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Insect Larva: The Culture Medium for Fungi Storage

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Authors' contributions

This work was carried out in collaboration between all authors. Author EIE designed the study, performed the statistical analysis, wrote the protocol, the first draft of the manuscript. and performed study on biochemical analysis. Author EEO managed the literature searches on Larvae. Author CIA managed the collection of larvae. Author EAOD managed fungal cultures isolation. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

The culture medium of *Oryctes monoceros* larva has nutrient composition and significant quantities of mineral elements required for fungi growth. The presence of these major mineral elements such as Na, Ca, K, Mg, Mn, Fe and Cu in the larva served as growth factor. The present study was carried out to examine the effect of culture media of *O. monoceros* larva and potato dextrose agar (PDA) in supporting growth and sporulation of fungi. The *O. monoceros* larva medium best supported growth and sporulation of *Ceratocystis paradoxa* (3.52), *Glomerella cingulata* (3.15), *Trichoderma harzianum* (4.80), *Fusarium oxysporium* (4.52) and *Byssochlamys nivea* (3.32) while potato dextrose agar was less suitable for growth and sporulation of *C. paradoxa* (2.30), *G. cingulata* (1.83), *T. harzianum* (3.41), *F. oxysporium* (2.72) and *B. nivea* (2.36) in spores/ml two weeks after incubation. However, six months after incubation PDA medium best supported growth and sporulation when compared with less suitable *O. monoceros* depleted medium. The ability of these fungi to break down the oil content in the larva for utilization means it could probably form a base for new culture medium for fungi storage. The cost of *O. monoceros* is cheaper compared with PDA. In the absence of PDA, *O. monoceros* would be the alternative medium for fungi storage.

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1. INTRODUCTION

All living organisms require a source of energy. A large variety and types of culture media have been developed with different purposes and uses. Microorganisms have been found in virtually all environments where there is liquid water, regardless of its temperature [1]. Culture media are employed in the isolation, maintenance of pure cultures and storage [1]. A defined medium will have known qualities of all ingredients, especially a defined carbon and nitrogen sources. An undefined medium has some complex ingredients which consist of a mixture of many chemical components in unknown proportions [2]. Potato dextrose agar is used for the cultivation of fungi. It is a general purpose medium for yeast and mold. Due to nutritional variation, some fungi may encounter poor growth or fail to grow or change in appearance from the original color [3].

Oryctes monoceros breeds in decaying organic matter, such as felled rotting palms [4]. An attack by *O. monoceros* predisposes the palm to secondary attack by *Rhyncophorus phoenix* [5]. Despite the destructive nature of *O. monoceros*, the larva is seen as food and delicacy in many parts of the tropics. It is cheaper to cultivate as food in decaying or felled palm trunks and trees.

Because of the nutritional value of *O. monoceros*, it becomes important to evaluate as it could form a base for new culture medium for fungi storage, and compare with the commonly used potato dextrose agar medium in growth and sporulation. Moreso, it is important to develop a relatively cheap medium using *O. monoceros* larva as alternative to the commercially available PDA that is presently becoming more expensive.

2. MATERIALS AND METHODS

The study was carried out in plant pathology and entomology laboratories of the Nigerian Institute for Oil Palm Research, Benin City, From March 2010 to July 2011.

2.1 Collection of Larvae

The *O. monoceros* larvae were collected from decaying oil palm trunks. They were transported from the field to the laboratory with a well ventilated container. The larvae were fed with pieces of sugar cane trunks for 48 hours before use.

2.2 Preparation of Larva as Culture Medium

The live larvae were rinsed with sterile distilled water and dried using sterile Whatman No 1 filter paper. The Petri dish plate and McCartney bottle had one larva each replicated ten times as one treatment. They were autoclaved at 121ºC for 15 minutes pressure ($1.2kg/cm^2$).

2.3 Fungal Cultures

Stock cultures of *Ceratocystis paradoxa* (Dade) C Moreau (Herbarium IMI No 314373), *Glomerella cingulata* (Herbarium IMI 283849), *Trichoderma harzianum, Fusarium*

oxysporium and *Byssochlamys nivea* Wasting IMI No. 396923 were collected from the NIFOR. They were re-suspended in PDA broth for 14 days for spores formation.

2.4 Inoculation of Larvae with Fungal Cultures

Suspension of equal amounts of the cultures of *C. paradoxa, G. cingulata, T. harzianum, F. oxysporium and B. nivea* were freshly prepared and the resulting fungi suspension containing about 10^4 spores/ 0.1ml was fed as droplets on the larvae using sterile syringes. The control treatment had sterile distilled water as droplets. Six treatments were carried out with five fungi suspensions. They were incubated aerobically at 24 ± 2ºC for 26 months.

2.5 Inoculation of Potato Dextrose Agar with Fungi

The pure cultures of *C. paradoxa, G. cingulata, T. harzianum, F. oxysporium and B. nivea* were each inoculated into slant McCartney PDA bottles with 2 mm agar disc. They were harvested for 2 weeks and 6 months after incubation.

2.6 Biochemical Analysis

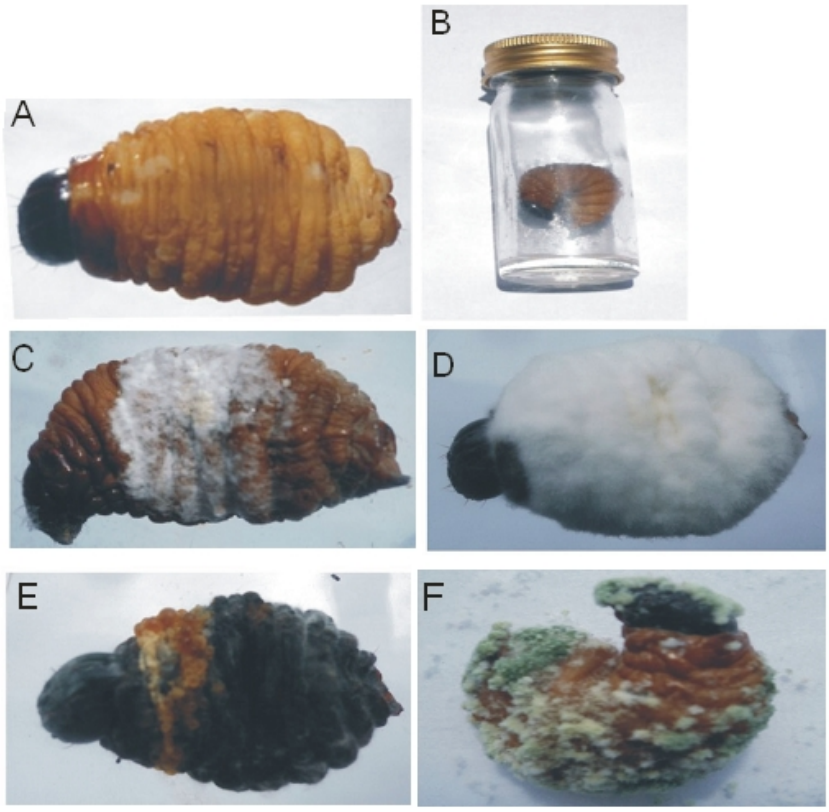
Fresh *Oryctes monoceros* weighing 8-9g each were dried in an oven at 60ºC for 72 hours to reduce moisture content to about 10-12% and ground with pestle in a porcelain mortar to a coarse state. The proximate analysis of the samples was carried out in triplicate according to standard methods [6]. Percentage protein was obtained by multiplying the total nitrogen by a factor of 6.25. Carbohydrate was computed by difference while the mean value of crude protein and carbohydrate respectively by 4, 9, 4 and taking the sum of the products expressed in kilocalories [7,8]. The mineral composition was determined as described by [6]. Calcium, potassium and sodium were determined by flame photometry on a Gallenkamp Digital Flame Analyzer while phosphorus and magnesium were determined by Atomic Absorption Spectrophotometer on Buck Scientific.

2.7 Statistical Analysis

The experiments were repeated three times. They were kept in a randomized manner. Data were analyzed using T-Test.

3. RESULTS AND DISCUSSION

The larva medium of *Oryctes monoceros* supported good mycelia growth of the fungi (Plate 1A-F). The proximate composition analysis of culture medium of *Oryctes monoceros* larva showed that it was a good source of nutrient with the moisture content quite high with 62.41% and 34.61%, protein 19.32% and 26.63%, lipid 22,51% 25.83%, carbohydrate 4.31% and 7.22% for wet and dry weights respectively (Table 1). The larva contained significant amount of important mineral elements. The sodium, magnesium, potassium and iron are major elements present (Table 2). The *O. monoceros* larva medium was found to be best suitable for the growth and sporulation of fungi and yeast two weeks after incubation with *T. harzianum* and *F. oxysporium* being most favoured (Table 3) while potato dextrose agar medium was suitable for growth and sporulation of fungi and yeast six months after incubation with *C. paradoxa* and *T. harzianum* being most favoured (Table 4).



Plates. 1A-F. Shows larva medium of *Oryctes monoceros*

A. Autoclaved *O. monoceros* larva medium.
B. Autoclaved *O. monoceros* larva medium in McCartney bottle.
C. Larva medium of *O. monoceros* inoculated with *Fusarium oxysporium* f.sp. *elaeidis* 5 days after incubation.
D. Larva medium of *O. monoceros* inoculated with *Byssochlamys. nivea* 5 days after incubation.
E. Larva medium of *O. monoceros* inoculated with *Ceratocystis paradoxa* 5 days after incubation.
F. Larva medium of *O. monoceros* inoculated with *Trichoderma harzianum* 5 days after incubation.

Table 1. Proximate Composition of *Oryctes monoceros* Larva

Nutrient (%)	Wet weight	Dry weight
Moisture	62.41 ± 0.18	43.61 ± 0.10
Ash	5.16 ± 0.08	5.82 ± 0.03
Protein	19.32 ± 0.18	26.63 ± 0.14
Lipid	22.51 ± 0.11	25.83 ± 0.70
Carbohydrate	4.31 ± 0.03	7.22 ± 0.05
Oil content	18.40 ± 0.09	23.11 ± 0.15

Results represent the mean ± SEM of five replications

Table 2. Mineral Elemental Composition of *Oryctes Monoceros* Larva

Mineral elements	Composition (mg/100g)
Sodium, Na	684.23 ± 0.48
Calcium, Ca	0.42 ± 0.03
Potassium, K	26.13 ± 0.12
Magnesium, Mg	136.27 ± 0.08
Manganese, Mn	2.36 ± 0.13
Iron, Fe	12.14 ± 0.04
Copper, Cu	1.09 ± 0.13

Values represent the mean ± SEM of five replicate results

Table 3. Sporulation of Fungi Grown on *Oryctes monoceros* Larva and PDA media two weeks after incubation

Fungi	Larva Mean sporulation (10^4/0.1	PDA Mean sporulation (2mm agar disc)
Ceratocystis paradoxa	3.52 ± 0.05 *	2.30 ± 0.03 *
Glomerella cingulata	3.15 ± 0.13 *	1.83 ± 0.11
Trichoderma harzianum	4.80 ± 0.07 *	3.41 ± 0.09 *
Fusarium Oxysporium	4.52 ± 0.22 *	2.72 ± 0.13 *
Byssochlamys nivea	3.32 ± 0.14 *	2.36 ± 0.09

*Values are mean ± SEM of five replicate results. Fungus (T. harzianum 4.80) with the highest sporulation was compared to others using t – test. Values followed by * in the same column indicate non significant difference at p = 0.05.*

Table 4. Sporulation of Fungi on *Oryctes monoceros* Larva and PDA media six Months after incubation

Fungi	Larva Mean sporulation (10^4/0.1	PDA Mean sporulation (2mm agar disc)
Ceratocystis paradoxa	1.11 ± 0.22	1.33 ± 0.09 *
Glomerella cingulata	0.63 ± 0.13	1.81 ± 0.03*
Trichoderma harzianum	1.76 ± 0.03 *	2.35 ± 0.11*
Fusarium Oxysporium	0.34 ± 0.09	1.72 ± 0.03*
Byssochlamys nivea	0.63 ± 0.11	1.25 ± 0.05*

*Values are mean ± SEM of five replicate results. Fungus (T. harzianum 2.35) with the highest sporulation was compared to others using t – test. Values followed by * in the same column indicate non significant difference at p = 0.05.*

The presence of Fe, Zn and Mn in the larva plays functional role and general functions in the cells of these funfi and yeast. This agrees with Toder 2009 who reported that the nutritional composition of a bacterium such as *Escherichia coli* consists of K, Mg, Fe, Mn and traces of Zn, Cu, Co and Mo. The presence of these major elements in larva not only serves as growth factor but makes it more suitable for the cultivation of various fungi. Sharma and Sharma [9] suggested that culture media significantly affected the growth, sporulation and conidia discharge. The high nutritional composition and mineral elements in the larva makes it a complex culture medium suggesting it stimulates growth and it could form a base for new culture medium for fungi and yeast storage. This agrees with Ekpo and Onigbinde [10] on

the nutritional potentials of the larva of *Rhyncophorus phoenicis*, Edijala et al., [11] on *Rhyncophorus phoenicis* and *Oryctes monoceros* and Nzikou et al., [12] on larva consumed in Congo Brazzaville.

The larva culture of *O. monoceros* with respect to cost is cheaper when compared with the industrially produced PDA. The larva can be obtained locally and rearing of mass larvae could also be done locally with the availability of decaying palm trunks. This agrees with Aisagbonhi, [4] who reported that *Oryctes monoceros* breeds in decaying organic matter, such as felled rotting palms. The reason why *O. monoceeros* larva was not favoured for sporulation six months after incubation was not studied but Eziashi et al., [13], had earlier reported the effect and production of metabolites by fungal species for mycelial growth inhibition. These metabolic compounds could support or retard growth and sporulation in culture media.

4. CONCLUSION

The larva medium of *O. monoceros* stimulates sporulation and it could form a base for new culture medium. While *O. monoceros* larva medium exhibited better sporulation two weeks after incubation, potato dextrose agar medium exhibited better sporulation six months after incubation. In the absence of PDA, it is cheaper to use *O. monoceros* larva as a medium for fungi storage.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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