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RESEARCH PAPER

Insecticidal properties of actinobacterial extracts against selected lepidopteran insect pests under laboratory and greenhouse condition

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Abstract: Microbial pesticides have evolved as an alternate technique to chemical insecticides due to their high target specificity and ecological safety. Investigations were carried out to explore the efficacy of 28 actinobacterial extracts against a day old second instar larvae of *Plutella xylostella*, *Spodoptera litura* and *Spodoptera frugiperda*. Among the actinobacterial extracts, DBT-64 recorded 78.85, 76.50 and 76.00 per cent mortality of *P. xylostella*, *S. litura* and *S. frugiperda* respectively followed by DBT-80 with 80.75, 75.25 and 77.50 per cent mortality. Further, DBT-59 has recorded 79.50, 78.25 and 75.25 per cent larval mortality of *P. xylostella*, *S. litura* and *S. frugiperda* respectively at 72 HAT under *in-vitro* conditions. Among the actinobacterial extracts, three potent extracts (DBT-64, DBT-80 and DBT-59) were taken forward to greenhouse conditions to test verify their efficacy against *P. xylostella*, *S. litura* and *S. frugiperda*. DBT-80 has caused highest larval mortality in *P. xylostella* (84%), *S. litura* (82.75%) and *S. frugiperda* (80.50%) followed by DBT-64 with 81.00, 80.00 and 79.50 per cent larval mortality in *P. xylostella, S. litura* and *S. frugiperda* respectively at 96 HAT under green house condition. Among the isolates, DBT-80 showed higher insecticidal activity against the test insects followed by DBT-64 and DBT-59. These potential actinobacterial extracts can be used in the Integrated Pest Management programmes of *Plutella xylostella*, *Spodoptera litura* and *Spodoptera frugiperda* which are found very effective under laboratory and greenhouse condition.

Key words: Actinobacterial extracts, Biocontrol, Insecticidal activity, Isolates

Introduction

In present-day agriculture, crop protection is becoming an unavoidable event to maintain productivity. Chemical pesticides are considered to be an excellent strategy for any given pest problem, but their overuse has produced many environmental concerns besides being ineffective due to resistance development. Microbial pesticides had evolved as an alternate technique at this point due to their high target specificity and ecological safety (Usta, 2013). Management of different groups of pests or insects is done by applying large varieties of microorganisms including fungi, bacteria viruses, protozoans, and nematodes as microbial pesticides. These pesticides are species-specific and are non-pathogenic to other useful microorganisms (Kumari *et al*., 2014). So, it is apparent that the selection of a strain of any given microbes for pest management is a function of the pesticidal metabolites it produces and their bioactivity against the target pest (Subbanna *et al*., 2020). Approximately 70,000 different species of insect pests damage food crops across the world (Vijayabharathi *et al*., 2014). Among them, species belonging to the order Lepidoptera are a major cause of crop loss (Pimentel, 2009). Among the microbes, actinomycetes are reported to produce about 45 per cent of the bioactive compounds and 80 per cent of antibiotics are available in the genera *Micromonospora* and *Streptomyces* sp. Several *Streptomyces* metabolites have been identified as possible protective agents against a variety of insect pests such as ivermectin, emamectin benzoate, polynactin, milbemycin and spinosad. Biopesticides have gained increased interest in recent years from those concerned with the production of environmentally sustainable and healthy integrated crop management with compatible pest management approaches and tactics. Microbial pesticides are more specific, have low relative cost and are more environmental friendly in nature, they have gained substantial attention (Castillo *et al*., 2000) in pest control programmes. Therefore, there is a need to evaluate the efficacy of the microorganisms for developing safe and eco-friendly alternatives to chemical insecticides as biocontrol agents. Several microorganisms produce bioactive secondary metabolites which activate the GABA (Gamma-aminobutyric acid) system of host insect or disruption of nicotinic acetylcholine receptors (Kirst, 2010) as seen in the mode of action of spinosad. Actinomycetes are important not only as degraders of organic matter but also known to produce enzymes like chitinase (*Streptomyces viridificans*) and this chitinase enzyme is very important in the biocontrol of insects (Reguera and Leschine, 2001). The different biochemical compounds exhibited diverse mode of action as they are acetylcholinesterase inhibitors, JH agonist, inhibit the synthesis of ecdysteroids, inhibitory effects on the digestive system and destroy respiratory functions. Hence, the present study is proposed to investigate the insecticidal properties of actinobacterial extracts against diamondback moth (*Plutella xylostella*), tobacco leaf eating caterpillar (*Spodoptera litura*) and fall armyworm (*Spodoptera frugiperda*) under laboratory and green house condition.

Material and Methods

Mass multiplication of test insects: Three species of insects (*Plutella xylostella* L*, Spodoptera litura* Fabricius and

Spodoptera frugiperda J. E. Smith) that were subjected to bioassay were reared in the Biocontrol Laboratory, Department of Agricultural Entomology, University of Agricultural Sciences, Dharwad during 2021-22.

Bioassay: Twenty-eight actinobacterial extracts were sourced from Microbial Genetics Laboratory, Department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad and were subjected for bioassay to ascertain insecticidal activity against insect pests. One actinobacterial extract (AUDT-240) and fungal extract (FIIRS2) which are potent against lepidopteran insect pests were included as standard check for comparison.

Preparation of actinomycetes culture for bioassay: Isolates were initially spotted on a starch casein agar (SCA) medium and purified by the four-way streaking method. Single pure colony were inoculated to the SCB (Starch casein broth) and incubated at 28±2^{0}C for 5-7 days on a rotary shaker (190 rpm/min). Then the incubated flasks were taken and placed in the dark room, after 10-12 days of incubation, the culture broth was filtered and centrifuged at 10,000 rpm at 4^{0}C for 10 min. The supernatant was subjected for the evaluation of its insecticidal activity against second instar larvae of *S. litura*, *S. frugiperda* and *P. xylostella* (EI-Khawaga and Megahed, 2012).

Insecticidal activity of actinomycetes isolates against test insects: The leaf dip bioassay method was followed. Leaf discs of 9 cm diameter covering either side of midrib were cut from fully expanded untreated soybean, cabbage and maize leaves. These discs were dipped in an aqueous solution of the test isolates for about 60 seconds. After draining off excess fluid, leaf discs were dried under shade for 10 minutes before transferring to Petri plates over a filter paper. Bioassay was done with two replications per treatment and ten second instar larvae released on each disc and Petri plates were closed using a rubber band. Leaf disc dipped in distilled water alone serve as a control and positive control with a spinosad. The larval mortality was recorded at 24, 48 and 72 hours after treatment under laboratory conditions. The potent actinobacterial isolates DBT-64, DBT-80 and DBT-59 were taken forward for greenhouse experimentation including spinosad 45 SC @ 0.2 ml/l as a standard check and control. The centrifuged and filtered supernatant of actinomycetes collected after the stationary condition was mixed with water in equal proportion and was used for spraying. Water based flowable (WBF) liquid formulation was prepared by mixing the supernatant of actinomycetes with adjuvants like Tween, Potassium sorbate, Xanthan gum (1.5: 0.2: 0.06 ml) in 100 ml water. The potent isolates were sprayed on the 25-30 days old seedlings of maize, soybean and cabbage. A day old ten second instar larvae of *P. xylostella*, *S. litura* and *S. frugiperda* were released. Each treatment was replicated four times. After the release of the larvae, each potted plant was caged with nylon mesh to avoid the escape of larvae. The larval mortality was recorded at 24, 48, 72 and 96 hours after treatment and data were subjected to analysis of variance after arcsine transformation and means were subjected to Duncan's Multiple Range Test (DMRT) (Duncan, 1955)

The per cent mortality was calculated by following the formula

$$\text{Per cent mortality} = \frac{\text{Number of dead larvae}}{\text{Total number of larvae treated}} \times 100$$

Results and discussion

In-vitro* evaluation of actinobacterial extracts against Diamondback moth, *P. xylostella

At 48 hours after treatment (HAT): Among the twenty eight actinobacterial extracts, DBT-80 has recorded more than 45 per cent larval mortality in the second instar larvae of *Plutella xylostella* at 40 ml/l (Table 1). All actinobacterial extracts tested against second instar larvae of the *P. xylostella* revealed that only 3 actinobacterial extracts (DBT-64, DBT-80 and DBT-59) have recorded more than 50 per cent mortality at 48 HAT with 50 ml/l. DBT-80 and DBT-64 recorded 59.50 per cent mortality of DBM larvae and were on par with DBT-59 (58.50%). The rest of the actinobacterial extracts recorded less than 40 per cent mortality. However standard check, spinosad 45 SC @ 0.2 ml/l caused highest mortality of 85.00 per cent under laboratory conditions (Table 1).

At 72 hours after Ttreatment (HAT): Observation on per cent larval mortality at 40 ml/l (Table 1) revealed that DBT-80 showed significant mortality of 56.50 per cent at 72 HAT. The actinobacterial extracts of DBT-59 caused 47 per cent mortality followed by DBT-64 (46.50%) which were statistically on par with each other. At 50 ml/l, DBT-64 (78.85%), DBT-59 (79.50%) and DBT-80 (80.75%) mortality and were statistically on par with each other. The insecticidal activity of remaining extracts was observed in the order of DBT-121 (46.50%) = DBT-55 (46.50%) > DBT-72 (46.00%) > DBT-70 (45.00%) > DBT-34 (44.50%) > DBT-47 (44.00%) which were statistically on par with each other. Standard check spinosad 45 SC @ 0.2 ml/l has recorded highest mortality of 95.00 per cent. The highest mortality of 78.85 per cent was recorded in DBT-64 which was followed by DBT-59 (79.50%) and DBT-80 (80.75%) (Fig. 1). The present findings are in line with the investigation of Gayatree (2011), who reported more than 50 per cent mortality of *P. xylostella* by the actinomycete isolates AUDT-210, AUDT-212, AUDT-240, AUDT-232, AUDT-217, AUDT- 299, AUDT-344 and AUDT-248. The crude filtrate extracts of DBT-96, DBT-70, DBT-72, DBT-55, DBT-53, DBT-47 and DBT-34 caused more than 40 per cent mortality of DBM larvae which is in line with the reports of Xuhong (2005) who reported a 45.22 per cent larval death of diamondback moth with the crude extract of *Streptomyces griseus* sub sp. *hangzhouensis*. Actinobacterial extracts DBT-64, DBT-59 and DBT-80 have registered 77.50 per cent larval mortality. Wang *et al*. (2015) found that *Streptomyces* isolate SN-336 collected from the soil samples caused 71.70 per cent mortality of diamondback moth after 48 HAT. In the present study, variation in the mortality obtained might be due to the strain difference in the actinobacterial extracts and also the difference in the insecticidal secondary metabolites they produce.

Table 1. Larval mortality of *Plutella xylostella* as influenced by different concentrations of actinobacterial extracts at 48 and 72 HAT under laboratory conditions

Sl. No.	Isolates / Conc	Per cent Mortality at			
		48 HAT		72 HAT	
		40 ml/l	50 ml/l	40 ml/l	50 ml/l
1	DBT-16	0	14.00 (21.92)^{f}	0	27.00 (31.30)^{g}
2	DBT-19	0	0	0	0
3	DBT-22	0	0	24.50 (29.66)^{g}	30.50 (33.52)^{f}
4	DBT-24	14.50 (22.37)^{g}	26.00 (30.65)^{e}	29.50 (33.57)^{f}	37.00 (37.96)^{e}
5	DBT-95	15.00 (23.12)^{g}	17.50 (24.72)^{f}	25.50 (30.32)^{g}	33.50 (35.36)^{f}
6	DBT-31	13.50 (21.52)^{g}	25.00 (30.45)^{e}	26.50 (30.97)^{g}	37.00 (37.46)^{e}
7	DBT-34	16.00 (23.56)^{g}	35.00 (36.67)^{d}	25.50 (30.32)^{g}	44.50 (41.84)^{d}
8	DBT-37	14.00 (22.46)^{g}	34.00 (36.25)^{d}	24.50 (30.76)^{g}	39.50 (39.86)^{e}
9	DBT-47	24.00 (29.40)^{e}	36.50 (37.16)^{d}	28.00 (32.43)^{g}	44.00 (41.50)^{d}
10	DBT-52	0	0	0	0
11	DBT-53	20.50(27.56)^{f}	37.00 (37.46)^{d}	25.50 (31.00)^{g}	42.50 (40.68)^{d}
12	DBT-54	0	0	0	0
13	DBT-55	30.50 (34.00)^{d}	37.00 (38.71)^{d}	40.50 (39.52)^{d}	46.50 (46.16)^{d}
14	DBT-61	0	25.50 (30.32)^{e}	0	31.00 (33.82)^{f}
15	DBT-64	36.50 (38.71)^{c}	59.50 (51.58)^{c}	46.50 (42.99)^{c}	78.85 (62.60)^{c}
16	DBT-67	0	0	0	0
17	DBT-70	25.50 (31.88)^{e}	29.50 (33.48)^{e}	35.50 (38.30)^{e}	45.00 (43.11)^{d}
18	DBT-72	25.50 (31.25)^{e}	36.50 (37.91)^{d}	37.00 (39.01)^{d}	46.00 (43.90)^{d}
19	DBT-78	0	0	0	0
20	DBT-80	46.00 (43.11)^{b}	59.50 (50.47)^{c}	56.50 (49.29)^{b}	80.75 (61.77)^{c}
21	DBT-96	26.00 (30.65)^{e}	36.00 (37.41)^{d}	30.00 (34.12)^{f}	40.50 (40.72)^{e}
22	DBT-84	0	14.50 (22.35)^{f}	0	35.00 (37.27)^{g}
23	DBT-59	37.00 (38.90)^{c}	58.50 (49.89)^{c}	47.00 (43.27)^{c}	79.50 (61.68)^{c}
24	DBT-90	25.00 (29.99)^{e}	36.00 (37.62)^{d}	35.50 (37.27)^{e}	39.50 (39.60)^{e}
25	DBT-92	0	14.00 (22.41)^{f}	0	22.50 (29.92)^{i}
26	DBT-121	26.00 (31.45)^{e}	37.00 (37.92)^{d}	29.50 (32.89)^{f}	46.50 (44.27)^{d}
27	DBT-93	0	0	0	0
28	AUDT-240	46.00 42.70)^{b}	68.00 (56.30)^{b}	55.00 (49.87)^{b}	90.00 (72.59)^{b}
29	FIIRS2	55.50 (49.51)^{b}	66.50 (55.20)^{b}	63.50 (53.55)^{b}	86.50 (68.44)^{b}
30	Spinosad 45 SC @ 0.2 ml/l	85.00 (79.24)^{a}	85.00 (78.43)^{a}	95.00 (77.77)^{a}	95.00 (84.22)^{a}
31	Untreated check	0	0	0	0
	S.Em. ±	0.73	1.13	1.00	1.34
	C.D.@5%	2.97	4.40	3.91	5.23
	C.V. (%)	5.16	5.55	5.61	5.32

Values in parentheses are the *arc sine* transformed values.
Means followed by the same letters in a column do not differ significantly (0.05) by DMRT
Sl.No: 1-28 are actinobacterial extracts, 29-Fungal extract HAT: Hour after treatment

In-vitro* evaluation of actinobacterial extracts against tobacco leaf eating caterpillar, *S. litura

At 48 hours after treatment (HAT): At 40 ml/l, 14 actinobacterial extracts could cause larval mortality with a mortality range of 10.00 to 50.00 per cent. Actinobacterial extracts DBT-19, DBT-22, DBT-47, DBT-52, DBT-54, DBT-61, DBT-67, DBT-70, DBT-72, DBT-78 DBT-96, DBT-84, DBT-92 and DBT-93 could not cause any larval mortality at 20, 30 and 40 ml/l. Further, at 50 ml/l except 3 extracts (DBT-19, DBT-54 and DBT-78), all the remaining actinobacterial extracts caused mortality with a range of 10.00 to 77.00 per cent. Among the isolates, DBT-80 (68%) caused higher mortality and remain effective against *S. litura* (Table 2).

At 72 Hours After Treatment (HAT): At 40 ml/l, extract DBT-80 documented 60.00 per cent mortality. The remaining actinobacterial extracts showed larval mortality below 50 per cent (Table 2). At a higher concentration of 50 ml/l, the results revealed that the some actinobacterial extracts were more effective against *S. litura*. Of the extracts, DBT-59 recorded highest of 78.25 per cent larval mortality and was statistically on par with DBT-64 (76.50%) and DBT-80 (75.25%). The remaining actinobacterial extracts showed comparatively less mortality which is below 50 per cent. Spinosad 45 SC @ 0.2 ml/l has recorded 95 per cent larval mortality. Among actinobacterial extracts, the highest larval mortality of 78.25 per cent was recorded by DBT-59 followed by DBT-64 (76.50%) and DBT-80 (75.25%) (Fig. 1). The present findings are in line with EI-Khawaga and Megahed (2012) who screened insecticidal activities of crude extract of twenty actinomycetes isolates collected from different locations of Egypt. The isolate No. A12 caused 60 per cent mortality of second instar larvae of cotton leaf worm, *Spodoptera littoralis.* AUDT-280, AUDT-257, ADUT-314 and CFE-62 (b) caused 66, 64, 64 and 60 per cent larval mortality respectively against *S. litura* (Srujana, 2015). Similarly, Shivukumar (2015) also reported the efficacy of few

Table 2. Larval mortality of *Spodoptera litura* as influenced by different concentrations of actinobacterial extracts at 48 and 72 HAT under laboratory conditions

Sl. No.	Isolates / Conc	Per cent Mortality at			
		48 HAT		72 HAT	
		40 ml/l	50 ml/l	40 ml/l	50 ml/l
1	DBT-16	20.00(18.43)^{e}	30.00 (32.93)^{f}	24.00 (29.32)^{f}	35.50 (37.36)^{e}
2	DBT-19	0	0	0	0
3	DBT-22	0	15.00 (23.29)^{i}	10.00 (19.39)^{h}	24.00 (30.61)^{i}
4	DBT-24	20.00 (28.46)^{e}	34.50 (36.25)^{f}	33.50 (36.00)^{e}	44.50 (43.06)^{d}
5	DBT-95	10.00 (18.43)^{f}	18.00 (25.09)^{h}	22.50 (29.36)^{f}	34.50 (35.96)^{e}
6	DBT-31	10.00 (19.89)^{f}	30.00 (33.20)^{f}	24.00 (29.32)^{f}	38.50 (39.10)^{e}
7	DBT-34	10.00 (19.44)^{f}	18.50 (25.88)^{h}	15.00 (23.12)^{g}	26.30 (30.88)^{i}
8	DBT-37	10.00 (19.94)^{f}	22.50 (28.30)^{g}	25.00 (31.11)^{f}	33.50 (36.06)^{e}
9	DBT-47	0	15.00 (22.74)^{i}	0	24.50 (30.05)^{i}
10	DBT-52	0	10.00 (19.44)^{j}	25.00 (29.99)^{f}	33.50 (35.35)^{e}
11	DBT-53	10.00 (18.43)^{g}	20.00 (27.56)^{h}	25.50 (30.32)^{f}	33.50 (37.66)^{e}
12	DBT-54	0	0	0	0
13	DBT-55	20.00 (27.55)^{e}	29.50 (33.75)^{f}	35.00 (36.27)^{e}	39.50 (40.60)^{d}
14	DBT-61		14.50 (22.71)^{i}	13.50 (21.52)^{g}	26.00 (30.65)^{g}
15	DBT-64	30.00 (33.21)^{d}	59.50 (51.08)^{d}	49.50 (44.71)^{c}	76.50 (63.44)^{c}
16	DBT-67	0	10.00 (18.43)^{j}	0	24.00 (30.05)^{g}
17	DBT-70	0	23.00 (28.86)^{g}	12.50 (20.67)^{g}	26.50 (30.97)^{g}
18	DBT-72	0	10.00 (19.43)^{j}	0	16.00 (25.06)^{h}
19	DBT-78	0	0	0	0
20	DBT-80	50.00 (45.00)^{b}	68.00 (56.25)^{c}	60.00 (51.82)^{b}	75.25 (63.64)^{c}
21	DBT-96	0	20.00 (26.56)^{g}	0	32.50 (35.56)^{f}
22	DBT-84	0	20.00 (27.64)^{g}	0	28.50 (33.70)^{f}
23	DBT-59	36.00 (37.41)^{c}	47.00 (43.27)^{e}	45.00 (42.12)^{d}	78.25 (62.38)^{c}
24	DBT-90	10.00 (18.43)^{f}	20.00 (27.56)^{g}	24.50 (30.05)^{f}	30.00 (35.07)^{f}
25	DBT-92	0	10.00 (19.33)^{j}	0	23.50 (30.16)^{g}
26	DBT-121	20.00 (26.56)^{e}	31.50 (34.12)^{f}	33.00 (35.70)^{e}	47.00 (44.13)^{d}
27	DBT-93	0	10.00 (18.43)^{j}	0	22.00 (28.86)^{g}
28	AUDT-240	49.00 (44.42)^{b}	77.00 (62.09)^{b}	60.00 (50.76)^{b}	84.00 (80.32)^{b}
29	FIIRS2	46.00 (42.70)^{b}	67.50 (55.24)^{c}	60.50 (51.06)^{b}	88.50 (70.59)^{b}
30	Spinosad 45 SC @ 0.2 ml/l	90.00 (72.05)^{a}	90.00 (72.05)^{a}	95.00 (77.92)^{a}	95.00 (77.77)^{a}
31	Untreated check	0	0	0	0
	S.Em. ±	0.63	1.08	0.90	1.40
	C.D.@5%	2.46	4.21	3.49	5.45
	C.V. (%)	5.68	5.33	5.48	5.04

Values in parentheses are the *arc sine* transformed values.
Means followed by the same letters in a column do not differ significantly (0.05) by DMRT
Sl. No: 1-28 are actinobacterial extracts, 29-Fungal extract HAT: Hour after treatment

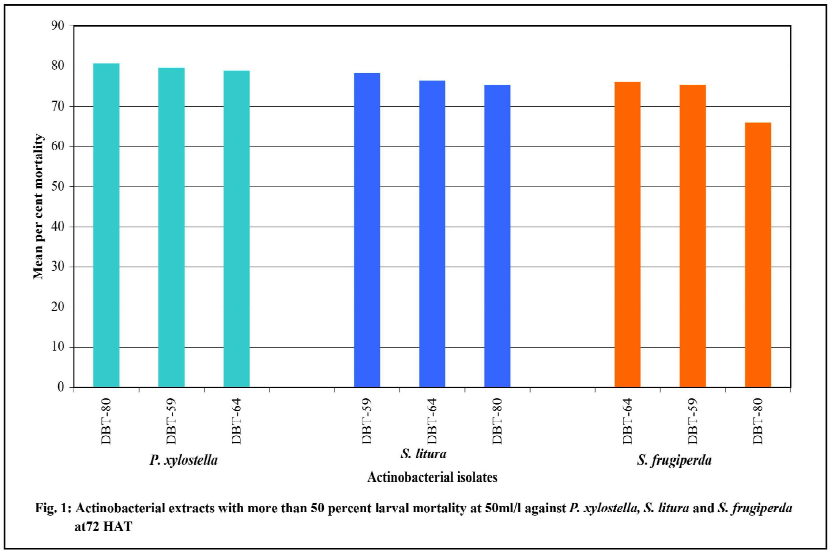

Fig. 1: Actinobacterial extracts with more than 50 percent larval mortality at 50ml/l against *P. xylostella*, *S. litura* and *S. frugiperda* at72 HAT

actinobacteria *viz.,* AUDT-732, AUDT-55, AUDT-741 and AUDT-755 which registered 63.30, 56.67, 50 and 46.67 per cent larval mortality, respectively against *S. litura*. The differential mortality observed in the present investigation compared to the previous findings might be due to the difference in biological, physiological, environmental conditions as well as the efficacy of the test isolates applied in the present finding. The larval biomass also plays an important role.

In-vitro evaluation of actinobacterial extracts against fall armyworm, *S. frugiperda*

At 48 hours after treatment (HAT): The higher larval mortality of 58.50 per cent was recorded in DBT-80 at 40 ml/l followed by DBT-64 (56.00%) which were statistically on par with each other.

DBT-59 with 47.00 per cent mortality and remaining actinobacterial extracts recorded mortality ranged from 13.00 to 26.50 per cent. At 50 ml/l, among the actinobacterial extracts, the highest larval mortality was observed in DBT-64 (67.00%) followed by 66.00 per cent in DBT-80 and both the extracts were statistically on par with each other. DBT-59 was recorded 56 per cent larval mortality. The remaining extracts could not exhibit larval mortality beyond 40 per cent in Table 3.

At 72 Hours after treatment (HAT): Observation on per cent mortality at 40 ml/l revealed that the highest mortality was recorded in DBT-80 with 65.40 per cent, while the next best extract was DBT-64 and DBT-59 which recorded 60.00 and 54.50 per cent larval mortality respectively (Table 3). The per cent larval mortality of *S. frugiperda* at 50 ml/l ranged from 24.00 to 77.75 per cent. Among twenty-eight actinobacterial extracts, DBT-80 was found superior over other extracts with 77.50 per cent and was on par with DBT-64 (76.00%) and DBT-59 (75.25%) larval mortality (Fig. 1). Since published information on the actinobacterial extracts against *S. frugiperda* was lacking, the literature related to other insect pests (*H. armigera, Sitophilus oryzae*, *Crocidolomia binotalis*) is discussed. Sridevi *et al.* (2004) found that Bt var. *kurstaki* (Bt k, Dipel 8L) at 4 days after the treatment caused 75.4 per cent larval mortality of third instar larvae *of H. armigera*. Sadalagi and Patil (2019) reported that metabolites of actinobacterial isolates *viz.,* BRSJ-1, AVC-48 and UPM-3 recorded the highest mortality of 100 per cent at a 1600 ppm at 48 hrs after treatment against *S. oryzae*. Similarly, Yadav (2007) reported 90 per cent mortality in native Sikkim Bt isolates 1707B/4, 1553/b, 1634/33/C and 1559/b against *C. binotalis*.

Bio efficacy of actinobacterial extracts against selected insect pests under greenhouse condition

***Plutella xylostella*:** Observation on per cent larval mortality of *P. xylostella* at 48 HAT presented in Table 4 revealed that highest

Table 3. Larval mortality of *Spodoptera frugiperda* as influenced by different concentrations of actinobacterial extracts at 48 and 72 HAT under laboratory conditions

Sl. No.	Isolates / Conc	Per cent Mortality at			
		48 HAT		72 HAT	
		40 ml/l	50 ml/l	40 ml/l	50 ml/l
1	DBT-16	14.50 (22.90)^{f}	26.50 (30.97)^{e}	24.50 (29.66)^{j}	35.50 (38.47)^{f}
2	DBT-19	16.00 (24.68)^{f}	25.00 (30.43)^{e}	26.50 (31.61)^{j}	26.50 (30.97)^{g}
3	DBT-22	16.05 (23.96)^{f}	34.00 (36.81)^{d}	26.20 (30.65)^{j}	36.50 (38.25)^{f}
4	DBT-24	16.00 (24.23)^{f}	34.00 (37.06)^{d}	23.50 (29.71)^{k}	45.50 (43.06)^{d}
5	DBT-95	26.00 (30.93)^{e}	30.00 (33.57)^{d}	37.00 (39.21)^{f}	40.00 (39.55)^{f}
6	DBT-31	0	13.50 (21.52)^{i}	0	26.00 (30.65)^{g}
7	DBT-34	0	16.50 (24.67)^{i}	0	27.00 (31.30)^{g}
8	DBT-37	26.00 (30.93)^{e}	36.00 (36.86)^{d}	30.50 (34.25)^{h}	45.00 (43.05)^{e}
9	DBT-47	0	24.50 (30.45)^{e}	0	36.00 (38.25)^{f}
10	DBT-52	0	24.50 (29.66)^{e}	0	35.00 (37.17)^{f}
11	DBT-53	26.50 (32.25)^{e}	30.00 (33.57)^{e}	33.50 (36.22)^{g}	37.50(38.51)^{f}
12	DBT-54	22.00 (27.96)^{f}	23.50 (29.76)^{g}	27.00 (31.30)^{i}	37.00 (38.66)^{f}
13	DBT-55	19.50 (26.20)^{f}	26.00 (30.65)^{e}	24.00 (30.05)^{k}	37.00 (39.51)^{f}
14	DBT-61	0	23.00 (29.32)^{h}	0	26.00 (31.72)^{g}
15	DBT-64	56.00 (48.44)^{b}	67.00 (55.94)^{b}	60.00 (53.51)^{c}	76.00 (61.56)^{b}
16	DBT-67	13.00 (21.67)^{g}	26.00 (30.65)^{e}	23.50 (30.66)^{k}	33.50 (36.31)^{f}
17	DBT-70	14.50 (22.85)^{g}	24.00 (29.32)^{e}	26.50 (30.98)^{j}	35.50 (38.11)^{f}
18	DBT-72	0	24.50 (30.65)^{e}	0	36.00 (37.91)^{f}
19	DBT-78	0	15.00 (22.77)^{h}	0	34.00 (36.25)^{f}
20	DBT-80	58.50 (50.53)^{b}	66.00 (54.64)^{b}	65.40 (54.40)^{b}	77.75 (61.68)^{b}
21	DBT-96	0	13.50 (21.86)^{h}	0	24.00 (29.32)^{g}
22	DBT-84	0	25.00 (30.39)^{e}	0	34.50 (38.11)^{f}
23	DBT-59	47.00 (43.36)^{c}	56.00 (49.71)^{c}	54.50 (48.93)^{d}	75.25 (56.75)^{b}
24	DBT-90	0	24.00 (29.32)^{e}	0	26.50 (39.56)^{g}
25	DBT-92	22.00 (27.96)^{f}	27.00 (31.61)^{e}	28.00 (32.49)^{i}	35.50 (38.86)^{f}
26	DBT-121	26.50 (30.98)^{e}	35.00 (36.67)^{d}	36.50 (37.77)^{f}	44.00 (41.55)^{e}
27	DBT-93	0	13.00 (21.12)^{h}	0	25.00 (31.39)^{g}
28	AUDT-240	45.50 (43.19)^{c}	55.50 (49.37)^{c}	55.50 (48.77)^{d}	66.00 (55.75)^{c}
29	FIIRS2	35.50 (37.02)^{d}	46.50 (42.99)^{d}	46.50 (43.97)^{e}	55.50 (48.77)^{d}
30	Spinosad 45 SC @ 0.2 ml/l	85.00 (69.29)^{a}	85.00 (69.33)^{a}	95.00 (76.45)^{a}	95.00 (79.24)^{a}
31	Untreated check	0	0	0	0
	S.Em. ±	0.77	1.29	0.94	1.49
	C.D.@5%	3.00	5.01	3.65	5.79
	C.V. (%)	5.30	5.43	5.49	5.22

Values in parentheses are the *arc sine* transformed values.

Means followed by the same letters in a column do not differ significantly (0.05) by DMRT

Sl.No: 1-28 are actinobacterial extracts, 29 – Fungal extract HAT: Hour after treatment

larval mortality was recorded in DBT-80 with 68.25 per cent followed by DBT-59 (67.00%) which were statistically on par with each other. The crude culture of DBT-64 caused 62.75 per cent mortality. Similarly, at 72 HAT, DBT-80, DBT-59 and DBT-64 registered larval mortality of 77.50 per cent. All the three actinobacterial extracts recorded more than 80 per cent mortality at 96 HAT. DBT-80 recorded the highest of 84 per cent mortality and was on par with DBT-64 (81.00%). The standard check, spinosad 45 SC @ 0.2 ml/l has recorded 97.50 per cent mortality at 48 HAT and 99 per cent mortality at 72 and 96 HAT against diamond back moth under greenhouse conditions. Irrespective of the insect pest, the mean per cent mortality caused by DBT-64 was 60.50, 76.66 and 80.16 at 48, 72 and 96 HAT respectively. DBT-80 caused 64.75, 74.33 and 82.41 per cent mortality whereas DBT-59 recorded 60.33, 74.33 and 80.25 per cent mortality at 48, 72 and 96 HAT respectively under greenhouse conditions (Fig. 2). The mean per cent larval mortality of three isolates against diamondback moth was 66.00, 79.70 and 81.66 per cent at 48, 72 and 96 HAT respectively (Fig. 3). The present results have strongly supported by the results of Srujana (2015) who reported that DBT-388, DBT-772, AUDT-240 and AUDT-258 greatly reduced the larval population of the diamondback moth. The results are partially in agreement with Chauhan and Sharma (2004) who reported that application of 0.007 per cent of Bt was found to be effective in controlling the diamondback moth population. Geeta (2011) found that Bt isolates UK762D and reference Bt strain HD1 caused significant mortality of *P. xylostella* under shade house conditions with cabbage as a test crop.

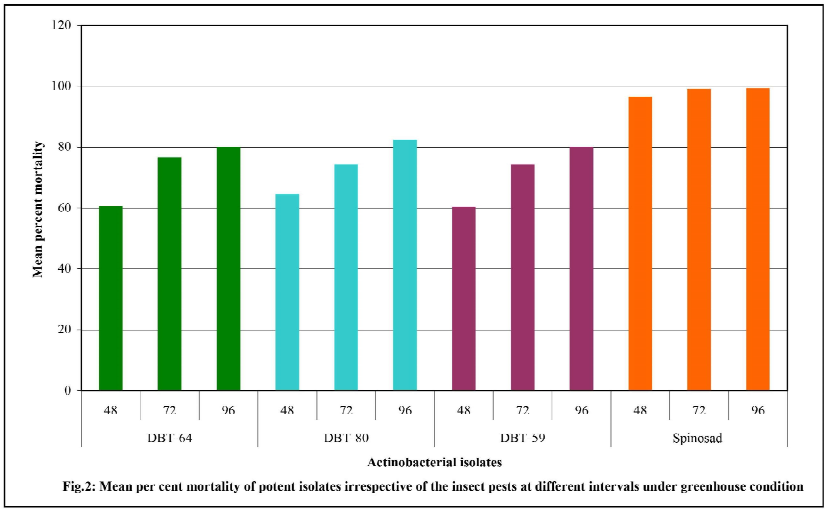

Fig.2: Mean per cent mortality of potent isolates irrespective of the insect pests at different intervals under greenhouse condition

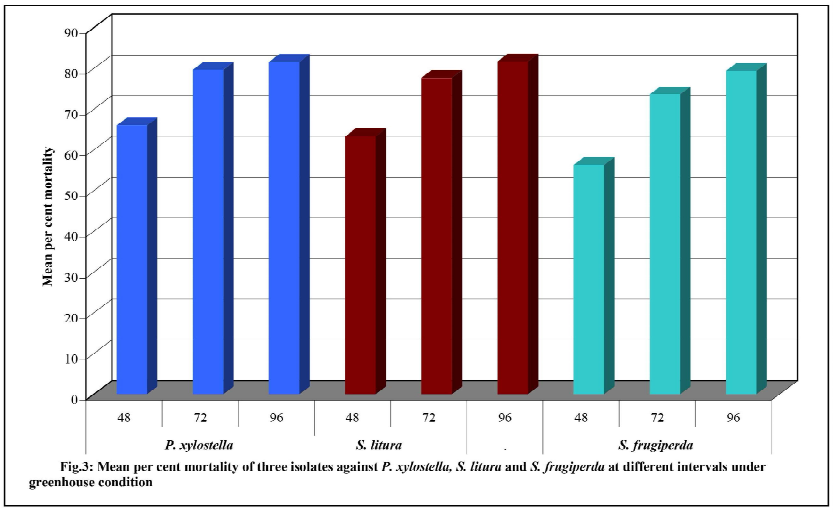

Fig.3: Mean per cent mortality of three isolates against *P. xylostella, S. litura* and *S. frugiperda* at different intervals under greenhouse condition

***Spodoptera litura*:** The three actinobacterial extracts DBT-64, DBT-59 and DBT-80 have recorded more than 55 per cent mortality on the second instar of *S. litura* (Table 4) at 48 HAT. DBT-80 was found superior over other extracts with 69.25 per cent larval mortality followed by DBT-64 (61.50%) and DBT-59 (59.25%). At 72 HAT, the mortality ranged from 77.00 to 78.50 per cent at 50 ml/l. Among the extracts, the highest larval mortality was recorded with DBT-80 (78.50%) followed

Table 4. Bio efficacy of actinobacterial extracts against *Plutella xylostella, Spodoptera litura and Spodoptera frugiperda* under greenhouse condition at 48, 72 and 96 HAT

Isolates	*Plutella xylostella*			*Spodoptera litura*			*Spodoptera frugiperda*		
	48	72	96	48	72	96	48	72	96
DBT-64	62.75	77.50	81.00	61.50	77.00	80.00	57.50	75.50	79.50
	(56.86)^{c}	(62.67)^{b}	(53.17)^{b}	(51.69)^{c}	(61.91)^{b}	(65.25)^{b}	(49.32)^{b}	(61.66)^{b}	(63.52)^{b}
DBT-80	68.25	77.50	84.00	69.25	78.50	82.75	56.75	77.50	80.50
	(55.81)^{b}	(63.30)^{b}	(54.65)^{b}	(56.36)^{b}	(60.58)^{b}	(67.42)^{b}	(48.45)^{b}	(63.35)^{b}	(65.42)^{b}
DBT-59	67.00	77.50	80.00	59.25	77.50	82.50	54.75	68.00	78.25
	(58.67)^{b}	(63.20)^{b}	(55.13)^{c}	(50.35)^{c}	(62.73)^{b}	(67.17)^{b}	(47.73)^{b}	(61.12)^{b}	(64.01)^{b}
Spinosad 45 SC @ 0.2 ml/l	97.50	99.00	99.00	96.75	99.25	99.50	95.50	99.25 ^{a}	99.50
	(81.35)^{a}	(85.93)^{a}	(68.21)^{a}	(79.71)^{a}	(87.42)^{a}	(89.38)^{a}	(77.85)^{a}	(86.79)	(88.74)^{a}
Untreated control	0 (0.29)	0 (0.29)	0 (0.29)	0 (0.29)	0 (0.29)	0 (0.29)	0 (0.29)	0 (0.29)	0 (0.29)
S.Em.± 1.37	1.56	1.21	1.23	1.40	1.51	1.14	1.23	1.41	
C.D. @ 5%	4.15	4.72	3.67	3.72	4.24	4.57	3.45	3.72	4.27
C.V. (%)	5.43	5.69	5.26	5.19	5.15	5.24	5.03	4.52	5.03

Values in parentheses are *arc sine* transformed values.

Means followed by the same letters in a column do not differ significantly (0.05) by DMRT, HAT: hours after treatment

by DBT-59 (77.50%) and DBT-64 (77.00%) which were statistically on par with each other. The per cent larval mortality of *S. litura* at 96 HAT ranged from 80.00 to 82.75 per cent. Among three actinobacterial extracts, DBT-80 was found superior over other extracts with 82.75 per cent and was statistically on par with DBT-59 (82.50%) and DBT-64 (80.00%). The standard check spinosad 45 SC @ 0.2 ml/l recorded 96.75, 99.25 and 99.50 per cent larval mortality at 48, 72 and 96 HAT respectively. Irrespective of isolate tested, the actinobacterial extracts (mean of DBT-64, DBT-59 and DBT-80) 63.33, 77.66 and 81.75 per cent larval mortality under greenhouse conditions at 48, 72 and 96 HAT respectively (Fig. 3). Since there is no literature available on the efficacy of actinobacterial extracts against *S. litura* under protected cultivation to compare the results of the present study, the efficacy of actinobacterial extracts on other insect pests is compared. Vijayabharathi *et al.* (2014) reported the bioactivity of 15 actinomycete isolates on the larvae of *H. armigera*, which exhibited 68-89 per cent mortality by extracellular metabolites and 59-71 per cent mortality by intracellular metabolites under greenhouse conditions. Plata-Rueda *et al*. (2020) reported the insecticidal activity of four Bt- strains *viz.,* HD-1 var. *kurstaki,* SA-12 var. *kurstaki,* ABTS-1857 var. *aizawai* and GC-91 var. *aizawai* which caused 35.62, 52.79, 23.12 and 51.37 per cent mortality against nettle caterpillar, (*Euprosterna elaeasa* Dyar) in field conditions.

***Spodoptera frugiperda*:** The insecticidal activity of actinobacterial extracts were tested against second instar larvae of *S. frugiperda* under greenhouse conditions. Results in Table 4 revealed that the efficacy of three culture filtrates at 48 HAT in descending order were DBT-64 (57.50%) > DBT-80 (56.75%) > DBT-59 (54.75%). The higher larval mortality of 77.50 per cent was recorded in DBT-80 at 72 HAT followed by DBT-64 (75.50%) and DBT-59 (68.00%) which were statistically on par with each other. At 96 HAT, three actinobacterial extracts *viz*., DBT-80, DBT-64 and DBT-59 documented 80.50, 79.50 and 78.25 per cent mortality respectively and were statistically on par with each other. Spinosad 45 SC @ 0.2 ml/l as a standard check recorded 95.50, 99.25 and 99.50 per cent mortality at 48, 72 and 96 HAT respectively against *S. frugiperda*. The mean per cent larval mortality of three isolates against second instar larvae of *S. frugiperda* was 56.33, 73.66 and 79.41 per cent at 48, 72 and 96 HAT respectively (Fig. 3). Since published information on the actinobacterial extracts against *S. frugiperda* was lacking, the literature related to other insect pests (*H.armigera*) discussed under protected condition. The bacterial strain, *Bacillus thuringiensis* isolate named Hr1 showed a 93 per cent larval mortality at 92 hours against first instar larvae of *H. armigera* under semi-controlled conditions on tomato plants (Bouslama *et al*., 2020). Under shade house conditions with cabbage as a test crop, Geeta (2011) revealed that Bt isolates UK762D and reference Bt strain HD1 produced significant mortality of *P. xylostella.* The bioactivity of 15 actinomycete extracts on the larvae of *H. armigera*, which showed 68-89 per cent death by extracellular metabolites and 59-71 per cent mortality by intracellular metabolites under greenhouse conditions as reported by Vijayabharathi *et al.,* 2014, which was partially in support of the present findings.

Conclusion

From the *In-vitro* and greenhouse studies it is concluded that the three actinobacterial extracts *viz.,* DBT-64, DBT-80 and DBT-59 had promising insecticidal activity against *P. xylostella, S. litura* and *S. frugiperda*, which serve as eco-friendly alternatives to chemical insecticides as biocontrol agents for the management of the lepidopteran insect pests under the investigation.

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