

glycosylation was taken to be at A-ring and most probably at C-6. A direct comparison of the compound with scutellarin (7-O- $\beta$ -D-glucuronyl scutellarein) (IR,  $R_f$  and co-PC) showed them as different and hence the new glycoside was identified as 6-O- $\beta$ -D-glucuronyl scutellarein, designated isoscutellarin. The other minor glycoside was identified as 6-O- $\beta$ -D-glucuronyl luteolin by  $\lambda_{max}$ ,  $R_f$ , acid and enzyme hydrolysis and direct comparison including co-PC with an authentic sample from *S. colorata*<sup>2</sup>.

The purple coloured flower petals were extracted with 0.01 N methanolic HCl and concentrated *in vacuo* below 40° C. The dark purple solid was crystallised from methanolic HCl-ether. It was homogenous on PC, did not melt upto 320°, and yielded cyanidin chloride and glucose on hydrolysis. It was identified as cyanidin-3-O-glucoside by  $R_f$  and co-PC with an authentic sample.

This is the first record of occurrence of 6-O- $\beta$ -D-glucuronyl scutellarein, an isomer of scutellarin; *S. foetida* containing isoscutellarin and 6-O- $\beta$ -D-glucuronyl luteolin resembles *S. colorata* containing the latter with scutellarein but differs from it in having significant quantity of the procyanidin glucuronide. The presence of 6-oxygenated flavones in *Sterculia* sp. is significant from the point of molecular taxonomy of the Natural Order Malvales.

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1. Scarpelli, D. G., *Science*, 1974, 185, 958.
2. Nair, A. G. R., Ramesh, P. and Subramanian, S. S., *Phytochem.*, 1976 (in press).

#### TOXICITY OF *PARTHENIUM HYSTEROPHORUS* L.

*Parthenium hysterophorus* L., an alien weed now growing wild in many parts of India is posing agricultural and health hazards. It has been shown to cause allergic contact dermatitis in humans<sup>1,2</sup>. Agriculturists in Maharashtra and Karnataka are expressing concern over the invasion of food and fodder crop fields by this weed. Since livestock grazing in open fields have an access to the weed it is of interest to know whether *Parthenium* has any after effects after ingestion. We have undertaken

a detailed investigation on the toxicology of *Parthenium* to animals and the present report describes our initial findings on the toxicity of the weed to livestock.

During our field survey it was found that goats graze on *P. hysterophorus* freely while cattle and buffaloes appeared to graze sparingly. Under laboratory conditions we found that both buffalo bull calves and cross bred bull calves freely feed on *Parthenium* either as such or after admixture with conventional green fodder. When nine buffalo bull calves and seven cross bred bull calves were fed on the weed mixed with green fodder *ad libitum*, they developed toxic symptoms resulting in the death of six buffalo bull calves and five cross bred bull calves within periods ranging from 8 to 30 days after feeding. All the buffalo calves developed severe dermatitis on face, base of the ears, shoulders, neck, back, loins and on legs extending down to hock and knee joints. Autopsy of the animals revealed gross and microscopic lesions in the gastrointestinal tract, liver and kidney. Control animals fed on conventional green fodder remained healthy and showed none of these external symptoms.

It is increasingly realized that parthenin, the major sesquiterpene lactone present in *P. hysterophorus* is responsible for allergic contact dermatitis of humans by this weed<sup>3</sup>. It is also reported that sesquiterpene lactones from certain members of Compositae family to which *Parthenium* also belongs, are toxic to livestock<sup>4</sup>. In our present studies it was found that subconjunctival and intracorneal tests<sup>5</sup> with an aqueous solution of parthenin (0.1% w/v) showed that the dermatitis manifested by *Parthenium*-fed animals was of allergic nature involving Type IV hypersensitivity. Further experiments to assess the possibility of excretion of parthenin and other toxic principles from the ingested weed by livestock through milk and their impact on human health are under way. The details of these studies will be published elsewhere.

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1. Lonkar, A., Mitchell, J. and Calnan, C. D., *Trans. St. John's Hosp. Derm. Soc.*, 1974, 60, 43.
2. Subba Rao, P. V., Mangala, A., Subba Rao, B. S. and Prakash, K. M., *Proc. Soc. Biol. Chem. (India)*, 1976, 45, 12.
3. Mitchell, J. C., *Recent Adv. Phytochem.*, 1975, 9, 119.
4. Kingsbury, J. M., *Poisonous Plants of the United States and Canada*, Prentice-Hall, Englewood Cliffs, New Jersey, U.S.A., 1964, p. 409.
5. Herbert, W. J., *Veterinary Immunology*, Blackwell Scientific Publications, Oxford, England, 1970, p. 83.

#### PRELIMINARY OBSERVATIONS ON THE PRODUCTION OF AMYLOGUCOSIDASE BY THERMOPHILIC FUNGI

KERR *et al* (1941) and Underkofler (1953) among others recognised that enzymes of amyloglucosidase type were present in preparations which produced fermentable sugars from starch. Philips and Caldwell (1951), Arima (1964), Nehira and Nomi (1956), Aschengreen (1969), Underkofler (1969), Mangallam (1973) and others studied the production of amyloglucosidase by species of *Aspergilli* and *Rhizopus*. Thus, in literature several reports of fungi producing this enzyme are available but none deals with thermophilic fungi. Therefore, a preliminary investigation on the production of amyloglucosidase by thermophilic fungi was carried out, with the hope to isolate a thermostable enzyme.

Four thermophilic fungi *viz.*, *Torula thermophila* Apinis; *Mucor miehe* Cooney and Emerson; *Humicola lanuginosa* (Griffon and Maublanc) Bunce; *Sporotrichum thermophile* Cooney and Emerson, which are unable to grow below 25°C and grow well above 50°C, thus satisfying the definition of Cooney and Emerson (1964) were screened for amyloglucosidase activity in the following manner.

100 ml. of the seed medium of the following composition (Tapioca starch 1%, CSL 1%,  $\text{NH}_4\text{NO}_3$  0.25%,  $\text{KH}_2\text{PO}_4$  0.2%,  $\text{MgSO}_4$  0.1%, pH 6) was distributed in 500 ml. Erlenmeyer flasks, plugged with non-absorbent cotton and sterilised for 45 mts., at 120°C and 15 lb./sq. in. pressure. After cooling they were inoculated with 2.5 ml. fungal spore suspension prepared from five-day old, heavily sporulating cultures grown on potato dextrose agar slants. They were incubated at 50°C for 48 hrs. on a rotary shaker with 280 r.p.m. and 2" throw.

Production of enzyme: Sterilized 500 ml. Erlenmeyer flasks containing 100 ml. production medium (Composition: Tapioca starch 2.5%,

Peanut meal 2%, ammonium nitrate 0.5%,  $\text{KH}_2\text{PO}_4$  0.25%,  $\text{K}_2\text{HPO}_4$  0.2%,  $\text{MgSO}_4$  0.1%, pH 6.5) were inoculated with 2.5 ml. seed. The mold was grown for 96 hrs. At the end of the fermentation cycle the mycelium of each species was separately harvested and filtered. The culture filtrate was assayed and quantitative estimation of the enzyme in terms of glucose was carried out by Hanes's method (1947). The conversion of starch to glucose by the enzyme was first identified by preparing glucosazone with phenyl hydrazine and later confirmed by paper chromatography. The enzyme is found to be stable for 60 mts. at 60°C. Mean of triplicate results are given in Table I.

TABLE I  
*Amyloglucosidase production by thermophilic fungi*

Sl. No.	Organism	Temp. (°C)	Age (Hrs)	pH	Filtrate activity* $\mu$ /ml.
1.	<i>Torula thermophila</i>	50	96	7.0	195.14
2.	<i>Mucor miehe</i>	50	96	6.7	102.7
3.	<i>Humicola lanuginosa</i>	50	96	7.0	75.6
4.	<i>Sporotrichum thermophile</i>	50	96	6.7	149.4

\* One amyloglucosidase unit is defined as the amount of enzyme releasing 1 mg. of glucose in one hour from a 4% starch solution at pH 4.5 at 60°C.

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1. Arima, K., In *Global Impacts of Microbiology*. Ed. by Starr, John Wiley and Sons, New York, 1964, p. 277.
2. Aschengreen, N. H., *Process Biochemistry*, 1969, 4, 23.
3. Cooney, D. G. and Emerson, R., *Thermophilic Fungi*, Freeman, San Francisco, California, 1964, p. 188.
4. Kerr, R. W., Meisel, H. and Schink, N. F., *Ind. Eng. Chem.*, 1941, 33, 1418.
5. Mangallam, S., *Ph.D. Thesis*, Poona University, Poona, India, 1973.
6. Nehira, T. and Nomi, R., *J. Ferm. Techno.* (Japan), 1956, 34, 391. 55
7. Hanes, C. S., *Practical Physiological Chemistry*, Ed. Philips, B. H., Publ. Blackstein Co., Inc., New York, 1947, p. 1.