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Assessment of the suitability of Tinopal as an enhancing adjuvant in formulations of the insect pathogenic fungus *Beauveria bassiana* (Bals.) Vuillemin

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Abstract

BACKGROUND: Biopesticides based on *Beauveria bassiana* (Bals.) Vuillemin hold great promise for the management of a wide range of insect pests. The conidia in the biopesticide formulation require an adjuvant to protect them from photoinactivation by sunlight. The suitability of Tinopal, an optical brightener used as sunscreen for baculovirus formulations, for use with *B. bassiana* was assessed. The aim was to study the effect of Tinopal on the growth and photoprotection of *B. bassiana*, and its effect on the susceptibility of insects to *B. bassiana*.

RESULTS: Tinopal was found to have no adverse effect on the growth of *B. bassiana*. It was found to confer total protection (~95% conidial germination at 10 g Tinopal L^{-1}) from sunlight up to 3 h of exposure, and a better survival rate than controls even up to 4 h. *Helicoverpa armigera* Hübner larvae fed on diet with 5 g kg^{-1} Tinopal were found to have reduced growth. The duration of the larval stage increased by 3–4 days in 1 and 5 g kg^{-1} Tinopal treatments. Among the moths that emerged from larvae fed on diet with 5 g kg^{-1} Tinopal, a significantly high number were malformed compared with controls. The larvae that were fed diet with Tinopal showed quicker and higher mortality and required a lower effective lethal dose (LC_{50}) than the controls. Tinopal was found to have a synergistic effect with *B. bassiana* in causing insect mortality.

CONCLUSIONS: Tinopal was found to be a suitable adjuvant for *B. bassiana*-based biopesticide formulations. It conferred tolerance to sunlight and caused stress in the insect, leading to a synergistic effect with *B. bassiana*.

Keywords: *Beauveria bassiana*; entomopathogenic fungus; Tinopal; UV protectant; sunscreen; synergistic effect; *Helicoverpa armigera*

1 INTRODUCTION

Biopesticide formulations based on *Beauveria bassiana* (Bals.) Vuillemin, a wide host range insect pathogenic fungus, are being marketed and used in insect pest management.[1] Although *B. bassiana* is highly virulent to several insect pests, its hyaline conidia are reported to lose viability quickly when exposed to sunlight,[2,3] and formulations must therefore contain sunscreens to counter this. Tinopal, a stilbene-derived optical brightener, has been found to be an effective UV protectant for entomopathogenic viruses and fungi, including *B. bassiana*.[3–5] There is, however, a conflicting report on the UV protecting role of Tinopal on *B. bassiana*.[6] With baculoviruses, Tinopal has been reported additionally to increase their infectivity, resulting in higher insect mortality.[7–10] However, the synergistic effect of Tinopal with *B. bassiana* on insect mortality has not been tested. Tinopal (Calcofluor white) has been reported to affect the growth of yeast and some fungal species.[11,12] The effect of Tinopal on growth of *B. bassiana* has not been studied. The authors tested the effect of Tinopal on the growth, UV protectant properties and its effect on insect growth and mortality with a virulent isolate of *B. bassiana*.

2 MATERIALS AND METHODS

2.1 Fungal isolate and culture

Beauveria bassiana isolate ITCC 4688 [Indian Type Culture Collection (ITCC); IARI, Delhi, India], which was found to be highly virulent to 15 insect species of diverse taxonomic orders in laboratory

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bioassays[13,14] (Uma Devi K, unpublished data), was chosen for the study. This isolate was found suitable for mass multiplication with a very fast growth rate and production of copious amounts of conidia when cultured on rice (Uma Devi K, unpublished data).

The fungal culture was initiated on Sabouraud dextrose yeast agar (SDAY) slants from conidia stored in 20% glycerol at −20 °C. The conidia from a 14-day-old culture were used in the experiments. The conidia were harvested from the culture by scraping with a sterile spatula. An aqueous suspension of conidia was made. The conidia from one culture slant were suspended in 5 mL water with 0.1 g L^{-1} Tween 80 (Sigma-Aldrich, India) and vortexed. The conidial concentration in the suspension was determined by counting with a hemocytometer. The suspension was diluted with water to achieve the required conidial concentration. The viability of conidia used in the experiments was checked as described by Varela and Morales.[15] Only the results from those experiments in which more than 95% conidial viability was observed were considered.

The effect of Tinopal UNPA-GX (Sigma Chemical Co., St Louis, MO) was tested at three different concentrations, 1, 5 and 10 g L^{-1}, for growth assay and experiments to study UV tolerance. For insect bioassays, Tinopal was tested at 1 and 5 g kg^{-1} in the diet. All assays were set up in triplicate. The assays were repeated 3 times with conidia from three different culture tubes. The control was set up without Tinopal.

2.2 Growth assay of *Beauveria bassiana* with Tinopal

The effect of Tinopal on the growth of *B. bassiana* was assessed by comparing its growth in liquid medium with and without Tinopal. Cultures were initiated by inoculating 1 mL of conidial suspension (~10^8 conidia) into 250 mL conical flasks containing 100 mL of SDY medium with different test concentrations of Tinopal. The growth assay was done as described by Uma Devi *et al*.[16]

2.3 Test of tolerance to UV radiation

Aqueous conidial suspensions with test concentrations of Tinopal were sprayed onto leaves and exposed to UV radiation to test if it conferred tolerance. Mature healthy leaves of *Solanum melongena* L. (aubergine, brinjal) grown in pots were used for the experiment. Two sources of UV radiation were tested – artificial [20 W UV-B fluorescent lamp (Philips, Eindhoven, Holland) with a light emission wavelength of 260–400 nm, with a peak at 313 nm] and natural (sunlight).

2.3.1 Exposure to a UV lamp

The tip of the short petiole of the cut leaf was wrapped with cotton dipped in water to prevent it from wilting. The leaf was placed in a plastic petri dish (150 mm diameter). Conidial suspensions (2 mL of 10^8 conidia mL^{-1}) with different test concentrations of Tinopal were applied to the entire surface of the leaves with a microsyringe. The petri dish was kept under an 85 cm long UV bulb at a distance of 50 cm. Under one UV light, 10 petri dishes were placed in two rows of five each. The room temperature during the experiment was 28 ± 1 °C. The conidia on the leaves were exposed for different intervals of time, from 30 min to 4 h.[3] After exposure, the conidia on the leaf surface were washed off with 10 mL of sterile distilled water into a glass beaker. An aliquot (200 μL) of this washoff was inoculated into Eppendorf tubes containing 1 mL of Sabouraud dextrose medium with 0.005 g L^{-1} carbendazim (Bavistin 500 g kg^{-1} WP; BASF India Ltd, Mumbai, India). The tubes were incubated at 25 °C. Carbendazim is used in the medium to slow down germ tube elongation, thus preventing the entangling of germ tubes from several conidia.[17] Counting of germinated conidia under the microscope is thus facilitated. Observations for conidial germination in control (no Tinopal) were made at hourly intervals starting from 14 h after incubation. Starting from the time when more than 90% conidia had germinated in the control, from every tube with Tinopal in the medium, 250 μL of medium with conidia was transferred to a new tube at hourly intervals up to 6 hours and immediately stored in a refrigerator (8 °C) to arrest growth until observations under the microscope could be made. From observations of the samples collected at different times, the time delay in germination, if any, on account of Tinopal in comparison with the control was assessed. To observe conidial germination, 60 μL of conidial suspension from the tube was dispensed onto a glass slide. A drop each of lactophenol cotton-blue (Hi-media, Mumbai, India) and glycerine were added to this, and a cover slip was placed over it. The number of germinated and ungerminated conidia were counted under a light microscope at 400× magnification. From each slide, counts were made in five different regions. Approximately 300 conidia were counted for each treatment.[18] For assessment, the germination count from the sample (collected at different time intervals after incubation in the culture medium) that showed the maximum germination was considered.

2.3.2 Exposure to UV radiation from sunlight

The method of Edgington *et al*.[6] was followed. The cut ends of leaf were immediately dipped in water in a test tube (2.5 × 15 cm). The leaf stood erect, resting on the mouth of the test tube. It was sprayed on both sides with conidial suspension (10^8 conidia mL^{-1}) of *B. bassiana* with different test concentrations of Tinopal. The suspension was applied with a microsyringe until the entire leaf lamina was wet. The tubes with the sprayed leaves were rested in a stand placed on the terrace of the laboratory building under direct sunlight. The experiment was conducted 3 times during September–October on bright sunny days between 10 a.m. and 2 p.m. The daytime temperatures during the experiments ranged between 32 and 35 °C. After

different exposure times, the leaves were removed and the viability of the conidia was tested as described in Section 2.3.1.

2.4 Insect bioassays

Second-generation larvae obtained from laboratory-bred (ICRISAT, Patancheru, India) *Helicoverpa armigera* Hübner (Lepidoptera; Noctuidae) established from field-collected insects were used in the experiments. The larvae were reared on semi-synthetic diet[19] with test concentrations of Tinopal. Control insects were fed the same diet without Tinopal. Neonate larvae of *H. armigera* from the laboratory colony were placed in a plastic dish (12 cm diameter) with a 2 mm thick layer of diet. About 100 larvae were placed on each dish. Immediately following the first moult, the larvae were transferred to a perforated plastic box (40 mL) with a lid. Each box contained 5 g diet with the appropriate concentration of Tinopal. A single larva was placed in each box. The larvae are cannibalistic and therefore kept single. For each treatment batch, 20 larvae were taken. The experiment was set up as a completely randomized block design[20] in an environmental chamber set to $26 \pm 2\,^{\circ}C$ and 90% humidity.

2.4.1 Susceptibility test

Second-instar larvae were treated. A range of conidial concentrations from 10^4 to 10^8 conidia mL^{-1} were tested. Each larva was treated with 100 µL of conidial suspension. The inoculum was dispensed with a micropipette (Eppendorf®) on the entire surface of the larva. Post-treatment mortality of the insects was recorded daily until all the larvae in the treatment batch either died or pupated. The dead larvae were transferred individually into petri dishes lined with moist filter paper to facilitate mycosis. The number of insect cadavers that developed mycosis was noted up to 1 week.

2.4.2 Insect growth assay

The effect of three different concentrations of Tinopal (1, 5 and 10 g kg^{-1}) on the growth and development of the *H. armigera* larvae was also investigated to validate its role as a stress-causing agent. The experiments were done as described by Marti'nez *et al*.[21] The rate of growth was evaluated by measuring larval weight at the end of each instar and the duration of each instar. The long-term effects of Tinopal were assessed by enumerating the number of adults that emerged, males, females and those with evident malformations.

2.5 Data analysis

The values in all experiments were arcsine percent square root transformed, and the mean ± SE in each treatment was back-transformed to normalize the data.[22] A homogeneity test was done to decide on pooling the data from the replicated experiments. The level of significance of differences among different treatments and treatments versus control was tested through ANOVA.

The number of insects that showed mycosis was estimated as a percentage of the total dead insects. Median lethal time was calculated from the cumulative mortality data on each day post-treatment using survival analysis with the Weibull distribution.[23] Median lethal concentration was estimated through probit analysis. The test for synergism of Tinopal was carried out as decribed by Borgert.[24] The expected effect of interaction of Tinopal and *B. bassiana* is computed as a product of the values (percentage proportion changed to decimals) of observed mortality with individual treatments. The observed effect is computed by adding the proportions (percentage proportion changed to decimals) of live insects in individual treatments and subtracting this from 1 (100%) to obtain the proportion of dead insects. When the observed value is higher than the expected value, the effect is concluded as synergistic. Statistical analysis was carried out using the statistics software packages Statistica[25] and SPSS.[26]

3 RESULTS

3.1 Effect of Tinopal on the growth of *Beauveria bassiana*

Tinopal was found to have no inhibitory effect on the growth of *B. bassiana*, as shown by the ANOVA test between test and control (Table 1).

3.2 Effect of Tinopal on *Beauveria bassiana* conidia when exposed to UV radiation

UV radiation from the UV lamp was found to have a more severe effect on conidial viability than natural sunlight (Table 2). When exposed to radiation from the UV lamp, conidial viability fell below 90% within 1 h of exposure in all the treatments as well as the control (Table 2). The viability decreased quickly, with conidial germination dropping below 50% after 4 h exposure (Table 2). Tinopal, however, conferred tolerance to UV radiation at all concentrations relative to the control. The effect was dose dependent, with greater tolerance at higher Tinopal concentration

Table 1. Assessment of growth of *Beauveria bassiana* (ITCC 4688) exposed to Tinopal UNPA-GX

Tinopal conc. (g L^{-1})	Biomass (g) (± SE)[a]	ANOVA test[b] versus control
10	0.59 (±0.02)	df = 3
5	0.57 (±0.01)	$F = 3.34$
1	0.59 (±0.02)	$P = 0.55$
0 (control)	0.57 (±0.02)	

[a] Dry weight of ten-day-old cultures. Values represent mean (± SE) of three experiments each set up in triplicate.

[b] All three treatments [the differences between replicates within an experiment, between different replicate experiments and between treatments were insignificant; therefore the data of all treatments and replicates ($3 \times 3 \times 3 = 27$) were pooled for ANOVA analysis].

Table 2. Conidial viability of the entomopathogenic fungus *Beauveria bassiana* as affected by UV radiation in the presence of Tinopal UNPA-GX

	Conidial germination (%) (± SE)[a] after different exposures					
Tinopal conc. (g L^{-1})	0 min	30 min	60 min	120 min	180 min	240 min
Artificial[b] *UV radiation*						
10	97.68 (±0.61)	96 (±0)	87.07 (±1.27)	75 (±0.38)	58.34 (±0.84)	40.98 (±1.21)
5	98.08 (±1.22)	96.04 (±0.85)	86.37 (±0.99)	73 (±0.37)	56.33 (±0.69)	38.31 (±1.19)
1	98.37 (±0.79)	94.67 (±0.4)	84.36 (±0.93)	69.33 (±0.41)	47.99 (±0.66)	23.64 (±0.8)
0 (control)	98.37 (±0.79)	91.67 (±0.34)	81.34 (±0.48)	60.34 (±0.85)	32.64 (±1.07)	17.32 (±0.5)
ANOVA	$F_{3,6} = 2.2$, $P = 0.18$	$F_{3,6} = 28.76$, $P < 0.05$	$F_{3,6} = 35.34$, $P < 0.05$	$F_{3,6} = 35.5$, $P < 0.05$	$F_{3,6} = 744$, $P < 0.05$	$F_{3,6} = 111$, $P < 0.05$
Natural[c] *UV radiation*						
10	98.81 (±0.81)	98.47 (±0.70)	97.34 (±0.34)	96.33 (±0.29)	95.45 (±0.40)	77.44 (±0.20)
5	99.17 (±0.99)	98.81 (±0.81)	96.67 (±0.30)	94.06 (±1.79)	92.23 (±0.40)	75.66 (±0.25)
1	98.81 (±0.81)	98.44 (±0.56)	95.93 (±0.87)	92.79 (±0.64)	91 58 (±0.76)	72.45 (±0.58)
0 (control)	99.22 (±0.35)	97.36 (±0.69)	91.34 (±0.51)	90.33 (±0.81)	73.94 (±1.38)	54.55 (±0.49)
ANOVA	$F_{3,6} = 0.5$, $P = 0.69$	$F_{3,6} = 2.1$, $P = 0.2$	$F_{3,6} = 28.7$, $P < 0.05$	$F_{3,6} = 25.21$, $P < 0.05$	$F_{3,6} = 88.2$, $P < 0.05$	$F_{3,6} = 618$, $P < 0.05$

[a] Values are angular transformed (arc sine $\sqrt{}$ percentage) before analysis, back-transformed and rounded. Values represent mean (± SE) of three experiments each set up in triplicate.
[b] UV lamp [20 W UV-B fluorescent lamp (Philips, Eindhoven, Holland) with light emission wavelength of 260–400 nm, with a peak at 313 nm] at 25 °C.
[c] Sunlight (between 10 a.m. and 2 p.m. on bright sunny days in September–October in South India, with temperatures at this time of between 32 and 35 °C).

(Table 2). Under natural sunlight, more than 90% conidia were viable in the control up to 2 h exposure (Table 2). By 3 h, the conidial viability in the control declined to ~74%, while conidia with Tinopal at all concentrations maintained more than 90% viability. After 4 h of exposure, conidial viability decreased in all test treatments (Table 2). However, in all treatments, significant protection from light was observed, with the effect being dependent on the concentration of Tinopal (Table 2). Germination of conidia incubated in SDY medium with different concentrations of Tinopal but not exposed to UV radiation (negative control) was similar to that of conidia in culture medium with no Tinopal. The germination in these negative controls was ~98% at all three Tinopal concentrations tested. Thus, Tinopal per se did not affect conidial germination.

3.3 Effect of Tinopal on the susceptibility of insects to *Beauveria bassiana* infection

No mortality was observed in the untreated controls, both the positive (Tinopal in diet) and negative (no Tinopal in diet) controls in all three repeated experiments, so no Abbott correction was necessary.

When treated with *B. bassiana*, larvae that were fed on diet with Tinopal at both tested concentrations showed higher mortality at all conidial concentrations compared with the larvae fed on diet with no Tinopal (Table 3). The differences in mortality between the test (1 and 5 g kg^{-1} Tinopal) and control were found to be significant in ANOVA ($F_{2,8} = 14.5$, $P = 0.00015$). The effect of Tinopal on larval susceptibility was more pronounced in the 5 g kg^{-1} treatment. Larvae fed on 5 g kg^{-1} Tinopal had much lower LC_{50} and LT_{50} values than larvae with no Tinopal in their diet (Table 3). Thus, larvae that ingested Tinopal were more susceptible to *B. bassiana* infection than those that were fed normal diet. Tinopal was found to be synergistic with *B. bassiana* at conidial concentrations of 10^6 conidia mL^{-1} and above (Table 3).

3.4 Effect on insect growth and development

The highest concentration of Tinopal tested, 10 g L^{-1}, was lethal to larvae, and with the two lower doses a reduction in larval growth was evident by the end of the third instar (Fig. 1a). With progression of instars, the effect on larval growth appeared more pronounced in the 5 g kg^{-1} Tinopal treatment, while the negative effect on growth with 1 g kg^{-1} Tinopal that was evident in the third and fourth instars was nullified by the end of the sixth instar (Fig. 1a). The larval growth was significantly lower than the control at the third and fourth instars for the 1 g kg^{-1} Tinopal treatment ($F_{1,4} = 456$, $P < 0.05$), while with 5 g kg^{-1} Tinopal it was significantly affected at all instars beyond the second ($F_{3,12} = 898.2$, $P < 0.05$) (Fig. 1a). The mean duration of every instar except the fourth increased in both test treatments compared with the control (Fig. 1b). The total duration of the larval stage was 17.5 ± 0.1 days in the control, while it was 20.7 ± 0.1 days and 21.5 ± 0.02 days in treatments with 1 and 5 g kg^{-1} Tinopal respectively. The difference in the duration of the larval stage between the two treatments was not significant ($F_{1,4} = 2.04$, $P = 0.17$), while the difference between treatments (1 and 5 g kg^{-1} Tinopal) and control was significant ($F_{2,8} = 47.47$, $P < 0.05$). Thus, insect growth was slowed down and the duration of the larval stage increased in Tinopal treatments.

Adult emergence was not affected by Tinopal treatment (Fig. 1c). Moths emerged from more than 95% of pupae in both control and test. The sex ratio

Table 3. Response of second-instar larvae of *Helicoverpa armigera* fed on diet with Tinopal UNPA-GX to treatment with conidia of the insect pathogenic fungus *Beauveria bassiana*

Tinopal conc. in diet (g kg^{-1})	Conidial conc. (L^{-1})	Mortality (%) (± SE)[a]	Mycosis (%) (± SE)[a]	LT_{50} (days) (95% CI)[b]	LC_{50} (conidia L^{-1}) (95% CI)[b]	Synergism test observed value/ expected value[c]
5	10^8	93.33 (±0)	95.36 (±1.53)	3.39 (2.43–4.41)	1.5×10^6 ($3.6 \times 10^5 - 4.2 \times 10^6$)	0.74/0.73
	10^7	92.29 (±1.57)	91.57 (±1.15)	3.48 (2.41–4.41)		0.71/0.70
	10^6	70 (±0)	71.21 (±0.95)	4.64 (3.97–5.6)		0.43/0.32
	10^5	48.88 (±1.27)	52.38 (±1.36)	7.35 (6.6–8.53)		–
	10^4	8.82 (±1.15)	61.11 (±3.24)	8.62 (7.3–13.84)		–
	0	0	–			
1	10^8	81.13 (±5)	95.94 (±4.88)	5.16 (4.73–5.63)	1.2×10^7 ($0.1 \times 10^7 - 3.2 \times 10^7$)	0.64/0.61
	10^7	79.89 (±1.59)	91.65 (±2.39)	5.48 (5.28–5.69)		0.61/0.57
	10^6	64.47 (±1.32)	96.66 (±6.14)	5.99 (5.75–6.27)		0.39/0.26
	10^5	32.21 (±0.68)	79.62 (±3.96)	7.73 (7.23–8.49)		–
	10^4	11.06 (±0.99)	69.44 (±1.75)	9.88 (8.62–12.8)		–
	0	0	–			
0	10^8	80 (±0)	92.59 (±0)	5.72 (5.54–5.93)	1.8×10^7 ($0.6 \times 10^7 - 4.2 \times 10^7$)	
	10^7	78.9 (±0.77)	90.19 (±1.22)	5.72 (5.53–5.92)		
	10^6	62.22 (±0.65)	53.5 (±0)	6.27 (6.01–6.58)		
	10^5	27.76 (±0.7)	51.3 (±0.5)	8.04 (7.44–8.97)		
	10^4	6.66 (±0)	0	11.83 (9.6–21.3)		
	0	0	–	–		

[a] Values are angular transformed (arc sine $\sqrt{}$ percentage) before analysis, back-transformed and rounded. Values represent mean (± SE) of three replicates.
[b] CI = confidence interval.
[c] Borgert.[24] Synergistic when observed value is higher than expected value.

was, however, affected in the treatment with 5 g kg^{-1} Tinopal, the females being fewer in number than the males (Fig. 1c). This reduction in the number of females was found to be significant when compared with the control ($F_{3,6} = 99.67$, $P < 0.05$). Moreover, the number of malformed moths was significantly higher in the 5 g kg^{-1} Tinopal treatment than in the control ($F_{3,12} = 108.5$, $P < 0.05$) (Fig. 1c). Thus, the number of healthy adults was decreased in addition to the reduction in the number of females with Tinopal treatment at 5 g kg^{-1}. Treatment of larvae with a lower (1 g kg^{-1}) concentration of Tinopal did not drastically affect the phenotype of the adults emerging from them (Fig. 1c).

4 DISCUSSION

Being a stilbene-derived optical brightener, Tinopal absorbs ultraviolet (UV) radiation and emits visible blue wavelengths.[21] Owing to this property, it is reported to bestow photoprotection to microbes when incorporated in the biopesticide formulation. Tinopal is a stable compound and reported to persist for a long time in the field.[27] Given the reports of Tinopal affecting growth of some yeast and fungi,[11,12] its effect on the growth of the entomopathogenic fungus with which it is intended to be formulated merits investigation. In the present study, Tinopal was found to have no adverse effect on the growth of *B. bassiana*. Tinopal was found to confer tolerance to sunlight to *B. bassiana* conidia. The UV rays from an artificial source were found to kill the conidia quickly, even in the presence of Tinopal. A similar observation was reported in *B. bassiana* by Edgington *et al*.[6] The conidia lost viability within 20 s of exposure to UV radiation from a UV bulb. The *B. bassiana* isolate tested in their study was reported to be sensitive to sunlight as well, even in the presence of Tinopal.

Susceptibility of insects to *B. bassiana* was found to increase when fed on diet containing Tinopal. In insect pest infested fields sprayed with biopesticide, the insects while feeding on plants simultaneously ingest the conidial formulation. Therefore, Tinopal was incorporated into the insect diet in the experiments. Increased infection by baculoviruses formulated with Tinopal has been reported on several occasions,[28–30] but not with entomopathogenic fungi. Tinopal is reported to slough the peritrophic membrane lining of the insect gut.[31] This membrane functions as a barrier to pathogens such as viruses that enter the system through the gut.[32] Therefore, loss of integrity of the gut membrane owing to Tinopal is believed to increase the infection with baculoviruses.[33] The main route of infection of *B. bassiana* is through the cuticle, and very little through ingested conidia.[20] Therefore, in the present experiment, the Tinopal-fed larvae were topically treated with *B. bassiana* conidia.

Tinopal is reported negatively to affect growth and development of insects and thereby their physiological fitness.[21,34] Destruction of the peritrophic membrane is also reported to effect physiological functions related to digestion and absorption of nutrients.[33] This is reflected in the slowing down of growth and increase in duration of the development cycle of the insect observed in the present study and also reported

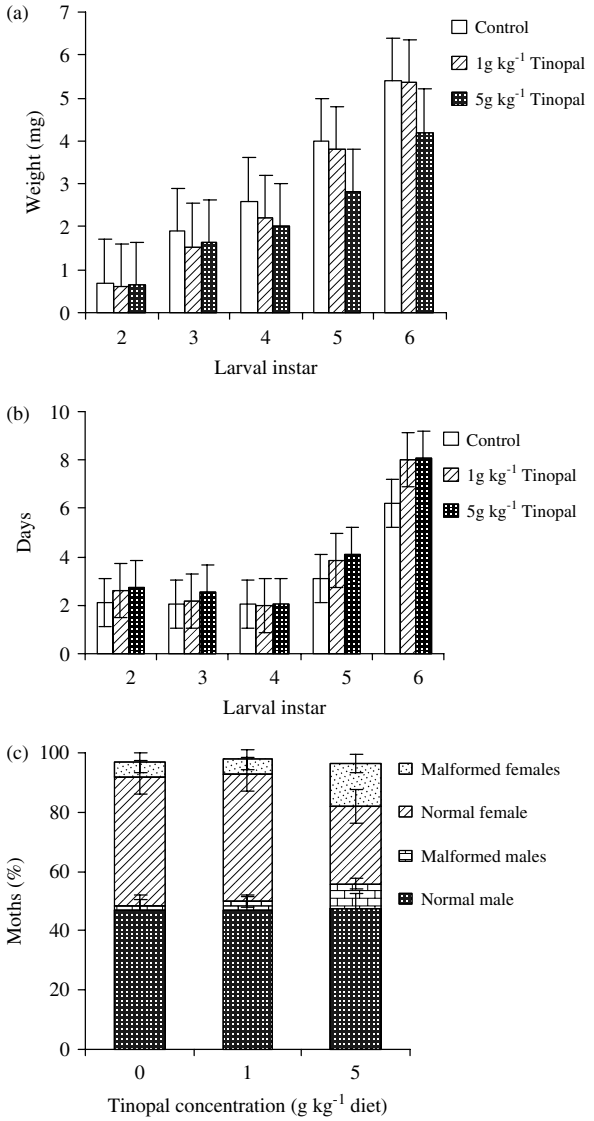

Figure 1. Effect of Tinopal on (a) larval growth, (b) rate of larval development and (c) adult phenotype of *Helicoverpa armigera* (Hübner). Each bar represents mean ± SE of three experiments. Each treatment batch consisted of 20 larvae.

earlier.[21,33] The increased susceptibility of insects fed on diet with Tinopal may have resulted from the physiological stress experienced by the insects owing to digestional disorders induced by Tinopal. Stressed insects are reported to be more susceptible to *B. bassiana* infection.[35,36] Tinopal, besides affecting the growth rate of the insect, was also found to cause abnormalities in the adult and tilt the sex ratio against females – the progenitors of future generations. A similar observation has been reported in another lepidopteran insect, *Spodoptera frugiperda* (Smith).[21]

There are reports of a negative effect of Tinopal on plant growth and insect pollinators.[37] The level of plant protection achieved with a Tinopal formulated microbial biopesticide and the extent to which plant growth and yield (because of decrease/absence of insect pollinators) are affected should be assessed in field applications. Some negative effects are likely to occur with most of the intervention practices. When benefits outweigh the disadvantages, the practice is adopted.

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