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POSSIBLE USE OF BANANA BUNCH RACHIS JUICE TO CONTROL THE FUNGUS SCLEROTIUM ROLFSII SACC.

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ABSTRACT

The present study reports the possible use of the Banana bunch rachis(BBR, a residue generaly lost or neglected in Guadeloupe and Martinique) as an organic amendment of the soil to control the stem blight fungus Sclerotium rolfsii.The soil amended with the residue at the dose of 4% (w:w) was suppressive to the fungus. In fact, the active part of the BBR was in the juice which was still effective after heating (boiling or autoclaving). The BBR juice decreased the soil infectious potential due to S. rolfsii and affected the growth of the fungus in laboratory experiments. In the field, it also reduced the population of viable S. rolfsii sclerotia. Non-toxic and toxic fractions (operating in different soil types) were extracted from the juice.

INTRODUCTION

In Guadeloupe and Martinique, after harvesting, Banana leaves and 'trunks' are cut and left in the field where they decompose before being incorporated into the soil during the tillage operations preceding the next plantation. On the contrary, after the recuperation of the 'hands', the Banana bunch rachis (BBR) are thrown at some place on the farm or 'spread' here and there alongside the accomodation roads of the cultivated fields. Thus, they are mostly considered as wastes and, thereby, it is an amount of more than 50000 t of organic residue which is lost, each crop,in Guadeloupe.

In an earlier study (TORIBIO, 1984),the BBR presented some interest as a soil organic amendment to control the stem blight fungus Sclerotium rolfsii.

The present study goes further into detail on the effects of the different BBR components on this fungus.

Experiments and Results

1- Préparation of the amendment.

The Banana bunch rachis were crushed in a JEFFCO crusher-grinder (Jeffress Bros.Ltd.,351 Melton Road,Northgate Brisbane,Q'ld) and separated into two lots. One lot was hand-pressed to get the banana bunch rachis juice (BBR juice) which was then kept refrigerated or autoclaved until use. The other lot was allowed to dry in the sunshine, then ground into little-sized fragments(less than 5mm) and kept dried.

2- Evidence of the effect of the BBR residue on S. rolfsii

The BBR solid residue was mixed in pots (7,5 x 7,5 cm) with an 'oxisol' collected at Duclos, Petit-Bourg (Guadeloupe) to get the final doses of 1,2, and 4% (w:w). Then, the soil was inoculated at 0-1 cm depth with sclerotia (45 per pot) produced on Bread fruit (Artocarpus altilis (Park), Forst.) leaves, moistened to moisture holding capacity and seeded with Lentil (Lens esculenta Moeneh.) pregerminated seeds. The check soil was not amended.

After 15 days of incubation in a 'climatic room' (temperatures of 30+/-2°C under light, the disease incidence (seedling mortality) was determined. Seven days later, the remaining viable (germinating) sclerotia were enumerated using a method previously described (TORIBIO,1977).

Table 1 shows that the BBR residue was effective on the disease incidence at the highest dose tested (4%), with about 37% of seedling mortality against 64% for the control. After 3 weeks, about 42% of the sclerotia remained viable in the control soil ; none was detected in the soil amended with 4% of BBR.

Since the banana bunch rachis is juicy, it was interesting to know whether the active components of this residue were in the fiber or in the juice.To test this, the infection by S. rolfsii of Lentil seedling 'protected' by different components of BBR, was followed. Four seedling were grown at two opposite sides in pots and two little vessels were placed in the soil, between them and 2 lines of 8 sclerotia of S. rolfsii.These vessels were filled with a) moistened ground BBR ; b) raw BBR juice ; c)moistened washed BBR fiber ; d) water extract of BBR ; e) reconstituted BBR : fiber + water extract. The pots were placed in mini-glass-houses and incubated in a climatic room 30 +/-2°C.

The sclerotia germinated on the soil surface. However,the fungus mycelium affected the seedling only in the case of the washed fiber (100% of mortality).It is evident from table 2 that the fiber sustained a good growth of the mycelium ; the water extract of BBR and the juice inhibited the fungus.

The juice was still active after boiling and autoclaving ; it could also be stored cold (refrigerated) without a pronounced loss of activity.

3- Effect of the juice on the soil infectious potential.

The soil, artificially infested with sclerotia of S. rolfsii (45 per 7.6 x 7. 6cm plastic pots), was moistened with a definite volume of juice not diluted (concentration 1) or diluted with distilled water to get the following concentrations : .1,.2,.3,.5 and .75. Then, pregerminated Lentil seeds were introduced in the pots (12 seeds/pot). After 15 days of incubation in the climatic room, the seedling mortality was determined. Fifteen days later,the remaining viable sclerotia in the soil were counted.

The figure 1 presents the effect of BBR juice on disease severity in different soils. It is observed that a phase of increased disease was followed by one of a rapid decline in the seedling mortality as the juice concentration augmented. Thus, for exemple, no disease was detected in the 'oxisol'at the concentration .3 and above. The relation between the disease incidence y and the concentration C of BBR juice was best described by the equations y=-28.9021-61.729 Log C (r=-.9554 ; ED50 = .0527),y = 1.9057-104.4007 Log C (r=-.9424 ; ED50 =.3183) and y =-3.2028 -143.2258 LogC (r =-.9619,ED50=.4251) for the 'oxisol', 'luvisol' and 'vertisol', respectively.

The remaining populations of viable sclerotia in the soils after 30 days followed the same evolution (fig.2). Finally, no sclerotium was detected at the concentrations.3 and above in the 'oxisol' and .75 in the 'luvisol' and the 'vertisol'.

4-Effect of BBR juice on the inoculum of S.rolfsii

Laboratory experiments

An 'oxisol', sterilized or not was moistened to moisture holding capacity with the same volume of BBR juice at different concentrations from .03 to 1 in Petri dishes. The pourcentage of germinating sclerotia was determined from 100 sclerotia produced on PDA, incorporated at the soil surface and distributed in 5 dishes (20 sclerotia/dish) for each concentration, after 48 hours of incubation in a growth chamber (30°C). The extension of the mycelium was measured from 5 out of 10 sclerotia in another 'set' of Petri-dishes kept in the same conditions.

It was observed that the percentage Y of germinating sclerotia in the two soils decreased with increased concentrations C of BBR juice ; however more sclerotia germinated in the sterilized soil, even at the highest concentration of the juice (fig.3,A). This relation could be described in the natural soil by the equation Y = 14.0749 - 72.9972 Log C (r = -.9668) which gives, in relation to the control (distilled water, instead of BBR juice) an ED_{50} of .1733. For the sterilized soil, the equation was Y = 44.2105 - 59.1947 Log C (r=-.9461) which gives an ED_{50} of .7984.

The same situation occured for the extension of the fungus mycelium (Fig 3.B) ; The relation between the mycelium extension Y and the concentration C of juice was best described by the equations y=1.2552-14.7474 Log C (r =-.9572, ED_{50} = .1558) and y=4.7706 - 9.9388 Log C (r=-.9561, ED_{50} = .5263) for the natural soil and the sterilized soil, respectively.

Field experiment.

The field experiment was conducted at INRA, Centre Antilles-Guyane, Duclos Petit-Bourg (Guadeloupe) with an 'oxisol'. Mini-plots were delimited by plastic pots (18x18x18 cm) deeped in the field and filled with the soil inoculated at the surface (0-2cm) with 720 mg of sclerotia (about 1750 sclerotia) produced on Breadfruit leaves. On February 16th 1988 (the day of inoculation) the soil was moistened with 400 ml of autoclaved or refrigerated BBR juice or water(control)or received 40 g of BBR solid residue spread at the soil surface. Another juice treatment was applied on March 23rd 1988. On March 4th 1988, a Bean (Phaseolus vulgaris L.,cv. 'Contender')crop was seeded in the pots (12 seeds/pot) and, on April 8th,soil samples were taken to the laboratory for the enumeration of viable sclerotia.The crop was harvested 15 days later.

Table 3 summarizes the effect of the treatments on viable sclerotia, disease incidence and fresh pod yield. The juice reduced one-half and the BBR solid residue increased almost 4-fold the population of viable sclerotia,comparatively to the water control. Only the BBR solid residue increased significantly the incidence of the disease in the field. The two juices increased significantly the yield over the control and the BBR solid residue.

5 - Active compounds of the juice

Phenolic compounds in the juice were eluted through an 'Amberlite CG50' ion exchange resin (Serva Feinbiochemica, Heidelberg,New York) in 3 stages controlled by using a Roucaire UA-5 Absorbance/Fluorescence Monitor' (Isco Inc.4700 Superior Lincoln Nebraska, USA) at 280 nm, according to the methodology of CABANNE (1980) slightly modified. The elution process used 200 ml of a)distilled water, b)ethanol (40%,v:v) and c)acetic acid (40%,v:v) in this order. The different fractions collected were evaporated under vacuum,diluted as necesaary by adding distilled water, and kept in the refrigerator untll use. Their effect on germinatlng sclerotia was tested in the same 3 soils. The soil was distributed in the honeycombed bottom of Api incubation trays (about 90 mg of dry soil/cavity),moistened with each juice fraction or water (control) and incubated under plastic bags in a growth chamber at 30°C. After 48th, the percentage of germinating sclerotia was determined.

The results are presented in Table 4. The sclerotia germinated totally when the different soils were moisten with distilled water (control) and the fractions obtained after elution with water (water fraction) or 40% ethanol (40% ethanol fraction). The fraction from elution with 40% acetic acid (40% acetic acid fractlon) permitted the germination of sclerotia (94.6%) only in the vertisol. However, in this case,the germination was limited mostly to individual hyphae and preceded or accompanied by a release of a whitish exsudate.

RESULTS AND DISCUSSION

The banana bunch rachis (BBR) residue, when incorporated in an 'oxisol' at the dose of 4% (w:w), decreased the severity of the disease caused by S.rolfsii in a population of Lentil seedlings grown in pots in a climatic chamber (Table 1). The residue totally suppressed the viable sclerotia within 3 weeks in these conditions. This dose corresponds to an amount of 40 t of dry residue per hectare on a depth of 10 cm of soil. Any method of drying the residue other than sunlight drying is uneconomic. Sunlight drying itself requires several sunny days in succession, and this is possible only at certain short periods of the year in Guadeloupe. If the drying problem is solved, another difficulty is to do an uniform spreading of the amendment in the soil.

The BBR is a juicy residue and,without any doubt, could be best applied as an aqueous amendment of the soil if the juice was effective on the fungus. We effectively demonstrated that the juice contained the active compounds of the BBR residue against S.rolfsii (Table 2). It reduced the gravity of the fungus on Lentil seedlings in climatic chamber experiments with different soils encountered in Guadeloupe : an 'oxisol' from Duclos Petit-Bourg, a 'luvisol'from Vieux-Habitants and a 'vertisol' collected at Saint-François, with some differences in its effectiveness (Fig.1). It also suppressed the number of remaining viable sclerotia in the soils after 4 weeks (Fig.2).

The BBR juice acted both on the germination of sclerotia and the extension of the mycelium. It was, however, more inhibitory for the mycelium than for the sclerotia as indicated by the ED50 values obtained (see the text).It was also more effective in the natural than in the autoclaved 'oxisol' (Fig.3).

Table 1. Disease incidence in an 'oxisol' amended with Banana bunch rachis (BBR) or not amended[a]

seedling mortality (%)[b]			
dose of BBR in the soil (w/w)			
0 (control)	1%	2%	4%
64.3 A[c]	84 B	76.6 B	37.3 C

a : The disease incidence was determined in a population of Lentil(Lens esculenta) seedlings grown in pots

b : The percentage of seedling mortality was calculated after 15 days from 5 pots containing 12 seedlings for each treatment. The data were 'corrected' by the Arsine Transformation before analysis.

c : The values followed by the same letter are not significantly different ($p = .05$, Newman-Keuls test).

Table 2. Infection of Lentil (Lens esculenta) seedlings by the germinating sclerotia across different substrates.

Substrate	Seedling mortality (%)[a]
ground BBR[b]	0
washed BBR fiber	100
BBR Juice	0
Water extract of BBR	0
Reconstituted BBR	0

a The seedling mortality was determined from 5 pots with 8 seedlings for each substrate.

b BBR = Banana bunch rachis

Table 3. Effect of the BBR juice and solid residue on viable sclerotia, plant mortality and yield in the field.

Treatment	germinating sclerotia/ 100g of soil[a]	S.rolfsii diseased Bean plants (%)[b]	green pod yield (kg/m^2)[c]
Autoclaved BBR juice	20.8 A[d]	23.7 A	5.324 A
Refrigerated BBR juice	19.8 A	14.4 A	5.246 A
Solid BBR residue	169 B	56.1 B	2.469 B
Control (water)	44 C	24.1 A	3.549 B

a The germinated sclerotia were determined from a composite soil sample collected at 0-5 cm dept in 4 pots for each treatment.

b The percentage of diseased plants was determined from 4 pots seeded with 12 Bean cv. 'Contender' each. The angular transformation was used.

c Only the marketable pods were considered.

d The values followed by the same letter within a column are not statistically different (p=.05, Newman-keuls test).

Table 4. Germination of S.rolfsii sclerotia in 3 soils moistened with different fractions extracted from the BBR juice[a]

Sol type	Germinating sclerotia (%)[b]			
	Control (water)	Water fraction	40% Ethanol fraction	40% Acetic acid fraction
'Oxisol'	100	100	100	0
'Luvisol'	100	100	100	0
'Vertisol'	100	98.2	98.2	94.6

a The different fractions were 2-fold concentrated, in comparison to the raw BBR juice.

b The sclerotia used were produced on Bread fruit leaves. The percentage of germinating sclerotia was determined from 4 replications of 28 sclerotia for each treatment.

The general shape of the different curves proposed suggests a toxic effect of the juice whose action may be also ' helped' by the biological environment. In fact,we observed that, in the natural soil treated with the juice, the sclerotia and the mycelium of S. rolfsii were invaded by fungi of the genus Gliocladium and Trichoderma at the lower concentrations and by actinomycetes and bacteria at the highest ones. This prevented the mycelium to form new sclerotia.

In the field, the BBR solid residue induced a severe attack of the Bean crop and left a considerable amount of viable sclerotia in the soil. This could be due to the washing by rain of the active part of the residue, the remaining cellulosic fiber being,then,easily colonized by S.rolfsii. On the contrary, the BBR juice reduced significantly the viability of the sclerotia and increased the green pod yield over the water control. Since the percentage of diseased plants is not significantly higher in the juice-treated plots, comparatively to the control, the juice may have acted also to reduce injuries caused by other soilborne pathogens and/or to stimulate the seedlings.

The juice obtained after grinding the Banana bunch rachis or the Banana 'trunk' was brown in color, probably because of the rapid intervention of polyphenol oxidase enzymes. It has been indicated that the polyphenoloxidases from Banana fruits are isoenzymes with both monophenolase and diphenolase activity (THOMAS & JANAVE,1986, and different authors cited by them). Although the action of such enzymes is likely to lead to the formation of 'classic' phenolic compounds which may be toxic for a given plant pathogenic fungus, the original composition of a plant organ in phenolic derivatives must also be taken into consideration in the suppression, as suggested by CABANE et al. (1977) and MARTIN-TANGUY et al. (1978). Thus, for example, the raw banana trunk juice is not effective against S. rolfsii (unpublished data) while the banana bunch rachis juice is suppressive to the fungus, even after autoclaving.

MARTIN-TANGUY et al. (1976) have found some hydroxycinnamic acid amides to have an antiviral effect. These susbstances have been identified as the main phenolic constituents in the reproductive organs of a range of flowering plants (MARTIN-TANGUY et al., 1978). A method developed in the same laboratory (CABANNE,1980) and modified by VANSUYT(unpublished) was used to extract phenolics from the BBR juice, since the rachis is also a part of the reproductive system of the Banana plant. The water fraction which probably contains acidic and neutral amino-acids, free phenol acids and diphenolamides, and the 40% ethanol fraction which is supposed to be rich in flavonoids and some neutral diphenolamides, did not affect the germination of S.rolfsii sclerotia. The 40% acetic acid fraction which might contain basic amino-acids and mono-phenolamides, and amines, prevented the germination of the sclerotia. It was very effective in the "oxisol" and the "luvisol".

Further attempts to identify the active compounds of the 40% acetic acid fraction are under way.

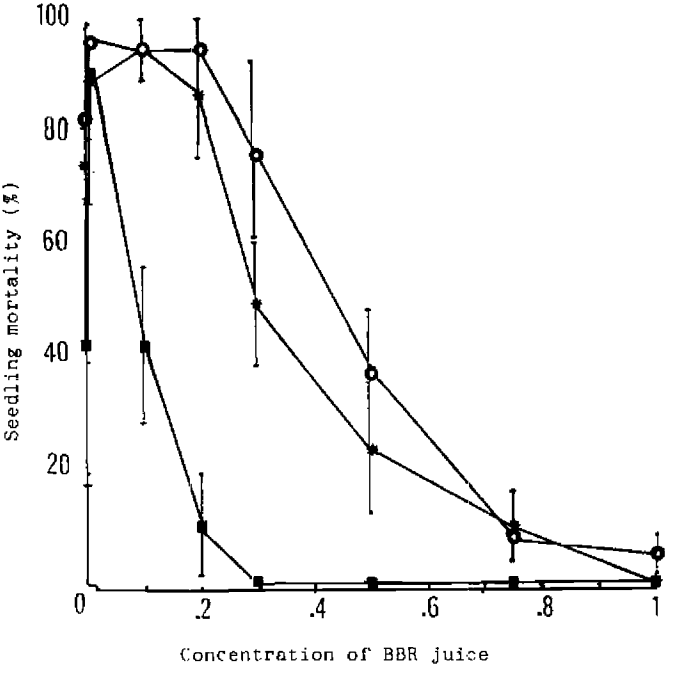

Fig.1. Evolution of Lentil seedling mortality with increased concentrations of BBR juice in 3 soils from Guadeloupe: ■ 'oxisol' from Duclos Petit-Bourg ; ✱ 'luvisol' from Vieux-Habitants ; ● 'vertisol' from Saint-François.
Shown at each concentration is the mean +/-the standard deviation. The sample size is five pots of 12 seedlings for each concentration.

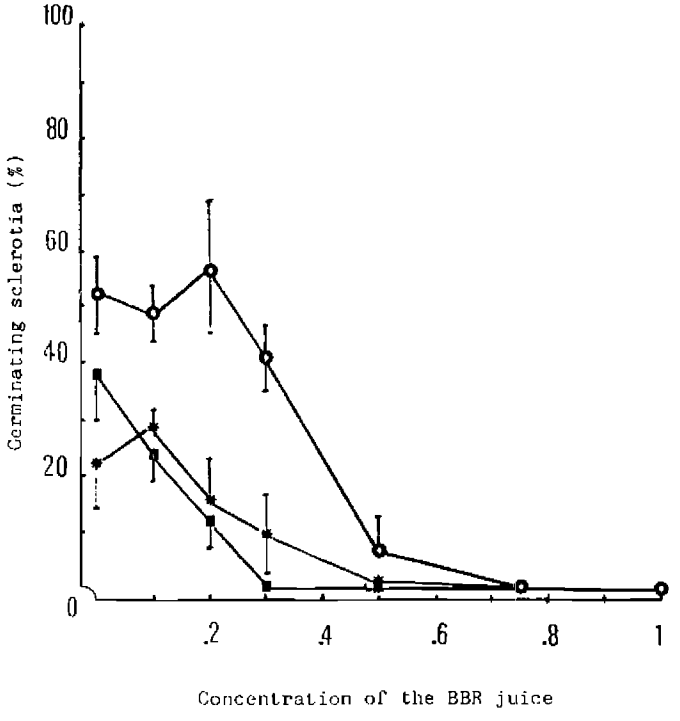

Fig.2. Evolution of the residual populations of viable sclerotia with increased concentrations of BBR juice in 3 soils from Guadeloupe ■ 'oxisol' from Duclos, Petit-Bourg ; ✱ 'luvisol' from Vieux-Habitants ; ● 'vertisol' from Saint François.

Shown at each concentration is the mean +/-the standard deviation. The sample size is 5 for each concentration.

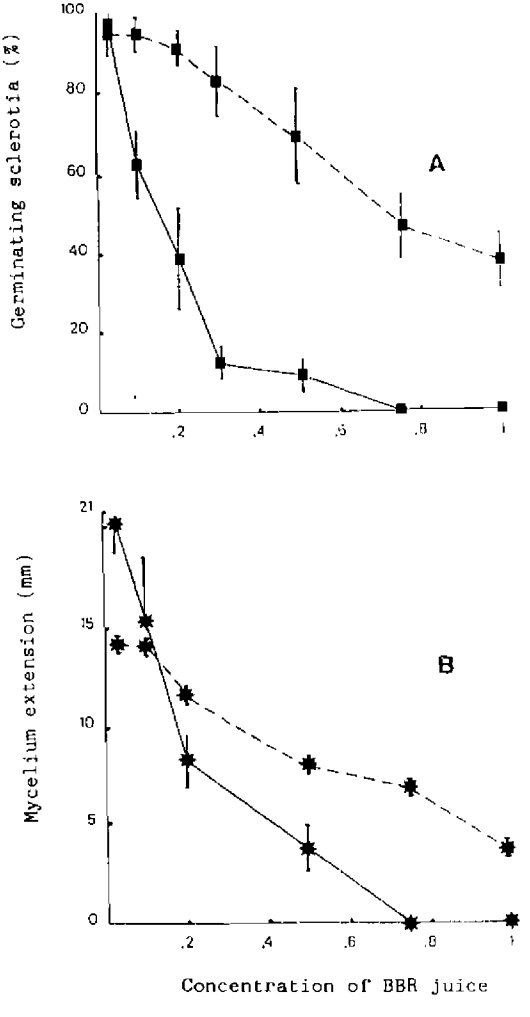

Fig.3. Effect of BBR juice on the inoculum of S.rolfsii in a natural (—) or an autoclaved (---) 'oxisol'
A. Effect on germinating sclerotia
B. Effect on mycelium extension

Shown at each concentration is the mean +/- the standard deviation.

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