Journal of Biological Control, 35(2): 82-87, 2021, DOI: 10.18311/jbc/2021/27761

Research Article

Influence of varying temperatures on toxicity of biorationals against diamondback moth, *Plutella xylostella* L.

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ABSTRACT: Effect of various temperatures on the efficacy of five biorationals, *viz*. *Beauveria bassiana* (1.15% WP), *Metarhizium anisopliae* (1.15% WP), *Bacillus thuringiensis* (0.5% WP), Azadirachtin (300 ppm) and Spinosad (45 SC) in terms of median lethal concentration (LC_{50}) value was evaluated against diamondback moth, *Plutella xylostella* larvae. The toxicity data for biorationals against *P. xylostella* on the basis of larval mortality revealed that *M. anisopliae* (1.15% WP), *B. thuringiensis* (0.5% WP), Azadirachtin (300 ppm) and Spinosad (45 SC) registered higher effectiveness (lower LC_{50} value) at 25°C whereas, increase in temperature led to declined efficacy of biorationals; whereas, use of entomopathogenic fungi, *B. bassiana* (1.15% WP) was most effective at 30°C and revealed lower effectiveness (higher LC_{50} value) at 25° and 35° C temperatures. Data on the influence of temperature on toxicity of biorationals to *P. xylostella* on the basis of adult emergence revealed lowest LC_{50} value for *B. thuringiensis* (0.5% WP) at 25°C and the efficacy decreased with increase in the temperature, whereas, *M. anisopliae* (1.15% WP), *B. bassiana* (1.15% WP), Azadirachtin (300 ppm) and Spinosad (45 SC) were most effective at 30°C and the higher LC_{50} value were evident at temperature above 30°C, indicating the reduced efficacy of biorationals with increase in temperature beyond 30°C or preference for lower temperature regimes under laboratory conditions.

KEY WORDS: Biorationals, diamondback moth, $LC_{50,}$ temperature regimes

(Article chronicle: Received: 02-12-2020; Revised: 27-05-2021; Accepted: 29-05-2021)

INTRODUCTION

Diamondback moth (DBM), *Plutella xylostella* is the dominant pest in cabbage and cauliflower ecosystem. DBM has been elevated to major pest status by current modern farming practices (Furlong, *et. al.* 2013). Factors like the ability of the pest to migrate long distances, high fecundity, short life cycle, absence of effective natural enemies, continuous *Brassica* cultivation and high selection pressure, particularly the widespread and often indiscriminate use of broad-spectrum synthetic insecticides has led to the development of resistance in diamondback moth to a wide range of insecticides.

Use of insecticides is the most common method employed to manage this dreaded pest. Unfortunately, indiscriminate use of insecticides has led to several adverse changes in environment as well as to the biotic balance, which lead to numerous problems like residues, resurgence and resistance (Deshmukh *et. al.,* 1973; Chawla and Kalra, 1976). To overcome these adversaries, use of Biointensive Pest Management (BIPM) strategies comprising of alternative controls, such as biological agents, plant extracts and botanical origin insecticides are being focused for the management of DBM. BIPM may be used either alone or in conjunction with other Integrated Pest Management tools for minimizing the use of chemical pesticides and exploiting the ecofriendly biorationals for the management of pest population below economic injury level.

Biological control with biorationals has been proved to be excellent alternative to chemical pesticides (Singh and Jalali, 1991). DBM is found susceptible to several entomopathogenic fungi including *Beauveria bassiana, Metarrhizium anisopliae* (Flenner and Belnxis, 1998). But higher temperature regime in Semi arid tropical region affects the efficacy of biorationals. Taking into consideration the potential of biorationals as ecofriendly alternatives of pest management and challenges posed by temperature, the

present study was framed to evaluate changes in median lethal concentration of biorationals at different temperature regimes against *P. xylostella.*

MATERIAL AND METHODS

The studies have been carried out at the Toxicology Laboratory, Department of Entomology, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. The insect, *P. xylostella* population was collected on cauliflower from Akola (Maharashtra) during 2015-16.

Mass rearing of *Plutella xylostella*

Plutella xylostella population was reared in the laboratory under controlled conditions of temperature 25^{0}C ± 2^{0}C, 75 ± 5 per cent relative humidity and photo period of 13 hours light and 11 hours darkness. The adults emerged from pupae were kept in oviposition chamber with mustard seedlings as an ovipositional substrate. The adults were provided with 10% fortified honey solution as diet. The mustard seedlings kept inside the rearing chamber were replaced by fresh seedlings on alternate days. The eggs were laid on the mustard seedlings and the neonate larvae after hatching, mined into the mustard seedlings and started feeding on mustard leaves. Later, the grown up larvae were reared on cauliflower leaves as natural food.

Biorationals

Readymade formulations of the biorationals *viz., Beauveria bassiana* 1.15 % WP, *Metarhizium anisopliae* 1.15 % WP, *Bacillus thuringiensis* 0.5 % WP, Azadirachtin 300 ppm and Spinosad 45 SC were used. The efficacy was assessed based on their toxicity against *Plutella xylostella* L. at pre decided temperature regimes in order to assess the temperature dependent changes in median lethal concentration pertaining to biorationals.

Bioassay

The F_1 population was used for the bioassay. The bioassays were conducted by standard leaf dip method (IRAC-7) using 5 cm diameter leaf disk. In general five concentrations of each biorational were used to generate dose mortality response for *P. xylostella*. At least two replications of 10-12 second instar (3 day old) larvae of *P. xylostella* were used for the bioassay per dose/treatment by leaf dip assay for assessment of median lethal concentration of biorationals. The leaf disk with water dip served as a control. This set of experiment was carried out under controlled temperature conditions at 25, 30 and 35^0 C using BOD chamber (the humidity was not controlled) to assess the temperature dependent changes in median lethal concentration of the biorationals against *P. xylostella* population.

The observations on the mortality of the larvae were recorded regularly at an interval of 24 hrs up to 5 days for data on larval mortality whereas, up to 15 days for assessment of toxicity up to adult emergence. Moribund larvae and deformed pupa or adult at any stage of the observation were considered as dead.

Data analysis

The mortality in control was corrected using Abbot's formula and was used for final analysis (Abbott, 1925). The dose mortality response was estimated using Probit analysis with a software (Probit EPA).

RESULTS

Effect of temperature on toxicity of biorationals to *P. xylostella* on the basis of larval mortality

The median lethal concentrations of biorationals (*M. anisopliae, B. thuringiensis*, Azadirachtin and Spinosad) against second instar *P. xyllostella* larvae were lowest at 25°C as compared to 30°C and 35°C whereas, *B. bassiana* was found to be most effective at 30°C as compared to 25°C and 35°C (Table 1).

Based on data in Tables 2, 3, 4 the LC_{50} value for *M. anisopliae* at 25^{o}C was 5.2x10^4 cfu/g (95% Fiducial limit - 1.73x10^4-2.26x10^5), which was lower than the values at 30^{o}C (1.04x10^5 cfu/g; Fiducial limit - 2.1x10^4-3.6x10^5) and 35^{o}C (2.2x10^5 cfu/g; Fiducial limit - 5.36x10^4-1.36x10^6). On the contrary, the LC_{50} value for *B. bassiana* was lowest at 30^{o}C (5.87x10^3 cfu/g; Fiducial limit - 9.0x10^2-1.9x10^4) than at 25^{o}C with LC_{50} value of 7.9x10^3 cfu/g (2.1x103-4.7x10^4) and 35^{o}C with LC_{50} value of 4.4x10^4 cfu/g (6.5x10^3-5.6x10^5), respectively. In case of *B. thuringiensis* the LC_{50} value at 25^{o}C was 1.1x10^5 cfu/g (95% Fiducial limit - 4.3X10^4- 3.3X10^5), which was lower than the values at 30^{o}C with LC_{50} value of 1.2x10^5 cfu/g (2.5X10^4-3.6X10^5) and 35^{o}C with LC_{50} value of 5.8x10^5 cfu/g (1.2X10^5-2.3X10^6), respectively. Similar trend was also evident in case of Spinosad and azadirachtin with higher efficacy i.e. lower LC_{50} value of 0.060 ppm (0.021-0.14) and 0.27 ppm (0.87-1.45) at 25^{o}C, respectively, whereas, higher LC_{50} value of 0.22 ppm (0.054-0.52) and 0.38 ppm (0.076-1.83) at 30^{o}C and 0.25 ppm (0.039-0.50) and 0.39 ppm (0.055-1.32) at 35^{o}C for Spinosad and azadirachtin, respectively.

Effect of temperature on toxicity of biorationals to *P. xylostella* on the basis of adult emergence

On the basis of adult emergence, the LC_{50} value (median lethal concentrations) of *M. anisopliae, B. bassiana,* Azadirachtin and Spinosad was lowest at 30^{o}C against II instar *P. xyllostella* larvae which was followed by LC_{50} values at 25 and 35^{o}C, whereas, *B. thuringiensis* was most effective

at 25°C as compared to 30°C and 35°C (Table 1).

Based on data in Tables 5, 6, 7 the LC_{50} value for *M. anisopliae* at 30°C was $2.45x10^3$ cfu/g (95% Fiducial limit - $3.7x10^3$-$8.4x10^4$) that was lower than the LC_{50} value at 30°C – 25°C $1.52x10^4$ cfu/g (95% Fiducial limit - $2.76x10^3$-$4.75x10^4$) and the LC_{50} value at 35°C - $8.21x10^4$ cfu/g ($2.34x10^4$-$2.32x10^5$), respectively. Similarly, the LC_{50} value for *B. bassiana* was lowest at 30°C - $1.53x10^3$ cfu/g ($2.6x10^2$-$4.82x10^3$) than at 25°C - $3.36x10^3$ cfu/g ($4.8 x10^2$- $1.24x10^4$) and 35°C - $9.7x10^3$ cfu/g ($1.57x10^3$-$4.2x10^4$), respectively. Similar trend was also evident in case of Spinosad and azadirachtin with higher efficacy i.e. lower LC_{50} value of 0.011 ppm (0.0022-0.032) and 0.047 ppm (0.0076-0.16) at 30°C, respectively, whereas, higher LC_{50} value of 0.025 ppm (0.0057-0.059) and 0.068 ppm (0.012-0.21) was at 25°C and 0.03 ppm (0.004-0.10) and 0.11 ppm (0.024-0.32) at 35°C for Spinosad and azadirachtin, respectively. In case of B.t. the LC_{50} value at 25°C was $2.0x10^4$ cfu/g (95% Fiducial limit - $3.1x10^3$-$7.0x10^4$), lower than that of 30°C - $2.6x10^4$ cfu/g ($4.7x10^3$- $8.0x10^4$) and 35°C - $1.6x10^5$ cfu/g ($2.0x10^4$-$7.0x10^5$), respectively.

DISCUSSION

According to the reports the efficacy of fungal conidia was influenced by conidial concentration and the temperature (Han *et al*., 2014). Unfortunately, present findings could not be compared with previous data sets for want of information and literature. Though, some of the cross references supports the present piece of work. The *M. anisopliae* isolate revealed 100% cumulative mortality against second instar larvae of *S. exigua* at 3 days after treatment at 20-30°C with 1×10^7 conidia/ml concentration. In the present investigation, the larval mortality with *M. anisopliae* was most effective (lower LC50 values) at 25°C whereas, on the basis of adult emergence the use of *M. anisopliae* was most efficacious at 30°C which is in line with the earlier reports. In an experiment on effect of temperature on third instars of differential grasshopper at temperatures ranging from 10 to 35°C, the activity of *B. bassiana* was greatest at 25°C and was adversely affected by high and low temperatures (Amarasekare, *et.al.* 2004), this finding is in corroboration with the present work.

The data suggest that temperature-dependent feeding and recovery did not contribute to quicker death at higher temperatures which could not be compared with present data set. Expression of the toxin itself appears to depend on temperature, possibly through the influence of temperature on metabolic rate of affected gut cells (van Frankenhuyzen and Nystrom, 2012), indicating higher preference for optimum temperature of 25oC.

CONCLUSION

The median lethal concentration of biorationals (*M. anisopliae*, *Bacillus thuringiensis*, Azadirachtin and Spinosad) against second instar larvae of *P. xyllostella* was lowest at 25°C over 30°C and 35°C whereas, *B. bassiana* was found to be most effective at 30°C as compared to 25°C and 35°C. On the basis of adult emergence, the LC_{50} value of *M. anisopliae, B. bassiana,* Azadirachtin and Spinosad was lowest at 30°C against II instar larvae of *P. xyllostella* that was followed by LC_{50} values at 25 and 35°C whereas, *B. thuringiensis* was most effective at 25°C as compared to 30°C and 35°C indicating the reduced efficacy of biorationals with increase in temperature beyond 30°C or preference for lower temperature regimes under laboratory conditions. The cropping season of cabbage and cauliflower in Vidarbha (Maharashtra) is generally in the month of August with temperature in the range of 25-30^{0}C with sufficient humidity which will be conducive for the biorationals though, it needs validation in the field.

ACKNOWLEDGEMENTS

Authors are thankful to the Associate Dean, Post Graduate Institute and the Head, Department of Entomology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola for providing necessary facilities.

Table 1. Variability in toxicity of biorational to *P. xylostella* as influenced by different temperatures

Bio-rational	On the basis of larval mortality			On the basis adult emergence		
	LC_{50} at 25°C	LC_{50} at 30°C	LC_{50} at 35°C	LC_{50} at 25°C	LC_{50} at 30°C	LC_{50} at 35°C
Metarhizium anisopliae (1.15 % WP)	$5.2x10^4$ cfu/g	$1.04x10^5$ cfu/g	$2.2x10^5$ cfu/g	$1.52x10^4$ cfu/g	$2.45x10^3$ cfu/g	$8.21x10^4$ cfu/g
Beauveria bassiana (1.15 % WP)	$7.9x10^3$ cfu/g	$5.87x10^3$ cfu/g	$4.4x10^4$ cfu/g	$3.36x10^3$ cfu/g	$1.53x10^3$ cfu/g	$9.7x10^3$ cfu/g
Bacillus thuringiensis (0.5 % WP)	$1.1x10^5$ cfu/g	$1.2x10^5$ cfu/g	$5.8x10^5$ cfu/g	$2.0x10^4$ cfu/g	$2.6x10^4$ cfu/g	$1.6x10^5$ cfu/g
Azadirachtin (300 ppm)	0.27 ppm	0.38 ppm	0.39 ppm	0.068 ppm	0.047 ppm	0.11 ppm
Spinosad (45 SC)	0.060 ppm	0.22 ppm	0.25 ppm	0.025 ppm	0.011 ppm	0.03 ppm

Tabular value at 0.05 level = 7.82

Table 2. Effect of temperature on toxicity of biorationals to *P. xylostella* on the basis of larval mortality (at 25ºC)

Biorationals	LC_{50}	FL (95%)	LC_{90}	FL (95%)	Slope	(x^2)
Metarhizium anisopliae (1.15 % WP)	$5.2x10^4$ cfu/g	$1.73x10^4$-$2.26x10^5$	$8.66x10^6$ cfu/g	$1.25x10^6$-$4.8x10^8$	0.57	2.97
Beauveria bassiana (1.15 % WP)	$7.9x10^3$ cfu/g	2.1x103-$4.7x10^4$	$1.3x10^6$ cfu/g	$1.53x10^5$-$1.9x10^8$	0.46	1.59
Bacillus thuringiensis (0.5 % WP)	$1.1x10^5$ cfu/g	$4.3x10^4$- $3.3x10^5$	$6.8x10^6$ cfu/g	$1.65x10^6$- $9.2x10^7$	0.71	3.06
Azadirachtin (300 ppm)	0.27 ppm	0.87-1.45	50.26 ppm	6.14-4779.52	0.56	3.11
Spinosad (45 SC)	0.060 ppm	0.021-0.14	2.15 ppm	0.72-14.67	0.82	3.96

Tabular χ^2 value at 0.05 level = 7.82

Table 3. Effect of temperature on toxicity of biorationals to P. *xylostella* on the basis of larval mortality (at 30ºC)

Biorationals	LC_{50}	FL (95%)	LC_{90}	FL (95%)	Slope	(x^2)
Metarhizium anisopliae (1.15 % WP)	$1.04x10^5$ cfu/g	$2.1x10^4$-$3.6x10^5$	$3.5x10^6$ cfu/g	$8.0x10^5$-$3.2x10^8$	0.83	1.40
Beauveria bassiana (1.15 % WP)	$5.87x10^3$ cfu/g	$9.0x10^2$-$1.9x10^4$	$2.65x10^5$ cfu/g	$6.4x10^4$-$8.3x10^6$	0.77	2.49
Bacillus thuringiensis (0.5 % WP)	$1.2x10^5$ cfu/g	$2.5x10^4$-$3.6X10^5$	$3.1x10^6$ cfu/g	$9.4x10^5$-$5.0x10^7$	0.91	0.63
Azadirachtin (300 ppm)	0.38 ppm	0.076-1.83	27.29 ppm	4.17-12124.9	0.69	2.56
Spinosad (45 SC)	0.22 ppm	0.054-0.52	1.98 ppm	0.81-13.29	1.34	2.25

Tabular χ^2 value at 0.05 level = 7.82

Table 4. Effect of temperature on toxicity of biorationals to *P. xylostella* on the basis of larval mortality (at 35ºC)

Biorationals	LC_{50}	FL (95%)	LC_{90}	FL (95%)	Slope	(x^2)
Metarhizium anisopliae (1.15 % WP)	$2.2x10^5$ cfu/g	$5.36x10^4$-$1.36x10^6$	$1.32x10^7$ cfu/g	$1.9x10^6$-$1.74x10^{10}$	0.72	1.6
Beauveria bassiana (1.15 % WP)	$4.4x10^4$ cfu/g	$6.5x10^3$-$5.6x10^5$	$8.4x10^6$ cfu/g	$6.29x10^5$-$6.3x10^{11}$	0.56	1.10
Bacillus thuringiensis (0.5 % WP)	$5.8x10^5$ cfu/g	$1.2x10^5$-$2.3X10^6$	$2.2x10^7$ cfu/g	$4.3x10^6$-$5.1x10^9$	0.81	1.48
Azadirachtin (300 ppm)	0.39 ppm	0.055-1.32	12.42 ppm	2.59-3022.71	0.82	1.32
Spinosad (45 SC)	0.25 ppm	0.039-0.50	3.34 ppm	1.18-24.32	1.02	1.29

Tabular χ^2 value at 0.05 level = 7.82

Table 5. Effect of temperature on toxicity of biorationals to *P. xylostella* on the basis of adult emergence (at 25ºC)

Biorationals	**LC_{50}**	**FL (95%)**	**LC_{90}**	**FL (95%)**	**Slope**	**(x^2)**
Metarhizium anisopliae (1.15 % WP)	$1.52x10^4$ cfu/g	$2.76x10^3$-$4.75x10^4$	$6.2x10^5$ cfu/g	$1.7x10^5$-$9.6x10^6$	0.79	0.96
Beauveria bassiana (1.15 % WP)	$3.36x10^3$ cfu/g	4.8 $x10^2$- $1.24x10^4$	$5.5x10^5$ cfu/g	$5.4x10^4$-$8.2x10^6$	0.68	0.43
Bacillus thuringiensis (0.5 % WP)	$2.0x10^4$ cfu/g	$3.1x10^3$-$7.0X10^4$	$1.1x10^6$ cfu/g	$2.9x10^5$-$1.6x10^7$	0.73	2.18
Azadirachtin (300 ppm)	0.068 ppm	0.012-0.21	2.57 ppm	0.69-46.96	0.81	0.19
Spinosad (45 SC)	0.025 ppm	0.0057-0.059	0.20 ppm	0.083-1.459	1.42	2.23

Tabular χ^2 value at 0.05 level = 7.82

Table 6. Effect of temperature on toxicity of biorationals to *P. xylostella* on the basis of adult emergence (at 30ºC)

Biorationals	**LC_{50}**	**FL (95%)**	**LC_{90}**	**FL (95%)**	**Slope**	**(x^2)**
Metarhizium anisopliae (1.15 % WP)	$2.45x10^3$ cfu/g	$3.7x10^3$-$8.4x10^4$	$1.3x10^6$ cfu/g	$3.1x10^5$-$4.0x10^6$	0.74	2.39
Beauveria bassiana (1.15 % WP)	$1.53x10^3$ cfu/g	$2.6x10^2$-$4.82x10^3$	$5.9x10^4$ cfu/g	$1.69x10^4$-$7.0x10^5$	0.80	2.00
Bacillus thuringiensis (0.5 % WP)	$2.6x10^4$ cfu/g	$4.7x10^3$- $8.0x10^4$	$8.4x10^5$ cfu/g	$2.5x10^5$-$4.4x10^7$	0.85	1.22
Azadirachtin (300 ppm)	0.047 ppm	0.0076-0.16	2.53 ppm	0.62-53.28	0.74	1.74
Spinosad (45 SC)	0.011 ppm	0.0022-0.032	0.22 ppm	0.077-16.88	0.99	4.24

Tabular χ^2 value at 0.05 level = 7.82

Table 7. Effect of temperature on toxicity of biorationals to *P. xylostella* on the basis of adult emergence (at 35ºC)

Biorationals	**LC_{50}**	**FL (95%)**	**LC_{90}**	**FL (95%)**	**Slope**	**(x^2)**
Metarhizium anisopliae (1.15 % WP)	$8.21x10^4$ cfu/g	$2.34x10^4$-$2.32x10^5$	$2.0x10^6$ cfu/g	$5.6x10^5$-$4.6x10^7$	0.92	1.24
Beauveria bassiana (1.15 % WP)	$9.7x10^3$ cfu/g	$1.57x10^3$-$4.2x10^4$	$1.0x10^6$ cfu/g	$1.6x10^5$-$1.5x10^8$	0.62	2.32
Bacillus thuringiensis (0.5 % WP)	$1.6x10^5$ cfu/g	$2.0x10^4$-$7.0X10^5$	$1.4x10^7$ cfu/g	$2.4x10^6$-$1.8x10^9$	0.66	0.42
Azadirachtin (300 ppm)	0.11 ppm	0.024-0.32	2.72 ppm	0.91-20.14	0.75	2.68
Spinosad (45 SC)	0.03 ppm	0.004-0.10	1.53 ppm	0.38-27.31	0.93	1.72

Tabular χ^2 value at 0.05 level = 7.82

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