THE POSSIBLE USE OF THE PLASMODIUM OF A MYXOMYCETE DIDYMIIUM ALBA EJALE AS A BIOLOGICAL CONTROL AGENT OF A CASSAVA LEAF SPOT DISEASE

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ABSTRACT

This study was carried out to evaluate the possible use of plasmodium of a myxomycete as a biological control agent of a cassava (Manihot utilissima) leaf spot disease [brown leaf spot disease] caused by Cercospora henningsii. Cercospora henningsii was isolated from a diseased cassava leaf collected from a cassava farm behind the University of Benin Health Service Centre. The Plasmodium of Didymium alba Ejale (the myxomycete) was cultured from the fructifications collected from the dead twigs of Ficus yoponensis Desdaux tree growing by the building housing the Department of Plant Biology and Biotechnology, University of Benin. The plasmodium of the myxomycete (Didymium alba) was tested in-vitro on the pathogen. During this study, it was observed that the plasmodium of the myxomycete adversely affected the growth of the fungal pathogen. This is a clear indication that the plasmodium of Didymium alba can be used to control Cercospora henningsii –the causal organism of cassava brown leaf spot.

Key words: Plasmodium, myxomycete, Manihot utilissima, biological control, leaf spot

INTRODUCTION

The myxomycetes often referred to by mushroom hunters as “slime molds” are fungus-like eukaryotes that are the largest subclass of mycetozoans. Traditionally, they are studied by botanists especially the mycologists. They are said to have evolved independently of the fungi from simple flagellate organisms (De Bary, 1887; Macbrille and Martin, 1934). The production of fruiting bodies and dispersal by spores make the myxomycetes similar to fungi. They are however, different from fungi in their mode of nutrition which is phagotrophic in the myxomycetes and autotrophic in fungi.

There are more than 700 recognized species of the myxomycetes (Stephenson et al.,2000). Myxomycetes occur in cool, shady, moist places. In the wood of decaying logs, dead leaves, bark of living tree, humus soil, even on green leaves and
fleshy fruits still attached to the plants (Farr, 1976; Alexopoulos and Mins, 1979; Martin et al., 1983; Decon, 1980; Stephenson et al., 2000; and Stephenson, 2003). Environmental conditions like: temperature, moisture, light, availability of food and pH have been reported to play an important role in determining the derivation of each stage [Nannenga-Bremkamp, 1991; Stephenson et al., 2001; Stephenson, 2003, Rajni, 2004]).

Figure 1: Life Cycle of a typical Myxomycete (Alexopoulos, 1969)
The use of the plasmodium of a myxomycete (Didymium sp) as a possible biological control agent of plant pathogenic organisms was brought about by their ability to feed on fungal and bacterial spores. As a group of organisms, the myxomycetes have not been implicated as disease causing agents which is in agreement with the aim of biological control methods to promote safety.

MATERIALS AND METHODS

Plate 1: A Cassava [Manihot esculenta L] leaf with symptoms of brown Leaf spots.[a and b]

Plate 2: Ficus yoponensis Desvaux..
Plate 3: Fructifications[a] of *Didymium alba* on the dead back[b] of *Ficus yoponensis* Desv.

Plate 4: The Plasmodium of *Didymium alba*[a] on dead leaves[b] of *Ficus yoporiensis*. 
Collection of materials

The plasmodium/fructifications of myxomycetes *Didymium alba* Ejale used for this study were collected from the Department of Plant Biology and Biotechnology, University of Benin, during the wet season. Identification was made using the keys of Martin and Alexopoulos, 1969, Farr, 1981, and Nannega- Bremekamp, 1991, and to species level using Ejale (1991).

Sterilization of materials

Glass wares used for this experiment were sterilized in an autoclave at 121°C and 1.1kgcm⁻² for 30 minutes. Conical flasks, inoculation pins, Petri dishes were wrapped in foil paper and were sterilized in oven for 60 minutes at 160°C. Other materials that could not be autoclaved were surface sterilized with near absolute alcohol (97% ethanol).

Medium

Malt Extract Agar was used for the study. The Malt extract Agar powder weighing 50g was dissolved in 1 litre of distilled water, swirled to mix, and autoclaved. The medium was allowed to cool (just before solidifying) before pouring it into the Petri dishes. Malt extract Agar was used as the medium to enhance the growth of the myxomycetes and the fungal pathogen.

Myxomycetes culture

Fructifications of the myxomycetes were inoculated on the solidified malt agar in the Petri dish with inoculating pin.

Growth from this medium was sub cultured to obtain a pure culture of the plasmodium of the myxomycete.

Isolation of pathogen from diseased materials

Diseased cassava leaf used for this work was collected from cassava farm behind University of Benin Health centre. The method for the isolation of fungal pathogen from the diseased leaves was as described by Harley and Waid (1995). The method entails the cutting of sections from diseased leaf with cork borer measuring 3mm in diameter inside a sterile Petri dish. The cut sections of the leaves were put into a sterile conical flask with 250ml sterile distilled water and washed 5 times by swirling the conical flask with the hand for one minute. The washed sections were then put on a wide sterilised filter paper for the water to drain from the cork borer used to cut the sections. The washed leaf sections were carefully placed on the solidified malt extract agar medium in the Petri dish. All the Petri dishes were put on the laboratory table at an average temperature of 29°C for observations. After 48 hours, the fungal growth from the cassava leaf section in the plates was sub-cultured to obtain a pure culture of the fungus.

Treatments

Set A

This consisted of 3mm in diameter of plasmodium of the myxomycete grown on the medium cut with sterile cork borer and placed at the centre of the plate on another malt
extract agar, with a sterile spatula. Each treatment was in three plates and the average of the growth of organism in the 3 plates was taken during measurement.

Set B

This treatment was agal block (3mm in diameter) of the fungus (*Cercospora henningsii*) cut with a sterile cork borer and placed at the centre of another plate of malt extract agar with a sterile spatula.

Set C

Agar blocks (3mm in diameter) of *Cercospora henningsii* and plasmodium of the myxomycete cut with a sterile cork borer were placed at the centre of another plate of malt extract agar with a sterile spatula. This served as the mixed culture

Set D.

All plates were incubated at an average room temperature (29°C). Daily observations were made on all plates. Measurements of *Didymium alba* and fungus were taken every 24 hours with aid of a metre rule (mm). The average of the three plates was recorded. The experiment was terminated after 5 days of inoculation when some plates were completely covered by the growth of the fungus.

Analysis of data

A paired T-test was used to carry out the statistical analysis. Statistical tools used include: mean, standard deviation, standard error of mean, variance (S) and critical value.

Plate 5. A photomicrograph of the fructification of *Didymium alba* showing sporangium (a) and stipe (b) and at x40 magnification.
Use of Plasmodium of a myxomycete as a biological control agent of cassava leaf spot

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Plate 6: Photo-micrograph of the sporangium of Didymium alba at dehiscence showing lime granules (a), spores (b), and capillitia threads (c) at x40 magnification.

RESULTS

The myxomycete grew better when cultured with the fungus while the fungus grew better when it was alone in culture. This indicates that the plasmodium of the myxomycete fed on the fungus.

Plate 7: Photomicrograph of Cercospora henningsis [x4 magnification] taken from the plate on which only the fungus was grown. The fungal hyphae[a] can be seen growing profusely.
Plate 8: Photomicrograph of a smear of the mixed culture of the plasmodium of *Didymium alba* and *Cercospora henningsii* at x40 magnification at the 5th day of inoculation. There is no sign of fungal hyphae.
Plate 10: The culture of *Cercospora henningsii* alone on malt extract agar at 5 days of incubation.

Plate 11: The plasmodium of *Didymium alba* alone on malt extract agar at 5 days of incubation.
Table 1: Effect of the plasmodium of *Didymium alba* on *Cercospora henningsii* showing growth in diameter of the plasmodium of *Didymium alba* in the mixed culture [GDPM]; growth in diameter of *Cercospora henningsii* in the mixed culture [GDCH]; growth in diameter of plasmodium alone in culture [GDPA]; and growth in diameter of *Cercospora henningsii* [GDCHA] alone in culture.

<table>
<thead>
<tr>
<th>Days</th>
<th>Temp (°C)</th>
<th>GDPM (mm)</th>
<th>GDCH (mm)</th>
<th>GDP (mm)</th>
<th>GDCHA (mm)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>28</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>2</td>
<td>29</td>
<td>10.00</td>
<td>4.40</td>
<td>5.00</td>
<td>36.00</td>
</tr>
<tr>
<td>3</td>
<td>28</td>
<td>15.00</td>
<td>**</td>
<td>8.50</td>
<td>58.00</td>
</tr>
<tr>
<td>4</td>
<td>27</td>
<td>35.00</td>
<td>—</td>
<td>11.00</td>
<td>60.00</td>
</tr>
<tr>
<td>5</td>
<td>28</td>
<td>52.00</td>
<td>**</td>
<td>16.00</td>
<td>73.00</td>
</tr>
</tbody>
</table>

** The colony of *Cercospora henningsii* indistinguishable from the plasmodium of *Didymium alba*.

Note: Mixed culture refers to the medium containing the plasmodium of *Didymium alba* and *Cercospora henningsii*.

**DISCUSSION**

Biological control is an existing science because it constantly incorporates new knowledge and technique (Leonard, 2004). It describes the normal state of affairs in natural, undisturbed ecosystem where populations of organisms exist in a dynamic equilibrium and species of individuals unable to compete or to find an ecological niche are replaced by those that can. Biological control uses one living organism’s natural enemy to control another living organism. The most commonly used organisms include insects and fungi. Almost anything that is of biological origin may be used as biocontrol agent (Gantam et al., 2003).

Other methods of disease control like chemical method also exist. Chemical control involves the use of synthetic chemicals and pesticides to eliminate pests or reduce their effects. However, several problems are associated with these other,
Table 2: Comparison of the Mean Growth in Diameter of plasmodium of *Didymium alba* in the Mixed Culture with Growth of plasmodium of *Didymium alba* alone.

<table>
<thead>
<tr>
<th>Growth of plasmodium in Mixed Culture (mm)</th>
<th>Growth of plasmodium alone (mm)</th>
<th>Cercospora henningsii alone (mm)</th>
<th>Significance (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>45.19</td>
<td>13.71</td>
<td>62.00</td>
<td>P&lt;0.05Highly significant</td>
</tr>
</tbody>
</table>

H₀ rejected P<0.05

control methods. These problems include: being harmful to wildlife, environment, humans due to the accumulation of toxic residues, many of these chemicals are expensive, non selective (adverse effects on beneficial species and non target organisms and the possibility of the organisms becoming resistant to chemical application (Leonard, 2004).

However, biological control as a method has an untapped potential, and it is being considered for an increasing number of crops and managed ecosystems due to its record of safety during the past 100 years (Khetan, 2000).

Reports showed that the rate of respiration in plants with bacterial and fungal infections tends to increase as epithelial tissues consume the carbohydrates reserves more quickly and cause several metabolic changes in plant (Agoris, 1997). However, Nieves-Riveral (2000), reported that when plasmodium of a myxomycetes (*Physarum cinereum*) was introduced into such infected plants, the acceleration rate which occurs shortly before fructification are formed continue to rise during sporulation, after which respiration lowers to normal level or below. Nieves-Rivern (2000), reported that myxomycetes fed on fungal and bacteria spores from infected plants, does not invade the internal tissues of the plant. This makes it an excellent biological control agent.

Ejale and Orumwense (2006) reported that the plasmodium of *Didymium alba* (a myxomycete) seemed to have potential for the treatment of diseases caused by three human pathogenic organisms (*Klebsiella pneumonia*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*). This was
noticed when the plasmodium of *Didymium alba* and each of the pathogenic bacteria were inoculated in the same medium (mixed culture). The plasmodium of myxomycetes *Didymium alba* had an inhibitory effect on the growth rate of each of the bacteria tested.

Shtienburg (1992) also carried out an investigation on the effect of myxomycetes on sooty moulds. He pointed out, however, that possibilities exist for protozoan plasmodia physically interfere with photosynthesis, transpiration, respiration (or the all), and they alter the plant’s normal physiological metabolism at a micro level e.g. affecting the chloroplast, preventing free folia gas exchange or altering the stomatal closure hence lowering the overall chlorophyll content of the leaf. When myxomycetes were introduced, it was observed that the photosynthetic activity of the meaning of chlorophyll seems to remain unaffected. This was demonstrated further using a myxomycete *Didymium cancellatum* in turf grass confirmed as asymptomatic condition in the plants. It has been on record that no microorganism or beneficial insect deliberately introduced or manipulated for biological control purpose has, itself, become a pest so far as can be determined (Khetan, 2000; Leonard, 2004 and Boss and Pond, 2005).

The objective of using myxomycetes as a possibly biological control agent plant cassava pathogenic organism (*Cercospora henningsii*) is necessitated by its ability to devour decomposing organic matters, bacterial and fungal spores (Martin and Alexopoulos, 1969; Ainsworth, 1976; Agoris, 1997 and Stephenson and Stempen, 2000).

From the results of this study, the growth of plasmodium was not very profuse when the plasmodium of *Didymium* sp was cultured alone in a medium. This could be attributed to the fact that the plasmodium of a myxomycete (*Didymium alba*) did not have much food to feed on. Under a suitable environmental condition, the plasmodium of myxomycetes survive for a very long period due to its ability to move about in the fashion of an amoeba, taking in food (bacterial and fungal spores) and increasing in size and under an unfavourable conditions the organism forms a resting stage (sclerotium) which is an inactive form as was observed during this study.

The plant pathogenic fungus used for this study is an obligate parasite. Leather (1959) also reported the occurrence of the disease in tree cassava (*Manihot glaziovii* Muell – Arg). *Cercospora henningsii* belongs to the family Mycosphaerellaceae, order Capnodiales, in the phylum Ascomycota. Members of this family form lesions, on both cassava and tree cassava, they form necrotic spots, circular (5-12mm) diameter in shape and dark brown spots on both surfaces of the leaf. Leaf of infected plants turn yellow and drop. Yields are greatly reduced. Biological control using organisms like the plasmodium of myxomycetes will help a great deal in solving this problem.
When *Cersospora henningsii* was grown alone on nutrient agar medium it grew profusely within a very short time. But when the plasmodium of *Didymium alba* and this cassava pathogenic fungus [*Cercospora henningsii*] were grown together in the same medium (mixed culture), the plasmodium of *Didymium alba* greatly reduced the growth of *Cercospora henningsii* as a result, the colonies of *Cercospora henningsii* became indistinguishable from the plasmodium of *Didymium alba* (the myxomycete) from the third day of inoculation. This clearly indicated that the plasmodium of *Didymium alba* fed on the fungus (*Cercospora henningsii*). This is in agreement with the observation of Martins and Alexopoulos (1969); Alexopoulos and Mins (1979), Nannenga-Bremekamp (1991); Stephenson and Stempen (2000) and Adl (2003). They reported that the plasmodium of the myxomycetes is a large coenocytic mass like a gigantic amoeba, feeding on organic particles as it moves along its substrum by phagocystism.

It must be noted that the antimicrobial effect of the plasmodium of the myxomycetes must not be underestimated (Alexopoulos and Mins, 1979, Stephenson and Stempen, 2000). In addition to feeding on the fungal spores, the plasmodium of *Didymium alba* also exerted a high inhibitory effect on the tested pathogenic fungus. This is attributed to the presence of enzymes, vitamins, steroids and other organic substances that have been isolated from various species of the plasmodium of myxomycetes (Alexopoulos and Mins, 1979; Stephenson and Stempen, 2000). The plasmodium of *Didymium alba* seemed to have had more antimicrobial effect in the mixed culture than when grown alone.

Since investigations were not carried out to determine the particular component responsible for the inhibition of this microorganism, a need for further research in this aspect is therefore recommended.

The plasmodium of myxomycetes can be easily collected from its natural habitat. The fructification can also be isolated and cultured so as to obtain the plasmodium, like *Ficus yoponensis*.

Using the plasmodium of *Didymium alba* (myxomycetes) as a biological control agent of this pathogenic organism will be very safe as they have not been known to possess components that are harmful to the plants on which they can grow neither they parasitic (Nannenga-Bremekamp, 1991 and Stephenson and Stempen, 2000). Myxomycetes have been known to exist completely unaffected or disturbed by the presence of other organism (De Bary, 1887, Ejale, 1991).

In this study biostatistical analysis was also used to further explaining the level of inhibitory effect of the plasmodium of *Didymium alba* on the tested plant pathogenic organisms.

**CONCLUSION**

The result of this study has shown that the plasmodium of *Didymium alba* seems to have a great potential for the treatment of diseases caused by *Cercospora henningsii* like brown
leaf spot of cassava (*Manihot esculenta*). Myxomycetes as a group of organisms have not been implicated as disease causing organisms (Agoris, 1997) and therefore its *[Didymium alba]* possibility as a biological control agent is promising. Further research work should be done to establish mode of application and dosage as well as

**REFERENCES**


