INVESTIGATIONS ON *GANODERMA LUCIDUM* (LEYS.) KARST CAUSING *ANABE* DISEASE OF ARECANUT

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ABSTRACT

The factors influencing the growth of the fungus, *Ganoderma lucidum* were studied *in vitro*. It was able to grow in a wide range of pH, from 2·0 to 9·0, the maximum growth was at pH 5·5. Among the various nitrogen sources studied, peptone supported the best growth. The fungus utilizes organic nitrogen only. Excellent growth was obtained when maltose was the carbon source. In general, the disaccharides supported good growth of the fungus. Formation of elongated sporophores was noticed in saw-dust-malt medium or saw-dust supplemented with Waksman's medium. Mercuric chloride, captan, difolatan and vitavax were lethal to the fungus.

INTRODUCTION

Anabe disease caused by the fungus *Ganoderma lucidum* (Leys.) Karst is one of the dreaded diseases of areca (*Areca catechu* L.). The earliest reference to this disease was made by Butler (1906) while recording the betelnut plague in Sylhet. The fungus also attacks coconut (Butler, 1906) and oil palm (Sharples, 1929) causing severe losses. Venkatarayan (1936) estimated that upto 5·0% arecanut trees died annually in an area while Naidu *et al.* (1966) recorded 1–2% loss on an average due to infection.

Bose (1930) brought the fungus into artificial culture from spore to spore. Venkatarayan (1936) found that the fungus grew best at pH 6·5. Nair and Rao (1962) and Menon (1963) studied the efficacy of some fungicides *in vitro* in arresting the growth of *G. lucidum*. Only little work has been done on the growth requirements of this fungus. Before a fungus can be studied in any detail, it is necessary to determine the conditions which affect the growth. The present study was taken up to fill in this lacuna.

MATERIALS AND METHODS

The isolate of the fungus was collected from an infected areca palm in Mettupalayam. Healthy fresh sporosphores were cut into small convenient pieces, sterilised in 0·1% $HgCl_2$ for 2 min and washed twice in sterile distilled water and plated on 2% malt extract-agar. Subcultures were maintained on the same medium.

For physiological studies, inoculum discs were cut out from a 10-day old culture in petri dish with the aid of a sterile stainless cork borer, 5 mm diameter. The discs were inoculated into 250 ml Erlenmeyer Pyrex flasks containing 50 ml Waksman's medium. The contents of the flasks were filtered at the end of an incubation period of 20 days. The mycelial mat was washed with distilled water and dried at 70° C. In all cases four replications were maintained. For testing the efficacy of different fungicides in inhibiting the growth of the fungus *in vitro*, the fungicides were mixed with sterile malt extract-agar (2%) as well as Waksman's liquid medium so as to obtain the desired concentrations. The fungicides used were (1) Benlate [Methyl 1-(butylcarbamoyl) 2-benzimidazole carbamate or Benomyl], (2) Captan (N-trichloromethylthio) 4-cyclohexene-1, 2-dicarboxymide), (3) Ceresan wet methoxyethyl mercuric chloride, (4) Leytosel (Phenylmercury urea), (5) mercuric chloride, (6) sulphur, (7) Thiram (tetramethylthiuram disulphide), (8) Aureofungin-sol (N-methyl-*p*-aminoacetophenone and mycosamine heptane), (9) mycostatin (Nystatin U.S.P.), (10) Difolatan [N-(1, 1, 2, 2-tetrachloroethylthio) cyclohex-4-ene-1, 2-dicarboxymide], and (11) Vitavax (5, 6-dihydro-2-methyl-1, 4-oxathiin-3-carboxanilide).

RESULTS AND DISCUSSION

1. *Growth of the fungus in different liquid media.*—The fungus was grown on five different

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liquid media to determine the best medium for growth. The mean dry mycelial weight per flask in mg was as follows: (1) Richard's solution: 15; (2) Czapek's solution: 62; (3) Asthana Hawker's medium: 115; (4) Elliott's medium: 60; and (5) Waksman's medium: 270. Thus Waksman's medium supported maximum growth of the fungus, the second best being Asthana Hawker's medium.

2. *Sporophore formation.*—While studying the best medium for growth of the fungus for purposes of inoculating the seedlings in pots for pathogenicity tests, profuse sporophore formation was observed in some culture bottles. The media used were (*a*) modified sand-oats medium (moist sand, 300 g; oats, 10 g; and 10% malt extract *plus* biotin at 5 ppm, 15 ml), (*b*) saw-dust malt-medium (moist saw-dust, 300 g and 10% malt extract *plus* biotin at 5 ppm, 15 ml), (*c*) and (*d*) the malt extract and biotin in media (*a*) and (*b*) were replaced by equal quantities of Waksman's medium. The media were taken in 500 ml bottles and autoclaved at 1 kg per cm^2 pressure for 90 min. The bottles were then inoculated with inoculum discs. Excellent vegetative growth of the fungus was observed in all the four media 10 days after inoculation. Sporophores were produced in saw-dust medium when the cultures were kept for more than 2 months. No sporophore was produced in sand-oats medium even after 4 months. This probably shows that the nature of substratum plays an important role in the production of sporophores. Menon (1963) obtained sporophores in cultures when biotin was added to PDA. Long and Harsch (1918) had opined that the character of the substratum played only a minor role in sporophore production.

The mycelium remained white for about 30–45 days after which it became pale yellow in colour. Drops of a colourless fluid were seen appearing during this time. The mycelium now turned yellowish brown in colour, and the mycelial mat thickened in some places. Lumps of mycelium sprang forth from this mat. They grew slowly, usually trailing on the wall of the culture bottle (Fig. 1). The bodies later turned russet in colour. Poriferous layers were visible on these bodies adpressed to the wall of the culture bottle. These sporophores are linear in shape as against the semicircular sporophores produced in nature.

3. *Effect of pH of medium on the growth of* G. lucidum.—The pH of the basal medium (Waksman's medium) was adjusted with dilute solution of NaOH or HCl to obtain pH values ranging from 1·5–9·0 (after autoclaving). The medium was then inoculated and incubated at room temperature. The mean dry mycelial weights obtained 20 days after incubation at different pH levels were as follows: (1) pH 1·5: nil; (2) 2·0: 18 mg; (3) 2·5: 68 mg; (4) 3·0: 155 mg; (5) 3·7: 203 mg; (6) 4·0: 228 mg; (7) 4.8: 233 mg; (8) 5·0; 235 mg; (9) 5·5: 276 mg; (10) 6·1: 187 mg; (11) 6·4: 175 mg; (12) 7·0: 155 mg; (13) 7·5: 154 mg; (14) 8·0: 131 mg; (15) 8·5: 98 mg; and (16) 9·0: 27 mg. The data thus show that the

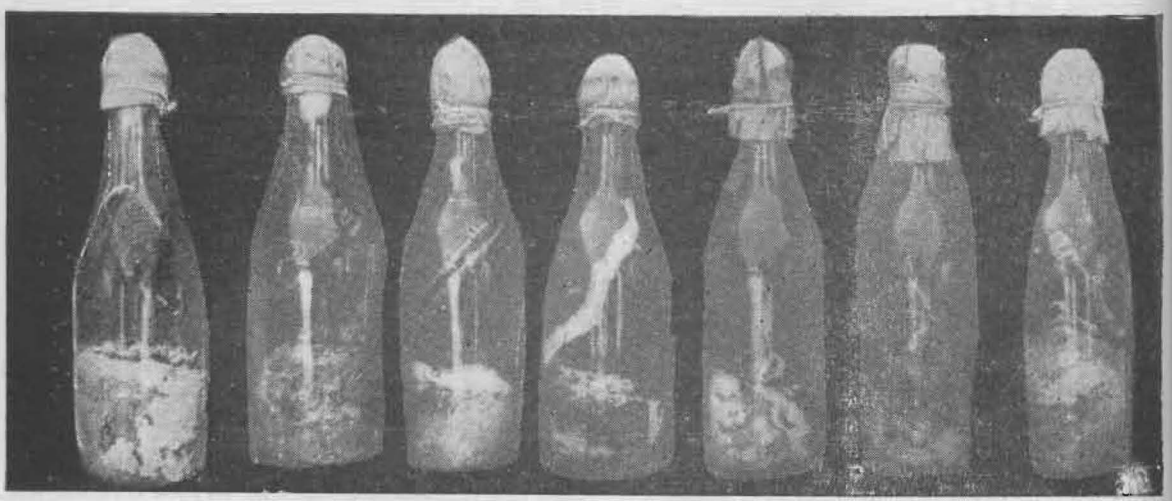

FIG. 1. Sporophore formation in culture.

fungus grows in a wide range of pH with the maximum growth occurring at pH 5·5.

Venkatarayan (1936) found that the fungus grew best at pH 6·5 and that the fungus was able to grow between pH 3·0 and 7·0. Hardly any growth was seen in alkaline media. In the present studies, however, mycelial growth was recorded up to pH 9·0. In this context, it is interesting to note that the disease occurs both in acid soils and in saline soils in various parts of Mysore State.

4. *Effect of different carbon sources on the growth of* G. lucidum.—Carbon is one of the key elements required by fungi for their growth. For studying the effect of different carbon sources on the growth of *G. lucidum*, glucose in the basal medium (*Waksman's* medium) was substituted with 17 different carbon sources to give the same amount of C as present in 5 g glucose in a litre of the medium. In the case of starch alone the same quantity as that of glucose was added. The results are given in Table I.

TABLE I

Effect of different carbon sources on the growth of Ganoderma lucidum

Sl. No.	Carbon source	Dry mycelial wt. in mg
1.	Arabinose ..	157
2.	Xylose ..	273
3.	Glucose ..	230
4.	Galactose ..	188
5.	Fructose ..	163
6.	Lactose ..	195
7.	Maltose ..	305
8.	Sucrose ..	218
9.	Starch ..	226
10.	Cellulose ..	30
11.	Tartaric acid ..	2
12.	Succinic acid ..	54
13.	Citric acid ..	43
14.	Malic acid ..	33
15.	Calcium carbonate ..	10
16.	Pottassium bicarbonate ..	2
17.	Sodium carbonate ..	2
18.	Control (No C) ..	22

The data show that the fungus growth was maximum when maltose was used as the carbon source. Among monosaccharides, xylose and glucose were best utilised. All the disaccharides supported good growth of the fungus. Significant growth was also obtained in the presence of the other carbon sources and especially of the carbonates of calcium, potassium, and sodium. Thus the fungus utilizes carbohydrates better than organic acids or carbonates.

5. *Effect of different nitrogen sources on the growth of* G. lucidum.—The concentrations of different nitrogen compounds were so adjusted as to contain the same amount of nitrogen as present in 5 g KNO_3 per litre of medium. In the case of peptone, 5 g of the substance was taken per litre of the medium. The results are given in Table II.

TABLE II

Effect of different nitrogen sources on the growth of G. lucidum

Sl. No.	Nitrogen source	Dry mycelial wt. in mg
1.	Ammonium acetate ..	36
2.	Ammonium carbonate ..	53
3.	Ammonium chloride ..	30
4.	Ammonium dihydrogen phosphate ..	32
5.	Ammonium nitrate ..	40
6.	Ammonium oxalate ..	23
7.	Ammonium sulphate ..	23
8.	Ammonium tartarate ..	29
9.	Calcium nitrate ..	23
10.	Lithium nitrate ..	30
11.	Magnesuim nitrate ..	41
12.	Peptone ..	330
13.	Potassium nitrate ..	34
14.	Sodium nitrate ..	57
15.	Sodium nitrate ..	23
16.	Urea ..	11
17.	Glycine ..	155
18.	Alanine ..	94
19.	Valine ..	76
20.	Isclucine ..	68
21.	Asparagine ..	51
22.	Glutamine ..	77
23.	Glutamic acid ..	149
24.	Arginine monohydrochloride ..	126
25.	Lysine ..	63
26.	Creatine ..	59
27.	Creatinine ..	83
28.	Cystine ..	45
29.	Cystine hydrochloride ..	69
30.	Methionine ..	46
31.	Phenylalanine ..	45
32.	Tyrosine ..	51
33.	Histidine monohydrochloride ..	82
34.	Hydroxyproline ..	82
35.	Control (No N) ..	82

The data show that peptone nitrogen supported the best mycelial growth of fungus. The growth was very poor with inorganic nitrogen compounds . The least growth was with urea. Among amino acids, glycine gave the maximum growth.

Fungi are known to be specific in their utilisation of nitrogen sources and this characteristic has been used by Robbins (1937) and Steinberg (1950) to classify fungi. From the present studies, *G. lucidum* was found to fall into the fourth category of Steinberg's classification, *viz.*, fungi able to utilize only organic nitrogen.

6. *Fungicidal evaluation.*—Screening of fungicides *in vitro* for their efficacy in killing/arresting the growth of the fungus was done employing both solid and liquid media. Inoculum discs (5 mm diameter) were placed in petri dishes containing 10 ml malt extract-agar poisoned with different fungicides or in Erlenmeyer flasks containing 50 ml Waksman's liquid medium *plus* respective fungicides. The petri dishes and flasks were incubated at laboratory temperature. The diameter of the colony in petri dish was measured daily. The mean diameter of the colony recorded on the third day and the dry mycelial weight recorded after an incubation period of 20 days are furnished in Table III.

The data show that in solid medium, complete inhibition of the fungus was obtained only with $HgCl_2$ (1,000 ppm). With liquid medium, $HgCl_2$ was fungicidal even at 250 ppm strength. Captan, thiram, and ceresan wet arrested the growth of the fungus completely in liquid medium at higher concentrations. Difolatan and vitavax also were efficient at the concentrations tried.

Another experiment was conducted to find out if the above chemicals are fungicidal or fungistatic in activity. Inoculation of mycelial discs was done in petri dishes containing poisoned agar. The discs were recovered 4 days after incubation, washed repeatedly in sterile distilled water and plated on agar medium. The plates were daily observed for fungal growth from the inoculum disc. The data recorded on the third day are furnished in Table IV.

It may be seen that only captan, $HgCl_2$, difolatan, and vitavax are fungicidal in action whereas ceresan wet, thiram, and leytosel are fungistatic.

The fungal growth was not arrested by sulphur even at 5,000 ppm strength. Menon

TABLE III

Effect of different fungicides on the growth of G. lucidum

Sl. No.	Fungicide	Growth in solid medium: Concentration of fungicides in ppm	Growth in solid medium: Colony dia. in mm	Growth in liquid medium: Concentration of fungicides in ppm.	Growth in liquid medium: Dry mycelial wt. in mg.
1.	Benlate	1000	52·5	1000	43·0
2.	Captan	2000	27·5	3000	0·0
3.	Ceresan wet	1000	40·0	2000	0·0
4.	Leytosel	1000	33·5	2000	0·0
5.	Mercuric chloride	1000	0·0	500 250	0·0 0·0
6.	Sulphur	2000	55·0	(2000 (5000	115·0 90·0
7.	Thiram	2000	15·5	3000	0·0
8.	Aureofungin sol	50	58·5	100 200	177·0 68·0
9.	Mycostatin	1 lakh unit per litre	57·0	..	..
10.	Difolatan	..	..	1000	0·0
11.	Vitavax	..	..	1000	0·0
12.	Control	..	58·0	..	225·0

TABLE IV

Effect of fungicides on the growth of G. lucidum

Sl. No.	Fungicide	Conc. in ppm	Growth in poisoned agar	Growth after transfer
1.	Captan	3000	—	..
2.	Ceresan wet	2000	—	
3.	Leytosel	2000	—	
4.	Mercuric chloride	250	—	—
5.	Thiram	3000	—	
6.	Difolatan	(1000 (2000	— —	
7.	Vitavax	100	—	—
8.	Control	—	+	+

\+ = Growth
— = No growth

(1963) also found that sulphur was inefficient in controlling the fungal growth. Though sulphur had been recommended in the control of *anabe* (Narasimhan, 1940), general observations in the field had shown that the disease cannot be controlled by sulphur application. Though $HgCl_2$ is a very efficient fungicide even at 250 ppm concentration, its high mammalian toxicity and exorbitant cost precludes its use as a general fungicide. Lalithakumari (1969) obtained complete inhibition of the fungus *in vitro* with cycloheximide at 2 ppm concentration. It may be worth testing cycloheximide under field conditions. Turner (1965) observed that fungicidal therapy of infected oil palm was unlikely to be successful and that emphasis should therefore be paid on phytosanitary measures. Perhaps our immediate effort must be to find out a systemic fungicide which when applied to the tree must be able to repel the invading pathogen. This of course should go hand in hand with phytosanitary measures to make the control measures easier and practicable.

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References

Bose, S. R. 1930. Artificial culture of *Ganoderma lucidum* Leys. from spore to spore. *Bot. Gaz.* **87**: 665–667.

Butler, E. J. 1960. Some diseases of palms. *Agri. J., India.* **1**: 299–310.

Lalithakumari. 1969. Studies *on Areca catechu* Linn. with special reference to *Ganoderma lucidum*, a fungal pathogen of areca palm. *Ph.D. thesis*, Indian Institute of Science, Bangalore.

Long, W. H. and Harsch, R. M. 1918. Pure culture of wood rotting fungi on artificial media. *J. Agri. Res.* **12**: 33–82.

Menon, R. 1963. Studies *in vitro* of *Ganoderma lucidum* Leys. Karst. *Phytopath. Z.* **48**: 434–438.

Naidu, G. V. B., Kumar, S. N. S. and Sannamarappa, M. 1966. *Ganoderma lucidum* (Leys.) Karst.—review and further observations. *Lalbagh* **20** (11): 14.

Nair, R. B. and Rao, K. S. N. 1965. A note on the efficiency of new fungicides on *Ganoderma lucidum*. *Arecanut J.* **16**: 6–9.

Narasimhan. M. J. 1940. Treatment against *anabe* roga of coconut and arecanut. *Mysore Agri. Calendar*, pp. 25–26.

Robbins, W. J. 1937. The assimilation by plants of various forms of nitrogen. *Amer. J. Bot.* **24**: 243–250.

Sharples, A. 1928. Annual report of the Mycologist for the year 1929. *Malay. Agri. J.* **17**: 294–303.

Steinberg, R. A. 1950. Growth of fungi in synthetic nutrient solution. *Bot. Rev.* **16**: 208–228.

Turner, P. D. 1965. Ganoderma disease of oil palm and some aspects of its control. Paper presented at the Symposium on "Diseases of oil palm," pp. 15–21. University of Malaya, Kuala Lumpur.

Venkatarayan, S. V. 1936. The biology of *Ganoderma lucidum* on arecanut and coconut palms. *Phytopathology* **26**: 153–175.

Discussion

Srivastava : Are you able to culture or isolate the fungus now ?

Nambiar : Yes. Isolation from arecanut is actually easier also than from coconut.

Wilson : It has been stated that $HgCl_2$ controls the growth of the fungus. What was the method tried here ? Are any antibiotics effective ?

Nambiar : Mercuric chloride was tested *in vitro* by the poisoned bait technique, using both solid and liquid media. The antibiotics, Aureofungin–Sol. and Mycostatin, were tried. They were not effective in inhibiting the fungus growth at the concentrations tried.