

Bioefficacy of geographical isolates of *Beauveria bassiana* for the management of *Paracoccus marginatus* infesting tapioca under field condition

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Abstract

To evaluate the bio-efficacy of various *B.bassiana* local isolates against *P. marginatus* in tapioca, two field trials were conducted during May - June, 2013 in tapioca crop (Var: Mulvadi) at farmer holding field, Onnipalayapudhur and Mathampalayam, near Coimbatore. The experiments were conducted in a Randomized Block Design (RBD) consisting of eight treatments including an untreated control which was replicated thrice with plot size of 5×4m². The dose of *B.bassiana* tested was 1×10⁸ spore/ ml concentration. The experimental results showed that at 10 days after first round of spraying, the highest mean per cent reduction in the mealybug population was 32.20% due to the application of UPI(Bb). After second round of spraying, the mean per cent reduction of population observed in UPI(Bb) was 56.98 per cent at Onnipalayapudhur. Whereas the mean per cent reduction of mealybug population after first round of spraying in Mathampalayam was 41.95% observed in UPI (Bb) treated plot. After second round of application, the mean per cent reduction of mealybug was recorded 72.76% in UPI(Bb).

Highlights

- The experiments consisting of eight treatments including an untreated control which was replicated thrice with plot size of 5×4m².
- The dose of *B.bassiana* tested was 1×10⁸ spore/ ml concentration.
- The highest mean per cent reduction in the mealybug population was 32.20% due to the application of UPI(Bb) after first spraying.
- After second round of spraying, the mean per cent reduction of population observed in UPI(Bb) was 56.98% at Onnipalayapudhur.
- The mean per cent reduction of mealybug population after first round of spraying in Mathampalayam was 41.95% observed in UPI (Bb) treated plot.
- After second round of application, the mean per cent reduction of mealybug was recorded 72.76% in UPI(Bb).
- Experiment results shows that UPI(Bb) isolate is highest efficacy against *P. Marginatus*.

Keywords: *Paracoccus marginatus*, tapioca, bioefficacy, *Beauveria bassiana*, microbial control

Papaya mealybug, *Paracoccus marginatus* (Williams and Granara de Willink), a pest of recent origin threatening the cultivation of papaya by causing extensive yield losses and has the ability to infest several field, fruit, ornamental plants and weeds. The papaya mealybug is believed to be the native of Mexico or Central America and was first described

in 1992 by Williams and Granara de Willink and re-described in 2002 by Miller and Miller. Papaya mealybug infestations are typically observed as clusters of cotton-like masses on the above-ground portion of plants. Colonization of mealybugs on papaya has been noted along the veins and the midribs of the older leaves and all areas of tender



leaves and fruits (Walker *et al.*, 2003). Papaya mealybug is a polyphagous pest that can damage a large number of economically important field crops, tropical and sub-tropical fruits, vegetables and ornamental plants. It has caused an estimated loss of about ₹ 300 crores in each state (Anonymous., 2010). It became serious pest of papaya in Tamil Nadu and nearly 820 ha in Erode and 576 ha in Coimbatore had been affected. Severely affected older leaves turn yellow and dry up. Tender leaves become bunched and distorted (Revathy, 2010). Heavy mealybug populations produce a large volume of honey dew, which causes black sooty mould on the infested fruits and vegetation (Meyerdirk *et al.*, 2004). *P. marginatus* feeds on the sap of plants by inserting its stylets into the epidermis of the leaf, fruit and stem, injects a toxic substance resulting in chlorosis, plant stunting, leaf deformation, early leaf and fruit drop, development of sooty mould on honey dew and death (Sakthivel, 2011; Regupathy and Ayyasamy, 2010).

Considering the adverse effect of insecticides, pest management through biological control is encouraged using parasite, predator, and pathogen. Among the many entomopathogenic fungi, *B. bassiana* is exploited in greenhouse and outdoor crops as tool for the management of papaya mealybug (Shah and Goettel, 1999). Hence a study was conducted to evaluate the field bioefficacy of *B. bassiana* against papaya mealybug infesting tapioca.

Material and Methods

Preparation of spore concentration of the fungal isolates

The fungal isolates were cultured in 2000 ml conical flask on yeast molasses medium composed of 5 gm of yeast, 30 gm of molasses and one litre of distilled water, incubated at room temperature for 10 days and ground into liquid spore suspension (plate 1). This was filtered through double layered muslin cloth to remove the mycelial mat. The suspension was shaken thoroughly with a drop of Teepol solution for uniform dispersion of the spores in the solution (Saranya *et al.*, 2010). The spore conidia count was determined by a double ruled Neubaur haemocytometer using phase contrast microscope (Goettel and Inglis, 1997). The suspension was filtrated through a double layer of muslin cloth and

the desired concentration for field application was obtained by the addition of sterile distilled water. Total spores were counted before application in the field using a haemocytometer.



Plate 1: Growth of *B. bassiana* on yeast molasses broth

To evaluate the bio-efficacy of various *B. bassiana* isolates of local strains against *P. marginatus* on tapioca, two field trials were conducted during May - June, 2013 in tapioca crop (Var: Mulvadi) at farmer holding field, Onnipalayapudhur and Mathampalayam, near Coimbatore (plate 2).



Plate 2: Infested tapioca plant with *P. marginatus*

The experiments were conducted in a Randomized Block Design (RBD) consisting of eight treatments including an untreated control which was replicated thrice with plot size of 5×4m². The dose of *B. bassiana* tested was 1×10⁸ spore/ ml concentration. The spraying was done during early morning hours in such a way to give uniform coverage of foliage, to

avoid drift and influence of UV fraction of sunlight. Two rounds of spraying were given at an interval of 10 days using knapsack hydraulic sprayer (Aspee®, Mumbai) with a spray fluid volume of 500 L ha⁻¹. Five plants were randomly selected from each plot avoiding those from margins. The observations on mealybug populations were taken on 3, 5, 7 and 10 days after spraying and measured in terms of 5 randomly tagged plants from each plot (plate 3).



Plate 3: *B. bassiana* infected *P. marginatus*

Second round of spray was taken up on 11th day after first spraying and pest count recorded on 10th day was taken as pre treatment count for second spray. A 10X hand magnifying lens was used for counting the mealybug population.

Results and Discussion

Field efficacy of *B. bassiana* isolates against *P. marginatus* at Onnipalayapudhur

The data on the field efficacy of fungal pathogens, *B. bassiana* isolates in comparison with the standard check viz., *B. bassiana* (B₂) talc based against *P. marginatus* showed significant variations among pre-treatment and post-treatment counts (Table 1). The pre-treatment population ranged from 75.66 to 80.10 per 3 leaf area. After the first spray, at 3 DAT, reduction in the mealybug population of about 19.56% and 15.79% was observed in UPI(Bb) and *B. bassiana* (B₂) talc based. ANR(Bb) and AVI (Bb) and KPI (Bb) showed significantly less population reduction of about 14.32 per cent, 12.26% and 12.11% respectively. PLR(Bb) and TMR(Bb) showed significantly least population reduction of about 8.99% and 7.24% respectively (Table 1).

Table 1: Effect of *B. bassiana* isolates against *P. marginatus* in tapioca at 1×10⁸ spore/ml concentration after first round of application (Location: Onnipalayapudhur)

Treatment	Dose (g or ml/ litre of water)	PTC No/3 leaves	Number of mealybug									
			3DAT	% reduction	5DAT	% reduction	7DAT	% reduction	10DAT	% reduction	Mean	% reduction
AVI (Bb)	10	79.00 ^a (8.91)	74.59 ^{abc} (8.66)	12.26	70.75 ^{bc} (8.43)	21.34	68.06 ^{cd} (8.27)	27.14	70.42 ^{cd} (8.41)	26.00	70.95	21.70
ANR(Bb)	10	78.55 ^a (8.88)	72.84 ^{abc} (8.56)	14.32	67.64 ^b (8.25)	24.80	63.33 ^{bc} (7.98)	32.20	65.99 ^{bc} (8.15)	30.66	67.45	25.49
KPI (Bb)	10	78.01 ^a (8.85)	74.72 ^{abc} (8.67)	12.11	71.92 ^{bc} (8.50)	19.54	69.68 ^d (8.37)	25.41	71.82 ^{cde} (8.50)	24.53	72.03	20.52
UPI(Bb)	10	77.68 ^a (8.83)	68.39 ^a (8.29)	19.56	61.44 ^a (7.86)	31.69	56.90 ^a (7.57)	39.09	58.55 ^a (7.67)	38.47	61.32	32.20
PLR(Bb)	10	79.35 ^a (8.93)	77.37 ^{bc} (8.82)	8.99	75.26 ^c (8.70)	14.69	72.77 ^{de} (8.55)	22.10	74.92 ^{de} (8.68)	21.27	75.08	17.17
TMR(Bb)	10	80.10 ^a (8.97)	78.86 ^{cd} (8.90)	7.24	77.04 ^c (8.80)	14.35	75.12 ^e (8.69)	19.58	76.99 ^e (8.80)	19.10	77.00	15.06
<i>B. bassiana</i> talc	10	78.08 ^a (8.86)	71.59 ^{ab} (8.48)	15.79	66.24 ^{ab} (8.16)	26.35	61.97 ^{ab} (7.90)	33.66	63.75 ^{ab} (8.01)	33.01	65.88	27.20
Control	—	75.66 ^a (8.72)	85.02 ^d (9.24)	0.00	89.95 ^d (9.51)	0.00	93.42 ^f (9.69)	0.00	95.17 ^f (9.78)	0.00	90.89	00.00

DAT- Days after treatment, *mean of three replications, figures in the parantheses are the values of $\sqrt{x+0.5}$. In a column, means followed by a common letter(s) are not significantly different (P= 0.05) by DMRT. AVI(Bb)- Avinasi, ANR(Bb) - Annur, KPI (Bb)- Kanchapalli, UPI (Bb)- Udumalipet, PLR (Bb)- Pongalore, TMR (Bb)- Thondamuthur, Bb- *Beauveria bassiana*

Table 2: Effect of *B. bassiana* isolates against *P. marginatus* in tapioca at 1×10^8 spore/ml concentration after second round of application (Location: Onnipalayapudhur)

Treatment	Dose (g or ml/ litre of water)	PTC No/3 leaves	Number of mealybug									
			3DAT	% reduction	5DAT	% reduction	7DAT	% reduction	10DAT	% reduction	Mean	% reduction
AVI (Bb)	10	70.42 ^{cd} (8.41)	65.86 ^{cd} (8.14)	33.19	61.84 ^{cd} (7.89)	38.40	58.69 ^{cd} (7.69)	42.70	61.15 ^d (7.85)	42.23	61.89	39.13
ANR(Bb)	10	65.99 ^{bc} (8.15)	60.93 ^{bc} (7.83)	38.19	56.39 ^{bc} (7.53)	43.82	52.93 ^c (7.30)	48.33	55.35 ^c (7.46)	47.71	56.40	44.51
KPI (Bb)	10	71.82 ^{cd} (8.50)	69.19 ^{de} (8.34)	29.82	66.37 ^{de} (8.17)	33.88	63.79 ^{de} (8.01)	37.72	66.21 ^{de} (8.16)	37.45	66.39	34.71
UPI(Bb)	10	58.55 ^a (7.67)	50.68 ^a (7.14)	48.59	44.06 ^a (6.66)	56.11	38.91 ^a (6.26)	62.01	41.06 ^a (6.43)	61.21	43.67	56.98
PLR(Bb)	10	74.92 ^{de} (8.68)	72.91 ^e (8.56)	26.04	70.55 ^{ef} (8.44)	29.72	68.62 ^{ef} (8.31)	33.01	70.62 ^{ef} (8.43)	33.28	70.67	30.51
TMR(Bb)	10	76.99 ^e (8.80)	75.62 ^e (8.72)	23.29	73.79 ^f (8.61)	26.49	71.86 ^f (8.50)	29.85	73.61 ^f (8.60)	30.46	73.72	27.52
<i>B.bassiana</i> talc	10	63.75 ^{ab} (8.01)	57.99 ^b (7.64)	41.18	51.77 ^b (7.22)	48.43	46.86 ^b (6.87)	54.25	49.01 ^b (7.03)	53.70	51.40	49.39
Control	—	95.17 ^f (9.78)	98.59 ^f (9.94)	0.00	100.39 ^g (10.04)	0.00	102.44 ^g (10.14)	0.00	105.86 ^g (10.31)	0.00	101.82	0.00

DAT- Days after treatment, *mean of three replications, figures in the parantheses are the values of $\sqrt{x+0.5}$. In a column, means followed by a common letter(s) are not significantly different ($P=0.05$) by DMRT. AVI (Bb)- Avinasi, ANR (Bb)- Annur, KPI (Bb)- Kanchapalli, UPI (Bb)-Udumalipet, PLR (Bb)- Pongalore, TMR (Bb)- Thondamuthur, Bb- *Beauveria bassiana*.

At 5 DAT, significant reduction in the mealybug population was observed in UPI (Bb) (31.69 %) and *B.bassiana* (B_2) talc based (26.35%) than ANR(Bb), AVI (Bb) and KPI (Bb) which recorded 24.80, 21.34 and 19.54% reduction respectively. PLR(Bb) and TMR(Bb) showed significantly least population reduction of about 14.69% and 14.35 % respectively. At 7 DAT, the same trend was observed in all the treatments. Mealybug population reduction was observed in UPI(Bb) (39.09 %) and *B.bassiana* (B_2) talc based (B_2) (33.66 %) than ANR(Bb), AVI (Bb) and KPI (Bb) which recorded 32.20, 27.14 and 25.41% reduction respectively. PLR(Bb) and TMR(Bb) showed significantly least population reduction of about 22.10% and 19.58% respectively.

At 10 DAT, the population build up was relatively more than at 7 DAT in all the treatments including UPI(Bb). The mean per cent reduction in the population after first round of treatments was 32.20, 27.20, 25.49, 21.70, 20.52, 17.17 and 15.06%, respectively in UPI(Bb), *B.bassiana* (B_2) talc based, ANR(Bb), AVI (Bb), KPI (Bb), PLR(Bb) and TMR(Bb). From the data obtained, UPI(Bb) was found to be superior among the other fungal isolates applied

under field conditions. The mealybug population on 10 DAT was taken as the pre-treatment count for the second round of treatments and it ranged between 58.55 to 95.17 per 3 leaf area. After second spraying, UPI(Bb), *B.bassiana* (B_2) talc based, showed a population reduction of 48.59 and 41.18% respectively at 3 DAT and ANR(Bb), AVI (Bb), KPI (Bb), PLR(Bb) and TMR(Bb) showed population reduction of 38.19, 33.19, 29.82, 26.04 and 23.29%. Similar trend was observed on 5 and 7 DAT (Table 2). Though, there was increase in population at 10 DAT, the UPI(Bb) was superior to all other treatments with a per cent reduction of 61.21. After second round of treatments, the mean per cent reduction in mealybug population at 10 DAT observed in UPI(Bb), *B.bassiana* (B_2) talc based, ANR(Bb), AVI (Bb), KPI (Bb), PLR(Bb) and TMR(Bb) were 56.98, 49.39, 44.51, 39.13, 34.71, 30.51 and 27.52%, respectively (Table 2).

Field efficacy of *B. bassiana* against *P. marginatus* at Mathampalayam

The pre treatment population of mealybug ranged from 51.04 to 55.28 per three leaves. This result

Table 3: Effect of *B. bassiana* isolates against *P. marginatus* in tapioca at 1×10^8 spore/ml concentration after first round of application (Location: Mathampalayam)

Treatment	Dose (g or ml/ litre of water)	PTC No/3 leaves	Number of mealybug									
			3DAT	% reduction	5DAT	% reduction	7DAT	% reduction	10DAT	% reduction	Mean	% reduction
AVI (Bb)	10	53.88 ^a (7.37)	49.77 ^{abc} (7.08)	20.36	45.90 ^{cd} (6.81)	30.32	42.60 ^{cd} (6.56)	38.41	44.95 ^{cd} (6.74)	38.71	45.80	31.92
ANR(Bb)	10	53.42 ^a (7.34) ^a	48.15 ^{ab} (6.97)	22.96	43.48 ^{bc} (6.62)	34.00	39.86 ^{bc} (6.35)	42.37	42.19 ^{bc} (6.51)	42.48	43.42	35.45
KPI (Bb)	10	52.15 ^a (7.25)	50.15 ^{abc} (7.11)	19.76	46.35 ^{cd} (6.84)	29.64	43.70 ^{cd} (6.64)	36.82	46.19 ^{cd} (6.83)	37.02	46.59	30.81
UPI(Bb)	10	54.46 ^a (7.41)	46.19 ^a (6.83)	26.09	38.62 ^a (6.25)	41.37	34.46 ^a (5.91)	50.18	36.53 ^a (6.08)	50.19	38.95	41.95
PLR(Bb)	10	53.22 ^a (7.32)	51.24 ^{bc} (7.19)	18.01	47.84 ^{de} (6.95)	27.38	45.28 ^{de} (6.76)	52.76	48.04 ^{de} (6.96)	34.50	48.10	28.60
TMR(Bb)	10	55.28 ^a (7.46)	53.93 ^c (7.37)	13.71	51.06 ^e (7.17)	22.49	49.48 ^e (7.06)	28.46	51.70 ^e (7.22)	29.51	51.54	23.54
<i>B.bassiana</i> talc	10	54.26 ^a (7.39)	47.64 ^{ab} (6.93)	23.77	41.53 ^{ab} (6.48)	36.96	37.55 ^{ab} (6.16)	45.71	39.57 ^{ab} (6.32)	46.05	41.57	38.12
Control	-	51.04 ^a (7.17)	62.50 ^d (7.93)	0.00	65.88 ^f (8.14)	0.00	69.17 ^f (8.34)	0.00	73.35 ^f (8.59)	0.00	67.72	0.00

DAT- Days after treatment, *mean of three replications, figures in the parantheses are the values of $\sqrt{x+0.5}$. In a column, means followed by a common letter(s) are not significantly different ($P=0.05$) by DMRT. AVI (Bb)- Avinasi, ANR (Bb)- Annur, KPI (Bb)- Kanchapalli, UPI (Bb) -Udumalipet, PLR (Bb)- Pongalore, TMR (Bb)- Thondamuthur, Bb- *Beauveria bassiana*.

revealed that there was no significant difference among the different treatments before application of *B.bassiana*. After first spraying, among the *B.bassiana* isolates, UPI(Bb) and *B.bassiana* (B_2) talc based recorded the mealybug population of 38.95 and 41.57 in three leaves corresponding to 41.95 and 38.12 mean per cent reduction of mealybug population over control on 3, 5, 7 and 10 days after spraying respectively (Table 3). This was followed by ANR(Bb), AVI(Bb) and KPI(Bb) which recorded a mean reduction of 35.45, 31.92 and 30.81%, respectively. PLR(Bb) and TMR(Bb) were comparatively less effective recording a mean population reduction of 28.60 and 23.54%, respectively. Second spraying was given ten days after the first application. Prior to second spraying, the mealybug population ranged from 36.53 to 73.35 in three leaves of a plant (Table 4).

Similar trend was observed with UPI(Bb), *B.bassiana* (B_2) talc based and ANR(Bb) which recorded a mean population reduction of 72.76, 65.23 and 59.84%, respectively. AVI (Bb), KPI(Bb), PLR(Bb) and TMR(Bb) were comparatively less effective

recording a mean population reduction of 52.73, 50.30, 47.04 and 41.67%, respectively (Table 4).

In the present study, there was significant difference in the pathogenecity caused by the pathogens. In the present study, under field condition UPI (Bb) was found to be effective against papaya mealybug, *P. marginatus* to an extent of 56.98 and 72.76% only after second spraying in Onnipalayapudhur and Mathampalayam respectively. This could be attributed to the variation in the relative humidity (RH) and temperature differences under field conditions. In contrast, Suresh *et al.* (2010) reported that *B. bassiana* caused 57.78% infestation in papaya at 5 days after treatment in a field trial conducted at Coimbatore and caused 77.0% mortality in *P. solenopsis* in cotton. Dorschner *et al.* (1991), reported 100% mortality in hop aphids exposed to *B. bassiana* in the laboratory but the fungus, did not show any infection in the field.

The results corroborate with the findings of Janaki *et al.* (2010) wherein *B. bassiana* caused 58.98% reduction in 10 days after second spraying against *P. marginatus* in brinjal. Suresh *et al.* (2010) found that

**Table 4:** Effect of *B. bassiana* isolates against *P.marginatus* in tapioca at 1×10^8 spore/ml concentration after second round of application (Location: Mathampalayam)

Treatment	Dose (g or ml/ litre of water)	PTC No/3 leaves	Number of mealybug								Mean	% reduction
			3DAT	% reduction	5DAT	% reduction	7DAT	% reduction	10DAT	% reduction		
AVI (Bb)	10	44.95 ^{cd} (6.74)	42.02 ^c (6.52)	46.07	38.48 ^d (6.24)	52.94	35.08 ^d (5.96)	57.99	37.70 ^d (6.18)	57.34	38.32	52.73
ANR(Bb)	10	42.19 ^{bc} (6.51)	37.88 ^b (6.19)	51.39	33.39 ^c (5.81)	59.16	29.35 ^c (5.46)	64.85	31.84 ^c (5.68)	63.97	33.11	59.84
KPI (Bb)	10	46.19 ^{cd} (6.83)	43.64 ^c (6.64)	44.00	41.28 ^{de} (6.46)	49.51	38.82 ^d (6.27)	53.52	40.50 ^{de} (6.40)	54.18	41.06	50.30
UPI(Bb)	10	36.53 ^a (6.08)	30.02 ^a (5.52)	61.47	22.59 ^a (4.80)	72.37	17.39 ^a (4.22)	79.17	19.41 ^a (4.45)	77.81	22.35	72.76
PLR(Bb)	10	48.04 ^{de} (6.96)	45.77 ^{cd} (6.80)	41.26	43.75 ^{ef} (6.65)	46.49	41.64 ^e (6.49)	50.14	43.93 ^{ef} (6.66)	50.29	43.77	47.04
TMR(Bb)	10	51.70 ^e (7.22)	50.04 ^d (7.10)	35.78	48.06 ^f (6.96)	41.22	46.35 ^f (6.83)	44.50	48.44 ^f (6.99)	45.19	48.22	41.67
<i>B.bassiana</i> talc	10	39.57 ^{ab} (6.32)	34.70 ^b (5.93)	55.47	28.70 ^b (5.40)	64.90	24.48 ^b (4.99)	70.68	26.64 ^b (5.20)	69.86	28.63	65.23
Control	—	73.35 ^f (8.59)	77.93 ^e (8.85)	0.00	81.77 ^g (9.06)	0.00	83.52 ^g (9.26)	0.00	88.39 ^g (9.42)	0.00	82.90	0.00

DAT- Days after treatment, *mean of three replications, figures in the parantheses are the values of $\sqrt{x+0.5}$, In a column, means followed by a common letter(s) are not significantly different ($P=0.05$) by DMRT. AVI (Bb)- Avinasi, ANR (Bb)- Annur, KPI (Bb)- Kanchapalli, UPI (Bb) -Udumalipet, PLR (Bb)- Pongalore, TMR (Bb)- Thondamuthur, Bb- *Beauveria bassiana*.

B. bassiana was moderately effective and caused 77% mortality of cotton mealybugs and 66.66% mortality of *P. marginatus* after 10 days of treatment. In contrast Sakthivel (2011), reported that *B. bassiana* recorded a mean population reduction of 43.23% of papaya mealybug in 14 days after spraying twice in papaya.

Conclusion

There was significant difference in the pathogenecity caused by the pathogens. In the present study, under field condition UPI (Bb) was found to be effective against papaya mealybug, *P. marginatus* to an extent of 56.98 and 72.76% only after second spraying in Onnipalayapudhur and mathampalayam respectively. Followed by, *B.bassiana* (B_2) talc based and ANR(Bb) which recorded a mean population reduction of 72.76, 65.23 and 59.84%, respectively. AVI (Bb), KPI(Bb), PLR(Bb and TMR(Bb) were comparatively less effective recording a mean population reduction of 52.73, 50.30, 47.04 and 41.67%, respectively (Table 4).

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