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STUDIES ON NATIVE ISOLATE OF *BACILLUS THURINGIENSIS* AGAINST DIAMOND BACKMOTH (*PLUTELLA XYLOSTELLA*) ON CABBAGE

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ABSTRACT: The single isolate of *B. thuringiensis* was obtained from the soil samples which were collected from Shahuwadi and Kagal tehsils of Kolhapur district of Western Ghat Zone of Maharashtra. The isolate was further subjected for morphological and biochemical characterization. The bioassay studies against 3rd instar larvae of DBM (*P. xylostella*) was conducted in laboratory and under pot culture condition and this isolate was compared with the commercial *Bacillus thuringiensis* product as standard check. Efficacy of five different concentrations of *Bacillus thuringiensis* isolate against 3rd instar larvae of DBM (*P. xylostella*) showed the mortality ranged from 21.66 to 90.10 per cent. LC_{50} values recorded for progressive 24 hrs, 48hrs and 72hrs were 979.70, 603.35 and 198.42ppm, respectively. Efficacy of *Bacillus thuringiensis* isolate with five different concentrations against DBM (*P. xylostella*) under pot culture condition using cabbage as a test crop recorded 19.66 to 81.66 per cent mortality.

KEYWORDS: *Bacillus thuringiensis*, DBM (P. xylostella), cabbage

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1.INTRODUCTION

Cruciferous vegetables are most important in our diet as they possesses nutritious value. Cabbage (*Brassica oleraceae* var. capitata L.) belongs to the Brassicaceae family and is a cool season crop (Best, 2000). The diamondback moth (DBM; *Plutella xylostella* L; Lepidoptera: Plutellidae) is an important pest of cruciferous crops and enjoys a worldwide distribution (CEI, 1967). It has become

the most destructive pest of cruciferous plants throughout the world (Talekar and Shelton, 1993). In India DBM infests important cruciferous crops, such as cabbage, cauliflower, kohlrabi, radish, turnip, beetroot, mustard.DBM causes more than 50% loss in marketable yield of cabbage (Chelliah and Srinivasan, 1985). Biological control of insect pests has become popular and provides a safer means to reduce insect damage (Dhaliwal and Arora, 1998). Among various options available, the use of soil bacterium *Bacillus thuringiensis* has become immense potential for use of biopesticide. India possesses a great diversity of natural ecosystem. One of the area rich in endemism is the Western Ghats which is recognized as one of the hot spot of biodiversity in the world (Khoshoo, 1994, Thakar and Sonawane 2013). *Bacillus thuringiensis* has been used as a successful biological insecticide for more than 40 years and is a uniquely specific, safe and effective tool for the control of a wide variety of insect pests (Nester *et al.,* 2002). Hence, the present research work, an attempt was made to isolate *Bacillus thuringiensis* from soil and evaluate their insecticidal activity against DBM.

2. MATERIALS AND METHODS

Totally 20 soil samples were collected from the Kagal and Shahuwadi Tehsils of Kolhapur district (Western Ghat Zone) of Maharashtra, for isolation of *Bacillus thuringiensis*. The isolation of *Bacillus thuringiensis* was carried out by following sodium acetate selection method (Travers *et al*.,1987).The isolate was subjected for morphological and biochemical characterization.

Morphological characterization was done by compairing the colony morphology with reference strain *Bacillus thuringiensis* strain HD1. The isolate was subjected for crystal staining as per the protocol given by Sharif and Alaeddinoglu (1988). The isolate was used for bioassay to ascertain he insecticidal activity against *Plutella xylostella.* The isolate obtained was tested for the efficacy against 3rd instar larvae diamond backmoth (*Plutella xylostella*) on cabbage as test crop. Diamond backmoth was mass cultured in the laboratory as per the method described by Liu and sun (1984) with little modification. The native *Bacillus thuringiensis* isolate was grown in modified glucose medium broth (MGM) (Aronson *et al.,* 1971) for 3 days at 28 ± 2ºC and used for conducting the Leaf dip bioassay. Cabbage leaf disc of 6 cm diameter were dipped in *Bt* culture of 5 different concentrations (50, 100, 250, 500, 1000 ppm respectively) including standard check Dipel (commercial *Bt* product) and control. The leaf disc were dried aseptically and feed to larvae. After feeding of leaf disc by larvae mortality was observed at 24 hr, 48 hr and 72 hr of treatment and data were subjected to analysis of variance after arcsine transformation and LC_{50} were obtained by probit analysis (Finney 1952). After analyzing per cent larval mortality of single native *Bacillus thuringiensis i*solate with commercial Dipel 8L was selected for further Pot culture evaluation on cabbage against 3rd instar larvae of *P. xylostella* and mortality was recorded at interval of 24, 48, 72 hrs respectively after treatment.

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3. RESULTS AND DISCUSSION

Single *Bacillus thuringiensis* isolate was obtained from the 20 soil samples which were collected from the Shahuwadi and Kagal Tehsil of Kolhapur district of Maharashtra. The isolate was examined for their colony morphology like colour, shape, size, nature of margin and surface appearance after 24 hr of incubation. The isolate showed creamy white coloured colony, circular shape, rough in nature, wavy margins (Table 1). Similar to that of the reference strain HD1.The diameter of the colonies ranged from 3mm.The isolate showed positive reaction for nitrate reduction, catalase production, Voges- Proskauer reaction and oxidase test, starch and casein hydrolysis respectively. But showed negative for acid and gas production, arginine hydrolysis, and esterase activity (Table 2). Similar observations made by Kaur *et al*. (2006) who reported that the strains of *Bacillus thuringiensis*, besides producing parasporal crystal bodies, were positive for catalase production, oxidase activity, nitrate reduction, starch and casein hydrolysis. Starr (1981) reported that the strains of *Bacillus thuringiensis*, were positive for catalase production, V-P reaction, nitrate reduction, starch and casein hydrolysis but were negative for acid and gas production. The isolate (Table 3) was gram positive rods, endospore forming and proved positive for the presence of crystal.The type of crystal was the bipyramidal. While the dominance of spherical crystals was observed by Arrieta *et al.* (2004) in coffee plantations, only bipyramidal inclusions were reported by Wangondu *et al.* (2003). Although the relationship between the type of crystal morphology and the level of insecticidal activity is not clear, it has been reported that the strains with bipyramidal crystals are more toxic to lepidopteran larvae (Obeidal *et al.,* 2004, Asokan and Puttaswamy, 2007, Monnerat *et al.,* 2007 and Opondo *et al*., 2010). The Leaf dip bioassay carried against third instar larvae of *P. xylostella*, the larval mortality under laboratory at lower concentration upto 48 hrs was nil, it increased with the increase in concentration and as well as time (Table 4). Among the treatments Dipel performed best and followed by the *Bacillus thuringiensis* isolate at 1000 ppm concentration was more effective. LC_{50} values recorded for progressive 24 hrs, 48hrs and 72hrs were 979.70, 603.35 and 198.42 ppm, respectively (Table 5). Ahmad *et al*. (1997) carried out Bioassay studies using leaf dip method. The leaf dip bioassay technique has been used frequently to assess susceptability of *P. xylostella* to microbial pesticides such as *Bacillus thuringiensis* (Tabashnik *et al*., 1990, Tabashnik *et al*., 1993 and Shelton *et al*., 1993). Based on the results of Leaf dip bioassay, 7 efficient treatments, were used against third instar larvae under Pot culture experiment trial with cabbage as test crop. The similar trend of result were observed under Pot culture condition. Among the treatments Dipel showed highest larval mortality followed by the *Bacillus thuringiensis* isolate at 1000 ppm concentration was more effective (Table 6). Goudar (2011) conducted Pot culture experiment and tested six different formulation of *Bacillus thuringiensis* against *P. xylostella* and reported 90.48 per cent mortality even after 5th days after

spraying in treatment with dipel formulation. The present study thus reported isolation of *Bacillus thuringiensis* is efficient to control the *Plutella xylostella*.

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Table1: Morphological characteristics of native *Bacillus thuringiensis* isolate

Sr. No.	Colony Morphology	
1	Shape	Circular
2	Colour	Creamy white
3	Nature	Rough
4	Margin	Wavy
5	Size	3mm
6	Elevation	Flat

Table 2: Biochemical characterization of the native *Bacillus thuringiensis* isolate

Sr.No.	Enzymatic test	Result
1	**Catalase**	+
2	**Starch hydrolysis**	+
3	**Casein hydrolysis**	+
4	**Nitrate reduction**	+
5	**V-P reaction**	+
6	**Acid and gas production**	-
7	**Arginine dihydrolase**	-
8	**Esterase avtivity**	-
9	**Oxidase test**	+

+ = positive Test, - = negative Test

Table 3 : Gram reaction, motility, endospore formation and crystal staining of native *Bacillus thuringiensis* isolate

Sr. No.	Microscopic Morphology	
1	Gram reaction	Gram positive
2	Motility	Motile
3	Endospore production	+
4	Crystal formation	+

Table 4:- Efficacy of native *Bacillus thuringiensis* isolate against 3rd instar larvae DBM (P. xyllostella) under Laborator condition

Sr. No	Concentration of Isolate	Mean per cent mortality at intervals after		
		24 hrs	48 hrs	72 hrs
1.	**50**	*0.00	0.00	21.66
2.	**100**	8.20	12.03	37.13
3.	**250**	12.50	20.43	49.43
4.	**500**	22.60 (26.41)	36.33 (37.07)	65.43 (53.99)
5.	**1000**	56.50 (48.74)	71.76 (57.84)	90.10 (71.67)
6.	**Dipel**	68.00 (55.55)	82.26 (65.10)	100 (90.00)
7.	**Untreated control**	0.00 (00)	0.00 (00)	0.0 (00)
	SE ±	0.86	0.28	0.59
	CD at 5%	2.61	0.86	1.80

Table 5: Median Lethal concentration (ppm) of Bt isolate against 3rd instar larvae of DBM (*P. xylostella*)

P. xylostella **at instar 3rd**	**LC 50 (ppm)**	**Fiducial limits**	**Probit equation**	**X^2 value**
24 hrs	979.70	545.73-6706.97	Y= 0.326 X + 1.781	10.50
48 hrs	603.35	369.67-1533.10	Y= 0.417X +1.948	10.77
72 hrs	198.42	161.77-241.35	Y= 1.399 X + 1.78	6.82

Table 6 :- Efficacy of native *Bacillus thuringiensis* isolate against 3 rd instar larvae of DBM (P. xyllostella) under Pot culture condition on Cabbage

Sr. no.	**Concentration of Isolate (ppm)**	**Mean per cent mortality at different intervals after treatment**		
		24 hrs	**48 hrs**	**72 hrs**
1.	**50**	*0.00 (0.0)	0.00 (0.0)	19.66 (26.32)
2.	**100**	6.61 (14.88)	11.35 (19.64)	35.10 (36.37)
3.	**250**	11.13 (19.49)	19.80 (26.42)	45.43 (42.38)
4.	**500**	18.83 (25.69)	31.60 (34.20)	60.33 (50.96)
5.	**1000**	49.46 (44.69)	68.90 (56.10)	81.66 (64.65)
6.	**Dipel**	65.43 (53.99)	79.66 (63.20)	99.53 (86.53)
7.	**Untreated control**	0.00 (0.0)	0.00 (0.0)	0.00 (0.0)
	SE ±	0.17	0.17	0.51
	CD at 5%	0.51	0.52	1.56