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DETERMINATION OF LETHAL CONCENTRATION AND LETHAL TIME OF ENTOMOPATHOGEN *Beauveria bassiana* (BALSAMO) VUILLEMIN AGAINST *Tetranychus urticae* KOCH

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Abstract- The present investigation was undertaken to determine the lethal concentration and lethal time of entomopathogenic fungus *Beauveria bassiana* against the two spotted spider mite *Tetranychus urticae*. Higher mortality percentage was observed in Direct Spray Bioassay for eggs, larvae, nymphs and adults as compared to Treated Food Bioassay. Mortality data fitted well to the probit mode as indicated by the small χ^2 values. The LC_{50} values in direct spray bioassay were 3.0×10^5, 1.4×10^6, 0.4×10^7 and 0.3×10^8 conidia ml^{-1} for eggs, larvae, nymphs and adults, respectively. The same values in treated food bioassay were 1.3×10^8, 5.1×10^9, 1.0×10^{10} and 2.6×10^{11} conidia ml^{-1}. This suggested that eggs and larvae were more susceptible to *B. bassiana* treatment than nymphs and adults of *T. urticae*. The LT_{50} values for eggs, larvae, nymphs and adults were 108.52, 52.12, 75.74 and 122.43 h, respectively in Direct Spray Bioassay and 155.09, 81.55, 86.13 and 141.53 h, respectively in Treated Food Bioassay. Lower LT_{50} values were obtained for larvae, followed by nymphs and adults. Direct Spray Bioassay is preferred over treated Food Bioassay on the basis of lower LC_{50} and LT_{50} values for *T. urticae* life stages.

Keywords- bioassay, entomopathogen, *Beauveria bassiana*, *Tetranychus urticae*, LC_{50}, LT_{50}

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Introduction

The two-spotted spider mite (TSSM), *T. urticae* is a member of the family Tetranychidae that contains many harmful species of plant-feeding mites. It was first described by Koch in 1836 and thought to originate from temperate climates [1]. It is a ubiquitous and economically important agricultural pest with a global distribution which feeds on a wide range of host plant species throughout the world [2]. *T. urticae* is known to attack about 1200 species of plants, of which more than 150 are economically important [3]. Pest status of *T. urticae* on greenhouse vegetables, ornamental and horticultural crops is well documented worldwide [4]. Short generation time, high fecundity and webbing by *T. urticae* are responsible for causing loss in number of fruits and yield in various crops. The major principles behind yield loss due to spider mite infestation in various crops have been established as biomass reduction, disturbance of water conduction, dry matter partitioning, CO_2 gas exchange, chlorophyll reduction and shedding of immature flowers [5,6]. The leaves infested by mites show white specks first which later coalesce and turn the leaf to rust red which later fall off to the ground. The reduction in leaves affecting the photosynthetic rate in turn reduces the number of fruits and yield [6]. Unfortunately, *T. urticae* is one of the most striking examples of polyphagy among herbivores with an unmatched ability to develop resistance to pesticides. Many aspects of the biology of this mite such as rapid development, high fecundity and haplo-diploid sex determination seem to facilitate rapid evolution of pesticide resistance [7].

Currently, great efforts are directed towards reduction in the use of traditional pesticides and increase in the use of Integrated Pest Management (IPM) strategies. Bio-pesticides based on microbes like bacteria, viruses, entomopathogenic fungi and nematodes offer considerable scope as protection agents against several insects and mites [8]. Microbial insecticides have some striking advantages in contrast to chemical insecticides. They tend to be host specific, safe, and have no toxic residues. They also ensure the survival of natural enemies and unlikely to stimulate resistance in target pests. Some microbial insecticides are compatible with chemical insecticides and can often be used in combination with them. These fungi invade the host by growing through the external cuticle, which is important for pests with sucking mouthparts such as, two spotted spider mite; can be formulated as myco-pesticides suitable for spraying using conventional chemical spraying equipment and are less harmful to non-target arthropods and mammals and thus ideal for integrated pest management (IPM) strategies [9]. The entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin is a ubiquitous pathogen of many pest arthropods such as aphids, leafhoppers, and whiteflies whose repeated fungal application has been found to control certain arthropod pests [10]. Thus, the present investigation was planned to determine the lethal concentration (LC_{50}) and lethal time (LT_{50}) of *B. bassiana* (strain Hb) against *T. urticae in vitro*

Material and Methods

The present investigation was chosen to foster the use of environmentally safe bio-control agents for the management of phytophagous pests and was carried out in Acarology laboratory in Department of Zoology, CCS HAU, Hisar during 2010-11.

Mite Culture

Two spotted mite, *T. urticae* were reared and maintained on potted okra plants (var. Varsha Uphar) in the screen house of the department since 2007. The copulating pairs of mites (10 pairs/ plant) picked from already maintained cultures on unsprayed infested plants were released on the potted plants at 5-6-leaf stage. After 10 -12 days of infestation, population increased to suppress the carrying capacity of plant. At this stage, a pot carrying uninfested plant was placed near the infested plant. Within 24 hours mites migrated from infested to uninfested plant.

Culturing of Entomopathogenic Fungus, *Beauveria bassiana*

Pure mother culture of the entomopathogenic fungus, *B. bassiana* (strain Hb- Hyderabad), isolated from *Helicoverpa armigera* was acquired from Dr. Vimala Devi, Biocontrol laboratory, Directorate of Oilseed Research, Hyderabad and maintained on Potato Dextrose Agar slants in the laboratory. Regular passaging and maintenance was done for further multiplication at 27±2ºC, > 90 % RH and 16: 8 (Light: Dark) photoperiod. Pure mother cultures in slants were stored under refrigerated conditions till further use.

Preparation of *Beauveria bassiana* Suspension

Aqueous conidial suspensions (100 ml) were made from conidia harvested from the slants prepared in conical flasks (250 ml) after 14 days of inoculation. Tween 80 (0.02%) was used to disperse the conidia. After the conidia come into the solution completely, it was filtered through a double layered muslin cloth. The number of conidia per ml was enumerated using haemocytometer. Highest required concentration (1×10^{12} conidia ml^{-1}) was prepared initially by adding some more spores to fungal suspension. This filtrate was treated as the stock solution and further lower concentrations (upto 1×10^{5} conidia ml^{-1}) were prepared from it by serial dilution technique.

Efficacy of *B*eauveria *bassiana* against *Tetranychus urticae* using Bioassay

Laboratory bioassays with varying concentrations of *B. bassiana* were carried out against *T. urticae* using Leaf-Disc Technique in a complete randomized block design. The bed was circled with moist cotton pad to prevent the escape of mites. Fresh uninfested leaf was plucked from the okra plant and washed with distilled water to eliminate the dust particles and infestation, if any. A disc (2cm dia.) was cut from this leaf and kept on the moist cotton pad in the centre of the Petri plate. Various dilutions (1×10^{5} to 1×10^{12} conidia ml^{-1}) of *B. bassiana* suspension were tested using Direct Spray and Treated Food Bioassay based on [10] for each stage (eggs, larvae, nymphs and adults) of *T. urticae*.

Direct Spray Bioassay

The leaf-disc with 20 eggs of *T. urticae*, as described above, was sprayed with *B. bassiana* @ 1×10^{12} conidia ml^{-1} with the help of a hand atomizer (0.2 mm dia.) containing 2ml suspension. The disc was air dried and then placed on moist cotton bed in Petri plate and covered. Likewise, different concentrations of fungus suspension were sprayed separately on leaf discs, each with 20 eggs. A total of eight *B. bassiana* concentrations were used in the experiment. Simultaneous control treatments, with sterile distilled water sprayed leaves and distilled water + Tween 80 (0.02%) were maintained. Separate such sets were prepared for larvae, nymphs and adults of *T. urticae*. Each treatment was replicated five times and each replication had 20 individuals of the appropriate test stage. The petri plates were incubated at 27±2ºC and 90 percent relative humidity in the BOD incubator.

Treated Food Bioassay

The leaf-discs (2cm dia.) were dipped in the required concentration of the fungus suspension (2ml) for two minutes, and allowed to dry on a filter paper for half an hour. The treated leaf-discs were placed on the moist cotton pad and kept in Petri plates. Twenty larvae of *T. urticae* were released on each of the five treated leaf-discs and Petri plates were covered. Separate sets were prepared for nymphs and adults of *T. urticae*. Each mite stage was evaluated against eight concentrations of *B. bassiana* and compared with two controls, *viz.,* distilled water-treated and distilled water + Tween 80 (0.02%) treated leaf-discs. Each treatment had five replications with 20 test stage/ replication. Petri plates used in this bioassay were kept under optimum conditions (27±2ºC and 90 % RH).

Assessment of Mite Mortality in Bioassays

Post-treatment observations were recorded daily (every 24h) and continued till the appearance of the next developmental stage or mortality in the test stage. In the case of eggs, observations were continued till all the eggs hatched in distilled water-treated control. Unhatched eggs were considered as dead. Observations on the number of eggs hatched were continued till 4 days. The number of motile stages of the mite (larvae, nymphs and adults) on each leaf-disc was counted after every 24h under a stereozoom microscope and compared with two control treatments. Before considering the mite stage as dead, it was probed lightly with the help of bird's feather pick to detect any movement. Live mites were considered as those that were mobile whereas, immobile mites failing to respond with leg movement were considered dead. All the dead mites, removed from the Petri plates, were kept in an incubator and the cadavers showing mycosis were considered to be killed as a result of infection by the fungus following Hall [11]. The observed mortality was converted into percent mortality and corrected mortality was obtained after deducting the mortality in control treatments. The observed mortality was converted into percent mortality and corrected mortality was obtained after deducting the mortality in control treatments. The observed data was used for the calculation of LC_{50} and LT_{50}.

Calculations and Statistical Analysis

The standard statistical tools were used for analysis of data recorded in different experiments.

The per cent mortality of mites was calculated by the following formula

$$\text{Per cent Mortality} = \frac{\text{Number of dead mites}}{\text{Total number of initial mites}} \times 100$$

Corrected per cent mortality was worked out after adjusting with the mortality in control using formula given by Abbott [12] and subjected to Probit analysis [13]. Data was generated for estimation of LC_{50} and LT_{50} values.

The corrected percent mortality was calculated by using the following formula:

$$\text{Corrected Percent Mortality} = \frac{P_t - P_c}{100 - P_c} \times 100$$

where,

P_t= Observed mortality in treatment;

P_c= Observed mortality in control

The procedure described above was used for the determination of LC_{50} and LT_{50}.values. Log concentration, probability regression including a control mortality correction as an offset for natural mortality [12] was estimated using Probit analysis. The LC_{50} i.e. lethal concentration required for 50 percent mortality of the test stage of the mite and LT_{50} i.e. Lethal time required for 50 percent mortality of the test stage of the mite were thus calculated. The statistical significance of data was assessed by two factorial analysis of variance (ANOVA). Critical difference (CD) was calculated between the treatments using Software 'OPSTAT', developed at the Computer Centre, College of Basic Sciences and Humanities, CCS Haryana Agricultural University, Hisar. For comparison of treatments Critical Difference (CD) was calculated at 5 percent level of significance.

Results

An analysis has been conducted to study the comparative efficacy of *B. bassiana* against different mite stages using both bioassays [Table-1], [Table-2]. Higher mortality percentage was observed in Direct Spray Bioassay for larvae, nymphs and adults as compared to Treated Food Bioassay [Table-1]. Mortality in terms of lower number of hatchings showed that eggs were also affected by the *B. bassiana* treatments [Table-2]. Among mobile stages, mortality decreased with increase in age of mite with larvae being the most susceptible to fungal infection than nymphs and adults [Table-1]. However, the difference between nymphs and adults at higher *B. bassiana* concentration (1×10^{12} conidia ml^{-1}) was negligible in Direct Spray Bioassay.

Determination of Concentration- Mortality Response (LC_{50})

LC_{50} values along with regression statistics were calculated using standard probit analysis method [Fig-1], [Fig-2] and are presented in [Table-3]. Mortality data fitted well to the probit mode as indicated by the small χ^2 values. The LC_{50} values in direct spray bioassay were 3.0×10^5, 1.4×10^6, 0.4×10^7 and 0.3×10^8 conidia ml^{-1} while in treated food bioassay these were 1.3×10^8, 5.1×10^9, 1.0×10^{10} and 2.6×10^{11} conidia ml^{-1} for eggs, larvae, nymphs and adults, respectively [Table-3]. The present study showed that direct spray bioassay was more effective because of lower LC_{50} values. The dose response for *B. bassiana* and mite mortality differed with the mite stage. LC_{50} value for eggs was the lowest followed by larvae, nymphs and adults. This suggested that eggs and larvae were more susceptible to *B. bassiana* treatment than nymphs and adults of *T. urticae*. The values of slope of regression lines were less than four probits for all the mite stages in both bioassays except for adults in treated food bioassay. This showed that in former case, an increase in concentration of *B. bassiana* would result in proportionately small increase in mortality of *T. urticae* while in the latter case, a slight increase in concentration of *B. bassiana* would lead to higher mortality in adults of *T. urticae*.

Table 1- Bioassay of *Beauveria bassiana* against mobile stages of *Tetranychus urticae in vitro*

Treatments (conidia ml^{-1})	Mortality (%)					
	Direct Spray			Treated Food		
	Larvae	Nymphs	Adults	Larvae	Nymphs	Adults
1×10^{12}	94 (77.43)^{a}	92 (73.74)^{a}	91 (72.87)^{a}	66.25 -60.06	64 -53.19	58.5 -53.19
1×10^{11}	90 (72.11)^{a,b}	88 (70.14)^{a,b}	86 (67.48)^{a,b}	62.25 (53.23)^{a}	57.5 (48.43)^{a}	52.75 (46.13)^{a}
1×10^{10}	87 (69.27)^{b,c}	84 (67.07)^{b,c}	83 (65.86)^{b}	60.6 (51.38)^{a}	56.75 (47.28)^{a,b}	52.25 (46.13)^{a}
1×10^{9}	80 (63.77)^{c,d}	77 (61.46)^{c}	70.5 (58.76) ^{c}	55.75 (45.55)^{b,c}	51 (46.13)^{a,b}	49.2 (43.83)^{a,b}
1×10^{8}	69 (56.73)^{d,e}	65 (53.81)^{d}	64 (53.23)^{c,d}	50 (48.45)^{a,b}	45 (43.25)^{b,c}	40.5 (39.74)^{b,c}
1×10^{7}	66 (54.38) ^{e}	61 (51.38)^{d}	59 (50.23)^{d,e}	47 (42.67)^{c,d}	43 (40.32)^{c,d}	39 (38.58)^{c}
1×10^{6}	60 (50.81)^{e,f}	57 (49.03)^{d}	50 (44.98)^{e,f}	42.85 (40.83)^{c,d}	40.25 (37.36)^{d}	37.25 (38.00)^{c}
1×10^{5}	51 (45.56)^{f}	46 -42.66	42 (40.34)^{e,f}	41.5 (39.73)^{d}	38.75 (38.00)^{d}	34.1 (35.75)^{c}
Control (Water+ Tween)	16 (23.41) ^{g}	14 (21.74)^{e}	12 (20.17)^{g}	14 (21.04)^{e}	11 (16.22)^{e}	8 (15.12)^{d}
Control (Water alone)	11 (19.06)^{g}	9 (17.09)^{e}	10 (18.52)^{g}	13 (21.79)^{e}	8 (19.06)^{e}	7 (16.22)^{d}
SE (m)	2.49	2.04	2.31	1.97	1.62	1.63
CD (p= 0.05)	7.17	5.87	6.62	5.64	4.65	4.67

Figures in parentheses are angular transformed values

Figures denoted by same superscript do not differ significantly

Table 2- Bioassay of *Beauveria bassiana* against Eggs of *Tetranychus urticae in vitro*

Treatments (conidia ml^{-1})	Pre treatment count	Average Number of hatchings after 96 hrs.	
		Direct Spray	Treated Food
1×10^{12}	20	2.00 (8.02)	8.80 (17.19)
1×10^{11}	20	3.00 (9.91)	9.80 (18.20)
1×10^{10}	20	5.00 (12.89)	10.40 (18.73)
1×10^{9}	20	5.20 (13.14)	12.40 (20.59)
1×10^{8}	20	9.00 (17.42)	13.20 (21.27)
1×10^{7}	20	11.80 (20.07)	11.80 (20.07)
1×10^{6}	20	14.00 (21.95)	12.40 (20.59)
1×10^{5}	20	15.20 (22.91)	13.40 (21.45)
(Water+ Tween 80) (Water alone)	20	18.60 (25.53)	18.80 (25.68)
Control (Water alone)	20	18.60 (25.53)	19.00 (25.83)
Mean		10.24 (17.73)	13.00 (20.95)

CD (Direct Spray) (p= 0.05) for Treatment (T) = (1.15); Duration (D) = (0.72); T x D = (2.29)

CD (Treated Food) (p= 0.05) for Treatment (T) = (1.10); Duration (D) = (0.69); T x D = (2.21)

Figures in parentheses are angular transformed values

Figures denoted by same superscript do not differ significantly

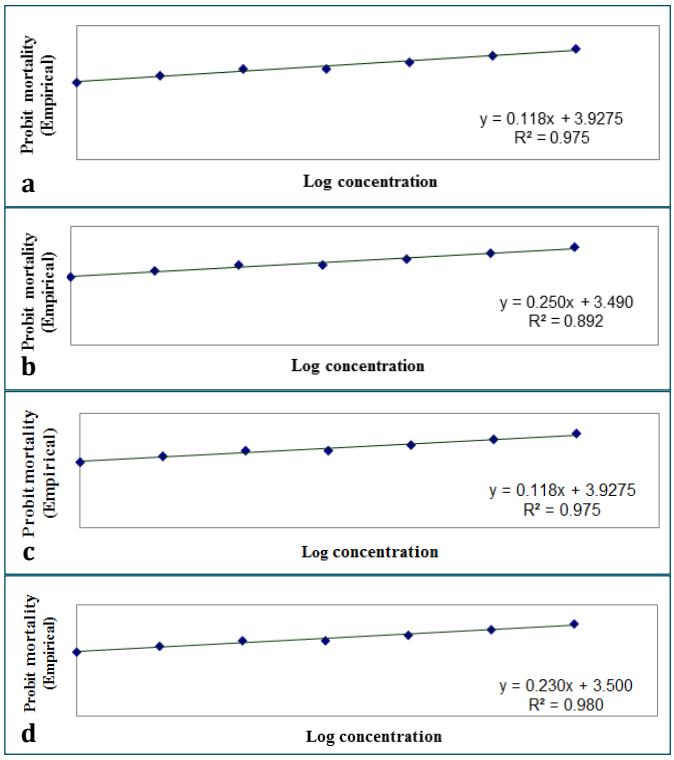

Fig. 1- Probit Analysis of Concentration- Mortality Response (LC_{50}) of *Beauveria bassiana* (strain Hb- Hyderabad) against Various *Tetranychus urticae* Stages using Direct Spray Bioassay [a: Eggs; b: Larvae; C: Nymphs; d: Adults]

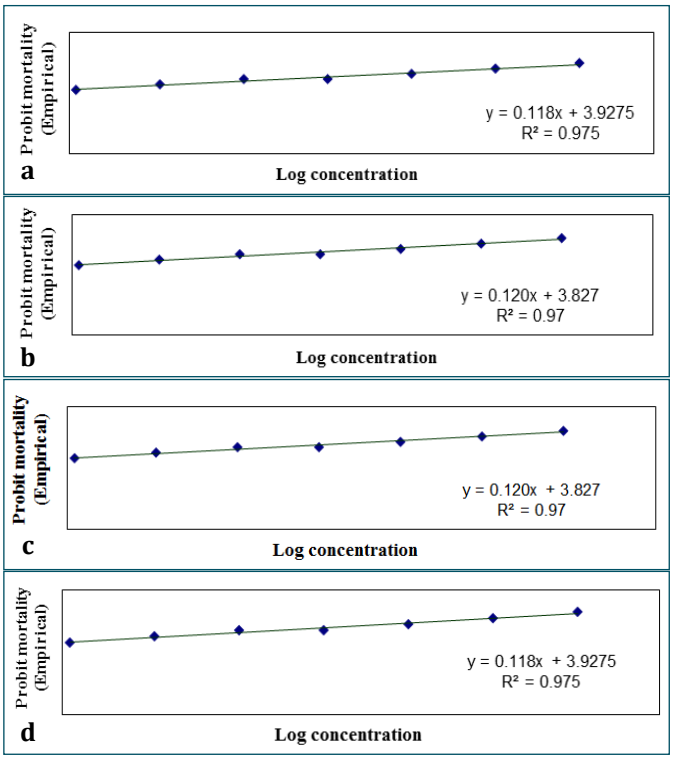

Fig. 2- Probit Analysis of Concentration- Mortality Response (LC_{50}) of *Beauveria bassiana* (strain Hb- Hyderabad) against *Tetranychus urticae* Stages using Treated Food Bioassay [a: Eggs; b: Larvae; C: Nymphs; d: Adults]

Table 3- LC_{50} of *Beauveria bassiana* against Different Stages of *Tetranychus urticae*

Mite stages	Direct Spray Bioassay					Treated Food Bioassay				
	n	χ2	df	Slope±SE	LC_{50} (conidia ml^{-1})	n	χ2	df	Slope±SE	LC_{50} (conidia ml^{-1})
Eggs	20	0.29	6	3.72± 0.23	3.0×10^{5}	20	0.1	6	3.87± 0.12	1.3×10^{8}
Larvae	20	1.62	6	3.53± 0.24	1.4×10^{6}	20	0.13	6	3.88± 0.11	5.1×10^{9}
Nymphs	20	0.39	6	3.44± 0.23	0.4×10^{7}	20	0.26	6	3.90± 0.10	1.0×10^{10}
Adults	20	0.15	6	3.41± 0.22	0.3×10^{8}	20	-2.02	6	4.08± 0.08	2.6×10^{11}

Table 4- LT_{50} of *Beauveria bassiana* against Different Stages of *Tetranychus urticae*

Mite stages	Direct Spray Bioassay					Treated Food Bioassay				
	n	χ2	df	Slope±SE	LT_{50} (h)	n	χ2	df	Slope±SE	LT_{50} (h)
Eggs	20	1.53	5	2.33±0.59	108.52	20	1.55	5	2.165±0.03	155.09
Larvae	20	6.52	4	1.69±0.10	52.12	20	4.46	4	1.638±0.09	81.55
Nymphs	20	5.34	4	1.82±0.06	75.74	20	4.65	4	1.638±0.05	86.13
Adults	20	5.34	4	2.16±0.08	122.43	20	12.58	2	1.766±0.12	141.53

Determination of Time- Mortality Response (LT_{50})

Probit analysis of the time- mortality response of mite stages of *T. urticae* to *B. bassiana* showed that eggs were almost two times more susceptible to various treatments when compared to larvae in treated food as well as direct spray bioassay. The results are presented in [Table-4] which was derived from the graphs [Fig-3], [Fig-4]. The LT_{50} values for eggs, larvae, nymphs and adults were 108.52, 52.12, 75.74 and 122.43 h, respectively in Direct Spray Bioassay and 155.09, 81.55, 86.13 and 141.53 h, respectively in Treated Food Bioassay [Table-4]. Lower LT_{50} values were obtained for larvae, followed by nymphs and adults. Regression statistics showed that the values of slopes of regression lines were less than four probits for all the mite stages in both bioassay methods.

The data on the comparison of lethal time (LT_{50}) in different bioassays are depicted in [Fig-5] which revealed superiority of Direct Spray method over Treated Food method. In all the *T. urticae* stages lower LT_{50} values were obtained in Direct Spray (108.52, 52.12, 75.74 and 122.43 h) as compared to Treated Food Bioassay (155.09, 81.55, 86.13 and 141.53 h), respectively for eggs, larvae, nymphs and adults.

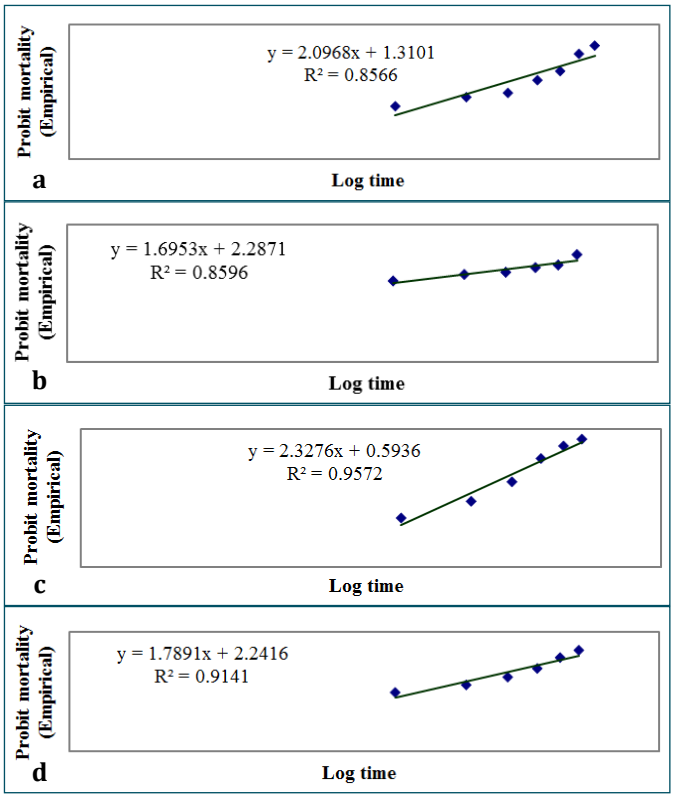

Fig. 3- Probit Analysis of Time- Mortality Response (LT_{50}) of *Beauveria bassiana* (strain Hb- Hyderabad) against *Tetranychus urticae* Stages using Direct Spray Bioassay [a: Eggs; b: Larvae; C: Nymphs; d: Adults]

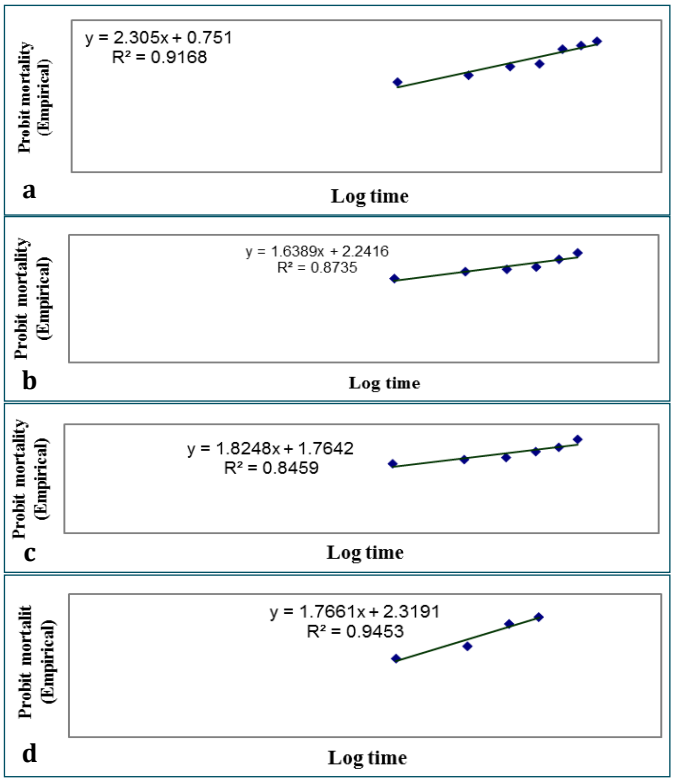

Fig. 4- Probit Analysis of Time- Mortality Response (LT_{50}) of *Beauveria bassiana* (strain Hb- Hyderabad) against *Tetranychus urticae* Stages using Treated Food Bioassay [a: Eggs; b: Larvae; C: Nymphs; d: Adults]

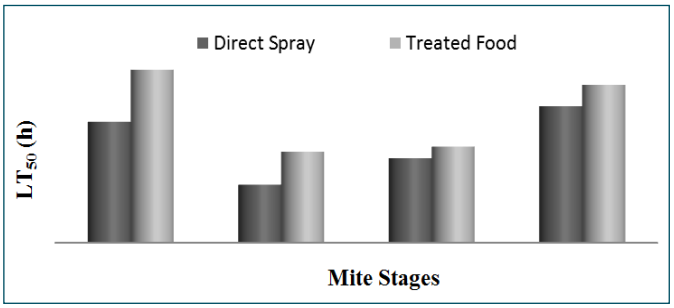

Fig. 5- LT_{50} of *Beauveria bassiana* (strain Hb- Hyderabad) against Various Stages of *Tetranychus urticae* using Different Bioassays

Discussion

The two types of bioassays i.e. Direct Spray and Treated Food Bioassay were used for the *in vitro* studies in the present investigation. Direct Spray Bioassay was found to be superior over Treated Food Bioassay in terms of lower LC_{50} and LT_{50} values. In present study, LC_{50} values in topical application (direct spray) was 3.0×10^5 to 0.3×10^8 conidia ml^{-1} in *T. urticae* stages i.e. eggs, larvae, nymphs and adults as compared to 1.3×10^8 to 2.6×10^{11} conidia ml^{-1} *B. bassaina* concentration under treated food bioassay. The percent mortality for eggs, larvae, nymphs and adults were 42 to 94 and 34.1 to 66.25 percent in Direct Spray and Treated Food bioassay, respectively. Hence, Direct Spray is considered as better method for conducting bioassay studies. Infection through the mouth parts has been reported by Veen [14] and Siebeneicher *et al*. [15]. Such infection could not be ruled out in the present study, as a portion of conidia may have attached to the mouth parts during feeding as the mode of action of the *B. bassiana* is through contact. Similar results on botanicals honey bee mites [16] and insects [17] were reported. The advantage of topical application technique is that the actual dose causing mortality in the different species or different life stages of a single species can be determined and compared [18]. Shi *et al*. [19] termed Treated Food bioassay as 'absorption test' and mortality caused in this bioassay was reported as systemic toxicity.

The lethal concentration required for the 50 percent mortality (LC_{50}) of the *T. urticae* was 3.0×10^5, 1.4×10^6, 0.4×10^7 and 0.3×10^8 conidia ml^{-1} for eggs, larvae, nymphs and adults, respectively in Direct Spray Bioassay while for the adults LC_{50} value of 0.3×10^8 conidia ml^{-1} was recorded in the present investigation. The same values in Treated Food Bioassay were 1.3×10^8, 5.1×10^9, 1.0×10^{10} and 2.6×10^{11} conidia ml^{-1} of *B. bassiana*. Lower LC_{50} values in Direct Spray Bioassay method indicated the higher toxicity of *B. bassiana* concentrations to *T. urticae*. The results were in agreement with Tamai *et al*. [20] who noted that conidia, blastospores and yeast-like cells of five isolates of *B. bassiana* were pathogenic against the *T. urticae* and LC_{50} values ranged from 4.95 to 82.1 $\times10^6$ cells ml^{-1}. Similarly, Irigaray *et al*. [21] evaluated the efficacy of Naturalis-L® (a *B. bassiana*-based commercial biopesticide) against the two-spotted spider mite and obtained lethal concentration values (LC_{50}) of 3184 viable conidia $ml^{-1}$for the juvenile stages and 1949 viable conidia $ml^{-1}$for the adults. With 17 isolates of *M. anisopliae* and two isolates of *B. bassiana* against *T. evansi*, Wekesa *et al*. [22] obtained LC_{50} values in the range of 0.7×10^7 and 2.5×10^7 conidia ml^{-1} which were also within the range observed in the present study. In another study, *B. bassiana* was found to be more effective (LC_{50} = 4.3×10^6 conidia ml^{-1}) than *Cladosporium cladosporioides* (LC_{50} = 5.27×10^6 conidia ml^{-1}) against *T. urticae*. Gatarayiha *et al*. [24] also reported LC_{50} values of 1.13 to 2.36 $\times10^5$ conidia ml^{-1} against *T.*

urticae on *Phaseolus vulgaris.*

During present study, the LT_{50} values for eggs, larvae, nymphs and adults were 108.52, 52.12, 75.74, 122.43 and 155.09, 81.55, 86.13, 141.53 h in Direct Spray Bioassay and Treated Food Bioassay, respectively. Tamai *et al*. [20] reported less values of LT_{50} (between 1.3 and 1.4 days) of *B. bassiana* against *T. urticae* as compared to our study. Wekesa *et al*. [22] obtained LT_{50} values in the range of 4.6 to5.8 days against *T. evansi*. Jeyarani et al. [23] noticed LT_{50} values of 63.80 and 110.30 h, respectively against *T. urticae* with *B. bassiana* and *Cladospoirum cladosporioides* fungi. Gatarayiha *et al*. [24] reported LT_{50} values of 5.5 to 8.6 days against *T. urticae* with 2×10^7 conidia ml^{-1} concentration. Lower LT_{50} values reported by above researchers indicated the rapid infection of the mite with the fungus, which is an important feature for selecting fungal strains as potential biocontrol agents. Thus, we conclude that Direct Spray Bioassay is preferred over Treated Food Bioassay on the basis of lower LC_{50} and LT_{50} values for *T. urticae* life stages.

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