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Toxic and repellent action of sugarcane bagasse-based lignin against some stored grain insect pests

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Abstract: Laboratory experiments were carried out to investigate the efficacy of sugarcane bagasse-based lignin against four stored grain insect pests *viz., Tribolium castaneum, Tribolium confusum, Sitophilus oryzae* and *Callosobruchus maculatus.* Mortality was assessed after 24, 48 and 72 h of treatment and LD_{50} values were calculated at the respective time intervals. It was observed that *T. castaneum* is more susceptible to the lignin than the other test insects. Repellent activity of the product was conducted for *T. castaneum* and *T. confusum*. All the concentrations of the product were found to be repellent to both the insect species. Strong repellent activity was observed at concentrations of 471.57 and 628.76 µg.cm^{-2} to *T. confusum* showing the repellency of class V activity.

Key words: Bagasse-based lignin, toxicity, repellency, *Tribolium castaneum, T. confusum, Sitophilus oryzae, Callosobruchus maculatus*

Introduction

Research into plant derived natural products for agriculture went into decline for a number of years, but this trend is now being reversed as it becomes evident that plant natural products still have enormous potential to inspire and influence modern agrochemical research (Benner, 1993). The plant kingdom can be a rich source of a variety of chemicals with the potential for development as successful pest control agents (Arnason *et al.,* 1989; Rahman *et al.,* 1999). Secondary compounds from plants include alkaloids, terpenoids, phenolics, flavonoids, chromenes and other minor chemicals can affect insects in several ways. They may disrupt major metabolic pathways and cause rapid death, act as attractants, deterrents, phago-stimulants antifeedants or modify oviposition. They may also retard or accelerate development or interfere with the life cycle of the insect in other ways (Smet *et al.,* 1986; Harborne, 1988; Bell, 1986; Houghton, 1996; Jurd & Manners, 1980). Morever, products from several floral species have been demonstrated to act as repellents, toxicants and antifeedants against a number of Coleoptera that attack stored products (Malik & Naqvi, 1984; Rahman & Schmidt, 1999; Raja *et al.,* 2001; Papachristos & Stamopoulos, 2002; Tapondjou *et al.,* 2002).

Sugarcane (*Saccharum officinarum*) bagasse is one of the most important sources of lignin. It contains about 20-22% lignin (Anon, 1965). To a wood pulp manufacturer, lignin represents a wood component, which must be removed from the pulp by alkali. On the basis of earlier research, lignin has been defined as incrusting material of the plant, which is built up mainly, if not entirely, of phenyl propane building stones. It carries the major part of the methoxyl content of the wood, unhydrolyzable by acids, readily oxidizable, soluble in hot alkali and bisulphite and readily condenses with phenols and thiocompounds (Anon., 1968).

Information is not available on the sugarcane bagasse-based lignin as a pesticide on stored grain insect pests. Local farmers reported that this product gave a good result by applying on such crops as mustard, onion, wheat, brinjal, etc. and proved to be promising from the pest control point of view. It also acts as a good fertilizer in the field. The properties and composition of lignin depends on the source and method of isolation. Keeping these in mind, the present study was therefore undertaken to test the efficacy of sugarcane bagasse-based lignin against four stored grain insect pests.

Materials and Methods

Sugarcane bagasse was collected from Rajshahi Sugar Mills adjacent to BCSIR Laboratories Rajshahi, dried and kept in room temperature. After drying, one kg bagasse was taken in a 10 l capacity stainless steel beaker containing 6 l of 10% sodium hydroxide solution. It was boiled for 4 h with continuous stirring. During boiling the volume of the mixture was kept constant by adding soft water. The mixture was stored for 24 h. The residual bagasse was separated by cloth filtering. The volume of the solution was reduced to 4 l by heating and then cooled. It was then acidified with HCl. The lignin emulsion was thus prepared and 250 g of urea was added to it. Then it was boiled, cooled and dried to crystalline form. Finally 500 g product was produced, kept in a reagent bottle (500 ml) and stored in room temperature. The above quantity of urea was added for the formation of ammonia in the medium. Ammonia reacts with water to form ammonium hydroxide (NH_4OH) solution which was weak alkaline in character. This alkalinity helps to solubilize the lignin as well as to develop fertility of the soil.

Stock cultures of the test insects were maintained in separate 1000 ml beakers at 30º ± 1ºC in an incubator. For testing beetle, mortality five doses were used including control (water). Ten to fifteen day-old adults of *T. castaneum, T. confusum, S. oryzae* and *C. maculatus* were used at 157.19, 314.38, 471.57 and 628.76 $\mu g.cm^{-2}$ concentrations. The doses were prepared by mixing the requisite quantities of the product with 01 ml water. After mixing properly the liquid was dropped in a petri dish (9.5-cm diameter). After drying by fanning and finally in an oven at 40ºC, 20 adults of each species were released in each petri dish. For each dose three replications were taken. The doses were calculated by measuring the weight of prepared product (µg) in 01 ml of water divided by the surface area of the petri dish and it was converted into $\mu g/cm^2$. Mortality was assessed after 24, 48 and 72 h of the treatment. The mortality was corrected by using Abbott's formula (Abbott, 1925) and LD_{50} values were determined by probit analysis (Busvine, 1971). The experiments were performed in the laboratory at 30ºC ± 0.5ºC.

Repellency test was conducted following McDonald *et al.* (1970) with some modifications. Substrata were prepared by cutting Whatman No. 40 filter paper disk (9-cm diameter) in half, then treated with the desired concentrations of the material so that deposits of 157.19, 314.38, 471.57 and 628.76 $\mu g.cm^{-2}$ were produced. The treated filter papers were then dried overnight at room temperature. Each treated half-disk was then attached lengthwise, edge to edge, to an untreated half-disk half with cellulose tape and placed in a petri dish. The orientation of the seam was changed in each replicate to avoid the effects of any external directed stimulus affecting distribution of the test insects. Ten adults of each species were released in the middle of each filter paper and a cover was placed on the petri dish. For each experiment three replications were made. Individuals that settled on each half-disk were counted at hourly intervals for 5 h (10:00-14:00 hrs). The average of the counts was converted to express the percentage of repulsion (R) as: R=2×(C-50), where C is the percentage of insects on the untreated half. Positive values expressed repellency and negative values attractancy. The averages were then assigned to different classes using the following scale (McGovern *et al.*, 1977; Talukder & Howse, 1993):

Class	% Repulsion (R)
0	>0.01 to <0.1
I	0.1–20
II	20.1 – 40
III	40.1 – 60
IV	60.1 – 80
V	80.1 – 100

Results and Discussion

The results of contact toxicity and statistical analyses have been presented in Table 1. For comparison of the susceptibility LD_{50} values and regression equation of the test material against the four insect species were determined. The probit analysis of percent mortalities in all the cases gave χ^2-values and the significant χ^2-values were adjusted for heterogeneity. Results demonstrate that this product was effective against the insect species at all the duration but it did not produce any effect on *C. maculatus* at 24 h interval. The susceptibility of the insect species was in the following order: *T. castaneum* > *T. confusum* > *S. oryzae* > *C. maculatus*, *T. castaneum* > *S. oryzae* > *T. confusum* > *C. maculatus* and *T. castaneum* > *S. oryzae* > *C. maculatus* > *T. confusum* at 24, 48 and 72 h interval respectively. It is striking to note that at all the duration, lowest LD_{50} values were observed for *T. castaneum*, which showed 100% mortality at 72 h treatment.

The present results are in conformity with the results of Liu & Ho (1999) who reported that *T. castaneum* adults were significantly more susceptible to the fumigant toxicity of the essential oil of *Evodia rutaecarpa* than the larvae. They also reported that the essential oil of *Evodia rutaecarpa* was toxic to *T. castaneum* and *S. zeamais* adults when applied topically to the insects. Our results are in agreement with the result of Shahjahan & Amin (2000), who reported the toxicity of water extract of akanda, bishkatali and neem against *S. oryzae*. The authors noted that the LC_{50} values for neem, bishkatali and akanda were 3.08, 3.38, and 3.51µl/insect, respectively at 24 h after treatment. At 48 h the LC_{50} value for neem was 2.40 µl/insect followed by bishkatali (2.54 µl/insect) and akanda (2.81 µl/insect) while at 72 h, the LC_{50} values for neem, bishkatali and akanda were 2.27, 2.19 and 2.54 µl/insect, respectively. The toxic effect of *A. reticulata* and *O. sanctum* leaves on *C. maculatus* produced 40 and 46.0 % adult mortality at 0.1 g/50 mungbean seeds, respectively (Rajapakse, 1996). He also reported that *Dillenia retusa* powder gave 46% mortality of *C. maculatus* at 0.1 and 0.2 g/50 mungbean seeds. Rajapakse *et al.* (1998) observed the highest mortality of *C. maculatus* with *Citrus lemon*, *Piper nigrum* followed by *A. reticulata* and *A. indica*. Mollah & Islam (2002) evaluated the toxic effect of akanda leaf, stem and root against *C. maculatus* adults in which the lowest LD_{50} value (538.35 $\mu g.cm^{-2}$) of petroleum spirit extract of leaves was recorded after 72 h exposure. The male adult showed highest mortality in all the solvent extracts. Khanam *et al.* (1995) reported the effect of *Thevetia neriifolia* leaf extract on *T. confusum* adults where acetone extract was found to be the most effective toxicant followed by ethyl acetate, petroleum ether and methanol extracts.

Table 1. χ^2-values, regression equations, LD_{50} and 95% confidence limits of sugarcane bagasse-based lignin against some stored grain insect pests after 24, 48 and 72 h of treatment

Hrs after treatment	Test insects	χ^2-values for heterogeneity*	Regression equations	LD_{50} ($\mu g.cm^{-2}$)	95% Confidence limits	
					Lower	Upper
24 h	*T. castaneum*	4.73	Y= 0.212 + 2.23X	139.38	99.06	196.11
	T. confusum	3.79	Y= -5.82 + 4.55X	239.54	213.92	268.23
	S. oryzae	6.97	Y= -4.78 + 3.87X	335.56	272.94	412.56
	C. maculatus	-	-	-	-	-
48 h	*T. castaneum*	1.28	Y= 2.26 + 1.61X	49.88	16.51	150.63
	T. confusum	6.47	Y= -5.79 + 4.60X	221.34	179.54	272.86
	S. oryzae	0.134	Y= 1.86 + 1.78X	57.43	22.75	144.96
	C. maculatus	1.06	Y= -0.469 + 2.24X	273.40	226.46	330.06
72 h	*T. castaneum*	100 % mortality				
	T. confusum	6.90	Y= -6.42 + 4.91x	215.86	175.47	265.54
	S. oryzae	0.27	Y= 3.14 + 1.33x	24.72	3.45	176.87
	C. maculatus	0.535	Y= 2.16 + 1.61x	57.74	22.04	151.42

*χ^2 = Goodness of fit. The tabulated value of χ^2 is 5.99 (df = 2, $P<0{\cdot}05$)

Table 2. Percent repulsion (R) of sugarcane bagasse-based lignin to *T. castaneum* and *T. confusum* adults using treated filter paper test

Test insects	Concentrations used	Average repellency (%) at hours after treatment					Mean	Repellency class*
		1	2	3	4	5		
T. castaneum	157.19 $\mu g.cm^{-2}$	40	80	60	40	60	56	III
	314.38 $\mu g.cm^{-2}$	40	80	60	80	80	68	IV
	471.57 $\mu g.cm^{-2}$	60	80	100	20	100	72	IV
	628.76 $\mu g.cm^{-2}$	60	80	100	80	60	76	IV
T. confusum	157.19 $\mu g.cm^{-2}$	60	100	20	100	100	76	IV
	314.38 $\mu g.cm^{-2}$	20	80	80	100	100	76	IV
	471.57 $\mu g.cm^{-2}$	60	100	100	80	80	84	V
	628.76 $\mu g.cm^{-2}$	40	100	100	100	100	88	V

*See Materials and Methods for details

The product (lignin) obtained from sugarcane bagasse showed strong repellent action on the flour beetles *T. castaneum* and *T. confusum* (Table 2). The results indicate that the repellent response of *T. confusum* is higher than that of *T. castaneum*. The results are in agreement with the work of Jilani & Malik (1973) who observed that water and ethanol extracts of leaves and seeds of neem repelled the red flour beetle, *T. castaneum*, the khapra beetle, *Trogoderma granarium* and the lesser grain borer, *Rhyzopertha dominica*. Rahman *et al.* (1999) also reported that water extract of urmoi and neem has repellent action on *S. oryzae* and *S. granarium*. These findings receive support from the results of Akou-Edi (1983) who reported the repellent action of neem oil against *T. confusum* and *S. zeamais*. Ho & Ma (1995) reported that chopped garlic and garlic extracts are repellent to *T. castaneum* and *S. zeamais*. The insecticidal property of the sugarcane bagasse-based lignin may be due to the presence of phenolic and alcoholic compounds. The present results suggest that the product may be effective against pest beetles in stores as well as in the fields.

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