Indian Journal of Agricultural Sciences **82** (12): 1079-82, December 2012

Bioherbicidal activity of *Streptomyces* spp isolated from tobacco rhizosphere against certain dicot and monocot weeds*

D V SUBHASHINI

Central Tobacco Research Institute, Rajahmundry, Andhra Pradesh 533 105

Received: 31 March 2012; Revised accepted: 12 September 2012

Key words: Bioherbicide, Biological control, Streptomyces, Weeds

Herbicides are chemicals marketed to inhibit or interrupt normal plant growth and development. There has been growing concern in recent years about excessive usage of chemical herbicides and its impact on nature and natural resources (Sinha 2012). Approximately 30 000 kinds of weeds are widely distributed in the world and yield losses caused by 1 800 kinds of weeds are approximately 9.7% of total crop production every year (Li *et al*. 2003). Herbicides provide cost-effective weed control with a minimum utilization of labour and mostly used on crops planted in large acreages, such as tobacco, cotton, corn and wheat (Randall 1996). Much work has been done on weed control measures through the application of weedicides (chemical control) and least through biological control, i.e. biotechnology, bioherbicides, mycoherbicides and integrated weed management (Setua *et al.* 2006). Crop-weed competition also severely affects the growth and yield of tobacco *(Nicotiana tabacum* L.). Although weedicides are effective for controlling weeds in tobacco at the initial growth stage but it is not possible to spray after 30 days growth of tobacco when the growth of weeds attained the peak. Besides, it is costly, toxic to soil health and beneficial microbial fauna, not eco-friendly and causes residual toxicity to the tobacco leaf which is the consumable product. Therefore the alternative measure of weed control was considered, as some of the noxious weeds are grown quickly in clay soil under high rainfall area. Allelopathy refers to any process involving secondary metabolites produced by plants, algae, actinomycetes, bacteria and fungi that influence the growth and development of agricultural and biological system. The exploitation of secondary metabolites produced by microorganisms is one of the recently emerging options for sustainable weed management (Kaul and Bansal 2005). Actinomycetes especially *Streptomyces* spp. are rich sources of bioactive natural products with potential applications as pharmaceuticals and agrochemicals (Atta 2009, Atta *et al.* 2009, Manteca *et al*. 2008). They are prolific producers of secondary metabolites: antibiotics, herbicides and pesticides (Atta and Ahmad 2009). Microbial herbicides can be divided into microbial preparations (microorganisms that control weeds) and microbially derived herbicides. The first microbial herbicide was independently discovered in Germany and Japan. A perusal of the literature shows that report on bioherbicides from actinobacteria is very minimal (Sondhia and Saxena 2003). The aim of the present investigation was to study the antagonistic effect of *Streptomyces* isolated from tobacco rhizosphere (*Nicotiana tabacum*) on four prominent weeds of world wide distribution, viz. *Euphorbia hirta, Solanum nigrum, Eleusine indica* and *Sorghum halepense.*

A pot experiment was conducted at Central Tobacco Research Institute, Rajahmundry during 2008 to evaluate the effect of culture filtrate of *Streptomyces* on two dicot and two monocot weeds. Soil samples were collected into sterile bags from 6-10 cm depth near rhizosphere of tobacco grown in alfisols and vertisols of Andhra Pradesh. The soil samples were pretreated with calcium carbonate (3 g/kg of soil) and then dried at 45^{0}C for 1 hr in order to reduce the incidence of bacteria and molds. This modified procedure was found to be suitable for the isolation and identification of *Streptomyces* spp. (Subhashini and Padmaja, 2009). Soil dilution plate technique was employed for the isolation and enumeration of *Streptomyces* spp using asparagine-glycerol salts agar (AGS) medium supplemented with streptomycin and amphotericin-B to suppress the growth of bacteria and molds. Pure cultures of *Streptomyces* isolated from soil were identified up to generic level by comparing the morphology of spore bearing hyphae of the spore chain as described in Bergey's manual. The slide culture method was employed for morphological studies on *Streptomyces* species (Narayana *et.al.* 2004).

About 52 total actinomycetes were isolated from rhizosphere of tobacco soils. The most abundant actinomycetes in soil are *Streptomyces* spp. *Streptomyces* abundantly inhabit the plant rhizosphere. Twelve isolates of

*Short note

Streptomycetes were screened for herbicidal principles. The germination and growth inhibiton assay were performed using selected weeds. Absorbent cotton was placed on petri dishes. Then the cotton was fully moistened with the cell free filtrate of the selected *Streptomyces* cultures. The seeds of weeds were placed on the absorbent cotton and incubated in a growth cabinet at 28°C. After 5 days of incubation, the herbicidal activity was observed (Dhanasekaran *et al*. 2011). Out of them two potent isolates (SI and SII) were selected for characterization and identification. Based on their morphological, biochemical and physiological characteristics the isolates were identified as *Streptomyces* species. These two isolates (SI and SII) were tested for herbicidal principle by growth inhibition assay. Four types of weeds were tested for herbicidal activity with *Streptomyces* isolates SI and SII that inhibited the growth of tested weeds.

Streptomyces spp. which were found to be dominant in rhizosphere of tobacco soils collected from different agroclimatic zones, were transferred to seed medium containing (g/L) glucose 4, yeast extract 4, malt extract 10, calcium carbonate 2, *p*H 7.00 and incubated at 27°C for one week.

After 24 hr 10 ml of the seed inoculum was transferred to 90 ml of fermentation medium comprising (g/L), glucose 40, proteose 5, peptone 9, calcium carbonate 6, ammonium sulfate 3.5, ammonium chloride 3, manganese sulphate 0.1, cobalt chloride, 0.005 (*p*H 7.00) and incubated at 27°C for one week to study the growth pattern at 24 hour intervals. 20 ml of chloroform extracts of culture filtrates were concentrated and used for testing their weedicidal activity which was ascertained by rolled paper towel assay (Dhanasekaran *et al.* 2010).

Out of the twelve isolates of *Streptomyces* spp., two isolates (SI and SII) were found to be dominant and grew well on AGS medium. Since the AGS medium was supplemented with streptomycin and amphotericin-B, the growth of bacteria and moulds were prevented and facilitated the development of *Streptomyces* alone. Both the isolates SI and SII were obtained from the Vertisols of tobacco. Isolate SII was isolated from the soil sample collected from farmyard manure treated plot of permanent manurial trial experiment of Vertisols at CTRI Farm, Katheru. The other three isolates from Vertisols and eight isolates from Alfisols were very slow growing and did not show any antagonism against the test weeds. Slide culture studies revealed the presence of substrate mycelium as well as aerial mycelium, supporting the observation of Subhashini (2010). Isolate SII appeared as white coloured, powdery mass and isolate SI was pale pink in colour and produced a dark brown pigment diffusing into the medium. The white colonies did not show any pigmentation.

The two isolates showed similar growth pattern except that the isolate SI was comparatively slow growing. In the isolate SI log phase lasted for 96 hr and the stationary phase

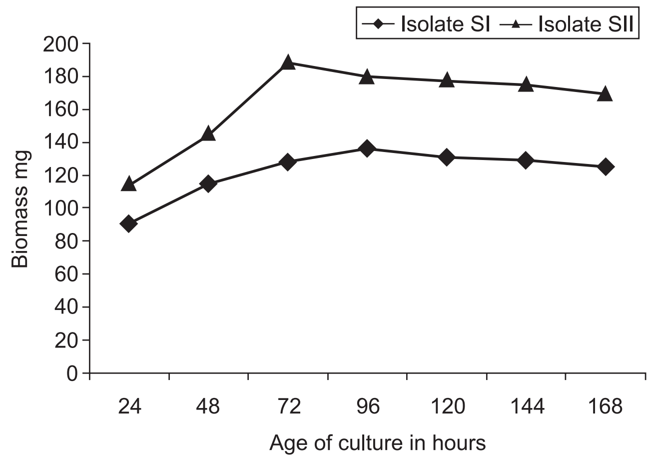

Fig 1 Growth pattern of *Streptomyces* isolates SI and SII

lasted up to 144 hr (Fig 1), while in case of isolate SII the log phase lasted up to 72 hr and declined later. This has confirmed the faster growth of isolate SII compared to the other.

Study on the herbicidal activity of 20 ml chloroform extract of the *Streptomyces* as foliar spray showed that 1-6 day old culture was also effective but the 9 d old culture showed inhibitory effect on the weeds. The increasing trend of the inhibitory effect lasted up to two weeks. The subsequent decline in weedicidal activity was commensurate with the decrease in the growth of the *Streptomyces*. The susceptibility of the weeds to the extracts of *Streptomyces* (isolate SII) was reflected in the drastic reduction in the dry weight compared to control (Fig 2 and Fig 3). There was a significant and progressive reduction in dry weight of all the weed species treated with the extracts of 3 to 9 day old cultures of *Streptomyces* isolate SI and SII. The experiment was laid out in a completely randomized design with 3 replications. The reduction in dry weight was better in case of isolate SII than

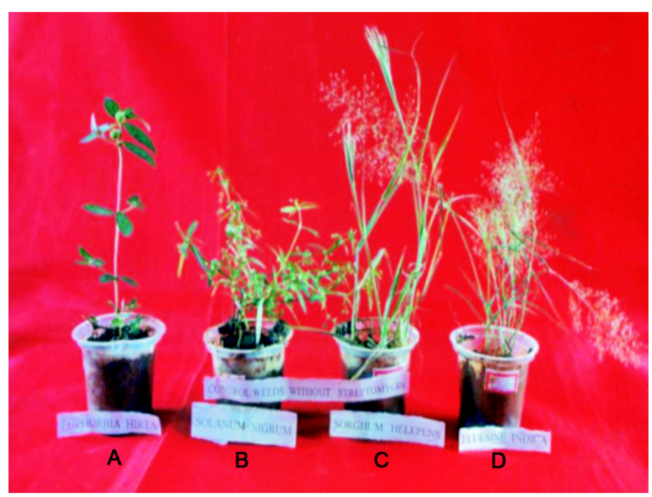

Fig 2 Untreated (control) weed plants
A. *Euphorbia hirta* B. *Solanum nigrum*
C. *Sorghum halepense* D. *Eleusine indica*

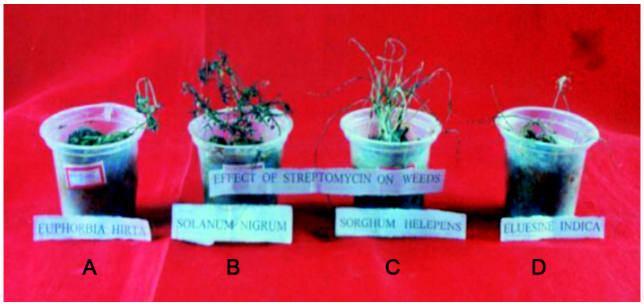

Fig 3 Weed plants treated with culture filtrate of *Streptomyces* isolate SII
A. *Euphorbia hirta* B. *Solanum nigrum*
C. *Sorghum halepense* D. *Eleusine indica*

isolate SI and the dry weight reduction was comparable with 2,4-D (1 mg/plant). Extracts from 9 to 15 day old cultures did not exhibit significant difference in dry weight as was the case with 2,4-D. All the extracts and 2,4-D were significantly superior to the untreated controls (Table 1). It could be concluded that extracts from 9 day old cultures can exert equal biological suppression as that of extracts from older cultures and extracts from cultures earlier to 9th day were not mature enough for biosuppression of the weeds. None of the treated weed species could produce seeds.

Wenping *et al*. (2009) studied the herbicidal activity of the metabolite SPRI-70014 from *Streptomyces griseolus* and identified the microbial metabolites as promising class of natural herbicides due to their effective control against weeds and a relatively low environmental impact and also reported on the potency and crop safety of a natural compound with herbicidal properties. The compound showed excellent herbicidal activities on various broadleaf and graminaceous weeds in both green house and field trials. Dhanasekaran *et al*. (2011) tested four types of weed seeds for herbicidal activity with *Streptomycetes* isolates. *Streptomyces* inhibited the growth of *Echinochloa crusgalli*, but it could not inhibit the growth of *Echinochloa colonum*, *Parthenium* sp. or *Ageratum conizoites*. Lee *et al.* (2003) reported that *Streptomyces* sp. 8E-12 produced a selective herbicidal metabolite which was identified as MHM. The metabolite showed stronger *in vivo* activity against monocotyledonous plants than dicotyledonous plants, and caused a bleaching (albino symptom) on some weeds including *Digitaria sanguinalis* and *Echinochloa crusgalli*.

Concern is growing among farmers and weed scientists regarding recent developments in weed control technology and there is great fear about the future situation and what solutions will be required if some species of weeds became well adapted. Hence, there is an urgent need for broad based screening of isolates of *Streptomyces* spp from unexplored habitats that will lead to identification of promising bioherbicide. An attempt was made to exploit the potential of *Streptomyces* as an effective biocontrol agent for the biological suppression of weeds. Further investigations are however necessary on the ecology of these strains and the development of an inexpensive and eco-friendly technology.

SUMMARY

A bioherbicide is a biologically based control agent for weeds that utilize such naturally occurring enemies, rather than depending on man-made chemicals. Out of 12 isolates of *Streptomyces* spp. obtained from tobacco rhizosphere two isolates (SI and SII) were found to be efficient in suppressing the weeds. Growth pattern of the isolates was studied. Isolate SII was found to be more effective against weeds. Weedicidal activity of *Streptomyces* was tested against two dicot and two monocot weeds of tobacco fields, viz. *Solanum nigrum, Sorghum halepense, Eleusine indica* and *Euphorbia hirta*. *Streptomyces* Isolate SII showed faster growth and effective bioherbicidal activity. There was a significant and progressive reduction in dry weight of all the weed species treated with the extracts of 3 to 9 day old cultures of *Streptomyces* isolate

Table 1 Weedicidal activity of chloroform extracts of Streptomyces-isolates SI, SII and 2,4-D on test weeds

Age of culture (days)	Dry weight of weeds (g)											
	Solanum nigrum			*Sorghum halepense*			*Eleusine indica*			*Euphorbia hirta*		
	S I	SII	2,4-D	S I	SII	2,4-D	S I	S II	2,4-D	S I	SII	2,4-D
3	7.47	7.48	7.48	7.42	7.48	7.5	7.37	7.47	7.55	7.48	7.73	7.85
5	7.17	6.1	6.38	6.83	6.17	6.23	6.23	6	5.93	6.4	6.23	6.3
7	6.17	5.4	5.52	6.2	5.53	5.65	5.63	4.57	4.25	6.1	6.17	6.17
9	5.77	4.75	4.85	5.75	4.9	5.03	4.76	3.43	3.47	5.53	4.93	4.96
11	5.55	4.55	4.72	5.71	4.35	4.6	3.63	2.22	2.56	5.32	4.66	4.68
13	5.58	4.47	4.72	5.73	4.5	4.58	3.63	2.23	2.5	5.32	4.58	4.63
15	5.62	4.47	4.7	5.65	4.53	4.53	3.63	2.28	2.52	5.38	4.55	4.55
Control8.96	8.95	8.92	9.03	9	9	9.03	9.03	9.02	8.93	9.13	9.12	
SEM ± 0.08		0.08	0.12	0.08	0.13	0.12	0.07	0.09	0.11	0.19	0.08	0.08
CD at 5%	0.26	0.35	0.24	0.39	0.37	0.2	0.26	0.32	0.36	0.24	0.26	0.23
CV %	2.24	3.5	2.29	3.36	3.59	1.97	2.72	3.96	4.33	2.14	2.44	2.21

SI and SII. The reduction in dry weight was better in case of isolate SII than isolate SI and the dry weight reduction was comparable with 2,4-D.

REFERENCES

Atta H M. 2009. An antifungal agent produced by *Streptomyces olivaceiscleroticus* AZ-SH514. *World Applied Sciences Journal* **6**: 1495–505.

Atta H M, Dabour S M and Desoukey S G. 2009. Sprasomycin antibiotic production by *Streptomyces* sp. AZ-NIOFD1: Taxonomy, fermentation, purification and biological activities. *Ammerican – Eurasian Journal of Agriculture and Environmental Sciences* **5**: 368–77.

Atta M A and Ahmad M S. 2009. Antimycin-A antibiotic biosynthesis produced by *Streptomyces* sp. AZ-AR62: Taxonomy fermentation, purification and biological activities. *Australian Journal of Basic Sciences* **3**: 126–35.

Dhanasekaran D, Thajuddin N and Panneerselvam A. 2010. Herbicidal agents from actinomyetes against selected crop plants and weeds. *Natural Product Research: Formerly Natural Product Letters* **24(6)**: 521–9.

Dhanasekaran D, Ambika K, Thajuddin N and Panneerselvam A. 2011. Allelopathic effect of actinobacterial isolates against selected weeds. *Archives of Phytopathology and Plant Protection* **25**(1): 1–17.

Kaul S and Bansal G L. 2005. Allelopathic potential of crofton weed (*Eupatorium adenophorum*) on wild sage (*Lantana camara*). *Indian Journal of Agricultural Sciences* **75**(11): 760–2.

Lee H, Kim C J, Hong K S and Cho K. 2003. A bleaching herbicidal activity of methoxyhygromycin (MHM) produced by an actinomycete strain *Streptomyces* sp. 8E-12. *Letters in Applied Microbiology* **36**: 367–91.

Li Y, Sun Z, Zhuang X, Xu L, Chen S and Li M. 2003. Research progress on microbial herbicides. *Crop Protection* **22**: 247–52.

Manteca A, Alvarez R, Salazar, N Yague P and Sanchez J. 2008. Mycelium differentiation and antibiotic production in submerged culture of *Streptomyces coelicolor*. *Applied Environmental Microbiology* **74**: 3877–86.

Narayana K J P, Ravi Kiran D and Vijayalakshmi M. 2004. Production of antibiotics from *Streptomyces* species isolated from virgin soils. *Indian Journal of Microbiology* **44**:147–8.

Randall J M. Weed control for the preservation of biological diversity. 1996. *Weed Technology* **10**: 370–83.

Setua G C, Setua M, Banerjee N D, Dutta A K, Debnaths S, Ghosh J K and Rajeurs S. 2006. Integrated weed management for sustainable leaf production in mulberry (*Morus alba*) under irrigated condition. *Indian Journal of Agricultural Sciences* **76**(4): 258–61.

Sinha B. 2012. Global biopesticide research trends: a bibliometric assessment. *Indian Journal of Agricultural Sciences* **82**(2): 95–101.

Sondhia S and Saxena N K. 2003. Allelopathic effect of *Xanthium strumarium* L. on some weeds. *Journal of Geobios* **30**(203): 173–6.

Subhashini D V and Padmaja K. 2009. Isolation of *Streptomyces* from tobacco soils that show antimicrobial activity. *Journal of Biological Control* **23**(4): 417–9.

Subhashini D V. 2010. Biological Control of damping-off (*Pythium aphanidermatum*) using *Streptomyces lavendulae* isolate 21 on tobacco seedbeds. *Journal of Biological Control* **24**(4): 338–42, 2010.

Wenping Xu, Liming Tao, Xuein Gu, Xiaoxia Shen and Sheng Yuan. 2009. Herbicidal Activity of the Metabolite SPRI-70014 from *Streptomyces griseolus*. *Weed Science* **57**(5): 547–53.