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# Mass Culturing of *Hirsutella thompsonii* Fisher a Fungal Pathogen of Eriophyid Mites

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**Abstract:** For the production of *Hirsutella thompsonii* Fisher isolates, a fungal pathogen of eriophid mites, the synthetic media and unprocessed materials were tested in submerged cultures. The liquid media, Sabouraud Maltose Broth (SMB), was found to be better in terms of mycelial production compared to other liquid media like Sabouraud Dextrose Broth (SDB), Yeast Extract Dextrose Peptone (YDP) and Potato Dextrose Broth (PDB). Maximum growth was observed in HtCRMB isolate of *H. thompsonii* in submerged culture followed by HtPDBC, HtCRMC, HtPMG, HtCRMK and HtEMC isolates. Mycelial growth on the synthetic medium SMB was about ten times than on media prepared using cheap, crude and unprocessed materials like Bengal gram, horse gram, red gram and maize as source of nitrogen, barley, sorghum, rice and wheat as source of carbon and combination of nitrogen and carbon sources. In submerged cultures more mycelial growth was observed in HtCRMB isolate of *H. thompsonii* than in other isolates.

**Key words:** *Hirsutella thompsonii*, mass production, biological control

## INTRODUCTION

The fungus, *Hirsutella thompsonii* Fisher, a fungal pathogen of eriophyid mites, causes spectacular natural epizootics in populations of citrus rust mite, *Phyllocoptruta oleivora* (Ashmead) (Acari: Eriophyidae) (Muma, 1955; McCoy, 1981).This mite is an important mite pest of citrus in most of the humid tropical regions of the world (McCoy and Albrigo, 1975; Childers *et al*., 1996). The efficacy of *H. thompsonii* to control citrus rust mite in USA, Surinam, Israel, and China has been recorded to be very promising (McCoy, 1978, 1996; Van Brucell, 1975; Gerson *et al*., 1979).

*H. thompsonii* has been cultured on agar media including potato dextrose, Modified Soil Fungus (SFM), Sabouraud-dextrose (McCoy and Kanavel, 1969); potato carrot (Kenneth *et al*., 1979); Sabouraud maltose-peptone grapefruit and egg yolk agar (Van Brussel, 1975). Growth and sporulation on these media are rather slow, maximum vegetative growth occurs on media high in carbon, and maximum condition on nutrient-deficient agar (McCoy and Kanavel, 1969). McCoy *et al.* (1972) reported that dextrose and sucrose at an optimum concentration of 5 and 10 mg $mL^{-1}$, respectively, were the most effective sources of carbon among sugars tested for use in the large-scale production in submerged culture. Inorganic nitrogen in a basal dextrose salts medium was unsuitable, but a combination of yeast extract and peptone at an optimum concentration of 5 and 0.5 mg $mL^{-1}$, respectively, was the most effective source of organic nitrogen.

McCoy *et al.* (1978) have reported that two percent molasses was the superior carbon source and 8.00% soybean meal the superior nitrogen source. In combination these gave significantly greater yield than other media including cerelose, sucrose and starch for carbon sources and soy flour, corn steep liquor, urea and yeast extract + peptone for nitrogen sources. Vey *et al.* (1993) reported that mean vegetative growth response of *H. thompsonii* was 1.8 g/100 mL (dry wt.) in Czapek-Dox plus 1% yeast extract, 0.6 g/100 mL in Czapek-Dox plus peptone and 0.3 g 100 mL in SFM broth after 10 days on a gyrator water bath shaker maintained at 25°C. According to the findings of above mentioned authors, *H. thompsonii* could be grew on media with different concentration of carbon and nitrogen sources and fungal growth has been recorded among them. The main objective of this study was using some grains as sources of carbon and nitrogen which are locally available, cheap, crude and more easily accessible in a cost effective manner on other substrates. Therefore the present investigation was carried out to comparative study on production of six isolates of *H. thopsonii* in different synthetic media and unprocessed materials in submerged culture.

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Table 1: Name and origin of the isolates of *H. thompsonii*

| Name | Origin of the isolates |
|---|---|
| HtCRMB | *P. oleivora* on citrus fruits in Bangalore, Karnataka |
| HtPMG | *Aceria guerreronis* on palmyra nuts in Govindapuram, Tamil Nadu. |
| HtCRMC | *P. oleivora* on citrus fruit in Chettahalli, Karnataka |
| HtCRMK | *P. oleivora* on citrus leaf in Kolkata, West Bengal |
| HtEMC | Obtained from the Microbial Technology Institute, Chandigarh, Panjab |
| HtPDBC | *A. guerreronis* on coconut, sprayed with Mycohit (product by Project Directorate Biological Control (PDBC)) in Bangalore, Karnataka |

Table 2: Dry mycelial weight (g/50 mL) of six isolates of *H. thompsonii* on the four different liquid media, twenty days after inoculation

| Isolate medium | HtCRMC | HtEMC | HtCRMK | HtPDBC | HtPMG | HtCRMB | Mean |
|---|---|---|---|---|---|---|---|
| SMB | 0.700 | 0.705 | 0.856 | 0.696 | 0.554 | 0.721 | $0.705^{a}$ |
| SDB | 0.678 | 0.423 | 0.469 | 0.732 | 0.551 | 0.852 | $0.618^{b}$ |
| PDB | 0.305 | 0.208 | 0.258 | 0.376 | 0.319 | 0.494 | $0.327^{d}$ |
| YDP | 0.525 | 0.190 | 0.239 | 0.771 | 0.531 | 0.629 | $0.480^{c}$ |
| Mean | $0.552^{f}$ | $0.382^{h}$ | $0.456^{g}$ | $0.644^{e}$ | $0.489^{fg}$ | $0.673^{e}$ | |

SMB : Sabouraud Maltose Broth; SDB : Sabouraud Dextrose Broth; PDB : Potato Dextrose Broth; YDP : Yeast extract Dextrose Peptone
Means with same superscript are not significantly different at p = 0.05 according to Duncan's multiple range test in column and row, separately

## MATERIALS AND METHODS

**Culturing of *H. thompsonii* isolates on synthetic media:** Six isolates of *H. thompsonii* (Table 1) were tested in four different liquid media, with three replication to determine the best medium for the growth of the fungus isolates as per the method described by Winkelhoff and McCoy (1984). These isolate were cultured on Potato Dextrose Agar (PDA) for two weeks in the incubator at 25°C and used as inoculation source of media, separately.

The different synthetic media were prepared with the following ingredients:

- Potato Dextrose Broth (PDB): potato dextrose powder (24 g), distilled water (1L)
- Sabouraud Dextrose Broth (SDB): sabourou dextrose powder (30 g), distilled water (1L)
- Sabouraud Maltose Broth (SMB): sabouraud maltose powder (50 g), distilled water (1L)
- Yeast Extract Dextrose Peptone (YDP): yeast extract (15 g), dextrose (20 g), peptone (0.5 g), $KH_2PO_4$ (1.5 g), $MgSO_4$ (0.5 g), $CaCl_2$ (0.1 g) , distilled water (1 L)

After the ingredients were mixed in the above proportions, the pH was adjusted to 6-7. Fifty milliliters of each medium was transferred to a 250 mL Erlenmeyer flask stoppered with cotton plugs and sterilized at 120°C for 15 min under 15 lb pressure. Following this, these flasks were inoculated with 5 mm dia. mycelial mat from the active growing region of the two weeks old fungus cultured on PDA, flasks were incubated at 28°C on a shaker at 160 rpm for 2 days, then the flasks were transferred to an incubator maintaining 28°C. After 11-20 days of incubation, the mycelia were harvested from the liquid medium by filtering the liquid through a filter paper (Watman No. 1) which had been dried for 24 h at 60°C and weighed.

**Culturing *H. thompsonii* isolates on grain extracts:** To know whether this fungus could be mass produced in a cost effective manner on other substrates the following studies were conducted:

In the first study with nine treatments and three replications, sorghum, wheat, barley, rice and maize formed the source of carbon, while bengal gram, horse gram and red gram, formed the source of nitrogen, these were compared with Sabouraud Maltose Broth (SMB). Five grams of each material in 250 mL distilled water was boiled for 15 min and filtered using a filter paper, the filtrate was collected and the volume was made up.

In the second study, the media in the above experiment which produced more mycelial mat were selected and a combination of the carbon and nitrogen sources including: sorghum + bengal gram, sorghum + barley, sorghum + soybean, soybean, soybean + bengal gram, soybean + barley, bengal gram + barley and SMB were tested with three replications. In both these experiments the method followed was same as mentioned above.

These experiments were conducted with the six isolates of the fungus, and three replications each. The data were analyzed statistically for comparing treatments following the analysis of variance (ANOVA) technique and the results are interpreted at five percent level of significance. This study was conducted at the Department of Plant Pathology, University of Agricultural Sciences, Bangalore, India during 2001-2002.

## RESULTS AND DISCUSSION

**Production of *H. thompsonii* on synthetic liquid media:** Maximum fungal growth (0.71 g dry wt./50 mL) was observed on SMB medium for submerged culture twenty days after inoculation (Table 2) compared to other liquid media like SDB (0.6 g/50 mL), YDP

Table 3: Dry mycelial weight (g/50 mL) of HtCRMB isolate of *H. thompsonii* on nine liquid media as submerged culture, eleven days after incubation

| Media | Mean dry wt.±SE |
|---|---|
| SMB | 0.273±0.016[a] |
| Bengal gram | 0.033±0.007[b] |
| Barley | 0.033±0.002[b] |
| Horse gram | 0.027±0.002[b] |
| Sorghum | 0.027±0.005[b] |
| Red gram | 0.023±0.002[b] |
| Rice | 0.023±0.002[b] |
| Wheat | 0.017±0.002[b] |
| Maize | 0.017±0.002[b] |

SMB: Sabouraud Maltose Broth, Means with same superscript are not significantly different at p = 0.05 according to Duncan's multiple range test

Table 4: Dry mycelial weight (g/50 mL) of HtCRMB isolate of *H. thompsonii* on eight liquid media of submerged culture 14 days after inoculation

| Media | Mean dry wt.±SE |
|---|---|
| SMB | 0.317±0.006[a] |
| Soybean + barley | 0.033±0.021[b] |
| Soybean + Bengal gram | 0.033±0.006[b] |
| Sorghum + Bengal gram | 0.030±0.010[b] |
| Soybean | 0.027±0.006[b] |
| Bengal gram + barley | 0.023±0.006[b] |
| Sorghum + barley | 0.023±0.006[b] |
| Soybean + sorghum | 0.023±0.006[b] |

SMB : Sabouraud Maltose Broth; Means with same superscript are not significantly different at p = 0.05 according to Duncan's multiple range test

(0.48 g/50 mL) and PDB (0.33 g/50 mL). Difference in fungal growth among the four different synthetic media were significant statistically. The results supported the views of McCoy *et al.* (1972) that found dextrose and sucrose at 5 and 10 mg $mL^{-1}$, respectively, were the most effective source of carbon (414.36 and 433.12 mg/50 mL mean dry wt. of mycelia) respectively, among sugars tested, for use in the production of *H. thompsonii* in submerged culture. A combination of yeast extract and peptone at 5 and 0.5 mg $mL^{-1}$, respectively was the most effective source of organic nitrogen (659.9 mg mean dry wt./10 mL). Although excellent growth occurred over a range of pH values from 6.0 (537 mg/100 mL) to 9.2 (573 mg/100 mL) the optimum pH appeared to be 7.5 (693.3 mg mean dry wt./100 mL). Vey *et al.* (1993) reported that by comparison mycelial production by *H. thompsonii* var. *thompsonii* in different culture media differed significantly after shaking for 10 days at 25°C. The mean vegetative growth was 1.8 g/100 mL (dry wt.) in Czapek-Dox Plus 1% yeast extract, 0.6 g/100 mL Czapek-Dox plus peptone and 0.3 g/100 mL in SFM broth, which supports present study.

The six isolates of *H. thompsonii* grew in these media successfully and there was a significant difference in fungal growth among them (Table 2). Growth in HtCRMB isolate of the fungus (0.67 g dry wt./50 mL) was better, followed by HtPDBC (0.64 g/50 mL), HtCRMC (0.52 g/50 mL), HtPMG (0.49 g/50 mL), HtCRMK (0.46 g/50 mL) and HtEMC (0.38 g/50 mL) isolates. These findings concor with those of Winkelhoff and McCoy (1984) who reported that fourteen isolates of *H. thompsonii* grew vegetatively in submerged cultures of various media, however, corn steep liquor medium produced 15.8 mg mean dry weight of mycelia per milliliter after ten days of incubation. A large scale method for producing this fungus in submerged culture by McCoy *et al.* (1975), in 12 liters of medium contained dextrose, yeast extract, a peptone add minera salts, an average of 400 grams (wet wt.) mycelia were produced after 96 h inoculation.

**Production of *H. thompsonii* isolate on grain extracts:** There was significant difference in mycelial growth of HtCRM isolate of *H. thompsonii* as submerged culture, eleven days after inoculation among the nine media (Table 3). Growth on SMB was best (0.27 g dry wt./50 mL) that on other eight submerged culture media. Fungal growth on other media did not differ significantly, as sources of carbon and nitrogen for the fungus. Among the nitrogen sources tested, bengal gram supported 0.033 g mycelia/50 mL and followed by horse gram (0.027), red gram (0.023) and maize (0.017 g/50 mL) and among the carbon sources barley was best (0.033 g dry wt mycelia/50 mL) followed by sorghum (0.027), rice (0.023) and wheat (0.017 g dry wt./50 mL). There was significant difference in fungal growth among the eight different submerged culture media fourteen days after inoculation. On SMB the fungal growth (0.32 g dry wt./50 mL) was higher than on the other seven media. The mycelial growth on other media which had combinations of carbon and nitrogen sources did not differ significantly, the mycelial growth was 0.033, 0.033, 0.030, 0.027, 0.023, 0.023 and 0.023 g dry wt./50 mL on soybean + barley, soybean + bengal gram, sorghum + bengal gram, soybean, bengal gram + barley, sorghum + barley and soybean + sorghum, respectively (Table 4).

Thus, SMB is a superior medium for production of *H. thompsonii* in submerged culture. HtCRMB isolate of *H. thompsonii* produced more mycelial growth than other isolates in submerged culture. Similarly the fungal growth on SMB liquid medium was better than on liquid culture media prepared using different sources of nitrogen and carbon. The results revealed for using grain extracts in this study were disagree to that reported by McCoy *et al.* (1978) for production of *H. thompsonii*, 2.00% molasses was the best carbon source and 8.00 percent soybean meal the best nitrogen source. In combination they gave a significantly greater yield (1200 mg/100 mL) than that in the dextrose-yeast-peptone medium (800 mg/100 mL) which had been earlier used in mass production by McCoy *et al.* (1972). Although, the used media were different in these studies.

The synthetic medium SMB encouraged mycelial growth which was about ten times than the media prepared using cheap, crude and unprocessed materials. But it would not be reasonable not to use these materials for culturing this fungus, although using synthetic media like SMB with respect to material cost is reasonable. Since the grains used are locally available, they are more easily accessible by small entrepreneurs. Hence, further studies are required to obtain more information. The present results suggest that sabouraud maltose broth as a suitable medium for rearing of *H. thompsonii* according to the described protocol can be a good method for mass production of this pathogen for using in biological control of citrus rust mite.

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## REFERENCES

Childers, C.C., M.A. Eastbrook and M.G. Solmon, 1996. Chemical control of eriophyoid mites. In: Lindquist, E.E., M.W. Sabelis and J. Bruin (Eds.), Eriophyoid mites-their biology, natural enemies and control. Elsevier sciences publ. Amsterdam, The Netherlands, pp: 695-719.

Gerson, U., R. Kenneth and T.I. Muttath, 1979. *Hirsutella thompsonii* a fungal pathogen of mites. II. Host-pathogen interaction. Ann. Appl. Biol., 91: 29-40.

Kenneth, R., T.J. Muttath and U. Gerson, 1979. *Hirsutella thompsonii* a fungal pathogen of mites. I. Biology of the fungus *in vitro*. Ann. Applied Biol., 91: 21-28.

McCoy, C.W. and L.G. Albrigo, 1975. Feeding injury to the orange caused by the citrus rust mite *Phyllocoptruta oleivora* (Prostigmata, Eriophyoidea) Ann. Ent. Soc. Am., 68: 289-297.

McCoy, C.W. and R.F. Kanavel, 1969. Isolation of *Hirsutella thompsonii* from the citrus rust mite, *Phyllocoptruta oleivora* and its cultivation on various synthetic media. J. Invertebr. Pathol., 14: 386-390.

McCoy, C.W., 1978. Activity of dimilin on the developmental stages of *Phyllocoptruta oleivora* and its performancee in the field. J. Econ. Entomol., 71: 122-124.

McCoy, C.W., 1981. Fungi: Pest Control by *Hirsutella thompsonii*. In: Burges H.D., (Ed.), Microbial Control of Insects, Mites and Plant Diseases. Academic Press, London, UK, pp: 499-512.

McCoy, C.W., 1996. Pathogens of eriophyoid mites. In: E.E. Lindquist, M.W. Sabelis and J. Bruin, (Ed.) Eriophyoid mites, their biology, natural enemies and control. Elsevier Sciences Publ., Amsterdam. The Netherlands, pp: 481-490.

McCoy, C.W., T.L. Couch and R. Weatherwax, 1978. A simplified medium for the production of *Hirsutella thompsonii*. J. Invert. Pathol., 31: 137-139.

McCoy, C.W., A.J. Hill and R.F. Kanavel, 1972. A liquid medium for the large-scale production of *Hirsutella thompsonii* in submerged culture. J. Invertebr. Pathol., 19: 370-374.

McCoy, C.W., A.J. Hill and R.F. Kanavel, 1975. Large-scale production of the fungus pathogen *Hirsutella thompsonii* in submerged culture and its formulation for application in the field. Entomophaga, 20: 229-2740.

Muma, M.H., 1955. Factors contributing to the natural control of citrus insects and mites in Florida. J. Econ. Entomol., 48: 432-438.

Van Brussel, E.W., 1975. Interrelations between citrus rust mite, *Hirsutella thompsonii* and greasy spot on citrus in Surinam. Landbouwproefst. Surinam Agric. St. Bull., 98: 43.

Vey, A., J.M. Quiot, I. Mazet and C.W. McCoy, 1993. Toxicity and pathology of crude broth filtrate produced by *Hirsutella thompsonil* var. *thompsonii* in shake culture. J. Invertebr. Pathol., 61: 131-137.

Winkelhoff, A.J.V. and C.W. McCoy, 1984. Conidiation of *Hirsutella thompsonii* var. *synnematosa* in submerged culture. J. Invertebr. Pathol., 43: 59-68.