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BIOCIDAL ACTIVITIES OF SOME TROPICAL MOSS POWDERS AGAINST *SITOPHILUS ZEAMAIS* MOTCH (COLEOPTERA: CURCULIONIDAE)

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ABSTRACT
Four bryophytes powders namely, *Calymperes afzelli* Sw., *Thuidium gratum* (*P. Beauv.)* Jaeg., *Bryum coronatum* Schwaeg and *Barbula lambarenensis* (Hook) Spreng. were evaluated for biocidal activities against maize weevil, *Sitophilus zeamais* Motch, at 5%, 10% and 20% w/w concentrations. All the moss plant materials showed encouraging biocidal principles in respect of toxicity, reduction in oviposition and F1 progeny emergence rate and prolongation of pre-imaginal duration. The bryophytes were very effective and prompt as toxicants and their order of efficacy was *B. coronatum*> *T. gratum*> *C. afzelli*>*B. lambarenensis. C. afzelli* and *B. coronatum* prolonged the pre-imaginal duration by 3 to 7 days with increasing treatment concentration. All the bryophyte powders reduced *S. zeamais* oviposition and F1 progeny emergence significantly ($P<0.05$), but these were not reflected in the capability of the individual to lay eggs or survive in the grain, respectively. A maize grain weight loss that may not be occasioned by a reduction in the food consumption rate of the pre-imaginal stages of *S. zeamais* was also recorded with the bryophyte treatments. The promise and desirability of the use of mosses as protectant against the maize weevil were highlighted.

KEY WORD: *Barbula lambarenensis*, Biopesticide, *Bryum coronatum*, *Calymperes afzelli*, Maize, *Sitophilus zeamais*, *Thuidium gratum*.

INTRODUCTION

Maize (*Zea mays*) is one of the principal sources of carbohydrate to humans and livestock. It also has numerous industrial uses (Arannilewa, 2007). Unfortunately its production is regimented and restricted to the rainy season of the year. This necessitates the need to store it for use when it is out of season. Storage pest activities have, however stood in the way of maximum utilization of maize. One of the major insect pests of maize is *Sitophilus zeamais* Motch (Coleoptera: Curculionidae). To enhance productivity of maize in the field and in the storage, there is a need to control the activities of pests. Various forms of control have been employed. The most popularly employed control tactic is the chemical method which relies heavily on the use of synthetic insecticide and fumigants. This, unfortunately, is costly, toxic to its users, and other non-target organisms, aids development of resistant strains and is generally not environmental friendly (Jembere, *et al*., 1995; Okonkwo and Okoye, 1996). Sourcing biopesticides against insect pests from plants has over the years become quite popular and acceptable, as quite a number of promising compounds that are safe and environmentally friendly have been identified (Anon, 1991). Bryophytes, for no notable reason have remained relatively untapped as a source of biocide for insect control despite the assertion by Bernerjee and Sen (1979) that bryophytes more commonly contain inhibitors against bacteria than fungi and the antifungal activity reported in many mosses (Ando and Matsuo, 1984).The objective of this research work was to find out if some common bryophytes have biocidal potentials against *S. zeamais*.

MATERIALS AND METHODS

Calymperes afzelli Sw., *Thuidium gratum* (P. Beauv.) Jaeg., and *Bryum coronatum* Schwaegr. located on palm tree stands on the campus of Adeyemi College of Education, Ondo, Nigeria, as well as *Barbula lambarenensis* (Hook) Spreng., located on a slab around the building of the Computer Science Department University of Ilorin, Ilorin, Nigeria, were collected separately and air-dried in a dark cupboard to constant weight. The dry bryophyte samples were subsequently grounded individually in an electric blender to fine powder and stored in dark labeled bottle with screw cap. *S. zeamais* adults were sourced from a culture raised on maize using a starter population obtained from the Nigeria Stored Product Research Institute (NSPRI), Ilorin, in the Entomology laboratory of the University of Ilorin. Clean maize cobs were obtained from the Biological garden, University of Ilorin, Ilorin Nigeria. The dried maize cobs were shelled and dried to constant weight in an oven at 30^0C to 35^0C. It was subsequently air dried for 2 hours, wrapped tightly in a polythene bag and stored in a deep freezer for 14 days. The grains were subsequently allowed 5 days of equilibration before use in bioassay.

BIOASSAY

Four replicates treatments were constituted at 0%(Control), 5%, 10% and 20% w/w treatment concentrations of pulverized bryophyte samples on 50g of the equilibrated clean maize by administering 0g, 2.5g, 5.0g and 10.0g, respectively of each pulverized bryophyte sample in a 300ml rearing plastic jar. The mixtures were stirred to ensure uniform coating of the grain by the

powder samples. Ten randomly selected newly emerged adult *S. zeamais* were subsequently introduced into each plastic jar from the *S. zeamais* culture for seven days. These were restricted to the jar with muslin cloth. Each jar was inspected daily for adult mortality and the dead individuals withdrawn. On the eighth day all surviving adults were withdrawn and the weight of each jar content noted. Daily mortality rates (%) were calculated as the proportion of dead insects in the total number of survivors the previous day. Absolute mortality rate (%) was calculated as the proportion of the 10 individuals that died after 7 days. Oviposition rate per jar *i.e*. Number of eggs per jar, was determined using the acid fuschin staining method. Ten maize grains from each jar was randomly selected on the tenth day, soaked in warm water for 2-3minutes, drained and subsequently immersed in 0.5% acid fushin stain for 2-5 minutes. The grains were rinsed in water and examined for cherry red gelatinous egg plugs. The number of egg plugs noticed on the ten grains was then extrapolated for the entire jar using an average number of 154 grains per jar. . The F1 adult emergence initiation time and total number of emergence per jar were recorded. Their respective means were calculated as duration of pre-adult life (Days) and emergence number per treatment jar. The weight of the content of each jar after F1 emergence was noted. Percentage weight loss by maize grain per jar was calculated as the difference between initial jar content weight and weight after F1 emergence as a proportion of the initial weight of the jar content.

DATA ANALYSIS

Mean values were obtained from the four replicate and expressed as X±SE. The total number of adult days recorded per jar was calculated as total number of day with an adult survivor during the seven days of adult exposure. Number of egg laid per adult day or per adult was calculated by sharing the egg load per jar over the number of adult a days and adult, i.e. 10, respectively. The survival rate was estimated as the proportion of eggs laid per jar that emerged in the F1 generation. The various data obtained were compared with control experiment using Analysis of Variance (ANOVA) test at P=0.05 level of significance.

RESULTS

Table 1 shows the level of toxicity of the four bryophyte samples to *S. zeamais* in terms of mortality. All the bryophyte samples recorded conspicuous and significantly higher mean absolute mortality values than the control experiment (12.50±0.48%). The least mean absolute mortality value of 57.50±0.75 % was recorded with 5% *B. lambarenensis* treatment. *C. afzelli*, *T. gratum* and *B. coronatum* recorded 100% mean absolute mortality with improved concentration and this encouraging fete was achieved at 10% concentration level with *T. gratum* and *B. coronatum*. The highest mean absolute mortality value recorded with *B. lambarenensis* at 20% concentration was 80.00±0.41%. *C. afzelli, T. gratum* and *B. coronatum* were quick acting as they recorded significantly higher mean daily mortality from the day of application. This was particularly noticeable with *B. coronatum* where 15.00 ±0.29% mean mortality was noticed with all treatment concentrations the first day. *T. gratum* however achieved 100% *S. zeamais* mortality earlier, i.e. day 5 at 10% and 20% concentrations. *B. lambarenensis* recorded a slightly significantly higher mortality value on the second day at higher concentrations of 10% and 20%. At the least tested concentration of 5%, however, *B. lambarenensis* mortality rates, though significantly higher on some days, were not distinctly different from the control readings in the first seven days after treatment. *C. afzelli* was quite gentle on *S. zeamais* with mortality spreading over the seven days, but relatively higher on days 3 to 5.

All the bryophyte treatments recorded significant ($P<0.05$) reduction in number of adult days per jar as compared with the 38.00±2.83 recorded in the control experiment (Table 2). These reductions also showed significant differences with increasing concentrations from 5% to 10%, it was however not sustained at a 20% concentration with all the bryophyte treatments. The order of efficiency regarding adult-day reduction was *B. coronatum*> *C. afzelli* =*T. gratum* >*B. lambarenensis*. Oviposition rates within each treatment jar showed that the number of eggs laid did not differ with bryophyte type or with treatment concentrations per jar. These were, however, significantly lower ($P<0.05$) than the 134.75±17.07 recorded in the control jar. The average number of eggs laid per adult as expected followed the pattern showed by oviposition rates per jar. This was however not the case per adult day. *S zeamais* exposed to bryophytes tend to show higher oviposition rate per adult-day. This was particularly evident with 20% *B. coronatum* and *T. gratum* where a higher and almost equal rate was recorded as in control experiment.

Adult emergence was not deterred by the bryophytes but its durations with bryophyte treatments were slightly prolonged than in the control experiment (35 days) and it showed a tendency towards an increase with increasing bryophyte concentrations. *C. afzelli* and *B. coronatum* were particularly good, as they delayed adult emergence by at least 3 days at the least concentration and for 5 and 7 days, respectively at 20% concentration. *B. lambarenensis*, however, could not delay adult emergence at lower concentrations but at 20% it did for 2 days. Adults emergence pattern again showed that significantly ($P<0.05$) lower number of adults were recorded from bryophyte treatments and these reduced slightly but significantly ($P<0.05$) with increasing concentrations. *T. gratum* and *B. coronatum* were particularly better in this respect. This, however, was a direct reflection of the oviposition level per treatment as survival rate of the egg in control (50.09%) was similar to all the treatments. *C. afzelli* and *T. gratum* treatments at 10% concentration enhanced the survival of pre-imaginal stages of *S. zeamais* substantially.

TABLE 1: Daily and absolute mortality rates of *S. zeamais* exposed to 0%, 5%, 10% and 20% pulverized dry samples of four bryophyte over seven days.

		Daily Mortality (%±S.E) Post Treatment Duration (Days)							Absolute Mortality (%±S.E)
Treatment	Conc. (%)	1	2	3	4	5	6	7	
Control	0	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	5.00±0.50^{ab}	7.50±0.48^{ab}	0.00±0.00^{a}	12.50±0.48^{a}
C. afzelli	5	2.50±0.00^{ab}	2.50±0.25^{a}	15.00±0.65^{abc}	20.00±0.71^{b}	12.50±0.25^{abc}	12.50±0.75^{ab}	12.50±0.48^{bc}	77.50±0.48^{cde}
	10	0.00±0.00^{a}	10.00±0.41^{ab}	20.00±0.71^{bcd}	15.00±0.65^{ab}	25.00±0.29^{c}	7.50±0.25^{abc}	7.50±0.25^{abc}	87.50±0.48^{ef}
	20	7.50±0.25^{bc}	12.50±0.75^{ab}	22.50±0.85^{bcd}	15.00±1.65^{ab}	20.00±0.71^{bc}	2.50±0.25^{ab}	2.50±0.25^{ab}	100.00±0.00^{f}
T. gratum	5	2.50±0.25^{ab}	17.50±0.25^{bc}	25.00±0.87^{cd}	12.50±0.75^{ab}	5.00±0.29^{ab}	2.50±0.25^{a}	10.00±0.41^{abc}	75.00±0.29^{cd}
	10	2.50±0.25^{ab}	30.00±0.41^{c}	27.50±0.48^{cd}	25.00±0.29^{b}	15.00±0.65^{sbc}	0.00±0.00^{a}	0.00±0.00^{a}	100.00±0.00^{f}
	20	12.50±0.25^{cd}	27.50±0.25^{c}	22.50±0.75^{bcd}	30.00±0.41^{b}	7.50±0.48^{ab}	0.00±0.00^{a}	0.00±0.00^{a}	100.00±0.00^{f}
B. coronatum	5	15.00±0.29^{d}	27.50±0.63^{c}	5.00±0.50^{ab}	17.50±0.48^{ab}	5.00±0.50^{ab}	7.50±0.48^{ab}	5.00±0.29^{ab}	82.50±0.48^{de}
	10	15.00±0.29^{d}	30.00±0.00^{c}	5.00±0.29^{ab}	22.50±0.48^{b}	25.00±0.50^{c}	2.50±0.25^{c}	0.00±0.00^{a}	100.00±0.00^{f}
	20	15.00±0.29^{d}	27.50±0.63^{c}	35.00±0.29^{d}	20.00±0.41^{b}	2.50±0.25^{a}	0.00±0.00^{a}	0.00±0.00^{a}	100.00±0.00^{f}
B. lambarenensis	5	0.00±0.00^{a}	2.50±0.25^{a}	15.00±0.29^{abc}	15.00±0.65^{ab}	7.50±0.25^{ab}	12.50±0.63^{ab}	5.00±0.50^{ab}	57.50±0.75^{b}
	10	0.00±0.00^{a}	7.50±0.48^{ab}	10.00±0.41^{abc}	15.00±0.65^{ab}	12.50±0.75^{abc}	12.50±0.75^{ab}	10.00±0.41^{abc}	67.50±0.48^{bc}
	20	0.00±0.00^{a}	5.00±0.29^{ab}	17.50±0.48^{abcd}	20.00±0.71^{b}	15.00±0.29^{abc}	5.00±0.50^{a}	17.50±0.63^{c}	80.00±0.41^{de}

Values are means of four replicates.

Means carrying same superscript along columns are not significantly different at P = 0.05.

TABLE 2: Mean number of adult-days, egg laid, hatchability rates, F1 progeny emergence of *S zeamais* and grain weight loss per jar with different bryophyte treatments.

						F1 emergence			
Treatment	Conc. (%)	Mean number of Adult- days per jar	Mean number of eggs laid per jar	Number of eggs laid per adult day	Mean number of egg laid per adult	Duration (days)	Number/jar	Survival rate (%)	%Weight loss
Control	0	38.00±2.83^{c}	134.75±17.07^{b}	3.55	13.48±3.42^{b}	35.0±2.5	67.50±2.92^{c}	50.09	4.88±1.27^{b}
C. afzelli	5	24.14±15.61^{abc}	34.65±9.69^{a}	1.44	3.47±1.94^{a}	39.0±3.5	19.50±0.65^{ab}	56.28	1.73±0.08^{a}
	10	19.29±16.11^{ab}	42.35±7.37^{a}	2.20	4.24±1.47^{a}	38.0±2.0	17.50±0.96^{a}	41.32	1.88±0.15^{a}
	20	17.29±16.21^{ab}	30.80±6.29^{a}	1.78	3.08±1.26^{a}	40.0±4.0	18.50±1.56^{a}	60.07	2.05±0.26^{a}
T. gratum	5	24.14±11.73^{abc}	46.20±10.89^{a}	1.91	4.62±2.18^{a}	36.0±2.0	24.50±2.22^{b}	53.03	2.45±0.27^{a}
	10	16.43±16.69^{ab}	23.10±4.45^{a}	1.41	2.31±0.89^{a}	38.0±2.5	17.25±1.44^{a}	74.68	1.98±0.14^{a}
	20	12.86±14.08^{ab}	42.35±7.37^{a}	3.29	4.24±1.47^{a}	38.0±3.5	17.50±1.19^{a}	41.32	2.15±0.44^{a}
B. coronatum	5	15.57±13.97^{ab}	34.65±7.37^{a}	2.23	3.47±1.47^{a}	38.0±4.0	19.75±0.48^{ab}	56.10	0.98±0.19^{a}
	10	11.57±13.25^{a}	34.65±3.85^{a}	2.99	3.47±0.77^{a}	38.0±2.0	16.50±1.19^{a}	47.62	1.38±0.26^{a}
	20	7.57±11.83^{a}	30.80±10.89^{a}	4.07	3.08±2.18^{a}	42.0±4.5	15.50±0.65^{a}	50.33	1.45±0.27^{a}
B. lambarenensis	5	29.71±8.20^{bc}	46.20±10.89^{a}	1.56	4.62±2.18^{a}	35.0±2.5	19.75±1.25^{ab}	42.75	2.25±0.27^{a}
	10	18.86±15.59^{ab}	38.50±4.45^{a}	2.04	3.85±0.89^{a}	35.0±2.0	18.00±2.20^{a}	46.75	2.48±0.53^{a}
	20	18.14±14.60^{ab}	42.35±3.85^{a}	2.33	4.24±0.77^{a}	37.0±3.5	18.00±2.20^{a}	42.50	2.20±0.41^{a}

Values are means of four replicates.

Means carrying the same superscript alphabet along columns are not significantly different at P=0.05.

Weight loss as a result of the activities of S. *zeamais* on maize grains showed a trend reflecting the number of adults that emerged from the respective treatment jars. Jars treated with bryophytes powders had significantly ($P<0.05$) lower weight loss when compared with the control experiment. This was however not due to a reduction in the consumption level of the pre-imaginal stages of *S. zeamais*, since weight losses per emerged individual in both cases were comparable and not significantly different.

DISCUSSION

All the bryophyte samples were toxic to *S. zeamais* at 5% w/w treatment and an optimum toxicity of 100% was recorded with *T. gratum* and *B. coronatum* at 10% w/w treatment level. The order of toxicity of the bryophyte was *B. coronatum*> *T. gratum*> *C. afzelli*>*B. lambarenensis*. The bryophytes also showed their toxic activities quite promptly with *C. afzelli*, *T. gratum* and *B. coronatum* recording significantly higher mean daily mortality the first day of application and absolute mortality was achieved within the first 5 days. This resulted in the sharp reduction in number of adult days recorded in the various treatments as compared with the control experiment. The order of efficacy regarding adult day reduction was *B. coronatum*> *C. afzelli* =*T. gratum* >*B. lambarenensis*. All the bryophytes reduced *S. zeamais* oviposition significantly ($P<0.05$) and these did not differ with type of bryophyte or it's in the capability of the individual to lay eggs. In fact treated *S. zeamais* recorded higher oviposition rate per adult-day in some cases. The reduction was however due to the paucity of the individuals laying the eggs and not a reduction.

The bryophytes did not deter adult emergence but it prolonged the duration of the *S. zeamais* pre-imaginal stages slightly. *C. afzelli* and *B. coronatum* prolonged the pre-imaginal duration by 3 days at 5% concentration and for 5 and 7 days, respectively at 20% concentration. The significantly ($P<0.05$) lower number of adult *S. zeamais* emergence noticed with bryophyte treatments may not have been due to mortality of the pre-imaginal stages, since survival recorded in the treatments and control experiments were comparable. Some of the bryophytes, e.g. *C. afzelli* and *T. gratum*, actually enhanced the survival of the pre-imaginal stages of *S. zeamais*.

Weight loss as a result of the activities of *S. zeamais* on maize grains showed a trend that reflected the number of adults that emerged from the respective treatment jars. Jars treated with bryophytes powders had significantly ($P<0.05$) lower weight loss when compared with the control experiment. Although a weight loss was recorded on the maize grain, this may however not be due to a reduction in the food consumption of the pre-imaginal stages of *S. zeamais*, since weight loss per emerged individual, in both cases, were comparable and not significantly different.

Although quite a number of plant materials have been reported to elicit grain protectant properties against *S. zeamais* (Okonkwo and Okoye, 1996; Okonkwo and Ewete, 1998; Lajide *et al*., 1998; Arannilewa, 2007), all these plants are spermatophytes. Biocidal activity of bryophytes is being reported against *S. zeamais* for the first time (?????). All the four lower plants (bryophytes) tested showed some promise as insecticidal materials. Further investigation in this regard is desirous as these plant materials abound in the ecosystem waiting to be tapped for constructive purposes.

CONCLUSION

The four bryophytes plants, *i.e. C. afzelli*, *T. gratum* and *B. coronatum* and *B. lambarenensis* reduced oviposition and F1 progeny emergence, prolonged the duration of pre-imaginal stages and elicited prompt toxic activity against *S. zeamais*.

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