# Fungitoxic Activity of *Solanum torvum* against *Fusarium sacchari*

*Shailendra Kumar GUPTA and Satish Chandra TRIPATHI*

*Natural Pesticide Laboratory, Department of Botany, D.D.U. Gorakhpur University, Gorakhpur, India*

**Abstract**

Gupta S.K., Tripathi S.C. (2011): **Fungitoxic activity of** *Solanum torvum* **against** *Fusarium sacchari*. Plant Protect. Sci., **47**: 83–91.

The leaf extract of *Solanum torvum* Sw. (Solanaceae) was evaluated for its fungitoxic activity against *Fusarium sacchari* (E.J. Butler & Hafiz Khan) W. Gams (1971). The leaf extract was found to inhibit completely the mycelial growth of the test fungus at minimum 0.5 ml/ml dose by the poisoned food technique. The nature of the extract was fungicidal at 0.5 ml/ml dose, since no further revival of growth was observed in inoculated plates even after incubation for 6 days after inoculation. The extract required minimally 5 min to kill the test fungus at its MIC. The extract also inhibited the growth of 12 other pathogenic fungi as well as 2 pathogenic strains of bacteria under test. It was found to be non-phytotoxic in nature and to show antifungal activity under *in vivo* conditions.

**Keywords:** *Solanum torvum* Sw.; *Fusarium sacchari;* antifungal activity; poisoned food technique

Sugarcane (*Saccharum officinarum* L., Poaceae) occupies about 4.2% of the total cultivated area in India (4.36 mill. ha) and is one of the most important cash crops, contributing about 7.5% of the gross value of agricultural production in the country (Anonymous 2009). Besides its main product – the white sugar, it also supports two important cottage industries, viz Gur (Jaggery) and Khandsari, which together produce about 10 +million tonnes of sweeteners (Gur and Khandsari sugar) consuming about 28–35% of the cane produced in the country (Anonymous 2009).

In addition to other factors like drought and unseasonal floods, sugarcane diseases cause significant losses in yield. Diseases caused by fungi, bacteria, viruses and phytoplasmas pose a serious threat to sugarcane cultivation in different sugarcane growing regions of India (Rao *et al.* 2002). Among the fungal diseases, red rot, wilt and smut are of major concern to sugarcane production.

Wilt disease is one of the most alarming fungal diseases of sugarcane and is next to red rot in causing economic losses (Agnihotri & Rao 2002). In recent years wilt has become an alarming problem in many commercial cultivars of India (Agnihotri & Rao 2002). Losses due to wilt, which are usually computed on the basis of the quantity of canes, dried or dead, found in the field after harvest, may vary from 2 to 10 t/ha (Parthasarthy 1972).

In view of the sett- and soil-borne nature of wilt pathogen, various types of control measures have been recommended to manage sugarcane wilt. So far, no single method has been known to control the wilt disease syndrome in sugarcane.

The efficacy of synthetic chemicals against wilt pathogens of sugarcane was demonstrated previously (Chattopadhaya & Nandi 1977; Srivastava *et al.* 2007; Tripathi & Pandey 2008). It is now well established that these synthetic chemical pesticides have potentially toxic effects on humans, wild life and the environment (De Waard *et al.* 1993). They are generally non-biodegradable and if degradable, their half-life period is much more prolonged (Sakurai *et al.* 2004).

Keeping in the view the above mentioned, the present investigation was undertaken to evaluate the antifungal efficacy of higher plant extracts in order to assess their toxicity against *Fusarium sacchari* (E.J. Butler & Hafiz Khan) W. Gams (1971) (syn. *Cephalosporium sacchari* Butler) and to study different aspects regarding its utility as 'sett protectant' for the control of Wilt Disease of Sugarcane.

## MATERIAL AND METHODS

***Plant materials***. Twenty-two plant species (Table 1) belonging to different families of angiosperms, collected from different parts of Gorakhpur and Maharajganj districts, were identified with the help of identification keys of floras (Hooker 1872–1897; Srivastava 1976). The identity of each collected plant was confirmed against their authentic herbarium specimens lodged in the herbarium of the Department of Botany, D.D.U. Gorakhpur University, Gorakhpur. A voucher specimen of all plants has also been deposited in the herbarium of the Department of Botany, D.D.U. Gorakhpur University.

***Preparation of crude leaf extract***. Twenty grams of freshly collected disease-free leaves of plants were surface sterilised with sodium hypochlorite solution (2%) for 5 min followed by washing with sterilized distilled water to remove all the traces of sodium hypochlorite. The sample was then chopped into small pieces and macerated to pulp with 20 ml (1:1 w/v) of sterilized distilled water using a sterilised pestle and mortar. The pulp was squeezed by double layered sterilised muslin cloth and the aliquot was filtered through Wattman's No. 1 filter paper. The crude extract thus obtained was subjected to antifungal testing against the test fungus *F. sacchari*.

***Preparation of inoculum***. The pure culture of *F. sacchari* (ITCC No. 4557) was obtained from the Indian Type Culture Collection (ITCC), Indian Agricultural Research Institute, New Delhi. The stock culture was maintained on potato dextrose agar (PDA) medium.

The test fungus was grown in Petri plates containing 10 ml of PDA medium. Mycelial discs of 5 mm

Table 1. Fungitoxicity of crude extracts of higher plants against *F. sacchari* using the Poisoned Food Technique

| Samples No. | Plant's name | Family | % inhibition of mycelial growth (mean ± SE) |
|---|---|---|---|
| 1 | *Abutilon indicum* G Don. | Malvaceae | 47.28 ± 0.21 |
| 2 | *Ageratum conyzoides* Linn. | Asteraceae | 70.21 ± 0.42 |
| 3 | *Allium cepa* Linn. | Liliaceae | 88.75 ± 0.72 |
| 4 | *A. sativum* Linn. | Liliaceae | 72.71 ± 0.21 |
| 5 | *Anagallis arvensis* Linn. | Primulaceae | 52.92 ± 0.21 |
| 6 | *Caesalpinia coriaria* (Jacq.) Willd. | Caesalpiniaceae | 76.46 ± 0.75 |
| 7 | *Capsicum annuum* Linn. | Solanaceae | 76.88 ± 0.36 |
| 8 | *Eupatorium odoratum* Linn. | Asteraceae | 48.33 ± 0.21 |
| 9 | *Lawsonia inermis* Linn. | Lythraceae | 66.84 ± 0.75 |
| 10 | *Leucas aspera* (Willd.) Spreng. | Lamiaceae | 72.92 ± 0.55 |
| 11 | *Mentha arvensis* Linn. | Lamiaceae | 49.17 ± 0.55 |
| 12 | *Momordica charantia* Linn. | Cucurbitaceae | 89.79 ± 0.55 |
| 13 | *Ocimum basilicum* Linn. | Lamiaceae | 82.29 ± 0.91 |
| 14 | *Peperomia pellucida* Linn. | Piperaceae | 56.88 ± 0.95 |
| 15 | *Putranjiva roxburghii* Wall. | Euphorbiaceae | 54.79 ± 0.21 |
| 16 | *Ricinus communis* Linn. | Euphorbiaceae | 26.25 ± 0.36 |
| 17 | *Solanum torvum* Sw. | Solanaceae | 100.00 |
| 18 | *Tagetes erecta* Linn. | Asteraceae | 47.92 ± 0.75 |
| 19 | *Terminalia chebula* Retz. | Combretaceae | 18.96 ± 0.75 |
| 20 | *Tridax procumbens* Linn. | Asteraceae | 20.21 ± 1.59 |
| 21 | *Vitex negundo* Linn. | Verbinaceae | 69.38 ± 0.36 |
| 22 | *Zizyphus mauritiana* Lam. | Rhamnaceae | 31.25 ± 0.36 |

diameter along with the adhering agar, cut from the periphery of seven days old culture with the help of flame sterilised cork borer, served as inoculum throughout the present study. Care was taken to ensure a regular supply of uncontaminated, seven days old culture until all the experiments were over. The cultures were maintained at 28 ± 1°C.

***Screening of crude plant extracts against*** *Fusarium sacchari*. The screening of the plants was done by Poisoned Food Technique of Grover & Moore (1962): For treatment sets, 10 ml of the prepared crude extract of each plant was mixed with 10 ml of molten PDA medium in a pre-sterilised Petri plate and the contents were agitated in a circular mode in order to mix the extract homogeneously. In control sets, a requisite amount of sterilised double distilled water was added in place of the extract. A fungal disc (5 mm in diameter) cut from the periphery of 7 days old culture of *F. sacchari* was inoculated aseptically in each assay plate and the plates were incubated for six days at 28 ± 1°C.

Colony diameters in mutual perpendicular directions were measured on the seventh day in assay plates. Fungitoxicity was recorded in terms of the % inhibition of mycelial growth and calculated using the following formula (Vincent 1947):

$$\text{Inhibition of mycelial growth (\%)} = \frac{dc - dt}{dc} \times 100$$

where:

*dc* – average diameter of fungal colony in control sets

*dt* – average diameter of fungal colony in treatment sets

The experiments were repeated twice and each set contained five replications. The results presented in Table 1 are based on the mean values of all replications.

***Determination of minimum inhibitory concentration (MIC)***. The MIC of the crude leaf extract required for absolute inhibition of mycelial growth of the test fungus, *F. sacchari*, was determined by the usual Poisoned Food Technique (Grover & Moore 1962). The crude leaf extract of *S. torvum* was prepared as described previously. Requisite amounts of the prepared crude leaf extract were added to pre-sterilised Petri plates containing 10 ml of molten PDA in order to obtain dilutions of 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, and 0.1 ml/ml of the crude leaf extract with respect to the volume of the medium. The contents of the plates were agitated in a circular mode to mix the extract in the medium homogeneously. In control sets, the same amount of sterilised distilled water was used in place of the extract. Fungal discs (5 mm in diameter) cut from the periphery of seven days old culture of *F. sacchari* were aseptically transferred in each Petri plate. The assay plates were incubated for six days at 28 ± 1°C. The observations were recorded on the seventh day in terms of the percent inhibition of mycelial growth and data presented in Table 2 are based on the averages of all the replications.

Table 2. Minimum inhibitory concentration (MIC) of the crude leaf extract of *S. torvum*

| Concentration of the extract (ml/ml) | Inhibition of mycelial growth (%) |
|---|---|
| 1.0 | 100.00 |
| 0.9 | 100.00 |
| 0.8 | 100.00 |
| 0.7 | 100.00 |
| 0.6 | 100.00 |
| 0.5 | 100.00 |
| 0.4 | 36.67 |
| 0.3 | 11.42 |
| 0.2 | 00.00 |
| 0.1 | 00.00 |

***Nature of fungitoxicity***. To determine the nature of fungitoxicity of the crude leaf extract of *S. torvum*, the experiments were designed according to the method described by Garber and Houston (1959).

The treatment sets were prepared by supplementing different doses of the extract in 10 ml of the molten PDA medium. In the control set, sterilised double distilled water was used in place of the extract. The assay plates were inoculated with fungal discs (5 mm in diameter) taken from the periphery of 7 days old culture of the test fungus and were incubated for six days at 28 ± 1°C. On the seventh day the fungal discs of the treatment sets exhibiting complete inhibition of mycelial growth were taken out, washed thoroughly with sterilised distilled water and re-inoculated in a separate set of Petri plates containing a fresh solidified PDA medium. The re-inoculated Petri plates were incubated at 28 ± 1°C for six days. The observations were recorded on the seventh day in terms of revival of the growth (Table 3). Experiments were repeated twice and each set contained five replications.

***Time required for fungicidal action***. The time required for the fungicidal action of the extract of

Table 3. The nature of fungitoxicity of the crude leaf extract of *S. torvum*

| Concentration of the extract (ml/ml) | Mycelial growth of *Fusarium sacchari* | |
|---|---|---|
| | T | R |
| 1.0 | – | – |
| 0.5* | – | – |
| 0.25 | + | + |
| 0.10 | + | + |
| 0.05 | + | + |
| 0.02 | + | + |
| Control | + | + |

*MIC; T – treated setts; R – re-inoculated setts; + presence of mycelial growth; – absence of mycelial growth

Table 4. Time required to kill the test fungus by the crude leaf extract of *S. torvum*

| Time (min) | Mycelial growth | |
|---|---|---|
| | T | C |
| 30 | – | + |
| 25 | – | + |
| 20 | – | + |
| 15 | – | + |
| 10 | – | + |
| 5 | – | + |
| 4 | + | + |
| 3 | + | + |
| 2 | + | + |

T – treatment setts; C – control setts ; + presence of mycelial growth; – absence of mycelial growth

*S. torvum* against *F. sacchari* was also determined. The Petri plates containing 10 ml of sterilised double distilled water were supplemented with the requisite amount of the crude extract so as to obtain 0.5 ml/ml dose of the crude leaf extract. Fungal discs (each of 5 mm in diameter) cut from the periphery of 7 days old culture were kept immersed in the prepared dilution of the crude leaf extract for different time periods. After requisite duration, the discs were taken out, washed thoroughly with sterilized distilled water and aseptically inoculated in Petri plates containing the fresh PDA medium. Mycelial discs immersed in sterilised distilled water instead of the extract for the same duration served as control. The assay plates were incubated at 28 ± 1°C for six days and mycelial growth was recorded on the seventh day. The experiment was repeated twice and each set contained five replications. The results, recorded in terms of the % inhibition of mycelial growth (Table 4) are based on the mean values of all replications.

***Range of antifungal activity***. The range of antifungal activity of the crude leaf extract at 0.5 ml/ml dilution was tested against twelve fungal pathogens, viz *Alternaria alternata* (Fr.) Keissler, *A. solani* (Ell. & Mart.) Jones & Grout., *Aspergillus niger* Von Tieghem, *Ceratocystis paradoxa* (Dade) C Mareau, *Colletotrichum falcatum* Went., *Curvularia lunata* (Walker) Boedijn., *Fusarium verticillioides* (Sacc.) Nirenberg, *F. udum* Butler, *Helminthosporium oryzae* Breda de Haan., *Penicillium italicum* Wehmer, *Pythium aphanidermatum* (Edson) Fitz., and *Rhizoctonia solani* Kuhn. using the Poisoned Food Technique as described previously. Experiments were repeated twice and

Table 5. Antifungal spectrum of the crude leaf extract of *S. torvum*

| Pathogen | % inhibition of mycelial growth (mean ± SE) |
|---|---|
| *Alternaria alternata* (Fr.) Keissler (MTCC 3880) | 66.46 ± 0.36 |
| *Alternaria solani* (Ell. & Mart.) Jones & Grout. (MTCC 2101) | 53.22 ± 0.91 |
| *Aspergillus niger* Von Tieghem (MTCC 2425) | 60.44 ± 1.08 |
| *Ceratocystis paradoxa* (Dade) C Mareau (MTCC 2122) | 51.42 ± 0.21 |
| *Colletotrichum falcatum* Went. (ITCC 430) | 42.54 ± 0.72 |
| *Curvularia lunata* (Walker) Boedijn. (MTCC 2030) | 73.56 ± 0.63 |
| *Fusarium verticillioides* (Sacc.) Nirenberg (MTCC 3322) | 100.00 |
| *Fusariun udum* Butler (MTCC 4238) | 58.24 ± 0.95 |
| *Helminthosporium oryzae* Breda de Haan. (ITCC 2537) | 54.82 ± 0.75 |
| *Penicillium italicum* Wehmer (ITCC 6424) | 57.75 ± 0.42 |
| *Pythium aphanidermatum* (Edson) Fitz. (ITCC 4746) | 22.56 ± 1.59 |
| *Rhizoctonia solani* Kuhn. (MTCC 4633) | 100.00 |

Table 6. Antibacterial spectrum of the crude leaf extract of *S. torvum*

| Bacteria | Zone of inhibition of bacterial growth (mm) (mean ± SE) | | |
|---|---|---|---|
| | *S. torvum* extract | positive control | negative control |
| *Escherichia coli* | 10.4 ± 47 | 11.8 ± 80 | 0.00 |
| *Bacillus subtilis* | 12.7 ± 32 | 12.0 ± 68 | 0.00 |

each set contained five replications. The results recorded in terms of the % inhibition of mycelial growth based on the mean values of all replications are given in Table 5.

***Range of antibacterial activity***. The range of antibacterial activity was tested using the Disc Diffusion Technique as described by Rios *et al.* (1988). Nutrient agar medium (NAM) was prepared, sterilised at 14 061.38 kg/m$^2$) pressure for 30 min, cooled to about 40°C and poured in Petri plates already containing 0.1 ml of a $10^{-2}$ dilution of the bacterial culture to be tested.

Filter paper discs of 6 mm in diameter were cut from Wattman's No. 44 filter paper. The crude leaf extract of *S. torvum* was prepared as described previously and diluted to 0.5 ml/ml dose. One ml of this diluted extract was impregnated in filter paper discs for treatment sets. For positive control sets, 30 µg of kanamycin was dissolved in 1 ml of sterilised double distilled water and the solution was impregnated in another disc. For negative control sets, 1 ml of sterilised double distilled water was impregnated in filter paper discs. One disc of each of treatment sets, positive control sets as well as negative control sets were placed equidistantly in Petri plate containing a medium seeded with bacteria to be tested, viz *Escherichia coli* and *Bacillus subtilis*. The assay plates were incubated at 37°C for 48 h and the zone of inhibition was recorded. Experiments were repeated twice and each set contained five replications. The results recorded in Table 6 are based on the mean values of all replications.

***Determination of phytotoxicity***. The phytotoxic effect of the crude leaf extract of *S. torvum* was studied with respect to sett germination and general health and morphology of the sugarcane plant.

***Sett germination***. The crude leaf extract of *S. torvum* was prepared as described previously. The healthy cane setts of one node length containing a single bud were cut obliquely with a secateur. These cane setts were soaked in the crude leaf extract of *S. torvum* for 1 h, 2 h, and 3 h separately in three lots. Some cane setts were also soaked in sterilised water for the same period for control setts. Treated as well as control cane setts were taken out after requisite time and dried in shade for two hours. After drying, these cane setts were sown into earthen pots containing sterilised garden soil. The earthen pots were irrigated regularly with water. The observations were recorded for germination of the bud on the 3$^{rd}$, 4$^{th}$, 5$^{th}$, 6$^{th}$, 7$^{th}$, 8$^{th}$, 9$^{th}$ and 10$^{th}$ day. Fifteen earthen pots, each containing three cane setts, were prepared. Five replications were maintained and the experiment was repeated twice. The data presented in Table 7 are based on the mean value of all replications.

***General health and morphology of the plant.*** The germinating cane setts of the previous experiment were allowed to grow. The general health and morphology of plants of treatment setts were compared with those of control setts at their different growth stages.

***In vivo efficacy.*** The preliminary *in vivo* efficacy of the crude leaf extract of *S. torvum* to control the wilt disease of sugarcane was assessed using sett treatment and soil amendment.

***Sett treatment***. The soil-maize meal medium of the following composition was prepared:

| | |
|---|---|
| Sieved field soil | 190 g |
| Ground maize meal | 10 g |
| Sterilised distilled water | 70 ml |

The contents of the flask were sterilised in an autoclave at 15 lb/sq inch pressure for 30 min for 2 consecutive days. The flask was then inoculated aseptically with 3 discs (each 5 mm in diameter) of *Fusarium sacchari*, obtained from the periphery of 7 day old culture. The flask was kept at 28 ± 1°C for 6 days. After 6 days of incubation the content of the flask was used as fungal inoculum for infesting the soil under experimentation.

Earthen pots (30 cm in diameter and 45 cm in height) were washed with water followed by 70% alcohol. The sterilized garden soil (sieved through 2 mm mesh, dried in the sun and autoclaved for two consecutive days) along with the fungal inoculum prepared previously was filled into the earthen pots (10:1 w/w) up to 25 cm height as recommended by Ghorbany and Salary (2005). The

Table 7. Effect of the crude leaf extract of *S. torvum* on the bud germination of *Saccharum officinarum* L. setts

| Germination period (days) | Percent germination of cane-setts | | | | | |
|---|---|---|---|---|---|---|
| | 1 h | | 2 h | | 3 h | |
| | C | T | C | T | C | T |
| 3 | 00.00 | 6.67 | 00.00 | 00.00 | 00.00 | 00.00 |
| 4 | 13.33 | 13.33 | 20.00 | 13.33 | 26.67 | 13.33 |
| 5 | 40.00 | 33.33 | 33.33 | 40.00 | 33.33 | 26.67 |
| 6 | 60.00 | 66.67 | 73.33 | 73.33 | 66.67 | 73.33 |
| 7 | 66.67 | 80.00 | 80.00 | 80.00 | 73.33 | 80.00 |
| 8 | 86.67 | 100.00 | 93.33 | 80.00 | 86.67 | 93.33 |
| 9 | 100.00 | 100.00 | 93.33 | 86.67 | 93.33 | 100.00 |
| 10 | 100.00 | 100.0 | 100.00 | 100.00 | 93.33 | 100.00 |
| *F*-value | | 0.85* | | 0.91* | | 0.69* |

C – control sets; T – treatment sets; *significance at 5% level

pot was then covered with sterilised garden soil up to 35 cm height (Ghorbany & Salary 2005). The crude leaf extract of *S. torvum* was prepared as described previously. Healthy cane setts were cut obliquely with a secateur and soaked:

- in the leaf extract of *S. torvum* for 2 h for treatment setts,
- in sterilised distilled water for 2 h for negative control setts,
- in 2.5 mg/ml dose of Carbendazim 50WP for 2 h for positive control setts.

Three cane setts, each of all the above three treatments, were separately sown equidistantly into earthen pots containing sterilised garden soil infested with the soil-maize meal medium and covered on top with sterilised garden soil up to 35 cm prepared as described previously and fifty assay pots of this type were maintained for each treatment sett and negative as well as positive control setts. The assay earthen pots were placed in open field conditions and watered, as and when required. For each sett, five replications were maintained. The experiments were repeated twice and the results presented in Table 8 are based on the mean value of all replications.

***Soil amendment***. The soil-maize meal medium infested with the test organism *F. sacchari* was prepared as described previously. The earthen pots (30 cm in diameter and 45 cm in height) were washed with water followed by 70% alcohol. The garden soil (sieved through 2 mm mesh, dried in the sun and autoclaved for two consecutive days) along with the soil-maize meal medium infested with *F. sacchari* (10:1 w/w) was filled into the earthen pots up to 25 cm height (Ghorbany & Salary 2005). The pots were then filled with sterilised soil up to 35 cm height, given light irrigation and left for 7 days.

After 7 days, the earthen pots with treatment setts were irrigated with 500 ml/day of the crude leaf extract for three alternate days. Negative control setts were irrigated with 500 ml/day sterilised distilled water in place of the extract for the same number of days whereas positive control setts were irrigated with 500 ml/day Carbendazim 50WP (2.5 mg/ml) in place of the extract for the same number of days.

The healthy cane setts of one node length containing a single bud were cut obliquely with a secateur. Three cane setts were sown equidistantly into the sterilised soil layer of each assay pot and fifty such assay pots were maintained for each treatment sett and negative as well as positive control setts. Thereafter the treatment and control setts were placed in the open field and the pots were watered, as and when required except the day of treatment. For each sett, five replications were maintained. The experiments were repeated twice and the results presented in Table 8 are based on the mean values of all replications.

## RESULTS AND DISCUSSION

Determination of the minimum inhibitory concentration (MIC) of a fungitoxicant is necessary in order to know its appropriate dose for the complete mycelial inhibition of test pathogen. High doses of a fungitoxicant may lead to a decrease in its rational value, increase its wastage and may also considerably deteriorate the quality of the crop treated. Although a large number of plants and their products have been

Table 8. Symptoms of wilting in sugarcane plants – setts dressed with the aqueous leaf extract of *S. torvum* and pots irrigated with the aqueous leaf extract of *S. torvum*

| Age of the plant (days) | Symptoms present in plants (No. of plants) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | | | II | | | III | | | IV | | |
| | T | PC | NC | T | PC | N | T | PC | NC | T | PC | NC |
| Setts dressed with the aqueous leaf extract of *S. torvum* | | | | | | | | | | | | |
| 45 | 150 | 150 | 150 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 60 | 150 | 150 | 150 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 75 | 150 | 150 | 150 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 90 | 150 | 150 | 150 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 105 | 150 | 150 | 135 | 0 | 0 | 12 | 0 | 0 | 3 | 0 | 0 | 0 |
| 120 | 135 | 141 | 81 | 12 | 9 | 30 | 3 | 0 | 21 | 0 | 0 | 18 |
| 135 | 111 | 129 | 60 | 12 | 15 | 30 | 24 | 3 | 27 | 3 | 3 | 33 |
| 150 | 105 | 111 | 12 | 9 | 21 | 30 | 15 | 9 | 48 | 21 | 9 | 60 |
| 165 | 81 | 105 | 0 | 0 | 21 | 12 | 21 | 15 | 60 | 48 | 9 | 78 |
| 180 | 78 | 99 | 0 | 3 | 6 | 0 | 12 | 6 | 30 | 57 | 39 | 120 |
| Pots irrigated with the aqueous leaf extract of *S. torvum* | | | | | | | | | | | | |
| 45 | 150 | 150 | 150 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 60 | 150 | 150 | 150 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 75 | 150 | 150 | 150 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 90 | 150 | 150 | 147 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| 105 | 147 | 150 | 99 | 3 | 0 | 21 | 0 | 0 | 21 | 0 | 0 | 9 |
| 120 | 141 | 150 | 72 | 6 | 0 | 30 | 3 | 0 | 33 | 0 | 0 | 15 |
| 135 | 129 | 141 | 39 | 9 | 6 | 39 | 9 | 3 | 45 | 3 | 0 | 27 |
| 150 | 111 | 129 | 9 | 9 | 9 | 36 | 21 | 9 | 42 | 9 | 3 | 63 |
| 165 | 99 | 120 | 0 | 15 | 12 | 9 | 15 | 6 | 51 | 21 | 12 | 90 |
| 180 | 87 | 105 | 0 | 9 | 9 | 0 | 12 | 12 | 21 | 42 | 24 | 129 |

I – absence of any symptom; II – yellowing of midribs; III – chlorosis of leaves; IV – complete wilting of crown; T – treatment; PC – positive control; NC – negative control

tested for their antifungal activity, only a few have been studied in detail with respect to their MIC. In the present study the MIC of the crude leaf extract of *S. torvum* was found to be 0.5 ml/ml, i.e. the crude leaf extract completely inhibited the mycelial growth of the test fungus at 0.5ml/ml dose but could not inhibit the mycelial growth completely at 0.4 ml/ml dose (Table 2).

The determination of fungicidal or fungistatic nature of a fungitoxicant evokes the idea whether the fungitoxic effect is temporary or permanent. Several plants and their products, viz *Aegle marmelos*, *Cleome gynandra*, *Croton roxburghii*, *Physalis peruviana* (Singh & Tripathi 1993); *Callistemon lanceolatus* and *Euphorbra hirta* (Raja & Kurucheve 1999), have been reported to possess fungicidal nature. However, the extract of *Schizozygia coffaeoides* has been reported to be fungistatic (Kariba *et al.* 2001). In the present study, the crude leaf extract of *S. torvum* exhibited fungicidal activity at 0.5 ml/ml dose indicating its Minimum Fungicidal Concentration (MFC) (Table 3).

It is desirable to know the time required to kill the test fungi for a chemical having fungicidal activity. In the present study, the crude leaf extract of *S. torvum* killed the test fungus within 5 min (Table 4).

The results presented in Table 5 reveal that the extract of *S. torvum* at 0.5 ml/ml dose completely inhibited the mycelial growth of *Fusarium verticillioides* and *Rhizoctonia solani*, while it significantly inhibited the mycelial growth of *Alternaria alternata*, *Aspergillus niger* and *Curvularia lunata*. However, it showed moderate or poor toxicity to the other fungi tested.

The results presented in Table 6 reveal that the extract of *S. torvum* at a concentration of 0.5 ml/ml

completely inhibited the growth of both tested bacteria.

A fungitoxicant may exhibit a broad range of toxicity inhibiting many fungi or may be effective against specific ones only. If a fungitoxicant possesses a narrow range of toxicity, it may not be successfully employed in controlling the disease incited by a complex of pathogens. Such fungitoxicants may be potentially valuable in controlling few diseases but their limited market value would make them ineffective for producers. In the present investigation the crude leaf extract of *S. torvum* exhibited a broad range of the antifungal spectrum. The crude leaf extract of *S. torvum* was found as effective as commercial antibiotic kanamycin when tested against two pathogenic strains of bacteria. This indicates that the active principle present in the leaves is effective against fungi as well as bacteria making the extract more valuable especially in the control of wilt diseases.

Wellman (1977) emphasised that any compound possessing fungitoxicity must be evaluated for its phytotoxicity before subjecting it to *in vivo* trials. Therefore it was decided to test the phytotoxicity of plant extracts exhibiting fungitoxicity. Previously, the extracts of *Allamanda cathartica, Lawsonia inermis* and *Eucalyptus citriodora, Ruellia tuberosa, Ricinus communis* were found to be non-phytotoxic by Tripathi *et al.* (1978), Dixit *et al.* (1982), and Sharma (2008), respectively. However, Fawcett *et al.* (1969) and Staron *et al.* (1969) reported that 'wyerone' from *Vicia faba* and 'anagalloside' from *Anagallis arvensis* were phytotoxic. The results shown in Table 7 reveal that soaking cane setts in the crude leaf extract of *S. torvum* for different time periods has no significant effect on its germination. This indicates that the extract of *S. torvum* has no phytotoxic effects as far as the sett germination of sugarcane is concerned.

The information about the fungitoxicity of plants or their extracts in laboratory conditions becomes useless unless the plant or its extracts exhibit significant *in vivo* efficacy in controlling plant diseases. Some publications clearly indicate the efficacy of plant crudes and their products in the control of various diseases in field conditions (Mosch & Zeller 1989; Alice & Sivaprakasam 1996). The control of soil-borne pathogens under *in vivo* conditions has generally been attempted by seed treatment or by soil amendment. Some researchers successfully cured many diseases of plants by treating the seeds with various plant extracts (Ghewande 1989; Singh & Tripathi 2000). Soil amendment with different plant parts for the control of soil-borne diseases has been demonstrated by various investigators (Singh & Rai 2000). Therefore in the present investigation, sett treatment and soil amendment with the crude leaf extract of *S. torvum* were done to evaluate the efficacy of the extract in controlling sugarcane wilt. The crude leaf extract of *S. torvum* was found to exhibit 52% wilt control when the sett treatment was done for 2 h (Table 8). However, the amendment of soil with the crude leaf extract of *S. torvum* (500 ml/day up to three alternate days) exhibited 58% control indicating its efficacy as herbal fungicide (Table 8). However, soil amendment proved better than seed treatment for the control of the wilt disease of sugarcane (Table 8).

Therefore the present investigation indicates that the crude leaf extract of *S. torvum*, on account of its fungitoxic, non-phytotoxic and *in vivo* antifungal efficacy, may be recommended for large-scale field trials in order to explore the possibility of its use as herbal fungicide to control the wilt disease of sugarcane.

## References

Agnihotri V.P., Rao G.P. (2002): A century status of sugarcane wilt in India. In: Singh S.B., Rao G.P., Easwaramoorthy S. (eds): Sugarcane Crop Management. SciTech LLC Publishing. Texas: 145–160.

Alice D., Sivaprakasam K. (1996): Fungicidal, bactericidal and nematicidal effect of garlic clove extract. Journal of Ecobiology, **8**: 99–103.

Anonymous (2009): Sugarcane Agriculture: Chapter III. Ministry of Food and Public Distribution, Government of India. Available at http://fcamin.nic.in/dfpd/EventDetails.asp? EventId=236&Section=Sugar and Edible Oil&ParentID=0& child_continue=1& child_check=0 (Accessed April 10, 2009)

Chattopadhyay N.C., Nandi B. (1977): Inhibition potential of auerofungin against *Cephalosporium sacchari*, an incitant of sugarcane wilt. Current Science, **46**: 240–241.

De Waard M.A., Georgopoulus S.G., Holloman D.W., Ishii H., Leroux P., Ragsdale N.N., Schwinn F.J. (1993): Chemical control of plant diseases: Problems and prospects. Annual Review of Phytopathology, **31**: 403–421.

Dixit S.N., Tripathi S.C., Ojha T.N. (1982): "Allamandoside" – A new fungitoxic substance from *Allamanda*

*cathartica* Linn. Journal of Antibacterial and Antifungal Agents, **10**: 197–199.

Fawcett C.H., Spencer D.M., Wain R.L. (1969): The isolation and properties of a fungicidal compound present in seedlings of *Vicia faba*. Netherland Journal of Plant Pathology, **75**: 72–81.

Garber R.H., Houston B.R. (1959): An inhibitor of *Verticillium arboratum* in cotton seeds. Phytopathology, **49**: 449–450.

Ghewande M.P. (1989): Management of foliar disease of groundnut (*Arachis hypogea*) using plant extract. Indian Journal of Agricultural Sciences, **59**: 133–134.

Ghorbany M., Salary M. (2005): Application of plant products to control some soil-borne fungal pathogens. In: Proceedings 4th World Congress on Allelopathy, Establishing the Scientific Base, Wagga Wagga, New South Wales, Australia, August 21–26, 2005.

Grover R.K., Moore J.D. (1962): Toxicometric studies of fungicides against brown rot organisms *Sclerotinia fructicola* and *S. laxa*. Phytopathology, **52**: 876- 880.

Hooker J.D. (1872–1897): The Flora of British India. William Clower & Sons Ltd., London.

Kariba R.M., Siboe G.M., Dorsaji S.F. (2001): *In vitro* antifungal activity of *Schizozygia coffacoides* Bail. (Apocynaceae) extracts. Journal of Ethnopharmacology, **74**: 41–44.

Mosch J., Zeller W. (1989): Control of fire blight (*Erwinia amylovora*) with selected plant extracts. Nachrichtenblatt des deutschen Pftanzenschutzdienstes, **41**: 149–151.

Parthasarthy S.V. (1972): Sugarcane in India. The K.C.P. Ltd., Madras: 685.

Raja J., Kurucheve V. (1999): Fungicidal activity of plant and animal products. Annals of Agricultural Research, **20**: 113–115.

Rao G.P., Viswanathan R., Singh S.B. (2002): Current situation of sugarcane diseases in India. In: Singh S.B., Rao G.P., Easwaramoorthy S. (eds): Sugarcane Crop Management. SciTech LLC Publishing. Texas: 1–9.

Rios J.L., Recio M.C., Villar A. (1988): Screening methods for natural products with antimicrobial activity: A review of the literature. Journal of Ethnopharmacology, **23**: 127–149.

Sakurai K., Todaka E., Saito Y., Mori C. (2004): Pilot study to reduce dioxins in the human body. Internal Medicine, **43**: 792–795.

Sharma S. (2008): Fungitoxic evaluation of some higher plants. [Ph.D. Thesis.] D.D.U. Gorakhpur University, Gorakhpur.

Singh J., Tripathi N.N. (1993): Efficacy of the plant extracts against *Fusarium oxysporum* f.sp. *lentis* on *Lens esculenta*. Journal of Indian Botanical Society, **72**: 51–53.

Singh J., Tripathi N.N. (2000): *Astabotrys hexapetalous*: A potential herbal agent for the management of *Fusarial* wilt of lentil. In: Maheshwari D.K., Dubey R.C. Prasad G. (eds): Microbes: Agriculture Industry and Environment. Publisher M/s. Bishen Singh Mahendra Pal Singh, Dehra Dun: 135–149.

Singh R., Rai B. (2000): Antifungal potential of some higher plants against *Fusarium udum* causing wilt disease of *Cajanus cajan*. Microbios, **102**: 165-173.

Srivastava T.N. (1976): Flora Gorakhpurensis. Today and Tomorrow's Printers and Publishers, New Delhi.

Srivastava A.K., Khare R.K., Singh H. (2007): Synthesis and fungicidal activity of some 3-(5-aryl-1,3,4-thiadiazol-2-yl)-1-(β-d-glucopyranosyl)-5-alkyl-2-thio-4-imidazolidinones. Indian Journal of Chemistry, **46B**: 875–879.

Staron T., Allard C., Heitz S., Billet D. (1969): Purification and antifungal properties of a triterpenic heteroside extracted from the stems and leaves of *Anagallis arvensis* var. *phoenicea*. Phytiat Phytopharm, **18**: 161–166.

Tripathi P., Pandey S. (2008): Synthesis, Cyclohexyl pyrazolines, Antifungal activity. Asian Journal of Chemistry, **20**: 808–810.

Tripathi R.D., Srivastava H.S., Dixit S.N. (1978): A fungitoxic principle from the leaves of *Lawsonia inermis* Linn. Experientia, **34**: 51–52.

Vincent J.M. (1947): Distribution of fungal hyphae in the presence of certain inhibitors. Nature, 159: 850.

Wellman R.H. (1977): Problems in development, registration and use of fungicides. Annual Review of Phytopathology, **15**: 155–163.

Received for publication October 26, 2010
Accepted after corrections April 13, 2011

*Corresponding Author;*

Shailendra Kumar Gupta, D.D.U. Gorakhpur University, Department of Botany, Natural Pesticide Laboratory, Gorakhpur 73009 India
tel.: + 91 993 568 89 80, e-mail: shailendra_450@yahoo.co.in