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DECOMPOSITION OF SUGARCANE BAGASSE BY THE BIRD'S NEST FUNGUS *CYATHUS*

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SUGARCANE bagasse represents a major agricultural byproduct whose cumulative deposition is a health hazard and causes an allergy termed "bagassosis". Major uses of bagasse include structural and acoustical wall boards, synthetic resins and light weight concrete and use as fuel[1]. Further microbial processing yields a variety of products chiefly alcohol and single cell protein[2,3]. This is impeded by the fact that lignocellulose is not easily amenable to decomposition. But it is established that nearly 70–80% of the residual plant material in the natural ecosystem is decomposed by the basidiomycetous fungi[4,5]. In earlier study with *Cyathus* on paddy straw yielded positive substrate decomposition[6]. It was therefore, considered worthwhile to explore the decomposition of bagasse by the species of *Cyathus*.

Cultures of *C. helenae* type (Brodie 1500), *C. helenae* Dick (Brodie 1500) and *C. striatus* (Nuds) wild ex. Press (Brodie 1500) were obtained from the National Collection of Fungal Cultures, Ottawa, Canada. *Cyathus* sp. was isolated from decaying mango wood collected locally. All cultures were maintained on Brodies agar slants[6].

Fermentation of bagasse was carried out at 28°C using 2 g dry powder in 50 ml Brodies broth (devoid of maltose, dextrose and glycerine) dispensed in 250 ml Erlenmeyer flasks. After autoclaving the contents of the flasks at 15 lb. sq. in. for 20 min, each were inoculated with three fungus-bearing discs (8 mm diam) taken from growing cultures of appropriate isolate on Brodie's agar; the normal broth and uninoculated bagasse media served as control. At suitable intervals, fermented residue with mycelial

Table 1 *Release of cellulase by species of Cyathus in a medium containing sugarcane bagasse*

Organism	*Cyathus sp.*		*Cyathus striatus*		*Cyathus helenae* Type		*Cyathus helenae* Dick	
	Incubation period (days)							
	50	62	50	62	50	62	50	62
CM cellulase								
EA	6.22	5.42	5.06	5.33	5.64	4.83	3.83	4.68
SEA	2.30	2.17	2.20	1.98	2.68	2.19	2.18	2.03
FP cellulase								
EA	1.11	1.81	0.94	0.78	1.64	1.11	1.47	1.89
SEA	0.41	0.72	0.41	0.29	0.78	0.51	0.34	0.82
Cotton activity								
EA	2.35	1.93	1.86	2.53	2.61	2.50	1.93	3.08
SEA	0.87	0.77	0.81	0.94	1.24	1.14	1.09	1.34

EA – enzyme activity, expressed as the release of 1 μmol of reducing sugar (as glucose) ml^{-1} hr^{-1}; SEA – specific enzyme activity, expressed as enzyme units per mg protein; values represent mean of atleast two replicates.

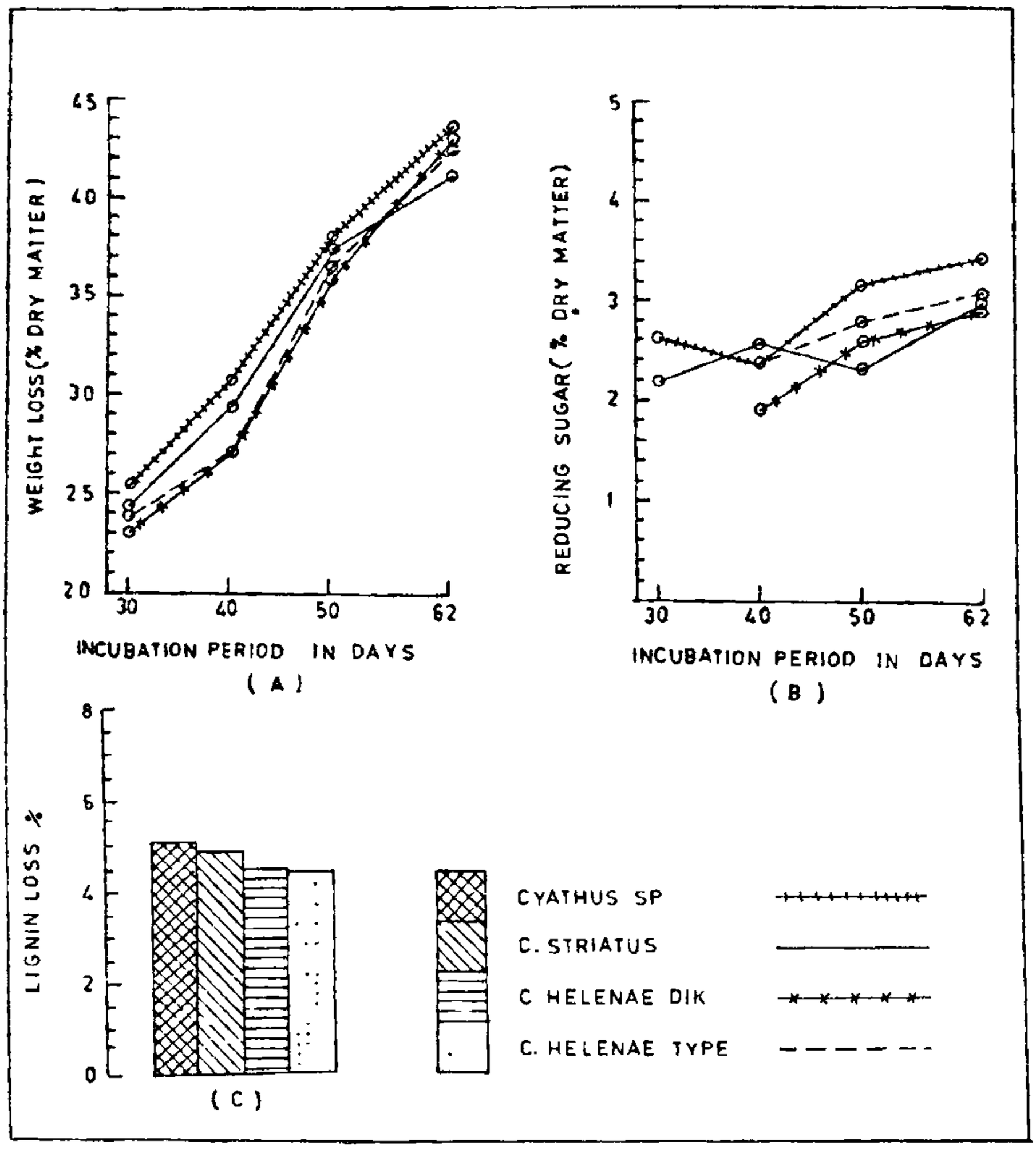

Figure 1. Decomposition of sugarcane bagasse in Brodies nutrient broth after 62-days of fermentation at 28°C by species of *Cyathus*. **A.** Wt loss of bagasse; **B.** Release of reducing sugar indicating cellulolysis; **C.** Loss of lignin.

mat as also the culture filtrate was subjected to analyses for wt loss[6] and lignin content[7]. Reducing sugar was estimated by dinitrosalicyclic acid reagent[8] and protein according to Lowry *et al*[9]. Cellulase activity was measured according to Ishaque and Kluepfel[10] and is expressed as μmol of reducing sugars (as glucose equivalent) per ml culture filtrate per hr.

The ability of isolates of *Cyathus* to degrade bagasse over a 62-day fermentation period is shown in figure 1. Weight loss in bagasse progressed linearly for all the four isolates but the relative level was not too different (figure 1A). The release of reducing sugar by *Cyathus* sp. (2.62-3.45%) was slightly greater than *C. helenae* type (2.37-3.10%) or *C. striatus* (2.19-3.0%); least release (1.87-2.50%) occurred from *C. helenae* Dick (figure 1B). *Cyathus* sp. decomposed more lignin in bagasse (5.2% of the dry matter) as compared to other species (figure 1C).

Culture filtrates of all the four *Cyathus* isolates were able to hydrolyse carboxymethyl cellulose (CMcase), filter paper (FP cellulase) and cotton (cotton activity). CM cellulase in *C. helenae* Dick increased with incubation period (table 1); this was also evident for FP cellulase and cotton activity.

However, for *C. striatus* this trend persisted only in the case of CMcase and cotton activity. No consistent pattern of cellulolysis was noted for *Cyathus* sp. and *C. helenae*. For all four isolates, higher specific CMcase activity was noted on the 50th day but for specific cotton and filter paper activity no definite pattern emerged (table 1).

Birds nest group of fungi, especially species of *Cyathus*, have attracted the attention as effective degraders of plant residues only recently[6,11]. However, their natural habitat has been indicative of this capacity[12]. Species of *Cyathus* degrade both lignin and cellulose suggesting that they could help in composting of plant material which might subsequently be utilized either for mushroom cultivation[5,13], or for increased digestibility and nutrient supplementation[4]. The latter application would, however, require more detailed investigation of these and other species of *Cyathus* besides the use of genetically improved strains.

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ANTIGENIC RELATIONSHIP AMONG BACTERIOCIN-PRODUCING AND NON-BACTERIOCIN-PRODUCING STRAINS OF *CAJANUS*-RHIZOBIA

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PIGEONPEA (*Cajanus cajan* L. Millsp), one of the most important pulse crop, is grown in the entire belt of semiarid tropics. Inoculation of this crop with *Rhizobium* is seldom successful. The major constraint being the poor or no nodulation by the inoculant strain due to severe competition with the indigenous strains, which outnumber the population of the former in soil and are usually poor in nitrogen fixation. Chauhan and Gaur[1] detected bacteriocinogeny among many strains of rhizobia isolated from pigeonpea (henceforth will be referred as *Cajanus*–rhizobia) and demonstrated ecological superiority of the bacteriocin–producing strain over non–bacteriocin–producing strain in liquid culture. The understanding of ecological superiority of such a strain in soil against indigenous population of the same host–rhizobia, demands a technique, which may not alter any of the intrinsic properties of the strain, and serology may be the only technique for this purpose. Chauhan and Gaur[1] also examined the antigenic constitution of 2 bacteriocin and 2 non-bacteriocin producing strains of *Cajanus*–rhizobia and observed complete antigenic homology among both the bacteriocin-producing strains. Non-bacteriocin-producing strains showed identity neither between each other nor with any of the bacteriocin-producing strains. Since the 2 bacteriocin-producing strains were isolated from different cultivars of pigeonpea, after growing the latter in soils collected from 2 distant locations in different years, it was of interest to know whether the antigenic identity among the bacteriocin-producing strains was common or a case of coincidence. Hence, the antigenic relationship of 6 bacteriocin-producing and 4 non-bacteriocin-producing strains was examined by using antisera of 2 bacteriocin–producing and 2 non–bacteriocin–producing other strains[1] (table 1) and the findings are reported here.

The origin and authenticity of all the strains of rhizobia, used in the study are described elsewhere[1]. The whole cell antigens were prepared according to