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Effect of herbal spray for coconut copra process, storage and Post-harvest disease management

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Abstract
India is the leading country in copra production and export. The process of copra making a fungal infection is the major problem and reduces the yield. Most of the copra suppliers using sulphur for fungal eradication. In the present study using herbal spray instead of sulphur for fungal eradication. Three types of fungus were isolated from infected copra. This fungus were identified as *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus stolonifer* under microscope using Anneline blue staining. The leaf extracts of *Peltophorum pterocarpum*, *Melia azedarach*, *Ocimum sanctum* leaves, *Terminalia chebula* seeds and juice of Allium *sativum* and Citrus *limon* juice were formulated with different ratio for poly herbal spray production. The formulated herbal spray (F1 to F6) was tested for antifungal activity against the isolated fungus like *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus stolonifer*. The formulation four and six were showed the maximum antifungal activity against the three fungal species. Hence these formulations were used for fungal eradication in copra production industries in future.

Keywords: coconut copra, medicinal plants, poly herbal spray, antifungal activity, agar well diffusion

1. Introduction
In India one third of population depends on the agricultural sector directly or indirectly. Agriculture remains as the main stray of the Indian economy since times immemorial. Coconut palms are grown in most of the zones expect subtropical and temperate regions, which include all states and three union territories in India. In Tamil Nadu, coconut farming is major cultivation practice in Coimbatore, Thanjavur and Dindigul districts. Among others Pollachi taluk in Coimbatore district is well known for coconut cultivation as the land cover in this zone is fully occupied by coconut groves.

Copra is the source of coconut oil. During the process of copra production, the coconut nuts are affected by microbial diseases and reduce the yield of the coconut oil product. Fungal contamination of stored copra is a very serious problem in tropical warm regions of the world. Many fungi have been found to be associated with copra. Only a few of them cause severe damage to the copra production. *Aspergillus niger, Aspergillus flavus, Penicillium italicum* and *Rhizopous stolonifer* and are the few important fungi which infect the copra. The fungal species *Aspergillus* and *Penicillium* are among the major reported genera having the ability to produce mycotoxins during copra storage [1, 2].

The copra needs to be preserved in good quality for storage purposes and it is important to identify and check fungal growth which are associated with the deterioration of coconut copra and investigate the possible control measures. Diseases of coconut palms are well documented [3] but little or no information available on the fungi of copra coconut and their deteriorative ability. Fungal infections are known to reduce the productivity of crops and its associated products. Like most nut meats, coconut meat contains less sugar and protein of high nutritive value. It is comparatively high in minerals like iron, phosphorus and zinc. Coconut oil (copra oil) extracted from the nutmeat of matured fruits white, glycerine rich and semi-solid stable in air and is used for cooking.

Nowadays most of the copra production industries in Pollachi area are using sulphur against the fungal infection in the coconut copra process and production. The sulphur treated copra are crushed to get oil and used for food preparation. Due to sulphur containing oil, people are suffering from ulcerative colitis. Sulphur rich foods may also cause inflammation of mucosa, increase blood pressure, skin disease, psoriasis and eczema. Currently, Integrated Disease Management (IDM) concept is increasingly adopted in many plant disease management programs with a view to protect the environment and to maintain the healthy food chain. The recent research also focuses on searching for safer and more compatible alternative methods for plant disease management. Plant derived bio-fungicides are now emerging as most important eco-friendly component of IDM [4]. The presence of inherent antimicrobial compounds in many higher plants has long been recognized as vital issue for illness resistance. Such compounds, being degradable and selective in their toxicity are considered as valuable ingredients for controlling plant diseases caused by fungi [5]. In the present study locally available medicinal plant extract at definite ratio were tested to evolve an antifungal herbal spray which can replace the sulphur for the process and production of copra.

2. Materials and Methods
2.1 Collection and preparation of herbal plant extract
The selected herbal plants were collected from Western Ghats of Tamilnadu, South India. The plants parts were washed with distilled water to remove the dust particles and

dried under shade for few days. After complete shade dried plant materials were powdered using pulverizer. The crude extracts were prepared using soxhlet extraction method with methanol solvent.

2.2 Formulation of herbal spray
Herbal spray was prepared by the definite ratio of selected plant extracts mixed with any one essential oil.

2.3 Isolation of fungi from infected copra
The infected copra was collected from coconut copra industry and it was inoculated to the PDA medium surface and incubated for 5-7 days. After 7 days the fungal colonies were grow at the PDA medium surface. Individual fungal colonies were sub cultured onto PDA medium to get pure culture [6].

2.4 Morphological identification of fungi:
The morphological characters of three pure culture fungi were identified by microscopic method. The Fungus was cultured on the PDA Media and incubated for 24 – 48 hours. Then the cultures were identified by using the stain Anneline blue. Pureculture of three strains were identified under microscope by the morphological characters [7].

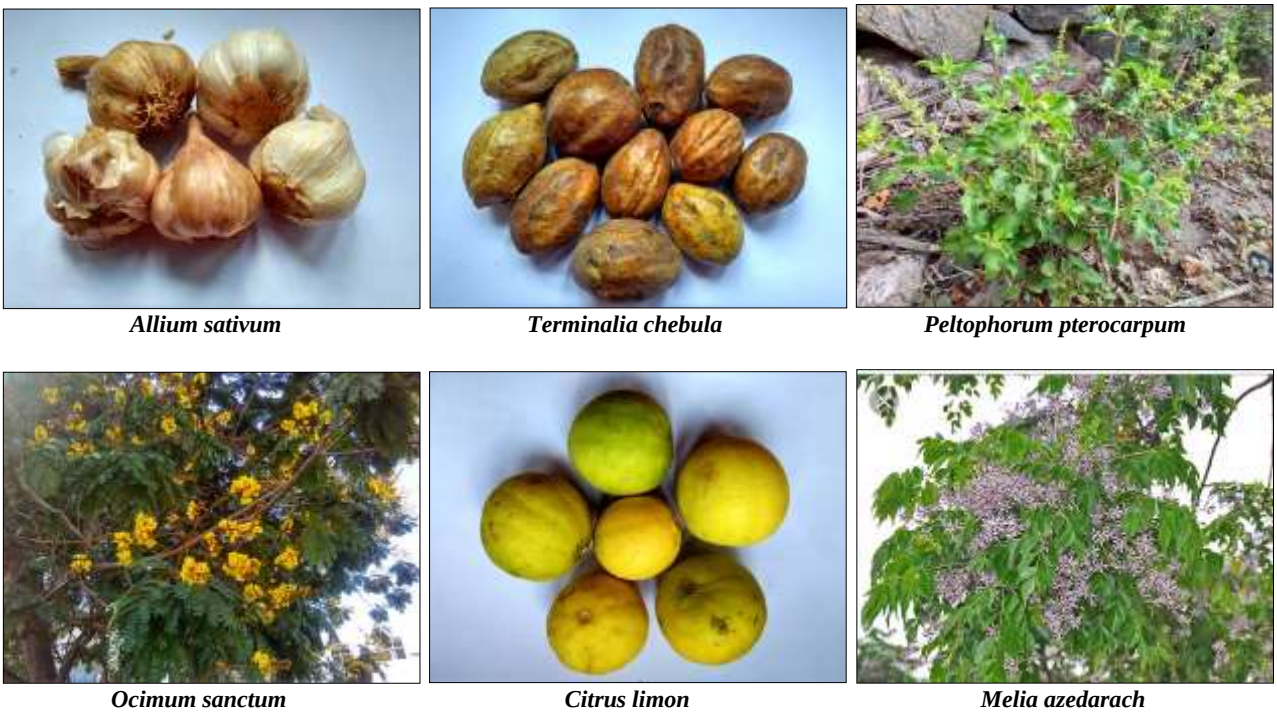
2.5 Antifungal Activity
The formulated herbal spray was tested for their antifungal activity by well diffusion method[8] against the isolated fungal pathogens. About 20-25 ml of potato dextrose agar medium was added to pre-sterilized plates. After this, 0.1 ml (1x10 [8] CFU/ml) of 12-16 hrs old culture of isolated three fungi was separately spread over the surface of agar plates. Petri plates were allowed to dry. Six wells in each plate of 6mm diameter were punched in agar surface with the help of sterilized cork borer. Each well filled with 100 µl of different formulated herbal spray extract. In another plate 3 wells were made with 6mm diameter. These three wells filled with different concentration of amoxicillin. The plates were kept in laminar air flow for 30 minutes for proper diffusion of the formulated extract and there after incubated at 37°C for 24 - 48 hours. After 48 hours of incubation the zone of inhibition was clearly visible and the diameter of the zone was measured.

3. Results
Poly herbal spray of various formulations were prepared using leaf extracts of *Peltophorum pterocarpum*, *Melia azedarach*, *Ocimum sanctum*, *Terminalia chebula* (seed), *juice of Allium sativum and Citrus limon*. Each formulation with definite ratios was given in (table 1).

Table 1: Formulations and composition of antifungal poly herbal spray

Plant Name	F1	F2	F3	F4	F5	F6
Peltophorum pterocarpum	20mg	10mg	50mg	30mg	40mg	60mg
Melia azedarach	40mg	60mg	10mg	20mg	30mg	50mg
Terminalia chebula	30mg	20mg	60mg	50mg	10mg	40mg
Ocimum sanctum	50mg	40mg	30mg	60mg	20mg	10mg
Allium sativum	600 µl	300 µl	400 µl	100 µl	500 µl	200 µl
Citrus lemon	100 µl	500 µl	200 µl	400µl	600 µl	300 µl
Clove oil	500 µl	500 µl	500 µl	500 µl	500 µl	500 µl

3.1 Isolation of fungi from infected copra
Infected copra was cultured in PDA medium and incubated for 3-5 days. After incubation period three types of fungal species were grown on agar plate (Figure-2). Based on the morphology each species was sub culture separately to the PDA medium and get three pure cultures of fungal species and named as Fungi 1, Fungi 2 and Fungi 3 (Figure-3)



Allium sativum ***Terminalia chebula*** ***Peltophorum pterocarpum***

Ocimum sanctum ***Citrus limon*** ***Melia azedarach***

Fig 1: Selected plants for poly herbal spray preparation

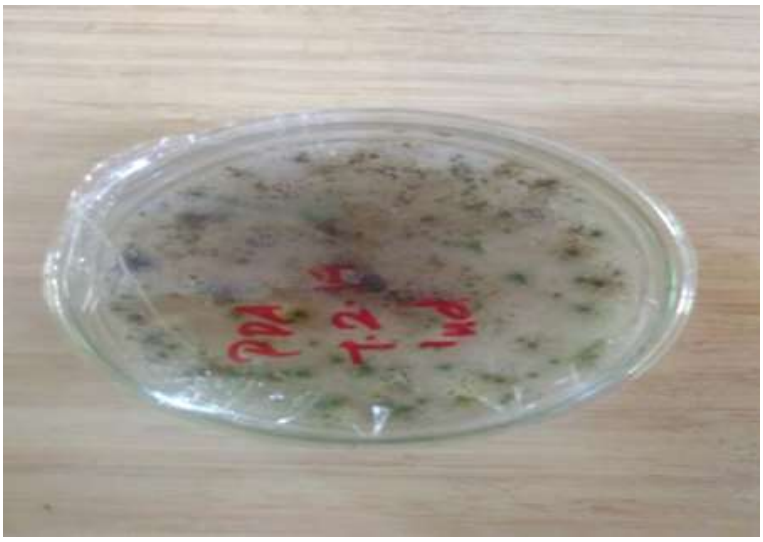
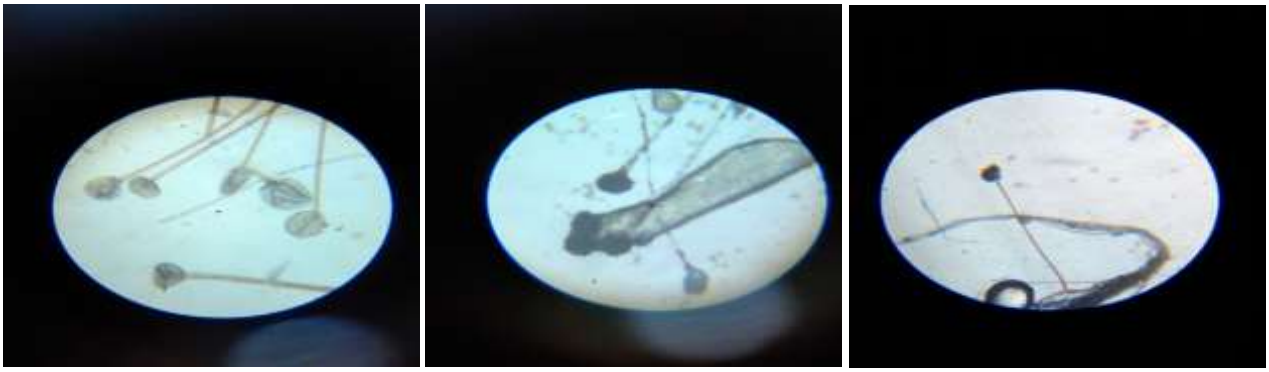


Fig 2: Three types of fungal species on PDA plate

3.2 Morphological identification of fungi

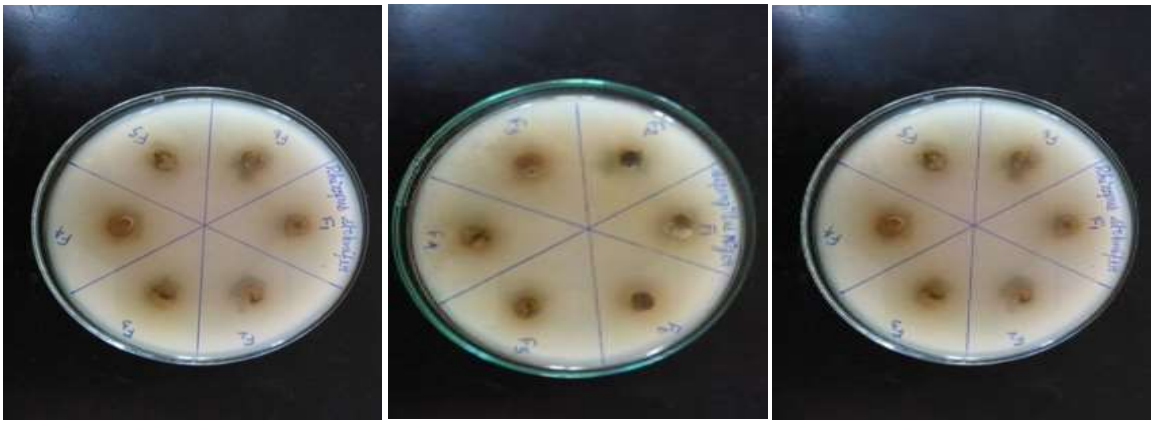
For identification of the fungi 1, fungi 2 and fungi 3 from the pure culture the mycelium was taken separately and mounted on a glass slide and stained with cotton blue and viewed under microscope. The character of fungi1 showed the conidia heads was globose and rectangular and it appears as light brown in colour. So it is confirmed as *Aspergillus niger*. In fungi 2, Condiophore was colourless and the heads are globular. So it is confirmed as *Aspergillus flavus.* In fungi 3, the sporangiophores bear sporangia and it is appears black in colour. So, the fungus was confirmed as *Rhizopus stolonifer* (Figure -4).

Fig 3: Pure cultures of fungal species on PDA plate



Aspergillus niger ***Aspergillus flavus*** ***Rhizopus stolonifer***

Fig 4: Morphological identification of isolated fungi



Aspergillus flavus ***Aspergillus niger*** ***Rhizopus stolonifera***

Fig 5: Antifungal fungal activity of Poly herbal spray

3.3 Antifungal activity

Evaluation of antifungal activity of formulated poly herbal spray was executed by agar well diffusion methods against copra infecting fungi. The result of antifungal activity of six different formulations was studied against three fungi namely *Aspergillus niger*, *Aspergillus flavus* and *Rhiopous stolonifer*. Six different formulations tried the formulation four and six showed the better antifungal activity against all the tested three organisms (table-2). The maximum zone of inhibition was observed in *Aspergillus flavus* (18mm) followed by *Aspergillus niger* (13mm) and *Rhizopous stolonifer* (10 mm) **(Figure-5). F1 formulation showed the antifungal activity against all the tested three organisms. The maximum zone of inhibition was observed in *Aspergillus flavus* (18mm) followed by *Aspergillus niger*** (13mm) and *Rhizopus stolonifer* (10mm). F2 formulation showed the maximum zone of inhibition was observed in *Aspergillus niger* (14mm) and *Aspergillus flavus* (14mm) followed by *Rhizopus stolonifer* (12mm). F3 formulation showed the maximum zone of inhibition in *Aspergillus flavus* (21mm) followed by *Aspergillus niger* (15mm) and *Rhizopus stolonifer* (11mm). F5 formulation showed the maximum zone of inhibition in *Aspergillus flavus* (19mm) followed by *Aspergillus niger* (13mm) and *Rhizopus stolonifer* (13mm).

Table 2: Antifungal activity of formulated herbal spray

Organisms	Zone of inhibition(mm)							
	F1	F2	F3	F4	F5	F6	Amoxycillin	DMSO
Aspergillus flavus	18	14	21	23	19	24	18	0
Aspergillus niger	13	14	15	17	13	16	19	0
Rhizopus stolonifer	10	12	11	14	13	14	15	0

4. Discussion

The microscopic characters of *Aspergillus flavus* are observed that conidial heads are typically radiate, conidiophore uncoloured, coarsely roughened less than 1mm long by 8-12µ wide with 1-2µ thick wall [9]. In the current study similar morphological character was observed. *Aspergillus niger* are observed that conidial heads biseriate, radiate, conidia in chains or detached and dispersed. Conidia heads were biseriate and globose in shape with wide spherical to globose vesicle that measured 37-52µm [10]. In the present study morphological character was similar to Beatrice *et al*. The microscopic characters of *Rhizopus stolonifer* are observed that hyphae broad, not or scarcely separate [11]. Our study also showed the significant result. Thus, it is clear from the above study that the plant extracts with different formulation have proved the good antifungal activity against the pathogenic fungi. In the similar studies, several reports stated that the extracts of medicinal plants play an important role in controlling many phytopathogenic fungi [12, 13]. The author reported that antifungal activity of the leaf extracts of some medicinal plants against numerous pathogens including *Alternaria sp*. and *Rhizopus sp* [14]. It is accepted that among of the foremost necessary fungi inflicting post-harvesting diseases of plant are *Aspergillus* spp., *Alternaria spp* and *Rhizopus stolonifer*. Mondall *et al*. reported that an inhibitory effect of neem leaf extracts on seed borne fungi *Aspergillus* and *Rhizopus* [15]. Raji and Raveendran, reported the antifungal activity of selected plant extracts against phytopathogenic fungi *Aspergillus niger* [12]. The present study indicated that the inhibitory effect of the formulated spray on these pathogenic fungi might be attributed to the presence of some partially effective antifungal ingredients in the formulation of all the test formulation. The alcoholic root, shoot and seed extract of *Eucalyptus australe* showed the antifungal activity against *Aspergillus niger* [16] (13mm, 12mm and 12mm). In the present study all the prepared formulations showed the significant antifungal activity against the isolated pathogens.

5. Conclusion

All the copra production industries in India were used sulphur for fungal eradication at the process of copra making. In our result state that formulated herbal extract showed higher antifungal activity against the pathogens isolated from infected copra. So the formulation 4 and 6 will be used for fungal eradication in copra production industries in future.

6. References

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