Insecticidal and growth inhibitory potential of *Streptomyces hydrogenans* DH16 on major pest of India, *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae)

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**Abstract**

**Background:** Destructive impacts of insecticides on non targeted populations necessitate the development of an eco friendly pest control method. *Streptomyces* spp. are rich source of bioactive secondary metabolites which may provide valuable alternatives to chemical insect-control agents as they can be less toxic and readily biodegradable. Because of its potent biocontrol attributes, ethyl acetate extract of *Streptomyces hydrogenans* DH16, a soil isolate, was tested to assess its anti-insect potential against polyphagous noctuid, *Spodoptera litura*.

**Results:** The secondary metabolites in the ethyl acetate extract of *S. hydrogenans* DH16 exhibited larvicidal and growth inhibitory activities. The results indicated that highest concentration of 1600 μg/ml was significantly effective as 70% larval, 66.66% prepupal and 100% pupal mortality was noticed. The metabolites also prolonged the larval developmental period. The LC50 and LC90 values were 1337.384 and 2070.516 μg/ml, respectively for the insect. Negative effects of *S. hydrogenans* were also observed on development of the insect. Significant decline in adult emergence, adult longevity, fecundity and % hatching was recorded at higher concentrations along with morphological abnormalities as compared to control. Significant decrease in relative growth and consumption rate, efficiency of ingested and digested food and increase in approximate digestibility in larvae reared on diet supplemented with ethyl acetate extract accounts for the toxic as well as anti-nutritive nature of extract.

**Conclusion:** Secondary metabolites in the fermentation broth from *S. hydrogenans* were toxic to the larvae at higher concentrations whereas lower concentrations significantly reduced the reproductive potential of *S. litura*. Therefore, these metabolites show considerable potential for incorporation in pest management programmes as new biopesticidal formulation.

**Keywords:** *Streptomyces hydrogenans*, *Spodoptera litura*, Larvicidal, Nutritional assay, Growth inhibitory

**Background**

According to the report of FAO, due to the attack from pathogenic organisms and insect pests, 20–40% decrease in crop yield occurs which results in loss of 120 billion US $ worldwide [1]. Pest insects, being plant disease vectors reduce crop output by 10–30% either by reducing the quality and quantity of the crop production, or by serving as vectors of plant diseases [2]. *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae), a polyphagous insect of cosmopolitan distribution, has a large host range of more than 150 host species [3] and is considered economically important in many countries including India, Japan, China and Southeast Asia [4]. This defoliating insect pest affects the yield of various cultivated crops, vegetables, weeds and ornamental plants by feeding gregariously on leaves and causes large economic losses of crop plants. It was reported as a major pest in groundnut in Andhra Pradesh, India and caused 28–100% yield loss depending upon crop stage and its level of infestation [5,6]. The management of *S. litura* to ensure the stable and high output of crops is a great
challenge in agricultural field and therefore, insecticide use
is most widely practiced for its control. However, there is
widespread concern over negative impact of insecticides
on environmental and human health due to accumulation
of insecticide residues as well as emergence of pesticide re-
sistance in the pests [7]. Application of chemical pesticides
also kills different varieties of pest predators and results in
ecological imbalance, thereby causing pest resurgence and a
greater outbreak of secondary pests [8]. Therefore, there
is a need for developing safe and eco-friendly alternatives
to chemical insecticides for pest control.

Biological control as a part of integrated pest manage-
ment has gained interest among researchers as it is an
environmentally friendly and a safe strategy for pest man-
age [9]. Natural products obtained from plants and
microorganisms have been used for insect control [10].
Azadirachtin (complex limonoids), a natural compound
isolated from Indian neem tree, *Azadirachta indica*
A. Juss (Meliaceae), is known to have lethal effects on
more than 400 insect species [11] and many workers have
used azadirachtin as positive control [12-14]. Recently, mi-
crobial insecticides have attracted considerable attention
[15] because they are more specific, have low relative cost
and are more eco-friendly [16-18]. Among the biological
control agents derived from different microbes, actinobac-
teria especially *Streptomyces* spp. are one of the most
important microbial resources which can provide potential
new bioactive compounds for use as insect-control agents
[19]. Many reports indicated the important role played by
actinobacteria in the management of *Spodoptera litoralis*
(Biosduval) [20], *S. litura* [21], *Musca domestica* (Linnaeus)
[22], *Culex quinquefasciatus* (Say) [23], *Drosophila melao-
gaster* (Meigen) [24], *Helicoverpa armigera* (Hubner)
[25], *Anopheles* mosquito larvae [26]. Bream et al. [20]
showed potent biological activity of secondary metabolites
of actinobacteria such as *Streptomyces* and *Streptoverti-
cillum* against *S. litoralis* which caused larval and pupal
mortality. Several metabolites from genus *Streptomyces*,
such as avermectin, prasinons, doramectin, milbemycin,
nanchangmycin, diamecamycin and spinosad have been
established as potential protective agents against a variety
of pest insects and are friendly to environment [27,28]. In
light of this and inspired by the remarkable pharmaceut-
ical and agricultural potential of bioactive metabolites
of actinobacteria, Kaur et al. [29] screened actinobacterial
isolates, recovered from different rhizospheric and non-
rhizospheric soils, for antifungal activity against fungal
phytopathogens and reported strong insecticidal activity
against *S. litura* in one of the isolates, *Streptomyces hydro-
genans* DH16 which also exhibited potent antifungal activ-
ity [30]. Present study was aimed at further systematic
evaluation of antifeedant, larvicidal, pupicidal and growth
inhibitory effect of solvent extract from *S. hydrogenans*
DH16 against *S. litura*.

**Results and discussion**

There is a long history of utilizing natural products pro-
duced by microbes for pharmaceutical and agricultural
purposes. Actinobacteria especially, *Streptomyces* spp.
have provided wide variety of secondary metabolites of
high commercial importance and continue to be rou-
tinely screened for new bioactive compounds. Present
work further corroborates the earlier findings and re-
ports that secondary metabolites from *S. hydrogenans*
showed deleterious effects on growth and development of *S. litura* larvae that survived the toxic effects of highest concentration. Significant increase in larval development period was ob-
served at all concentrations over the control (*P* ≤0.05). At
highest concentration (1600 μg/ml), larval period pro-
longed by 6.24 days in comparison to control group
(Table 1). Our result coincided with the findings of Arasu et al. [21] who reported larvicidal and growth inhibitory
activities of a novel polyketide metabolite isolated from
*Streptomyces* sp. AP-123 against *H. armigera* and *S. litura*.
The metabolite also prolonged the larval–pupal duration
of the insects at all the tested concentrations as compared
to control. The delayed larval period observed in the
present study could be due to low consumption of diet by
the larvae of *S. litura* indicating the antifeedent effect of
the extract. Pupal period decreased significantly with treatment (*P* ≤0.01) however, at highest concentration pupae
formed from treated larvae remained in pupal stage till
the termination of experiment. The total development
period from larva to adult of *S. litura* differed but remained non significant (Table 1). The LC50 and LC90
values were 1337.384 and 2070.516 μg/ml, respectively
for *S. litura* (Table 2). No larval mortality was observed
in lowest concentration as well as in control but when
larvae were fed on highest concentrations of 800 and
1600 μg/ml, larval mortality of 20 and 70%, respectively
was recorded and was statistically significant compared
to control (*P* ≤0.01). Similar study on soil bacterium
metabolite 12-epi-Hapalindole J isonitrile isolated from
*Cyanobacterium terium* showed 100% larval mortality
against dipteran *Chironomus riparius* (Meigen) at 26 μM
concentration within 48 h of exposure [31]. Similarly,
anti-insect activity of crude ethanolic extracts from
*Streptomyces* sp. in terms of larval mortality had been
reported by Rishikesh et al. [32]. The isolate showed a
marked insecticidal activity against *Sitophilus oryzae* in
a dose dependent manner with 100% mortality at con-
centration of 24 mg/ml. Later, Arasu et al. [21] docu-
mented 68.41% and 60.02% larvicidal activities by
polyketide metabolite from *Streptomyces* sp. AP-123
against *H. armigera* and *S. litura*, respectively at
1000 ppm. Azadirachtin showed a more toxic effect to-
wards *S. litura* as compared to the crude extract of
S. hydrogenans as 100% mortality was noticed at higher concentrations.

Prepupal mortality (66.66%) was also higher at the highest concentration (P ≤ 0.01) (Table 3). Diet supplemented with extract of S. hydrogenans induced 48–100% pupal mortality. As compared to control, significantly higher mortality of more than 50% was recorded at highest concentrations (P ≤ 0.01) (Table 3). Similarly, dose dependent (125–1000 ppm) pupal mortality (18–62%) was reported by Arasu et al. [21] and documented that prolonged larval–pupal durations were directly proportional to the increase in pupicidal activities. The adverse effect of solvent extract was also observed on emergence and performance of adults emerged from treated larvae. Adult emergence was significantly lower when larvae were reared on diet amended with extract (P ≤ 0.01) and the decrease was found to increase with increase in concentration (Figure 1). Maximum adverse effect was observed at highest concentration where no adult emergence occurred. Also, adults emerged at lower concentrations were small in size with varied abnormalities. Xiong et al. [33] found that out of 40 isolates from marine micro-organisms, Streptomyces sp.173, similar to avermectin B1 possessed strong insecticidal potential against H. armigera. In another study, Xiong et al. [34] reported strong inhibitory activity of Streptomyces avermitilis strain 173 isolated from marine source against Heliothis zea (Boddie), Plutella xylostella (Linnaeus), Spodoptera exigua (Hübner) and aphids.

Adult survival time was also influenced by the S. hydrogenans as longevity of emerged adults declined significantly from 11.50 days in control to 4.33 days at 800 μg/ml (P ≤ 0.01) (Table 4). Fecundity in emerged adults from treated larvae was also significantly inhibited. It declined from 1500 eggs/female (control) to 150.20 eggs/female at 400 μg/ml concentration (P ≤ 0.01). The viability of these eggs was also negatively affected as the eggs failed to hatch whereas in control 87.66% hatching of eggs was observed (Table 4). No egg laying was recorded at 800 μg/ml concentration. Abouelghar et al. [35] also demonstrated the negative effects of sublethal concentrations of spinosad on development, fecundity and food utilization in the cotton leafworm, S. littoralis (Boisd.).

**Morphological abnormalities**

The inhibitory effect of extract was further manifested in the form of deformed adults which emerged from the larvae fed on S. hydrogenans extract supplemented diet. The deformed adults had crumpled and underdeveloped wings as well as were half emerged from pupa. These adults were also smaller in size with varied abnormalities. Xiong et al. [33] found that out of 40 isolates from marine micro-organisms, Streptomyces sp.173, similar to avermectin B1 possessed strong insecticidal potential against H. armigera. In another study, Xiong et al. [34] reported strong inhibitory activity of Streptomyces avermitilis strain 173 isolated from marine source against Heliothis zea (Boddie), Plutella xylostella (Linnaeus), Spodoptera exigua (Hübner) and aphids.

### Table 1 Influence of ethyl acetate extract of S. hydrogenans and azadirachtin on various developmental parameters of S.litura

| Treatments                        | Concentrations (μg/ml) | Larval period (in days) (Mean ± S.E.) | Pupal period (in days) (Mean ± S.E.) | Total developmental period (in days) (Mean ± S.E.) |
|-----------------------------------|------------------------|---------------------------------------|--------------------------------------|---------------------------------------------------|
| Streptomyces ethyl acetate extract| 400                    | 17.30 ± 0.19<sup>a</sup>              | 10.36 ± 0.40<sup>b</sup>            | 27.66 ± 0.40                                      |
|                                   | 800                    | 19.97 ± 2.15<sup>b</sup>              | 8.03 ± 0.76<sup>c</sup>             | 28.00 ± 0.93                                      |
|                                   | 1600                   | 22.00 ± 2.13<sup>b</sup>              | -                                   | -                                                 |
|                                   | f- value               | 3.30*                                 | 5.83**                              | 0.62<sup>NS</sup>                                 |
|                                   | R²                     | 0.99                                  | 0.82                                | 0.57                                               |
| Azadirachtin                      | 400                    | 16.66 ± 0.33<sup>c</sup>              | 7.00 ± 0.36<sup>c</sup>             | -                                                 |
|                                   | 800                    | -                                     | -                                   | -                                                 |
|                                   | 1600                   | -                                     | -                                   | -                                                 |
|                                   | f- value               | -                                     | -                                   |                                                    |
|                                   | R²                     | -                                     | -                                   |                                                    |

Mean ± SE followed by different letters (superscript) within a column are significantly different. Tukey’s test P ≤ 0.05, NS = Non Significant, R² = Coefficient of determination, *Significant at 5% level, **Significant at 1% level.

### Table 2 Regression equation, lower as well as upper 95% confidence limits for LC<sub>50</sub> and LC<sub>90</sub>

| Regression equation | LC<sub>50</sub> (μg/ml) | LC<sub>90</sub> (μg/ml) |
|---------------------|--------------------------|-------------------------|
|                     | Lower                   | Upper                   |
| Streptomyces ethyl acetate extract | Y = 6.751X-16.107 | 1164.962<sup>a</sup> | 1562.021<sup>a</sup> |
|                     |                         |                         | 1337.384 | 2070.516 |
| Azadirachtin        | Y = 3.866X-9.344        | 1729.403<sup>d</sup>    | 2989.165<sup>d</sup>    |
|                     |                         | 32.516<sup>c</sup>     | 363.252<sup>c</sup>     |
|                     |                         | 320.121                | 560.390                |

*Lower and upper 95% confidence limits for LC<sub>50</sub> for Streptomyces ethyl acetate extract, <sup>a</sup>Lower and upper 95% confidence limits for LC<sub>90</sub> Streptomyces ethyl acetate extract, <sup>c</sup>Lower and upper 95% confidence limits for LC<sub>50</sub> for azadirachtin, <sup>d</sup>Lower and upper 95% confidence limits for LC<sub>90</sub> for azadirachtin.
deformities in adults were recorded only at 400 and 800 μg/ml concentrations (Figure 2).

Food utilization assay

The diet utilization experiments indicated significant effect of *S. hydrogenans* solvent extract on *S. litura*. As is apparent from Table 5, there was significant decrease in relative growth and consumption rate of *S. litura* as well as efficiency of conversion of ingested and digested food. Diet supplemented with extract resulted in 13–49% reduction in RGR over the control (P ≤ 0.01). Food consumption rate reduced to half of that in control at highest concentration (P ≤ 0.01).

A concentration dependent decrease in ECI and ECD was observed in the larvae of *S. litura* (Figures 3 and 4). The diet amended with extract caused 18–67% decline in ECI and 17–72% decline in ECD over the control. Approximate digestibility increased by 43% at 1600 μg/ml in comparison to control as shown in Table 5 (P ≤ 0.01). The reduction in diet utilization suggests that reduced growth and development might have resulted from both behavioral and physiological effects. It is likely that this decrease in consumption rate (RCR) could be due to the antifeedant nature of the extract and accounts for the majority of the decrease in growth rate (RGR). The *Streptomyces* extract also altered food utilization indices in *S. litura* and revealed less conversion of ingested (ECI) and digested (ECD) food to body biomass. The extract also influenced AD of larvae fed on amended diet as it increased with increase in concentration but the increase in AD could not compensate for the decrease in ECD, which consequently led to reduced growth rate. ECI is an overall measure of an insect’s ability to utilize the food that it ingests for growth and development and ECD is a measure of the efficiency of conversion of digested food into growth [36]. A drop in ECI indicates more food is being metabolized for energy purpose and less for conversion to body substance. ECD also decreases as the proportion of digested food metabolized for energy increases. Thus, decreased ECI and ECD values in the present studies indicate that ingested crude extract of *Streptomyces* does exhibit some chronic toxicity against *S. litura* [37].

**Table 3 Effect of ethyl acetate extract of *S. hydrogenans* and azadirachtin on mortality rate of different developmental stages of *S. litura***

| Treatments                        | Concentrations (μg/ml) | Larval mortality (%) | Prepupal mortality (%) | Pupal mortality (%) | Corrected Pupal mortality (%) |
|-----------------------------------|------------------------|----------------------|------------------------|---------------------|-------------------------------|
| Control                           | -                      | -                    | 13.80 ± 0.67<sup>a</sup> | -                   | -                             |
| *Streptomyces* ethyl acetate extract | 400                    | -                    | 48.26 ± 1.01<sup>b</sup> | 39.98 ± 1.40<sup>a</sup> | -                             |
|                                   | 800                    | 20.00 ± 0.00<sup>a</sup> | 20.00 ± 4.47<sup>a</sup> | 57.13 ± 2.09<sup>c</sup> | 50.26 ± 0.45<sup>b</sup>     |
|                                   | 1600                   | 70.00 ± 12.40<sup>b</sup> | 66.66 ± 0.38<sup>b</sup> | 100.00 ± 00<sup>d</sup> | 100.00 ± 0.00<sup>f</sup>     |
| f- value                          | 16.30<sup>**</sup>    | 107.79<sup>**</sup>  | 863.97<sup>**</sup>    | 1436.26<sup>**</sup> |
| R<sup>2</sup>                     | 0.80                   | 0.81                 | 0.94                   | 0.94                 |
| 400                               | 76.66 ± 1.59<sup>c</sup> | -                   | 85.70 ± 1.22<sup>c</sup> | 83.41 ± 0.45<sup>d</sup> |
| 800                               | 96.66 ± 0.42<sup>d</sup> | -                   | -                     | -                   |
| 1600                              | 100.00 ± 00<sup>e</sup> | -                   | -                     | -                   |
| f- value                          | 146.19<sup>**</sup>   | -                   | -                     | -                   |
| R<sup>2</sup>                     | 0.85                   | -                   | -                     | -                   |

Mean ± SE followed by different letters within a column are significantly different. Tukey’s test P ≤ 0.05, R<sup>2</sup> = Coefficient of determination, **Significant at 1% level.

Conclusions

Present study reports growth inhibitory activities of metabolites of *S. hydrogenans* on *S. litura*. The metabolites in the extract showed strong antifeedant, larvicidal, pupicidal and toxic activities against major pest *S. litura*. Diet utilization experiments clearly revealed the growth inhibitory impact of extract. However, the toxic effect of
the extract was less as compared to the positive control, azadirachtin, which could be due to the purified nature of the plant compound. These findings indicate that the extract has considerable potential to control insect pest populations and can further be used for development of novel insecticidal formulation as an alternative to toxic chemicals for the management of field pests.

Methods

**Streptomyces hydrogenans** DH16 (GenBank: JX123130) was isolated from soil, procured from Dalhousie, Himachal Pradesh, India and identified using polyphasic taxonomic approach [29]. Culture was maintained on starch casein nitrate agar (SCNA, starch: 10 g/l, NaCl: 2 g/l, KNO₃: 2 g/l, K₂HPO₄: 2 g/l, CaCO₃: 0.02 g/l, MgSO₄: 0.05 g/l, FeSO₄: 0.01 g/l, casein: 0.3 g/l and agar: 20 g/l) slopes at 4°C and as mycelial fragments and spores in 20% v/v glycerol at −80°C.

**Production and extraction of bioactive metabolites from Streptomyces**

Production and extraction of solvent extract of *S. hydrogenans* was carried out by the method of Kaur and Manhas [29]. The isolate was cultured on starch casein nitrate agar medium at 28°C. After 7 days of incubation, the growth was scrapped and transferred aseptically into the seed medium (SCN broth) and incubated for 48 h to develop inoculum. The seed culture, at a concentration of 2%, was inoculated into production medium having same composition as seed medium and fermentation was carried out at 28°C at 180 rpm for optimum production of bioactive

| Concentrations (μg/ml) | Longevity (in days) (Mean ± S.E.) | Fecundity (No. of eggs laid/ female) (Mean ± S.E.) | Percent Hatching (Mean ± S.E.) |
|------------------------|----------------------------------|-----------------------------------------------|-------------------------------|
| Control                | 11.50 ± 0.76⁰                    | 1500 ± 151.00⁰                                | 87.66 ± 0.91                  |
| 400                    | 5.00 ± 0.77⁰                    | 150.20 ± 22.40⁰                              | -                            |
| 800                    | 4.33 ± 0.66⁰                    | -                                             | -                            |
| 1600                   | -                               | -                                             | -                            |
| f-value                | 28.89**                         | 78.64**                                       | -                            |
| R²                     | 0.91                            | 0.67                                          | 0.60                         |

Mean ± SE followed by different letters within a column are significantly different. Tukey’s test P ≤ 0.05, R² = Coefficient of determination. **Significant at 1% level.

Table 4 Effect of ethyl acetate extract *S. hydrogenans* on longevity, fecundity and percent hatching of *S.litura* adults

**Figure 2** Developmental stages of *S.litura* reared on control diet (a,c,f) and abnormalities in different stages fed on diet supplemented with different concentrations of ethyl acetate extract of *S. hydrogenans* (b,d,e,g,h).
metabolites. After three days, the flasks were harvested and the biomass was separated from the culture broth by centrifugation at 10000 rpm for 20 min at 4°C. After centrifugation, the active metabolites in the cell free fermented broth were extracted in ethyl acetate and organic phase was concentrated under vacuum to yield a brown colored extract which was re-dissolved in dimethyl sulfoxide (DMSO) and was stored at 4°C for further use.

Insect culture

*Spodoptera litura* is a widely spread species and is found in much of the Asia and Oceania regions [3]. For rearing, egg masses of *Spodoptera litura* were collected from cauliflower planted in the fields around Guru Nanak Dev University, Amritsar (Punjab), India. The culture was maintained in the B.O.D. incubator at a temperature of 27 ± 2°C, relative humidity 60% and photoperiod (L16:D18) on castor (*Ricinus communis*) leaves in battery jars (l15 × d10 cm). The leaves were washed with sodium hypochlorite solution (1%) and changed regularly till pupation. The pupae were separated and kept in pupation jars provided with moist sterilized sand. After adult emergence, adult moths were transferred to oviposition jars in the ratio of 1 male: 2 females and covered with muslin cloth. The jars, containing cotton soaked in 20% sugar solution, were lined with filter paper to aid egg laying. The eggs were kept in small Petri plates having a moist cotton swab. After hatching, the larvae were fed on artificial diet (bran: 6 g, kidney bean flour: 30 g, yeast extract: 3 g, agar: 3 g, vegetable oil: 375 μl, streptomycin: 0.3 g, vitamin-B complex: 0.6 g, formaldehyde: 600 μl and distilled water 195 ml) [12]. Bran, kidney bean flour, vegetable oil and formaldehyde were mixed together. Agar was boiled separately in 100 ml of distilled water in beaker. The dissolved agar was poured into the above said mixture and stirred for 4–5 mins. Rest of the diet contents were added at last to the mixture and mixed thoroughly. The whole diet was poured

Table 5 Effect of ethyl acetate extract of *S. hydrogenans* and azadirachtin on food utilization and feeding of *S. litura*

| Treatments                          | Concentrations (μg/ml) | RGR (mg/mg/day) (Mean ± S.E.) | RCR (mg/mg/day) (Mean ± S.E.) | AD (%) (Mean ± S.E.) |
|-------------------------------------|------------------------|-------------------------------|-------------------------------|----------------------|
| Control                             | 2.17 ± 0.07<sup>a</sup> | 6.97 ± 0.39<sup>a</sup>       | 28.35 ± 1.05<sup>a</sup>       |
| 400                                 | 1.88 ± 0.03<sup>b</sup> | 7.29 ± 0.26<sup>a</sup>       | 30.00 ± 0.29<sup>b</sup>       |
| 800                                 | 1.66 ± 0.10<sup>c</sup> | 6.99 ± 0.38<sup>a</sup>       | 51.96 ± 0.44<sup>b</sup>       |
| *Streptomyces* acetate extract      | 1600                   | 1.10 ± 0.11<sup>bc</sup>     | 3.53 ± 0.29<sup>b</sup>        | 66.00 ± 1.32<sup>c</sup> |
| f-value                             | 26.45**                | 27.53**                       | 416.91**                      |
| R<sup>2</sup>                       | 0.95                   | 0.59                          | 0.92                          |
| 400                                 | 1.54 ± 0.20<sup>c</sup> | 3.92 ± 0.80<sup>b</sup>       | 43.56 ± 9.37<sup>d</sup>       |
| 800                                 | 1.39 ± 0.10<sup>d</sup> | 3.57 ± 0.80<sup>b</sup>       | 49.00 ± 10.76<sup>d</sup>      |
| Azadirachtin                        | 1600                   | -                             | -                             |
| f-value                             | -                      | -                             | -                             |
| R<sup>2</sup>                       | -                      | -                             | -                             |

Mean ± SE followed by different letters with in a column are significantly different. Tukey’s test P ≤ 0.05, R<sup>2</sup> = Coefficient of determination, *Significant at 5% level, **Significant at 1% level.

Figure 3 Effect of (a) ethyl acetate extract of *S. hydrogenans* and (b) Azadirachtin on ECI of *S. litura*. Columns and bars represent the mean ± SE. Different letters above the columns representing each concentration indicate significant differences at Tukey’s test P ≤ 0.05.
into sterilized Petri plates while still hot. The diet was allowed to cool at room temperature for 24 h and stored at 4°C before giving to larvae. Control diet was prepared without extract and treated diet had different concentrations of the extract.

Bioassay studies
Bioassay studies were carried out to evaluate the effect of ethyl acetate extract from *S. hydrogenans* on growth and development of *S. litura*. For this, the artificial diet was supplemented with three concentrations (400, 800 and 1600 μg/ml) of extract as well as respective controls. Then, 2nd instar (5 to 6 days old) larvae were starved for 2–3 h and transferred individually to plastic containers (49 × 6 cm) containing cubical pieces of treated and control diets. The experimental trays were kept in B.O.D incubator maintained under controlled conditions and observed daily for various parameters such as larval period, pupation time, number of pupae formed, the number of adults emerged, fecundity and percent hatching. Larval, prepupal and pupal mortality was also recorded. The diet was changed regularly. Each experiment was replicated six times with 5 larvae/replication (n = 120). Abbott’s formula was used to correct mortality in the control group (only for % pupal mortality) as given below:

\[
\text{Mt} - \frac{\text{Mt} - \text{Mc}}{100 - \text{Mc}} \times 100
\]

Where Mt: % age mortality in treated group, Mc: % age mortality in control group

For the fecundity assay, ten pairs of moths that emerged on the same day from control and 2–3 pairs from treatment group were collected and put into a battery jar lined with filter paper to facilitate egg laying and absorbent cotton soaked in a 10% sugar solution was provided for moth nutrition. The egg-masses laid were counted daily under stereomicroscope (Magnüs, 10X) and removed individually to a petri dish for further observation. To evaluate the fertility, egg-masses obtained from control and treatment group were observed daily for hatching, and then the hatch percent was calculated.

Nutritional indices
The nutritional indices of *S. litura* were determined by following the procedure of Koul et al. [38]. To find out weight gain, food consumption and feces produced, gravimetric technique was used. All weights were measured in milligrams (mg) using a monopan balance (Citrizen) accurate to 0.1 mg. Newly molted 2nd instar larvae were starved for 1–2 h to clear their digestive tracts. After measuring the initial weight of the larvae carefully with the help of brushes, they were individually introduced into experimental plastic containers containing weighed quantities of control and treated diet. The larvae (30 larvae/concentration including control, 6 replicates) were allowed to feed for a period of three days on diet supplemented with extract as well as control. After this feeding period, larvae were again weighed and weights of larvae, uneaten diet and faecal matter were taken at the end of the experiment. The net gain or loss in terms of body weight (wet) of individual larvae, food ingested by larva and faecal matter of larvae were calculated by subtracting the initial weight from the final weight at the end of the experiment. Dry weights of larvae were taken by incubating the larvae at the end of experiments at 60°C for 72 h inside an incubator. Similarly dry weights of different samples of diet and faecal matter were also taken. The dry weight readings indicate water loss under control conditions. From the results the following nutritional indices were obtained as proposed by Waldbauer [39] and all indices were calculated using dry weights.

RGR and RCR were calculated on dry weight basis after 3 days of feeding as G/I (G = change in larval dry
weight/day and \( I \) = starting larval dry weight) and \( C/I \) (\( C \) = change in dry weight/day and \( I \) = starting larval dry weight), respectively. Both were calculated as mg/mg/day. Index of food conversion efficiency (ECI) was calculated as \( 100 \times G/C \), where \( G \) = dry weight gain of insect and \( C \) = dry weight of food consumed. Approximate digestibility (AD) and efficiency of conversion of digested food (ECD) were calculated as \( C - F/C \times 100 \) (where \( C = \) change in diet dry weight/day and \( F \) = dry weight of frass/day) and \( G/C - F \times 100 \) (where \( G = \) change in larval dry weight/day, \( C = \) change in diet dry weight/day and \( F = \) dry weight of frass/day, respectively. ECI, AD and ECD were calculated as percent.

Statistical analysis

Data collected from the above experiments were subjected to statistical analysis where values were represented as their mean \( \pm \) SE. To compare difference in means, one way analysis of variance (ANOVA) was performed using Minitab (version 14). Tukey's post hoc test was done with the help of ASSISTAT (7.7 beta). Linear regression analysis was performed to know coefficient of determination (R\(^2\)) with Microsoft office excel 2007 (Microsoft Corp., USA). To calculate LC\(_{50}\) SPSS software for windows version 16.0 (SPSS Inc., Chicago) was used.

Competing interests

The authors declare that they have no competing interest.

Authors' contributions

Conceived and participated in the design of the experiments and supported the execution of the experiments: SKS RKM TK AV. Performed the experiments: SKS RKM TK AV. Conceived and participated in the design of the experiments and supported the execution of the experiments: SKS RKM TK AV. Wrote the manuscript: TK AV RKM. Reviewed and approved the final manuscript: TK AV RKM.

Acknowledgements

We duly acknowledge the funding provided by University Grants Commission, New Delhi, India.

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Received: 10 May 2014 Accepted: 19 August 2014 Published: 28 August 2014

References

1. Zhou CN. A progress and development foresight of pesticidal microorganisms in China. Pesticides 2001, 40:8–10.
2. Ferry N, Edwards MG, Mulligan EA, Emami K, Petrova AS, Frantescu M, Davison GM, Gatehouse AMR. Engineering Resistance to Insect Pests. In Handbook of plant. Volume 1. Edited by Christou P, Chichester HK. UK: John Wiley and Sons Ltd; 2004:373–394.
3. Rao GV, Wrightman JA, Rao DR. World review of the natural enemies and diseases of Spodoptera litura (F.) (Lepidoptera: Noctuidae). Insect Sci Appl 1993, 14:273–284.
4. Anonymous: Distribution Maps of Plant Pests, Spodoptera litura (F.), Map. No. 61. Wallingford, UK: CAB International. 1967.
5. Ayyanna T, Anjanarao P, Subbaratnam GV, Krishna Murthy Rao BH, Narayana KL: Chemical control of Spodoptera litura F. groundnut crop. Pestology 1982, 16(8):19–20.
6. Dhe BC, Mohapatra HK, Senapathi B. Assessment of crop loss in groundnut due to tobacco caterpillar, Spodoptera litura (F.). Indian J. Plant Protect 1992, 20:215–217.
7. Armes NH, Wightman JA, Jadhav DR, Rao GV. Status of insecticide resistance in Spodoptera litura in Andhra Pradesh, India. Pest Sci 1997, 50:240–248.
8. Jang L, Ma CS. Progress of researches on biopesticides. Pesticides 2000, 16:75–77.
9. Leonard GC, Julius JM. Review biopesticides: a review of their action, applications and efficacy. Pest Manag Sci 2000, 56:651–676.
10. Hu QB, Ren XB, An XC, Qian MH. Insecticidal activity influence of deoxynuxins on the pathogenicity of Pseudomonas javanicus against Spodoptera litura. J Appl Entomol 2001, 131:262–268.
11. Mordue AJ, Blackwell A. Azadirachtin: an update. J Insect Physiol 1993, 39:903–904.
12. Ioul O, Singh G, Singh R, Singh J. Bioefficacy and Mode-of-Action of Aglarox B and Aglaroxin C from Aglaia elaeagnoides (syn. A. roxburghiana) against Helicoverpa armigera and Spodoptera litura. Biopestic Int 2005, 11(1):54–64.
13. Tang W, Wei X, Xu H, Zeng D, Long L: 13-Decoytrol A, a new insecticidal isoylamodane diterpene from the seeds of Itea orientalis. Fitoterapia 2009, 80:286–289.
14. Jaysankar A, Raja N, Ignacimuthu S. Insecticidal compound isolated from Syzygium lineane Wall. (Myrtaceae) against Spodoptera litura (Lepidoptera: Noctuidae). Saudi J Biol Sci 2011, doi:10.1016/j.sjbs.2011.01.003.
15. Demain AL, Sanchez S. Microbial drug discovery: 80 years of progress. J Antibiot 2009, 62:S–16.
16. Cantillo MA, Moya P, Herna ñdez E, Primo-Yuñera E. Susceptibility of Ceratitis capitata Wiedemann (Diptera: tephritidae) to entomopathogenic fungi and their extracts. BioControl 2000, 19:274–282.
17. Sh Yi: Advances of insecticidical microorganisms. Plant Prod 2000, 26:32–34.
18. Xie MJ: The perspective of the studies on microbial insecticides. J Liaoning Normal Uni (Natural Science) 1998, 21:326–329.
19. Oka Y, Kohai H, Bar-Eyal M, Mor M, Sharon E, Chet I, Spiegel Y: New strategies for the control of plant-parasitic nematodes. Pest Manag Sci 2000, 56:983–988.
20. Bream AS, Ghosal SA, El-Azz ZKA, Ibrahmy SY. Insecticidal activity of selected actinomycetes strains against the Egyptian cotton leaf worm Spodoptera littoralis (Lepidoptera: Noctuidae). Meledenica Facultad Landbovuwkdunhe en Toegesapte Biologische Wesenshappen Universiteit Gent 2001, 66(2a):503–544.
21. Arasu MV, Al-Dhabi NA, Saritha V, Duraiappanay V, Muthukumar C, Kim SJ. Antifeedant, larvicidal and growth inhibitory bioactivities of novel polyketide metabolite isolated from Streptomyces sp. AP-123 against Helicoverpa armigera and Spodoptera litura. BMC Microbiol 2013, 13:105.
22. Hussain AA, Mostafa SA, Ghosal SA, Ibrahmy SY. Studies on antifungal antibiotic and bioseccessicidal activities of some actinomycete isolates. African J Mycol Biotechnol 2002, 10:63–80.
23. Sundarapandian S, Sundana MD, Tholakanppan P, Balasubramanayan V: Mosquitocidal properties of indigenous fungi and actinomycetes against Culex quinquefasciatus Say. J Biol Control 2002, 1689–91.
24. Gadelhalk GG, El-Tarabily KA, Al-Kabi FK. Insect control using chitlinolytic soil actinomycetes as biocontrol agents. Int J Appl Bio 2005, 7:263–633.
25. Osman G, Mostafa S, Mohamed SH. Antagonistic and insecticidal activities of some Streptomyces isolates. Pak J Biotechnol 2007, 4(1):2165–71.
26. Dhansakaran D, Sakti V, Thajuddin N, Paneersevaram A: Preliminary evaluation of Anopheles mosquito larvicidal efficacy of mangrove actinomycetes. Int J Appl Biol Pharm Technol 2010, 1:374–381.
27. Montesinos E. Development, registration and commercialization of microbial pesticides for plant protection. Int Microbiol 2003, 6:245–252.
28. Omura S. Sequence: 25 years and still going strong, Int J Antimicrob Agents 2008, 31:91–98.
29. Kaur T, Sharma D, Kaur A, Manhas RK. Antagonistic and plant growth promoting activities of endophytic and soil actinomycetes. Arch Phytophathol Plant Protect 2013, 46(14):1756–1768.
30. Kaur T, Manhas RK. Antifungal, insecticidal, and plant growth promoting potential of Streptomyces hydrogenius DH16. J Basic Microbiol 2013, http://dx.doi.org/10.1002/jobm.201300086.
31. Becher PG, Keller S, Jung G, Sussmuth RD, Juttner F: The perspective of the studies on microbial insecticides. Fitoterapia 2009, 80:286–289.
33. Xiong L, Li J, Kong F: *Streptomyces sp.* 173, an insecticidal micro-organism from marine. *Lett Appl Microbiol* 2004, 38:32–37.
34. Xiong L, Jian-zhong L, Hui-li W: *Streptomyces avermitilis* from marine. *J Env Sci* 2005, 17(1):123–125.
35. Abouelghar GE, Sakr H, Ammar HA, Yousef A, Nassar M: Sublethal effects of spinosad (tracer\(^*\)) on the Cotton leafworm (lepidoptera: noctuidae). *J Plant Protect Res* 2013, 53(3), doi:10.2478/jppr-2013-0041.
36. Nathan SS, Kalaivani K, Murugan K, Chung PG: Efficiency of Neem limnoids on *Cnaphalocrocis medinalisi* (Guenee) (Lepidoptera: Pyralidae) the rice leaffolder. *Crop Protect* 2005, 8:760–763.
37. Wheeler DA, Isman MB: Antifeedant and toxic activity of *Trichilia americana* extract against the larvae of *Spodoptera litura*. *Entomol Exp Appl* 2001, 98:9–16.
38. Koul O, Shankar JS, Mehta N, Taneja SC, Tripathi AK, Dhar KL: Bioefficacy of crude extracts of *Aglaia* species (Meliaceae) and some active fractions against lepidopteran larvae. *J Appl Entomol* 1997, 121:245–248.
39. Waldbauer GP: The consumption and utilization of food by insects. *Adv Insect Physiol* 1968, 5:229–288.