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Short Scientific Reports

Mass Production of the Entomopathogen *Metarhizium anisopliae* in Coconut Water Wasted from Copra Making Industry*

The fungus *Metarhizium anisopliae* (Metsch.) Sor. is an important pathogen of several insect pests including *Oryctes rhinoceros* (L.). Field application of the fungus effected successful suppression of the coconut rhinoceros beetle population in the south Pacific Islands (Catley, 1969; Marschall, 1969). Previously, the fungus was mass cultured in conventional laboratory media, such as, potato dextrose broth (PDB), Sabouraud's dextrose, grains of different cereals (Marschall, 1969; Latch, 1976) and used for field application. Till date, the cheapest medium used for mass production of *M. anisopliae* was cassava chips mixed with rice bran supplemented with urea or waste fish meal extract as nitrogen source (Mohan and Pillai, 1982). Coconut water, a still cheaper and easily available agricultural waste from copra making industry was tested for mass production of the fungus.

One hundred ml of each of the following coconut water media and potato dextrose broth (PDB) taken in 500 ml conical flasks were sterilized at 1.1 kg/cm^2 pressure in autoclave (unless otherwise mentioned). The coconut water used for the experiments had a pH 5.5 and the same was not altered unless it is specifically mentioned. The media used for the study were :

1. Potato dextrose broth (PDB): Composition : potato 200 g, glucose 20 g, distilled water 1000 ml, pH 6.5.

2. Aseptically drawn out coconut water from surface – sterilized mature nuts.

3. Coconut water collected from mature nuts from copra industry.

4. Coconut water collected from mature nuts of copra industry adjusted to pH 6.5.

5. Coconut water from copra making industry, sterilized by passing through G5 grade scintered–glass filter or 45 grade millipore filter.

6. Coconut water from copra making industry without sterilization, but mixed with filter (G5) sterile antibiotics *e. g.*, penicillin 100 IU/l, streptomycin 10mg/l, chloramphenicol 200 mg/l, gresiofulvin 20 mg/l, cyclohexiamide 200 mg/l either singly or in combinations. Parallel sets of controls for all the experiments were maintained.

Each flask was inoculated with a disc (5 mm dia.) of fungal mat of four–

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day-old plate cultures and incubated at 28 ± 2°C as still culture. Batches of 5 flasks were taken from each set of experiments at d10 intervals and 1 ml teepol was added to each. Flasks were shaken and spores counted under microscope with the help of a haemocytometer. Fungal growth was filtered on a preweighed filter paper disc and dried to a constant weight at 80°C. Data were corrected from the results obtained from appropriate controls.

Vegetative growth of *M. anisopliae* was highest (0.73 g/100 ml) in autoclaved coconut water (pH 5.5), followed sequentially by filter sterile coconut water, autoclaved coconut water (pH 6.5), aseptically drawn out coconut water and PDB on d10 incubation (Table I). But, on d10, highest number of spores (10.75×10^6/ml) was produced in filter sterile coconut water, followed by aseptically drawn out coconut water (Table II). Remaining media produced only about one half or less number of spores (Table II).

After d20, maximum vegetative growth was recorded in autoclaved coconut water, pH 5.5 (1.00 g/100 ml), followed by aseptically drawn out coconut water (0.97 g/100 ml), autoclaved coconut water, pH 6.5 (0.91 g/100 ml), filter sterile coconut water and PDB (0.79 g/100 ml) (Table I). Maximum and minimum number of spores were produced in aseptically drawn out coconut water (29.74×10^6/ml) and PDB (7.17×10^6/ml) on d20 (Table II). Sporulation in the remaining media did not vary much (Table II).

On d30, both vegetative growth (1.56 g/100 ml) and sporulation (46.66×10^6/ml) were highest in aseptically drawn out coconut water (Tables I, II). Though vegetative growth of the fungus in other coconut water media (except that of pH 6.5) did not vary greatly from that of the former medium (Table I), production of spores in the latter media was comparatively less (Table II). The vegetative growth (Table I) and sporulation (Table II) in autoclaved coconut water (pH 6.5) were not parallel to those of the aseptically drawn out coconut water (Tables I, II). Vetetative growth (1.15 g/100 ml) and spore production (18.72×10^6/ml) in PDB were too less *i. e.*, about 0.74

Table I. *Growth (g dr. wt. of mycelium / 100 ml medium) of* M. anisopliae *in different medium in different days*

Medium	Days			Mean
	10	20	30	
Autoclaved CW (pH 5.5)	0.73	1.00	1.46	1.06
Filter sterile CW (pH 5.5)	0.65	0.83	1.45	0.98
Aseptically drawn out CW (pH 5.5)	0.59	0.97	1.56	1.04
Autoclaved CW (pH 6.5)	0.60	0.91	1,33	0.95
Potato dextrose broth (pH 6.5)	0.50	0.79	1.15	0.81
Mean	0.62	0.90	1.39	0.97

SE/Plot = 0.04 CV(%) = 3.96
CD (P = 0.05) for medium = 0.026
CD (P = 0.05) for days = 0.020
CD (P = 0.05) for medium × days = 0.045

Results are the means of 5 replications. Unsterile coconut water containing antibiotics (See text) did not support growth of the fungus.

CW = Coconut water. pH 5.5 = natural pH of coconut water.

Table II. *Spore production (× 10⁶/ml) of* M. anisopliae *in different media on different days*

Medium	Days			Mean
	10	20	30	
Autoclaved CW (pH 5.5)	5.55	24.25	34.68	21.49
Filter sterile CW (pH 5.5)	10.75	27.18	41.03	26.32
Aseptically drawn out CW (pH 5.5)	9.56	29.74	46.66	28.65
Autoclaved CW (pH 6.5)	4.32	24.07	33.62	20.60
Potato dextrose broth (pH 6.5)	4.48	7.17	18.72	10.12
Mean	6.93	22.48	34.90	21.22

SE/Plot = 0.078 CV(%) = 3.65
CD (P = 0.05) for medium = 0.571
CD (P = 0.05) for days = 0.442
CD(P = 0.05) for medium × days = 0.989
Others same as table I.

and 0.40 times, respectively, than those of the aseptically drawn out coconut water.

Both coconut water and potato are sources enriched with all common types of carbohydrates, proteins, fats, amino acids, vitamins and minerals (Aykroyd, 1963), So, superiority of coconut water over PDB for both mycelial growth (Table I) and sporulation (Table II) of *M. anisopliae* could be due to qualitative variations of some cofactors or minor chemicals, which are preferred by the fungus. Unlike PDB, the metabolites of coconut water are either in solution or suspended in liquid endosperm. So, easy availability of common metabolites also can enhance vegetative and reproductive growth of the fungus. Oppositely, any organism requires to produce number of enzymes to utilize the complex medium like PDB and lesser growth is not unlikely. Coconut water contains, about 2.5% (average) total sugars (Aykroyd, 1963) comparable to 2% glucose added to PDB. It suggests that though potato after storage contains 3–10% sugars (Aykroyd, 1963), they are not freely available in the medium, otherwise, addition of glucose would have been avoided. The same idea could be extended for other constituents also. Like *M. anisopliae,* other microorganisms *e. g.,* yeast also can excellently use coconut water and is exploited for commercial production of single cell protein (Grimwood, 1975). Generally, sporulation of any organisms is initiated after cessation of active vegetative growth due to depletion of nutrients. So, variation of vegetative growth and sporulation in different media within any stipulated time (Tables I, II) suggests that nutritional status of different media for *M. anisopliae* is variable. Similar relationship of variation of nutrition and production of reproductive bodies is well known in fungi *viz., Cordyceps militaris* produced perithecia on dead pupae, but not on autoclaved one (Shanor, 1936) and *Beauvaria bassiana* produced higher numbers of spores on Sabouraud maltose agar, followed by Mollisch medium, blood agar, Raulin-Thomb and Czapek-Dox, and PDB and cornmeal agar (Macleod 1954). In *M. anisopliae* also almost the same trend was noticeable (Table II).

Though vegetative growth of the fungus did not vary much in different coconut water media (Table I) production of lesser numbers of spores in

autoclaved coconut water than those in aseptically drawn out coconut water or filter sterile coconut water (Table II) indicates that the chemicals which favour sporulation are vulnerable to autoclaving. Less growth and sporulation in autoclaved coconut water at pH 6.5 than those at pH 5.5 (Tables I, II) suggest that increase in pH of coconut water has more detrimental effect on the nutritional quality of the medium for *M. anisopliae*. Similarly, weaker performance of filter sterile coconut water than aseptically drawn out coconut water (Tables I, II) might be due to loss of some of the suspended constituents during filtration. Highest number of spores produced in aseptically drawn out coconut water (150% over PDB) than in all other coconut water media on d30 (Table II) supports the view. These results clearly reveal that aseptically drawn out coconut water is superior and the cheapest medium for mass production of the fungus *M. anisopliae*.

REFERENCES

AYKROYD, W. R. 1953. *The Nutritive Value of Indian Foods and Planning of Satisfactory Diets.* (Revised 6th. edn. by Gopalan, C. and Balasubramanian, S. C). ICAR, New Delhi, pp. 255.

CATLEY, A. 1969. The cocount rhinoceros beetle *Oryctes rhinoceros* L. *Pans.* **15**: 18–30.

GRIMWOOD, B. E. 1975. *Coconut Palm Products–Their Processing in Developing Countries. FAO agric. Dev. Paper, No. 99.* FAO, Rome, pp. 261.

LATCH, G. C. H. 1976. Studies on the susceptibility of *Oryctes rhinoceros* to some entomogenous fungi. *Entomophaga.* **21**: 31–38.

MACLEOD, D. M. 1954. Investigations on the genera *Beauveria* Vuill. and *Tritirachium* Limber. *Can. J. Bot.* **32**: *818–890.*

MARSCHALL, K. J. 1969. Report of the insect pathologist (Project area) UNDP (SF)/SPC project for research on the control of the coconut palm rhinoceros beetle. Semi-annual report of the Project Manager for the Period June, 1969–November, 1969 (Mimeographed) pp. 18–26.

MOHAN, K. S and PILLAI, G. B. 1982. A method for laboratory-scale mass cultivation of *Metarhizium anisopliae. Folia Microbiologica.* **27**: 281–283.

SHANOR, L. 1936. The production of mature perithecia of *Cordyceps militaris* (Linn.) Link. in laboratory culture. *J. Elieha Mitchell Sci. Soc.* **52**: 99–104.

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