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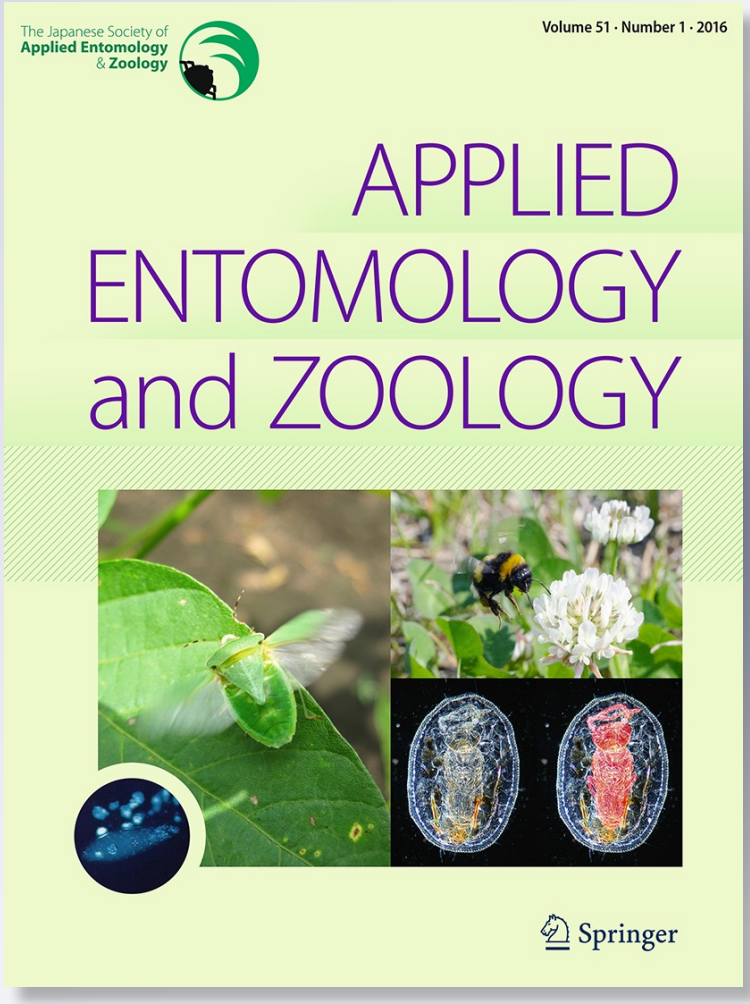

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ORIGINAL RESEARCH PAPER

Assessment of a diketopiperazine, cyclo(Trp-Phe) from *Streptomyces griseoplanus* SAI-25 against cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae)

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Abstract Microorganisms produce a range of metabolites with varying pest control properties. With this concept, we earlier identified 15 *Streptomyces* spp. strains with insecticidal activity against *Helicoverpa armigera* (Hübner), *Spodoptera litura* (Fabricius), and *Chilo partellus* (Swinhoe). In recent studies, we evaluated an insecticidal compound purified from the extracellular extract of *S. griseoplanus* SAI-25 by bioactivity-guided fractionation against *H. armigera*. Spectral studies by infrared (IR), nuclear magnetic resonance (NMR), and electron spray ionization mass spectroscopy (ESI-MS) confirmed that the purified compound was cyclo(Trp-Phe) of the diketopiperazines class. Cyclo(Trp-Phe) exhibited antifeedant (70 %), larvicidal (67 %), and pupicidal (59 %) action against *H. armigera* in a dose-dependent manner. The lethal dose for 50 % of the group (LD_{50}) and LD_{90} values for larvicidal effect were 619 and 2750 ppm, respectively. In addition, the purified compound prolonged larval (10.3–11.1 days) and pupal (10.9–11.8 days) periods compared with the untreated control (larval duration 9.8 days, pupal duration 10.6 days). This is the first report on the presence and biological activity of cyclo(Trp-Phe) isolated from the genus *Streptomyces*.

Keywords *Helicoverpa armigera* · *Streptomyces* · Diketopiperazine · Cyclo(Trp-Phe) · Pest management

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Introduction

Crop production is limited by various biotic and abiotic factors; however, the magnitude of crop loss depends on the causative agent (i.e., weeds, pests, pathogens, or environmental factors) and crop species. Insect pests are one of the major constraints to crop production, and during 2001–2003, they caused 7.9, 9.6, 12.3, and 15.1 % loss in crop yield in wheat, maize, cotton, and rice, respectively. Annual losses of major crops due to insect pests have been estimated to be 10.8 % (Oerke 2006), and in India, losses have been estimated to be 17.5 %, valued at Rs. 863,884 million during 2007–2008 (Anonymous 2010; Dhaliwal et al. 2010). Among the insects pests, *Helicoverpa armigera* (Hübner) is a polyphagous pest that feeds on >200 plant species, including cereals, grain legumes, vegetables, and fruit crops (Sharma 2005). Though it is mainly distributed in Asia, Africa, Oceania, and European Plant Protection Organization (EPPO) regions, it is widely distributed in India and China (Kranthi et al. 2002). Crop losses by *H. armigera* on soybean and cotton in Brazil leads to losses of ~US $ 500 million during 2012–2013 (Czepak et al. 2013; SEAGRI 2013).

Chemical pesticides play a vital role in enhanced crop protection. Their usage is higher in the current scenario because of arising pest and pathogen attacks and outbreaks (Dhaliwal and Arora 2001; Paras Nath 2007). Genetic engineering of plants for insect resistance may reduce the use of pesticides; however, this technology is insect and/or crop specific. A report by Benbrook (2012) on pesticide usage in the USA revealed that herbicide-resistant technology increased pesticide use by 239 million kilograms during 1996–2011; meanwhile, *Bt* crops reduced insecticide application by 56 million kilograms. This increase is

an indication for sensitivity loss by pests/crops, meaning they likely acquired pesticide resistance through exposure to large amounts and/or repeated doses of pesticide applications. The cotton bollworm, *H. armigera*, is resistant to range of conventional insecticides, such as organochlorines, organophosphates, carbamates, cyclodienes, and pyrethroids (Kranthi et al. 2002; Yang et al. 2013). Connections between problems of crop damage, yield loss, pest resistance, natural-enemy loss, and degradation of ecosystem components have necessitated the role of biopesticides that are environmentally benign and ecofriendly products with targeted activity (Crowder and Harwood 2014).

Considerable research has focused on biopesticides by exploring plant extracts/secondary metabolites; however, this has had limited success (Koul 2012). The diverse and ubiquitous nature of microbes creates an almost infinite pool for novel metabolites with medicinal, agricultural, and industrial importance. Among them, actinomycetes, especially *Streptomyces*, which produces secondary metabolites with unique structure and mode of action, are the major options for biocontrol. Approximately 17 % of biologically active secondary metabolites (7600 of 43,000) have been characterized from streptomycetes (Berdy 2005). The chemical diversity of metabolites synthesized by streptomycetes ranges from simple amino acid derivatives to peptides and high-molecular-weight proteins, as well as simple lactones to condensed macrolactones. These metabolites have documented biological activity for antagonistic, pharmacological, and agrobiological traits. However, many biologically important traits may have been missed, as many of the streptomycetes have been evaluated primarily for antimicrobial activity. Evaluation of metabolites from streptomycetes for pharmacological and insecticidal activity has recently become an area of active interest (Tarkka and Hampp 2008).

Various agriculturally important products of microbial origin with bactericidal/fungicidal/insecticidal traits have been reviewed by Copping and Duke (2007). Among those reviewed; spinosad, a biorational pesticide obtained from *Saccharopolyspora spinosa* (Mertz and Yao), is effective against several insect classes, including Lepidoptera, Diptera, and Coleoptera, and it has been registered in 37 countries for 150 crops (Cleveland et al. 2002). Despite its efficacy, spinosad has started to create resistance and has been documented in several pests (Levot and Sales 2008) and natural enemies at lower concentrations (Schneider et al. 2004). Therefore, it is evident that the agricultural sector is in need of newer insecticidal products of biological origin.

Based on this information, we identified 15 *Streptomyces* strains with insecticidal activity. During the course of screening, we found that extracellular and intracellular extracts of *S. griseoplanus* SAI-25 has broad-spectrum insecticidal activity against lepidopteran insects *H. armigera*, *Spodoptera litura* (Fabricius), and *Chilo partellus* (Swinhoe) under laboratory and/or greenhouse conditions (Vijayabharathi et al. 2014). Therefore, we extended the study to isolate, identify, and characterize an insecticidal compound from *S. griseoplanus* SAI-25.

Materials and methods

Chemicals

Azadirachtin, phorbol 12-myristate-13-acetate, Diaion HP-20, and C_{18} were obtained from Sigma Chemicals (St. Louis, MO, USA). All chemicals used were of analytical grade.

Extraction and purification of active compound

Streptomyces griseoplanus SAI-25 was cultured on starch casein broth at 28 °C for 7 days at 120 rpm. At the end of the incubation period, the culture was centrifuged at 10,000 g for 10 min at 4 °C, and the supernatants were collected. The supernatants (cell-free culture filtrates) were fractionated on Diaion HP-20 adsorptive resin column (20 × 2.5 cm). The fraction obtained before elution was termed the unbound (nonadsorbed) fraction. Residues of unbound materials in the column were washed with water. The resin adsorbed with culture filtrate was eluted with 100 % methanol (MeOH) and termed the bound (adsorbed) fraction. Both unbound and bound fractions were assayed for efficacy against *H. armigera* by diet impregnation assay. The active fraction was further subjected to C_{18} column chromatography (23 × 3.6 cm) and eluted with MeOH:H_2O gradient (5, 10, 20, 40, 60, 80, and 100 % MeOH). All fractions were assayed by diet impregnation and detached-leaf assay. The active fraction was further purified on high-performance liquid chromatography (HPLC) [Agilent 1100, diode array detector/220 nm, Agilent Zorbax C_{18}, 5 µm column, 250 × 4.6 mm, flow rate 0.5 ml min^{-1}, acetonitrile:water 30:70 (v/v)] and subjected to structural identification studies.

Characterization of purified compound

Chemical characterization of the pure compound was performed through infrared (IR), nuclear magnetic resonance (NMR), and electron spray ionization mass spectroscopy (ESI-MS) analysis. IR spectrum was recorded by Fourier transform infrared (FTIR) spectrophotometer (Jasco FTIR-420, USA). Hydrogen 1 nuclear magnetic resonance (^{1}H NMR) and carbon 13 (^{13}C) NMR spectra of the compound were recorded in dimethylsulfoxide (DMSO) at room temperature using tetramethylsilane as an internal standard

on an NMR spectrophotometer (Bruker Avance 400 MHz, Bruker, Billerica, MA, USA). Mass spectrum was recorded on a Q-ToF Micromass spectrometer with electrospray ionization (Micromass Manchester, UK).

Rearing and maintenance of *H. armigera*

Larvae of *H. armigera* were reared using chickpea-flour-based semisynthetic diet, as per the standard protocols of Narayanamma et al. (2007). The rearing conditions were maintained at a temperature of 27 ± 3 °C, with a relative humidity of 65–70 %.

Diet impregnation assay

In brief, 2 ml of the artificial diet was poured into a 24-well plate and allowed to dry. At the end of drying, 300 µl of the test sample was added to the diet in each well and air dried (3 h). Larvae of *H. armigera* were prestarved for 6 h before being released (one each) into the treated 24-well plates. There were three replications per trial, and trial was repeated twice. For each replication, 24 larvae were used. Insect mortality was recorded on days 2, 4, and 6 after treatment (DAT).

Detached leaf bioassay

The detached-leaf bioassay was performed as per Sharma et al. (2005): 10 ml of 3 % agar was poured into plastic cups positioned at an angle of 45°. Chickpea terminal branches with four leaflets along with the terminal bud were washed thoroughly in distilled water to avoid interference of exudates released by the plant. Branches were dipped in 5 ml of the samples for 5 min, allowed to dry, and inserted into agar. Healthy larvae (prestarved for 6 h) of similar weight were taken for the experiment. There were three replications per trial and the trial was repeated twice. For each replication, ten larvae were used. Observations were recorded on DAT 2, 4, and 6.

Antifeedant activity by leaf-disc no-choice method

Antifeedant activity for purified and standard compounds was tested at different concentrations against third instar larvae of *H. armigera* according to the methods of Arasu et al. (2013). Fresh and young cotton leaves were collected and cleaned thoroughly with distilled water and wiped with soft tissue towels to remove excess moisture; 3-cm-diameter leaf discs were prepared using a cork borer. The surface area of the leaf discs was measured using leaf area meter (Li-Cor Area Meter 3100, Nebraska, USA). The standard and test compounds were tested at three different concentrations with a geometrical progression factor of 2. Leaf discs were dipped in test/standard solutions for 1 min and allowed to dry. Similarly, leaf discs dipped with water and solvent were considered for control treatments. Treated leaf discs were transferred into plastic cups, lined with wet filter paper to avoid early drying. A single, healthy, third instar *H. armigera* larva was introduced into each plastic cup. There were three replications per trial and the trial was repeated twice. For each replication, 15 larvae were used. Consumption of leaf discs were monitored after 24 h, and the antifeedant index was calculated as per the following formula: Antifeedant index $= [(C-T)/(C + T)] \times 100$, where C is the leaf area consumed in control and T is the leaf area consumed in test.

Larvicidal activity

Larvicidal activity was studied using the leaf-disc no-choice method, as per the above-mentioned similar experimental conditions for antifeedant activity analysis. After 24 h, larvae were fed continuously with nontreated leaves, and every 24 h, fresh diet was provided. Larval mortality was recorded after 96 h. Mortality percent was calculated and corrected with Abbott's formula (Abbott 1925) as, Abbott's corrected mortality percent $=1-[(n$ in T after treatment)/(n in C after treatment)$] \times 100$; where n is the number of larvae, T test/standard samples, and C the control. With the corrected data, lethal dose for 50 % of the group (LD_{50}) and LD_{90} were calculated by probit analysis.

Pupicidal activity

Larvae that survived were continuously fed with control diet until they became pupa and adults. Pupicidal activity was calculated by the number of emerged adults from the total number of pupa.

Larval and pupal durations

Larval duration is the time taken by treated larvae to become pupae; pupal duration is the duration taken for adult emergence from pupae.

Statistical analysis

Data for antifeedant, larvicidal, pupicidal, larval, and pupal durations were subjected to one-way analysis of variance (ANOVA) followed by post hoc Tukey's test. LD_{50} and LD_{90} values were calculated by probit regression analysis. All statistical analyses were conducted using SPSS (Statistical Package for the Social Sciences) version 13.0 (SPSS Inc., Chicago, IL, USA). Values were expressed as mean ± standard error (SE).

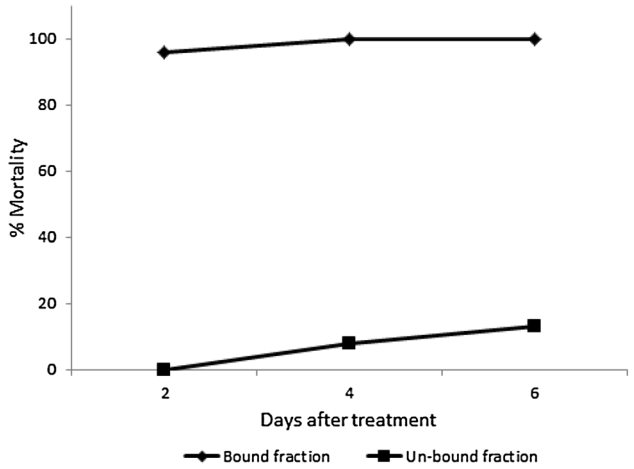

Fig. 1 Larvicidal activity of Diaion HP-20 fractions of *Streptomyces griseoplanus* SAI-25 against second instar *Helicoverpa armigera*. Each *point* represents the mean of three replicates

Results

Isolation and purification of bioactive compound

Larvicidal activity of Diaion HP-20 bound and unbound fractions of *S. griseoplanus* SAI-25 extracellular extract on second instar *H. armigera* is depicted in Fig. 1; the bound fraction showed 100 % at 3 DAT, and the unbound fraction registered either nil or residual activity (4–13 %), even at 6 DAT.

Further fractionation of Diaion HP-20 bound fraction by C_{18} open-column purification gave seven fractions. Larvicidal activity of these fractions against second- and third-instar *H. armigera* larvae is shown in Fig. 2a–d. The highest larvicidal activity of 100 % was recorded in 100 % MeOH fraction on 2 DAT, 80 % MeOH on 4 DAT, followed by 60 % MeOH with 95.8 % on 6 DAT by diet-impregnation bioassay in second instars *H. armigera* (Fig. 2a). Other fractions showed significantly lower larvicidal activity (4–25 % mortality at 6 DAT). Comparable activity was observed on third instar *H. armigera* in the MeOH fractions of 100 (100 %/4 DAT), 80 (100 %/6 DAT), and 60 % (87.5 %/6 DAT) (Fig. 2b).

In the chickpea detached-leaf assay, the different fractions showed marginally lower larvicidal activity than in the diet-impregnation assay. Highest larvicidal activity of 100, 90, and 73.3 % was observed in 100, 80, and 60 % MeOH fraction on 6 DAT against second-instar *H. armigera* (Fig. 2C). Though the third-instar larvae showed lower susceptibility than second-instar larvae, they still exhibited 90 % larvicidal activity (Fig. 2d).

Identification of bioactive compound

Based on the above results, the 100 % MeOH fraction was further subjected to HPLC using C_{18} column in which an active compound with a retention time of 2.872 min was documented. The purified compound was subjected to spectral studies, including IR, NMR, and ESI-MS. The IR spectrum of the compound exhibited a sharp and strong band at 1638 cm^{-1}, which corresponded to the vibrational stretching frequency of C=O, indicating the presence of a carbonyl group. A broadband was observed in the region of 3201 cm^{-1}, which indicated the presence of the N–H group. ^{1}H NMR data in Table 1 not only indicated an indole ring related to proton signals (δ 6.96, 7.47, 6.99, 7.15, 7.33) but also implied a monosubstituted benzene ring related to proton signals (δ 7.17, 6.71). The proton resonances at δ 7.71 and δ 7.91 combined with carbon resonances at δ 166.66, δ 166.29, δ 55.77, and δ 56.29 (Table 1) conformed the structure to a diketopiperazine skeleton. Finally, the structure of the isolated compound was determined to be cyclo(Trp-Phe) by comparison with reported spectral data of Kimura et al. (1996). Based on experimental data, the molecular formula was determined to be $C_{20}H_{20}N_3O_2$, with the molecular ion of 356.3, [M+Na]+, which was shown in ESI-MS. The compound was identified as cyclo(Trp-Phe), a diketopiperazine (DKP). The ESI-MS spectrum of purified compound and the structure of cyclo(Trp-Phe) are depicted in Figs. 3 and 4, respectively.

Bioactivity of cyclo(Trp-Phe)

Antifeedant activity of cyclo(Trp-Phe)

Antifeedant activity of the purified compound cyclo(Trp-Phe) is given in Table 2. Cyclo(Trp-Phe) showed dose-dependent antifeedant activity of 70.9 %/1000 ppm > 47. 0 %/500 ppm > 26.9 %/250 ppm. Standards azadirachtin (58.0–88.8 %) and phorbol ester (34.9–76.2 %) also showed dose-dependent activity, but with significantly higher antifeedant activity.

Larvicidal activity of cyclo(Trp-Phe)

The isolated compound cyclo(Trp-Phe) showed dose-dependent larvicidal activity of 23.3, 39.4, and 67.6 % at 250, 500, and 1000 ppm concentration, respectively (Table 2). The standards azadirachtin and phorbol ester showed highest activity: 97.8 and 86.3 % at 1000 and 200 ppm, respectively. Cyclo(Trp-Phe) showed significantly lower activity than the standards. The LD_{50} and LD_{90}

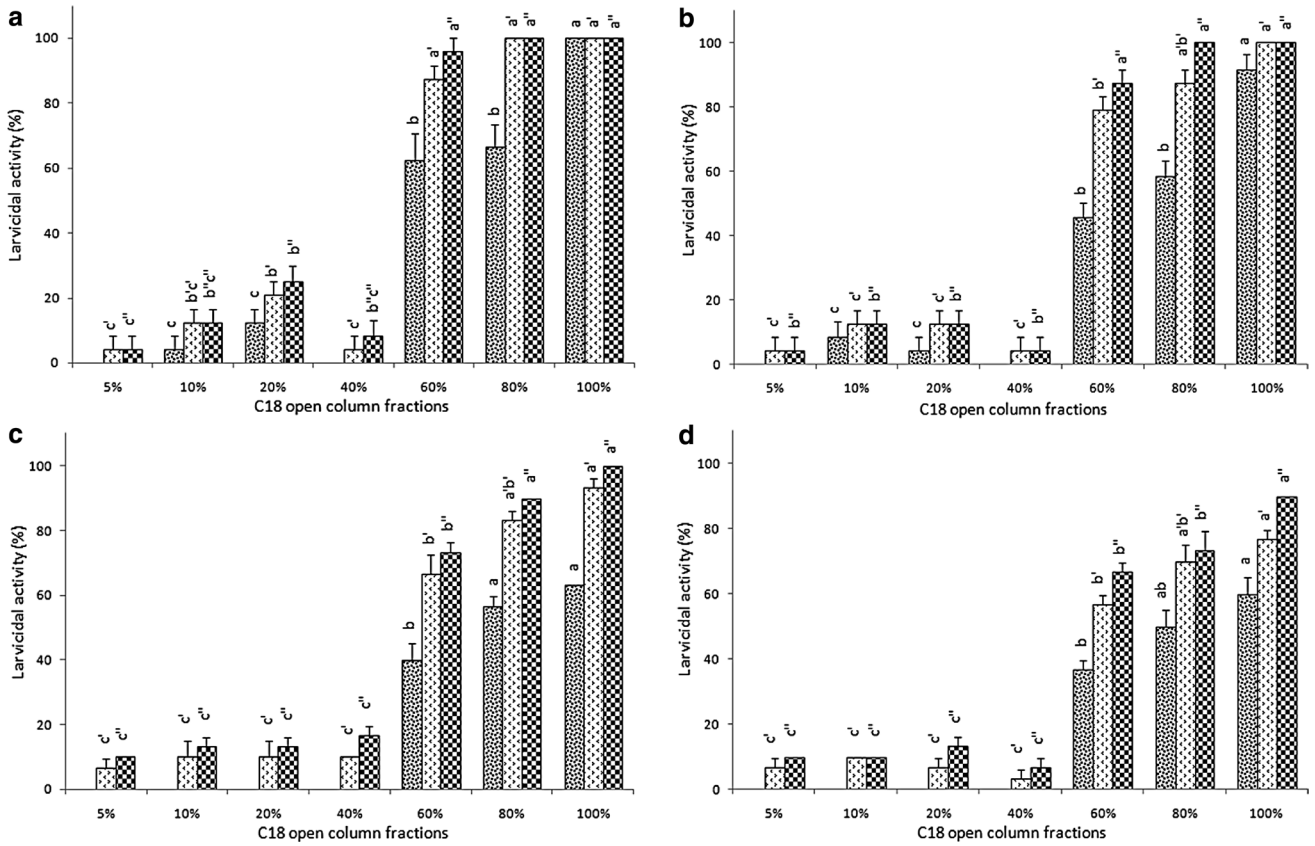

Fig. 2 Larvicidal activity of C_{18} open-column fractions of *Streptomyces griseoplanus* SAI-25 against second and third instar *Helicoverpa armigera*. Each *bar* depicts the mean of three replications for larvicidal activity by diet-impregnation bioassay on second instar (**a**) and third instar (**b**) *H. armigera,* and by chickpea detached-leaf bioassay on second instar (**c**) and third instar (**d**) *H. armigera*. *Error bars* indicate standard error. *Lower-case letters a–c*, *a′–c′* and *a″–c″* indicate significant difference between larvicidal activity of fractions on second (▨), 4th (▩) and 6th (▩) days after treatment, respectively ($p < 0.05$)

value of cyclo(Trp-Phe) was 619 and 2750 ppm (Table 3), respectively. Cyclo(Trp-Phe) required a 2.7- and 4.4-fold higher concentration to exert larvicidal activity similar to azadirachtin, and 8.3- and 11-fold higher concentrations for LD_{50} and LD_{90}, respectively, for phorbol ester.

Pupicidal mortality due to cyclo(Trp-Phe)

As with antifeedant and larvicidal activity, pupicidal activity was also correlated with concentration of cyclo(Trp-Phe) (Table 2). Pupicidal activity in increasing order was 21.9 %/250 ppm, < 36.5 %/500 ppm, < 59.3 %/1000 pp m. Azadirachtin showed pupicidal activity only at 250 and 500 ppm, and showed none 1000 ppm, as it resulted in 100 % larval mortality.

Effect of cyclo(Trp-Phe) on larval and pupal durations of *H. armigera*

The effect of cyclo(Trp-Phe) and standards on larval and pupal durations of *H. armigera* is given in Table 4. The lowest larval duration was 9.8 days in the untreated control. The highest larval duration was observed with azadirachtin (12.7–13.9 days), followed by phorbol ester (10.9–12.8 days) and cyclo(Trp-Phe) (10.3–11.1 days). Although the extended larval duration in larvae fed on cyclo(Trp-Phe)-treated diets was lower than on standards, it was still significantly higher than the control. Similar results were observed for pupal duration by azadirachtin (14.3–15 days) > phorbol ester (11.6–12.4 days) > cyclo(Trp-Phe) (10.9–11. 8 days). Cyclo(Trp-Phe) prolonged the pupal period significantly at 1000 ppm (11.8 days) only. The prolongation of larval and pupal durations was dose dependent for cyclo(Trp-Phe) and standards.

Discussion and conclusions

Microbial metabolites are often produced in low quantities with complex mixtures, and therefore, purification of the desired metabolite is a sequential process. Since greater larvicidal activity was observed in extracellular

Table 1 Comparative data of nuclear magnetic resonance (NMR) with a previous report on cyclo(Trp-Phe)

Position	^{13}C		^{1}H	
	Experiment	Literature[a]	Experiment	Literature[a]
1	–		10.89 (1H, s)	10.89 (1H, s)
2	121.36	120.89	6.96 (1H, d, 2.21)	6.96 (1H, d, 2.20)
3	109.28	108.85	–	–
4	119.23	118.42	7.47 (1H, d, 7.7)	7.48 (1H, dd, 7.17, 1.00)
5	118.89	118.76	6.99 (1H, m)	6.98 (1H, ddd, 7.17, 7.45, 1.45)
6	124.88	124.41	7.15 (1H, m)	7.08 (1H, ddd, 7.73, 7.45, 1.00)
7	111.80	111.33	7.33 (1H, d, 8.03)	7.32 (1H, dd, 7.73, 7.45)
8	127.99	127.54	–	–
9	136.52	136.07	–	–
10	30.03	29.69	2.48 (1H, dd, 14.42, 5.72)	2.52 (1H, dd, 14.47, 5.68)
			2.80 (1H, dd, 14.42, 4.26)	2.81 (1H, dd, 14.47, 4.46)
11	55.77	55.29	3.98 (1H, m)	3.98 (1H, m)
12	–	–	7.91 (1H, d, 2.07)	7.91 (1H, d, 2.00)
13	166.66	166.22	–	–
14	56.29	55.64	3.86 (1H, m)	3.89 (1H, m)
15	–	–	7.71 (1H, d, 2.30)	7.71 (1H, d, 2.00)
16	167.29	166.88	–	–
17	39.47	39.89	1.85 (1H, dd, 13.41, 7.05)	1.85 (1H, dd, 13.49, 7.02)
			2.46 (1H, dd, 13.41, 4.68)	2.45 (1H, dd, 13.49, 4.70)
18	136.99	136.56	–	–
19	127.99	128.03	7.17 (1H, m)	7.16 (1H, m)
20	130.17	129.70	6.71 (1H, m)	6.71 (1H, m)
21	126.83	126.36	7.17 (1H, m)	7.17 (1H, m)
22	130.17	129.70	6.71 (1H, m)	6.71 (1H, m)
23	128.50	128.03	7.17 (1H, m)	7.16 (1H, m)

J values (Hz) shown in parentheses

[a] Kimura et al. (1996)

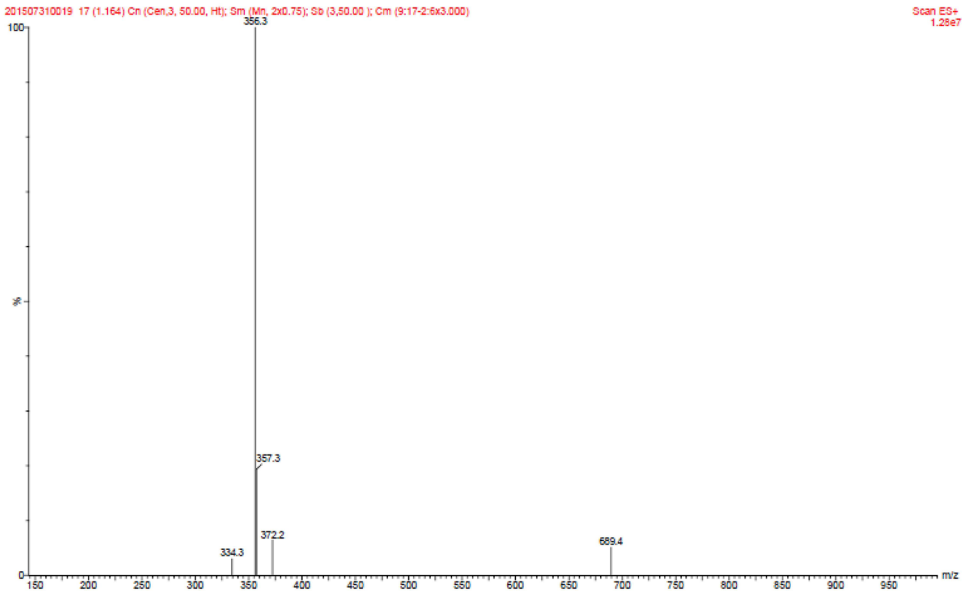

Fig. 3 Electron spray ionization mass spectroscopy (ESI-MS) of the purified compound. *Peak with [M+Na] = 356.3* cyclo(Trp-Phe)

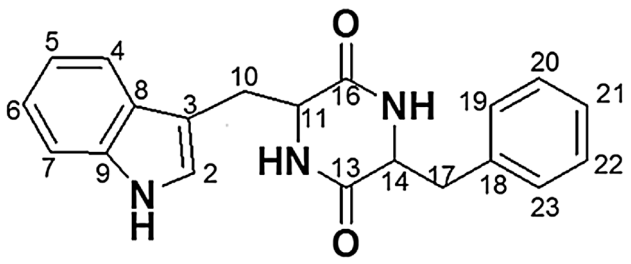

Fig. 4 Structure of the purified compound cyclo(Trp-Phe)

Table 2 Antifeedant, larvicidal, and pupicidal activity of cyclo(Trp-Phe) against third-instar *Helicoverpa armigera*

Compounds (ppm)	Antifeedant (%)	Larvicidal (%)	Pupicidal (%)
Cyclo(Trp-Phe)			
250	26.9 ± 0.8^{g}	23.3 ± 2.7^{e}	21.9 ± 0.7^{cd}
500	47.0 ± 1.2^{e}	39.4 ± 5.5^{de}	36.5 ± 3.6^{bc}
1000	70.9 ± 0.4^{c}	67.6 ± 3.8^{bc}	59.3 ± 5.3^{ab}
Standards			
Azadirachtin			
250	58.0 ± 1.0^{d}	54.6 ± 4.1^{cd}	48.7 ± 3.1^{abc}
500	69.2 ± 0.6^{c}	81.9 ± 4.3^{ab}	78.9 ± 14.9^{a}
1000	88.8 ± 0.3^{a}	97.8 ± 2.2^{a}	–
Phorbol ester			
50	34.9 ± 0.9^{f}	33.9 ± 3.3^{e}	21.7 ± 2.9^{cd}
100	56.2 ± 1.1^{d}	59.0 ± 3.9^{c}	35.1 ± 4.8^{bcd}
200	76.2 ± 1.4^{b}	86.3 ± 3.9^{ab}	62.9 ± 12.6^{ab}

Values are the mean of three replicates ± standard error. Values followed by different lower-case superscript letters significantly different ($p < 0.05$)

Table 3 Lethal dose for 50 %/90 % of the group (LD_{50} and LD_{90}) values of cyclo(Trp-Phe) compound against third-instar *Helicoverpa armigera*

Compounds	LD_{50} ppm (95 % LFL–UFL)	LD_{90} ppm (95 % LFL–UFL)	$\chi^2_{(df)}$	*P* value
Cyclo(Trp-Phe)	619 (516–776)	2750 (1765–6431)	$15.9_{(7)}$	0.026*
Standards				
Azadirachtin	233 (164–286)	627 (511–895)	$23.9_{(7)}$	0.001*
Phorbol ester	75 (63–88)	249 (194–375)	$15.5_{(7)}$	0.030*

LFL lower fiducial limits, *UFL* upper fiducial limits, *df* degree of freedom

* χ^2 values are significant at $p < 0.05$

than intracellular extracts, purification and identification of the bioactive compound was carried out on extracellular extracts. Initial fractionation by Diaion HP-20 identified larvicidal activity in the bound fraction. Earlier reports on microbial metabolites/antibiotics such as tubelactomicin A, pentostatin, calphostin, and teicoplanin A2 using Diaion HP and/or SP series have proved the efficacy of Diaion in natural product isolation (Sterner 2012). Subsequent fractionation by C_{18} yielded seven fractions, with the highest activity in the 100 % MeOH fraction. Final purification on HPLC identified the active compound with the RT of 2.872 min. Spectral studies on the purified compound identified it as a diketopiperazine (DKP)—called cyclo(Trp-Phe).

DKPs are the smallest known cyclic peptides synthesized by organisms, including mammals. Amongst these, 2,5-DKPs have recently received attention due to their biological activities and their peculiar heterocyclic system in natural product constituents. DKPs have antiviral, antifungal, antibacterial, hypoglycemic, antidepressant, and neuroprotecting properties (Wang et al. 2013a). The DKP reported in this study—cyclo(Trp-Phe)—was first isolated from *Penicillium* sp., (Link) (Kimura et al. 1996) and later from a fungal species EF8 (Ding et al. 2008), sponge-associated fungi *Aspergillus versicolor* TS08 (Vuillemin) (Chu et al. 2011), and an entomopathogenic nematode associated bacterium, *Comamonas testosteroni* (Marcus and Talalay) (Nishanth Kumar et al. 2014). This is the first report on cyclo(Trp-Phe) in an actinomycete, *S. griseoplanus* SAI-25. Other DKPs, such as maremycin A and B from marine *Streptomyces* sp., (Balk-Bindseil et al. 1995); cyclo(L-Leu-L-Pro), cyclo(L-Phe-L-Pro), cyclo(L-Val-L-Pro), cyclo(L-Trp-L-Pro), and cyclo(L-Leu-L-Val) from *Streptomyces fungicidicus* (Li et al. 2006); and vinylidene substituted diketopiperazines from *Streptomyces* sp., FXJ7.328 (Wang et al. 2013b), were reported in the genus *Streptomyces*.

This is the first report on insecticidal activities of cyclo(Trp-Phe), though some other DKPs have been reported to have insecticidal properties. In this study, we used two compounds, azadirachtin and phorbol ester, as standards for comparative analysis of isolated bioactive compound, cyclo(Trp-Phe). Azadirachtin is a known biopesticide from a neem tree (Koul 2012). Phorbol ester is one of the major toxic components of *Jatropha*, and it has broad-spectrum bioactivity on various insects because of its antifeedant, oviposition deterrent, and ovicidal properties. Though reports on natural enemies, mammalian systems, and mode of action are scarce, we have considered this as a standard, as it is a candidate for pesticide research by various researchers (Devappa 2012).

Antifeedants are the first encounter between an insect and its host. They kill the insects through starvation rather than through direct toxicity. In addition, antifeedants protect the crops until the slow-acting natural pesticides exert their antifeedant effect; therefore, investigations on antifeedants against polyphagous pests are gaining increased attraction (Isman 2002). The purified compound cyclo(Trp-Phe) exhibited antifeedant activity in a dose-dependent (70.9 %/1000 ppm > 47.0 %/500 ppm > 26.9 %/250 ppm) manner. Feeding cessation leads to

Table 4 Effect of cyclo(Trp-Phe) compound on larval and pupal duration on third-instar *Helicoverpa armigera*

Compounds (ppm)	Larval duration (days)	Pupal duration (days)
Cyclo(Trp-Phe)		
250	10.3 ± 0.1	10.9 ± 0.1
500	10.9 ± 0.0*	11.2 ± 0.1
1000	11.1 ± 0.1*	11.8 ± 0.2*
Standards		
Azadirachtin		
250	12.7 ± 0.1*	14.3 ± 0.2*
500	13.9 ± 0.3*	15.0 ± 0.0*
1000	–	–
Phorbol ester		
50	10.9 ± 0.2*	11.6 ± 0.1*
100	12.2 ± 0.20*	12.4 ± 0.1*
200	12.8 ± 0.2*	12.3 ± 0.3*
Control	9.8 ± 0.1	10.6 ± 0.1

Values are the mean of three replicates ± standard error

* Values are statistically significant at $p < 0.05$ compared with control group

larval weight reduction, which was visually observed but not measured in this study. Similarly, 17 and 30 % larval weight reduction of *H. zea* (Boddie) has been observed in case of isopentenylated 2,5-DKPs, such as *N*-methyl *epi*-amauromine, and *epi*-amauromine isolated from *Aspergillus ochraceus* (Wilhelm). Another DKP cycloechinulin showed 33 % weight reduction on *Cimex hemipterus* (Fabricius) (de Guzman et al. 1994). Similar results have been reported by Arasu et al. (2013) on a polyketide metabolite isolated from *Streptomyces* sp. AP-123 and Baskar and Ignacimuthu (2012a) on violacein isolated from *Chromobacterium violaceum* (Schröter), which showed 78 and 72 % antifeedant activity at 1000 ppm against *S. litura*. In comparison with standards, cyclo(Trp-Phe) showed lower larvicidal activity but comparable activity with a polyketide metabolite from *Streptomyces* sp., AP-123 (68 % at 1000 ppm) (Arasu et al. 2013). In addition, cyclo(Trp-Phe) showed equivalency with plant secondary metabolite ononital monohydrate (63 %/1000 ppm) (Baskar and Ignacimuthu 2012b). Cycloechinulin, a DKP from sclerotia of *A. ochraceus*,, showed effective control of coleopteran and lepidopteran insects, such as *H. zea* and *Carpophilus hemipterus* (Linnaeus) (de Guzman et al. 1993). The LD_{50} (619 ppm) and LD_{90} (2750 ppm) value of cyclo(Trp-Phe) are similar to previously reported LC_{90} values for violacein (Baskar and Ignacimuthu 2012a). Three DKPs containing isopentenyl substituted indole moiety, which were isolated from the fungus *Eurotium cristatum* (Raper and Fennell), were found to have insecticidal activity against *Artemia salina* (Linnaeus), with LD_{50} values of 19.8, 27.1 and 19.4 μg ml^{-1}, respectively (Chinese Patent No: CN102675293 2012b; Chinese Patent No: CN102669110, 2012a).

Pupal mortality was directly correlated with cyclo(Trp-Phe) concentration. In addition to interfering with the development of *H. armigera* larvae, cyclo(Trp-Phe) showed a postexposure effect (i.e., pupicidal activity and decreased adult emergence). These findings are contrary to those observed with *C. violaceum* metabolite violacein, which showed 20–24 % mortality across concentrations (125–1000 ppm) against *S. litura* (Baskar and Ignacimuthu 2012a).

The onset of insect metamorphosis depends on both endocrine and nonendocrine functions. The driving force behind this process is larval nutrition, which provides growth, development, and hormonal balance (Johnson et al. 2014; Telang et al. 2007). The extended larval and pupal periods in insects treated with cyclo(Trp-Phe) suggested changes in physiological processes in the insect, possibly because of antifeedant effects. The extended developmental period will enhance the exposure time of insects to predators and parasitoids in natural environments (Akhtar et al. 2012).

Here we report for the first time purification of an insecticidal compound cyclo(Trp-Phe) from *S. griseoplanus* SAI-25. The exhibited insecticidal properties, such as antifeedant, insecticidal, and pupicidal activity, against *H. armigera* reveal the importance of microbes for the exploration of new insecticidal compounds and their development into an ingredient for biopesticide formulation. This warrants further studies on cyclo(Trp-Phe) in relationship to its mode of action and efficacy trails under greenhouse and field conditions.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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