The Pharma Innovation Journal 2023; 12(9): 2280-2284

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(9): 2280-2284
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www.thepharmajournal.com
Received: 09-06-2023
Accepted: 13-07-2023

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Effect of *Streptomyces* strains on non-target organisms

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Abstract
Streptomyces is a gram-positive, prokaryotic and multicellular organisms. The secondary metabolites of *Streptomyces* were used in the pharmaceutical as well as in the agricultural industry, as bio-control agents. Soil samples were collected from the various regions of Western Ghats and isolation of *Streptomyces* were carried out by serial dilution and pour plating technique. The isolates were purified and the strains *S. katrae* (ST 1), *S. acidiscabies* (ST 3), *S. andamanensis* (ST 5) and *S. cerasinus* (ST 7) were cultured on International Streptomyces Project (ISP 2) broth and also screened against the non-target organisms such as Indian honeybee, silkworm, green lacewing and egg parasitoid, *Trichogramma chilonis* Ishii. The strain, *S. katrae* (ST 1) and the consortium of *S. katrae*, *S. acidiscabies*, *S. andamanensis* and *S. cerasinus* (ST 1 + ST 3 + ST 5 + ST 7) were also safer to Indian honeybee, *A. cerana indica* as compared to other target organisms *viz*., *B. mori*, *C. carnea* and *T. chilonis.*

Keywords: Safety, *Streptomyces*, Indian honeybee, mulberry silkworm, green lacewing, *Trichogramma* egg parasitoid

Introduction
Streptomyces is the largest genus of Actinomycetota, and the type genus of the family Streptomycetaceae. Over 700 species of Streptomyces bacteria have been described. *Streptomyces* is a gram- positive, multicellular and prokaryotic organisms (Hwang *et al.*, 1994) [1] and it possess both bacterial and fungal characters, they are commonly refereed as ray fungi (Kutovaya and Watson, 2014) [2]. Berdy (2005) [3] documented more than 17 biologically active secondary metabolites in *Streptomyces* and they are widely used as the pharmacological, agrobiological, antagonistic traits. More than 10,000 microbial compounds were characterized and they are used in agricultural, medicinal and veterinary fields (Sundarapandian *et al.*, 2002) [4]. About 75 percent of *Streptomyces* antibiotics were used in commercial and medicinal purposes and 60 percent in agriculture use. Tarkka and Hampp (2008) [5] documented the entomopathogenic activity of *Streptomyces* active metabolites against insect pests. *Streptomyces* plays an important role in the management of *Culex quinquefasciatus* (Say) (Sundarapandian *et al.*, 2002) [4], *Musca domestica* (Linnaeus) (Hussain *et al.*, 2002) [6], *Drosophila melanogaster* (Meigen) (Gadelhak *et al.*, 2005) [7], *Helicoverpa armigera* (Hubner) (Osman *et al.*, 2007) [8], *Anopheles* mosquito larvae (Dhanasekaran *et al.*, 2010) [9], *Spodoptera litura* (Fab.) (Arasu *et al.*, 2013) [10] *etc.* The bioactive metabolites derived from *Streptomyces* spp. possessed entomopathogenic activity against insect pests with less toxicity to the non-target organisms such as silk worm, *Bombyx mori* (Linnaeus) (Koga *et al.*, 1987) [11] and also possess nematicidal activity (Takatsu *et al.*, 2003) [12].
The present study was conducted to study the safety of *Streptomyces* strains against non-target organisms such as Indian honey bee, *Apis cerana indica* (Fabricius), *mulberry* silkworm, *Bombyx mori* (Linnaeus), green lacewing, *Chrysoperla carnea* (Stephens) and egg parasitoid, *Trichogramma chilonis* (Ishii).

Materials and Methods
(i) Isolation and purification of *Streptomyces*
The soil samples were collected from various regions of Western Ghats, Kerala and Tamil Nadu, India for isolation of *Streptomyces*. The isolation was performed by serial dilution and pour plating technique (Kumar *et al.*, 2010) [13]. The serial dilution for *Streptomyces* were made upto 10^{-4}. The plates were incubated for 7 days and after incubation period, white coloured powdery colonies were observed. The individual colonies were picked and streaked on the International Streptomyces Project (ISP 2) media for purification (Narayana and Vijayalakshmi, 2009) [14].

The purified plates were kept at room temperature for incubation. The purified isolates were identified by 16S rDNA sequencing using NCBI BLAST program and named it as *Streptomyces katrae* (ST 1), *S. acidiscabies* (ST 3), *S. andamanensis* (ST 5) and *S. cerasinus* (ST 7).

(ii) Safety of *Streptomyces* strains and consortium of *Streptomyces* on non-target organisms

Isolated native strains were tested against non-target organisms like Indian honey bee, silkworm, green lacewing and *Trichogramma* egg parasitoid.

a. Indian honeybee, *Apis cerana indica* (Fabricius)

The bioassay was conducted to study the safety of *Streptomyces* strains against Indian honey bee, *A. cerana indica*. Adults were fed with sugar syrup which was mixed with 800 µl of fermentation/broth cultures. The Indian honeybees were released into a petridish along with cotton wool pads which was dipped in 50 percent sugar syrup contaminated with suspensions of isolates. The safety was conducted for individual effective strains as well as for the consortium of effective strains. Ten adults were used per replication and three replications per treatment were maintained and observations were recorded at 12, 48 and 72 hours (Worsley *et al.*, 2020) [15].

b. Mulberry silkworm, *Bombyx mori* (Linnaeus)

Fresh mulberry leaves and first instar silkworm larvae were bought from Demonstration cum Training Centre, Dept. of Sericulture, V.M. Chatram, Tirunelveli, Tamil Nadu, India. Isolated strains of *Streptomyces* were used for conducting bioassay on mulberry silkworm. Leaves were washed with distilled water and allowed to air dry. After air drying leaves were coated with 800 µl suspension of broth culture and then leaves were air dried. First instar larvae were fed with treated mulberry leaves. The biosafety was conducted for individual effective strains as well as for the consortium of effective strains. For each replication, ten larvae were used and three replications per treatment were maintained. Observations were recorded at 12 hours interval upto 72 hours (Subramanian *et al.*, 2010) [16].

c. Green Lacewing, *Chrysoperla carnea* (Stephens)

Freshly emerged adults *C. carnea* were allowed in separate petriplates. The concentration of 800 µl suspension of broth culture were mixed with 10% of sucrose solution and were given to the emerged adults. The control was maintained with 10% of sucrose solution. The safety study was conducted for individual effective strains as well as for the consortium of effective strains. The mortality percentage and longevity of adults were recorded (Govindan *et al.*, 2012) [17].

d. Egg parasitoid, *Trichogramma chilonis* Ishii

A total of 800 µl broth culture of *Streptomyces* strains were sprayed on the egg card and allowed to air dry at laminar air flow chamber. The safety study was conducted for individual effective strains as well as for the consortium of effective strains. Three replications per treatment were maintained and observations were recorded based on the parasitization rate from parasitized cards (Prakash *et al.*, 2022) [18].

Statistical analysis

The mortality percentage as well as the parasitization rate was corrected by using Abbott's (1925) [19] formula. The data on mortality of non-target organisms by different isolates were directed to Analysis of Variance (ANOVA) analysis (Gomez and Gomez, 1984) [20].

Results and Discussion

a. Indian honeybee, *A. cerana indica*

In Indian honeybee, the individual *Streptomyces* isolates showed less significant difference among all the isolates. The isolate, *S. katrae* (ST 1) showed the least effect on honey bees i.e. 8.66 percent mortality followed by *S. andamanensis* (ST 5) (15.66%), *S. acidiscabies* (ST 3) (19.33%) and *S. cerasinus* (ST 7) (24.00%) (Table 1 and Fig. 1). The standard culture showed 27.00% of mortality. Grubbs *et al.* (2021) [21]reported that the strains *Streptomyces* sp. ICBG1323 and *Micromonospora* sp. ICBG1321 showed no effects on population rate of honey bees but the feed utilization bioassay caused 30 percent mortality in the treated honey bees.

b. Silk worm, *B. mori*

In silk worm, the isolate *S. katrae* (ST 1) exhibited the lowest mortality of 30.33% whereas the isolates *S. acidiscabies* (ST 3) (33.33%) and *S. andamanensis* (ST 5) (43.33%) were on par with each other. The standard culture showed the highest mortality of 44.67% (Table 1 and Fig. 2). There was no mortality were showed in the control. Chatterjee (2009) [22]studied the effect of microbial insecticides such as Vertimec (*Streptomyces avermitilis*-1.8% w/v) against third instar larvae of silk worm and the Vertimec showed somewhat less mortality percentage of 30% in the third instar larvae of silk worm, *B. mori*.

Table 1: Safety of *Streptomyces* species on Indian honey bees and Silkworm larvae

Treatments	Isolates	Mortality (%)	
		Cerana indica	***Mori***
T1	*S. katrae* (ST 1)	8.66 (17.09)^{c}	30.33 (33.41)^{c}
T2	*S. acidiscabies* (ST 3)	19.33 (26.00)^{ab}	33.33 (35.17)^{bc}
T3	*S. andamanensis* (ST 5)	15.66 (23.07)^{bc}	43.33 (41.11)^{ab}
T4	*S. cerasinus* (ST 7)	24.00 (29.22)^{ab}	30.66 (33.57)^{c}
T5	Standard culture (*S. griseus*)	27.00 (31.05)^{a}	44.67 (41.93)^{a}
T6	Control	0.00 (0.29)^{d}	0.00 (0.29)^{d}
	CD (0.05)	6.98	6.22
	SEd	3.20	2.86
	CV	18.56	11.32

c. Green lace wing, *C. carnea*

The isolate, *S. andamanensis* (ST 5) showed the lowest percentage of mortality i.e. 24.33 percent followed by *S. cerasinus* (ST 7) (30%), *S. katrae* (ST 1) (32.00%) and ST 3 (40%) were on par with each other. The isolate ST 5 showed lower percent of mortality (24.33%) (Table 2). The standard culture exhibited 29.00% of mortality of adults of *Chrysoperla carnea*. Govindan *et al.* (2012) [17]reported that 30-40 percent of second instar grubs of green lacewing by the application of *Streptomyces* species. Karthikeyan and Ayysamy (2017) [23] documented that the application of emamectin benzoate (product of *S. avermitilis*) 1.9 EC was safe to the *C. carnea*.

d. Egg parasitoid, *T. chilonis*

The isolate ST 3 exhibited higher percentage of parasitization rate (65.00%) on tricho egg cards. The isolates ST 1 (64.22%), ST 5 (52.67%) and ST 7 (64.66%) were on par with each other (Table 2). The standard culture showed 44.67% of parasitization rate. Parsaeyan *et al.* (2018) [24] evaluated the effect of emamectin benzoate on *T. brassicae* (Bezdenko) and exhibited 72.00 percent mortality of *T. brassicae*. Ashtari *et al.* (2020) [25] studied the effects of emamectin benzoate (product of *S. avermitilis*) on *T. chilonis* and the egg parasitoid showed 40-50 percent parasitism rate and the longevity of insects were also increased.

Table 2: Safety of *Streptomyces* on green lace wing, *C. carnea* and egg parasitoid, *T. chilonis*

Treatments	Isolates	*C. carnea* mortality (%)	*T. chilonis* Parasitization rate (%)
T1	*S. katrae* (ST 1)	32.00 (34.37)^{ab}	64.22 (53.28)^{a}
T2	*S. acidiscabies* (ST 3)	40.00 (39.23)^{a}	65.00 (43.18)^{b}
T3	*S. andamanensis* (ST 5)	24.33 (29.32)^{b}	52.67 (46.54)^{b}
T4	*S. cerasinus* (ST 7)	30.00 (33.16)^{ab}	64.66 (53.56)^{a}
T5	Standard culture (*S. griseus*)	29.00 (32.52)^{b}	44.67 (41.93)^{b}
T6	Control	0.00 (0.29)^{c}	0.00 (0.29)^{c}
	CD (0.05)	6.18	6.12
	SEd	2.83	2.80
	CV	12.34	8.64

Safety of *Streptomyces* consortium on non-target organisms

a. Indian honeybee, *A. cerana indica*

The combination of all the *Streptomyces* species (ST1 + ST 3 + ST 5 + ST 7) exhibited 9.66 percent mortality of adult honeybees. The mortality range varied from 9.66-14.55 percent. The other combinations of *Streptomyces* species were on par with each other (Table 3 and Fig. 1).

b. Silk worm, *B. mori*

The lowest mortality (25%) of silkworm larvae were recorded on the *Streptomyces* consortium of (ST 1 + ST 7) followed by (ST 3 + ST 5), (ST 1 + ST 5), (ST 3 + ST 7), (ST 1 + ST 3), (ST 1 + ST 3 + ST 5 + ST 7) and (ST 5 + ST 7) which were on par with each other. The mortality range of silkworm larvae varied from 25-39.33 percent (Table 3 and Fig. 2).

Table 3: Safety of consortium of *Streptomyces* species on *A. cerana indica* and *B. mori*

Treatments	Isolates	Mortality %	
		Cerana indica	***A. mori***
T1	ST 1 + ST 3	10.89 (19.19)^{a}	31.11 (33.89)^{b}
T2	ST 1 + ST 5	10.00 (18.38)^{a}	25.67 (30.33)^{b}
T3	ST 1 + ST 7	14.00 (21.86)^{a}	25.00 (29.93)^{b}
T4	ST 3 + ST 5	12.33 (20.34)^{a}	25.33 (30.11)^{b}
T5	ST 3 + ST 7	14.55 (22.27)^{a}	28.00 (31.89)^{b}
T6	ST 5 + ST 7	13.19 (21.16)^{a}	39.33 (38.82)^{a}
T7	ST1 + ST 3 +ST 5 + ST 7	9.66 (18.11)^{a}	31.66 (34.24)^{ab}
T8	Control	0.00 (0.29)^{b}	0.00 (0.29)^{c}
	CD (0.05)	4.99	4.91
	SEd	2.35	2.31
	CV	16.31	9.88

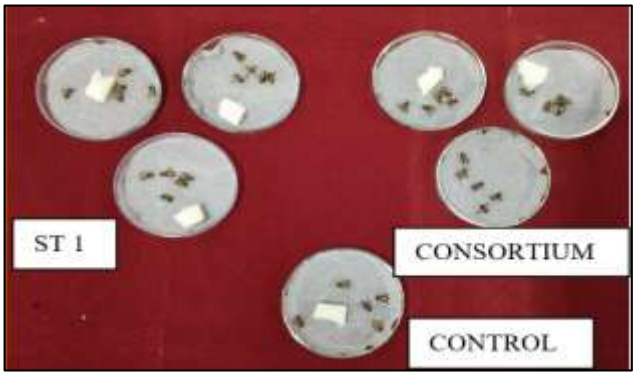

Fig 1: Safety of *Streptomyces* on Indian honey bee, *A. cerana indica*

Fig 2: Safety of *Streptomyces* species on silkworm larvae, *B. mori*

c. Green lace wing, *C. carnea*

The consortium of *S. katrae*, *S. acidiscabies*, *S. andamanensis* and *S. cerasinus* (ST 1 + ST 3 + ST 5 + ST 7) showed 31.33 percent of mortality whereas the combination of *S. katrae* + *S. cerasinus* (ST 1 + ST 7) exhibited the lowest mortality i.e. 24 percent (Table 4 and Fig. 3).

d. Egg parasitoid, *T. chilonis*

The consortium of *S. katrae*, *S. acidiscabies*, *S. andamanensis* and *S. cerasius* (ST 1, ST 3, ST 5 and ST 7) exhibited 64.33 percent parasitization whereas the combination of *S. katrae* and *S. andamensis* (ST 1 + ST 5) showed the lowest parasitization rate of 45 percent (Table 4 and Fig. 4).

Table 4: Safety of consortium of *Streptomyces* species on green lacewing and egg parasitoid, *T. chilonis*

Treatments	Isolates	*C. carnea* mortality (%)	*T. chilonis* Parasitization rate (%)
T1	ST 1 + ST 3	30.00 (33.20)^{ab}	58.33 (49.83)^{ab}
T2	ST 1 + ST 5	24.00 (29.29)^{bc}	45.00 (51.84)^{a}
T3	ST 1 + ST 7	22.33 (28.08)^{c}	47.67 (43.65)^{b}
T4	ST 3 + ST 5	26.00 (30.57)^{abc}	60.66 (51.19)^{a}
T5	ST 3 + ST 7	33.00 (34.99)^{a}	64.67 (53.57)^{a}
T6	ST 5 + ST 7	32.33 (34.61)^{a}	53.67 (47.15)^{ab}
T7	ST1 + ST 3 +ST 5 + ST 7	31.33 (34.03)^{ab}	64.33 (53.35)^{a}
T8	Control	0.00 (0.29)^{d}	0.00 (0.29)^{b}
	CD (0.05)	4.82	7.39
	SEd	2.27	3.48
	CV	9.89	9.74

Fig 3: Safety of *Streptomyces* on green lacewing, *C. carnea*

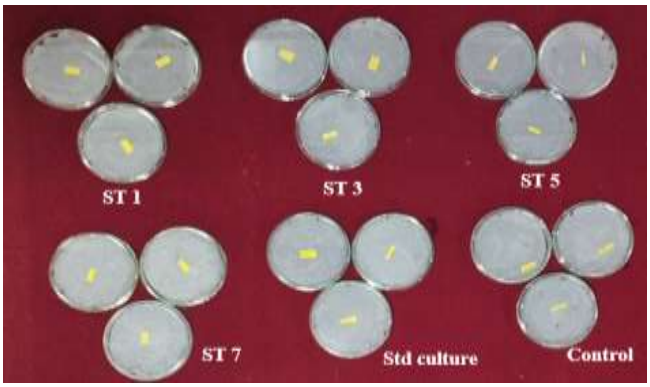

Fig 4: Safety of *Streptomyces* species on Egg parasitoid, *T. chilonis*

Conclusion

These findings denote that the fermentation broth of *S. katrae* (ST 1) and the consortium of *S. katrae*, *S. acidiscabies*, *S. andamanensis* and *S. cerasinus* (ST 1 + ST 3 + ST 5 + ST 7) were safer to Indian honeybee, *A. cerana indica* as compared to other target organisms *viz*., *B. mori*, *C. carnea* and *T. chilonis.*

Acknowledgement

We would like to thank Dr. V. S. Saravanan, Assistant Professor (Microbiology), Indira Gandhi College of Arts and Science, Pondicherry for his academic help in gene sequencing and molecular work.

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