray)

Treatments	PTC (No. of mites/plant)	No. of mites/plant at different days after spraying					Mean	Reduction over control (%)	Pooled mean	Cumulative reduction over control (%)
		3	5	7	10	14				
Groundnut oil based formulation without fungus	16.25 (4.09)	17.42 <sup>c</sup> (4.23)	18.46 <sup>c</sup> (4.35)	20.49 <sup>c</sup> (4.58)	22.76 <sup>c</sup> (4.82)	21.46 <sup>c</sup> (4.68)	20.12	6.31	17.03 <sup>c</sup> (4.19)	5.17
Groundnut oil based fungal formulation	8.26 (2.76)	7.92 <sup>b</sup> (2.90)	6.29 <sup>b</sup> (2.61)	4.62 <sup>b</sup> (2.25)	4.10 <sup>b</sup> (2.13)	7.23 <sup>b</sup> (2.77)	5.51	74.33	5.97 <sup>b</sup> (2.64)	64.98
Crude fungal suspension	7.60 (2.86)	7.12 <sup>b</sup> (2.76)	6.23 <sup>b</sup> (2.59)	5.62 <sup>b</sup> (2.46)	3.26 <sup>b</sup> (1.94)	5.32 <sup>b</sup> (2.41)	6.03	71.90	6.42 <sup>b</sup> (2.55)	62.16
Fenazaquin 10 % EC (1.5 mL L <sup>-1</sup> )	4.72 (2.20)	3.21 <sup>a</sup> (1.89)	2.63 <sup>a</sup> (1.74)	2.41 <sup>a</sup> (1.69)	1.97 <sup>a</sup> (1.57)	6.25 <sup>a</sup> (2.57)	3.29	84.65	3.84 <sup>a</sup> (2.08)	77.20
Control	16.14 (4.08)	17.43 <sup>c</sup> (4.23)	19.72 <sup>c</sup> (4.49)	20.73 <sup>d</sup> (4.61)	23.75 <sup>d</sup> (4.92)	25.73 <sup>d</sup> (5.12)	21.47		18.00 <sup>c</sup> (4.30)	
SE(d)	-	0.18	0.18	0.18	0.16	0.10	-	-	0.19	
C.D. (0.05)	NS	0.41	0.41	0.39	0.35	0.22	-	-	0.41	

Dose of fungus - 10<sup>8</sup> spores mL<sup>-1</sup>PTC- Pretreatment count; Figures in parentheses are  $\sqrt{x + 0.5}$  transformed values

In a column, means followed by a common letter (s) are not significantly different at P = 0.05 by LSD

NS – Non - significant

**Fig 1.** Compatibility of *Beauveria bassiana* (MK918495) with different concentrations of groundnut and sunflower oil



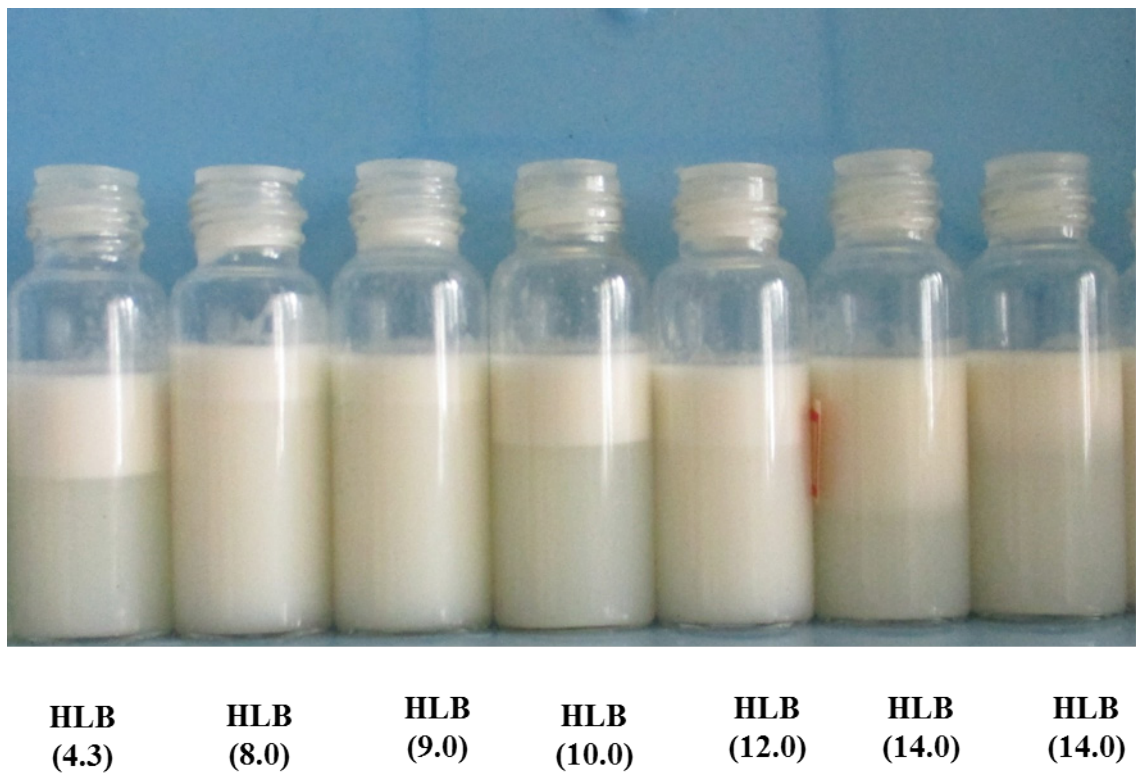


Fig 2. Oil in water formulation of *Beauveria bassiana*(MK918495)

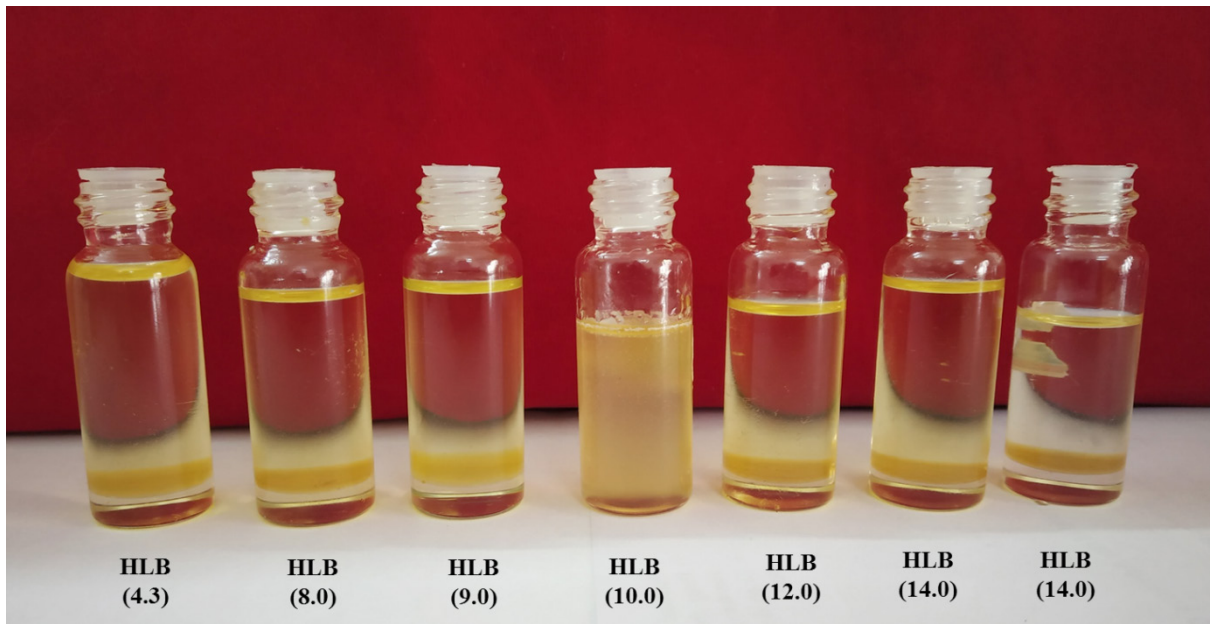


Fig 3. Oil based formulation of *Beauveria bassiana*(MK918595)

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