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Management of mycoflora in copra using chemicals and biocontrol agents

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Coconut palm (*Cocos nucifera* L.), a perennial horticultural crop, is a symbol of national and international integration involving more than 93 producing countries with an area of 12.8 million ha and production of 10.9 million tones of copra equivalent and more than 140 consuming countries (Madhavan *et al*., 2010). Coconut is mainly grown for the high oil content of the endosperm (copra) which is an important source of vegetable oil used in the manufacturing of edible oils, margarine, cooking fat and soaps (Van Dam, 2005; Rajarajan *et al.,* 2009). A number of value added products are manufactured from coconut. At present, India is the largest producer of coconut in the world with an annual production of 1,49,40,000 MT (2011-12) (http:/www.coconutboard.gov.in/stat.htm). Coconut oil accounts for about 7.8 per cent of the total edible oils production in the country with a production of 4.4 lakh tones. In addition, India imports around 2000-7000 tonnes of crude coconut oil. Before extracting oil form the copra, it has to be dried, but drying in the winter poses problems like the growth of molds and subsequent rotting of the copra. Common salt is widely used to smear the copra to arrest the mold growth. In the present study, attempt was made to assess the effect of some chemical preservative and biocontrol agents on the growth of mycoflora of copra. The outcome of this study will largely benefit the resource poor small farmers and small sized copra processing and oil extracting units throughout the coconut producing countries in the world.

Copra samples were collected from the farm of Coconut Research Station, Veppankulam, Tamil Nadu, India. The samples were collected from the cultivar, East Coast Tall (ECT). The mycoflora associated with copra were isolated using potato dextrose agar (PDA) medium. Copra was sliced into small pieces, washed in sterile distrilled water and plated in Petri dishes containing PDA medium. The plates were incubated at 26±2 ^{o}C for 7 days in incubator.

Fungal growth from the copra slices were transferred to PDA plates and were identified on the basis of colour of hyphae, shapes of the spores and morphology of the spores and morphology of the mycelium produced, using light microscope. Pure cultures of the fungi were prepared and preserved in PDA slants in the refrigerator for further studies.

Mycelial dics of 6 mm diameter of mycoflora of copra were placed in Petri dishes (90 mm dia) containing PDA medium using flame sterilized cork borer in one end and the same diameter mycelia disc of *Trichoderma viride* was placed at the opposite end of the plate and incase of *Pseudomonas fluorescens* a loopful of culture was streaked. Plates without biocontrol agents served as control. Five replications were maintained for

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each mycoflora and for each biocontrol agent. The plates were incubated at room temperature (28 ± 2 °C) for 7 days and the growth of mycoflora and biocotnrol agents were measured. Per cent inhibition was worked out using the following formula

$$\text{Per cent inhibition} = \frac{\text{Control} - \text{Treated}}{\text{Control}} \times 100$$

Twenty five milli litre of one week old culture filtrate (10^6 spores mL^{-1}) of *T. viride* was poured into conical flasks (250 mL capacity) containing 100 mL of sterilized molasses yeast extract broth for testing the efficacy of culture filtrate of *T. viride* in arresting the biomass production of mycoflora of copra and for *P. fluorescens* 25 mL of three days old bacterial suspension ($2x10^8$ cfu mL^{-1}) was poured into conical flasks (250 mL capacity) containing 100 mL of sterilized King's Broth. Six millimeter mycelial discs of one week old mycoflora of copra were inoculated into the flasks and incubated at room temperature for one month. Flasks without inoculation of culture filtrate of *T. viride* and bacterial suspension of *P. fluorescens* and inoculated with mycelial discs of mycoflora of copra served as control. Five replications were maintained for each mycoflora and for each biocontrol agent. The biomass was filtered, dried and the dry weight was recorded and the per cent inhibition was worked out.

Citric acid, acetic acid and sodium chloride were incorporated in PDA medium at 1000 ppm concentration and poured into the Petri plates. Six millimeter diameter mycelia discs of one week old mycoflora of copra were placed in the centre of the plates and incubated at room temperature. Plates without the incorporation of preservatives and inoculated with respective mycoflora served as control. The plates were incubated until the control plates were fully grown by the mycoflora, the growth was measured and the per cent inhibition was worked out. Five replications were maintained for each preservative and for each fungus. All the experiments were conducted in a factorial manner in completely randomized design (CRD). The results were analysed by means of analysis of variance (ANOVA).

The results on the management of mycoflora of copra with the help of biocontrol agents and chemical preservatives are presented in Tables 1, 2 and 3. *Aspergillus flavus, A. niger, Botryodiplodia theobromae*, *Penicillium* sp and *Rhizopus stolonifer* were the common mycoflora isolated from copra. The fungal biocontrol agent, *T. viride* inhibited the growth of all the mycoflora isolated from copra in the present study. The inhibitory effect was more pronounced in liquid medium than in solid medium. In solid medium, the highest inhibition of 67.8 per cent was observed with respect to *Penicillium* sp. as against the lowest of 55.6 per cent in case of *B. theobromae* (Table 1). However, the inhibition of growth of various mycoflora of copra by *T. viride* does not differ significantly from each other. On the other hand, the inhibitory effect is statistically significant with regard to various mycoflora. Maximum of 93.5 per cent inhibition of growth of *A. flavus* was observed in liquid medium by *T. viride*.

Table 1. Effect of *T. viride* on the growth of mycoflora of copra

Name of the mycoflora	Solid medium			Liquid medium		
	Mycelial growth (mm)		Inhibition	Dry weight biomass (g)		Inhibition
	Treated	Control	(%)	Treated	Control	(%)
A. flavus	38	90	57.8	0.06	0.92	93.5
A. niger	33	90	63.3	0.16	1.08	85.2
B. theobromae	40	90	55.6	0.07	0.89	92.1
Pencillium sp.	29	90	67.8	0.19	1.34	85.7
R. stolonifer	32	90	64.4	0.15	0.91	83.7
CD (P=0.05)	NS	NS	NS	0.02 **	0.12 **	2.2 **

Table 2. Effect of *P. fluorescens* on the growth of mycoflora of copra

Name of the mycoflora	Solid medium			Liquid medium		
	Mycelial growth (mm)		Inhibition	Dry weight biomass (g)		Inhibition
	Treated	Control	(%)	Treated	Control	(%)
A. flavus	21	55	60.5	0.20	1.80	88.7
A. niger	22	56	60.8	0.11	2.34	95.2
B. theobromae	19	60	67.5	0.23	0.92	74.6
Pencillium sp.	22	57	61.3	0.17	1.99	91.5
R. stolonifer	20	50	60.0	0.15	0.37	59.3
CD (P=0.05)	NS	NS	NS	0.02	0.11	4.6

Table 3. Effect of chemical preservatives on the growth of mycoflora of copra

Name of the mycoflora	Mycelial growth (mm)				Growth inhibition (%)		
	Citric acid	Acetic acid	Sodium chloride	Control	Citric acid	Acetic acid	Sodium chloride
A. flavus	53	66	70	90	41.1	26.7	22.2
A. niger	51	60	73	90	43.3	33.3	18.9
B. theobromae	41	55	72	90	54.4	38.9	20.0
Pencillium sp.	48	70	78	90	46.7	22.2	13.3
R. stolonifer	60	64	80	90	33.3	28.9	11.1
CD (P=0.05)	NS	9.20	NS	NS	NS	10.2	NS

The effect of the bacterial biocontrol agent, *P. fluorescens* is depicted in Table 2. The inhibitory effect was more in liquid medium compared to solid medium. In solid medium, 67.4 per cent inhibition was noticed in the growth of *B. theobromae* whereas; in liquid medium 95.2 per cent inhibition was recorded in *A. nige*r. The above results indicate that these two biocontrol agents can be effectively used for managing the mycoflora of copra. All the three chemical preservatives namely citric acid, acetic acid and sodium chloride showed inhibitory effect (Table 3). Per cent inhibition of growth of various mycoflora by citric acid ranged from 33.3 per cent (*R. stolonifer*) to 54.4 per cent *(B. theobromae*). Acetic acid caused a maximum of 38.9 per cent inhibition of *B. theobromae* while, sodium chloride showed 22.2 per cent inhibition of *A flavus.* Onuegbu and Ihuanne (1994) showed a reduction in growth of *A. niger, Pencillium* sp. and *Clalosphorium* sp., isolated from palm oil, by common salt. Similar to the observations made in the present study, acetic acid and citric acid exhibited varying degrees of inhibitory action against *A. niger, A. flavus, Rhizopus* sp. and *Penicillium* sp. under *in vitro* conditions (CPCRI, 2009).

A. flavus, A. niger, B. theobromae, Penicillium sp and *R. stolonifer* are the common mycoflora associated with copra. Biocontrol agents, *P. fluorescens* and *T. viride* were found to be promising in inhibiting the growth of mycoflora of copra. Chemical preservatives *viz.,* acetic acid, citric acid and sodium chloride can be used for the management of mycoflora of copra.

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