Postharvest diseases of perishables in west Bengal (India). Control of spoilage of grapes due to *Aspergillus niger* VAN TIEGH. and *Botryodiplodia theobromae* PAT.

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MALADIES APRES RECOLTE DES DENREES PERISSABLES DANS L'OUEST DU BENGALE (INDE).
CONTROLE DE L'ALTERATION DES RAISINS DUE A *ASPERGILLUS NIGER* VAN TIEGH ET *BOTRYODIPLODIA THEOBROMAE* PAT.

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RESUME - Sur dix champignons dont la présence est commune dans l'ouest du Bengale (Inde), *Aspergillus niger* et *Botryodiplodia theobromae* sont ceux occasionnant les dégâts les plus graves sur les raisins. Des pertes de 6 à 60 p. 100 ont été notées pendant la principale saison des arrivages sur les marchés, dues à la fois à la présence des pathogènes et à des conditions physiologiques, climatiques ou variétales, pour l'ensemble des vendeurs et des détaillants.
Aucun traitement effectué avant l'inoculation n'a été efficace contre *A. niger* alors qu'un post-traitement avec 500 ppm de bavistine par trempage pendant 10 minutes, a donné de bons résultats. Le métabisulfite de potassium (2,5 p. 100) a été très efficace avant inoculation réduisant l'infection de *B. theobromae* de 93,3 p. 100. Le thiabendazole et la bavistine pourraient réduire cette même infection par traitement après inoculation. A ce stade, la lutte contre *A. niger* et *B. theobromae*, grâce à plusieurs combinaisons de bavistine + métabisulfite de potassium, a donné de bons résultats, les meilleurs étant ceux obtenus avec des doses respectives de 0,1 p. 100 + 2,5 p. 100 de ces produits.

Le stockage des denrées maintenu à 3-5,5ºC jusqu'à 35 jours en réfrigérateur, suivi par un stockage à l'air libre pendant 10 autres jours, a été efficace.
En supposant une perte moyenne de 23 p. 100, la perte annuelle calculée en Inde a été de 90.000 tonnes et 160 m dollars. Le coût du traitement a été calculé à 0,20 dollar pour 10 kg, ce qui permettrait de réduire les pertes d'au moins 20 p. 100.

In India, grapes are grown in diverse climatic conditions in North India (one crop in Mid-April to June) and in South and South Central India (two-season crop in Dec to Jan and Feb to Mid-April). To meet internal demands from August to October it is necessary to import some amount of grapes from Afganisthan and Pakisthan. Grapes contain high sugar and vitamins in the juice and, as a necessary evil, it provides a very good substrate for pathogenic growth. PATHAK (1972) mentioned that more than thirty fungi were recorded till then to cause spoilage in grapes and *Botrytis cinerea* PERS., *Cladosporium herbarum* (PERS.) LINK, *Elsinoe ampellina* (DE BARY) SHEAR, *Guignardia bidwelli* (ELLIS) VIALA and RAVAZ, and *Penicillium* spp. were considered to be the most destructive of them on a global basis. Besides these, *Phoma macrostoma* MONT., *Cladosporium tenuissimum* COOKE, *Aspergillus niger* VAN TIEGH., *Penicillium citrinum* THOM and RAPER, *Pseudostemphylium radicinum* were also recorded to be serious in India (BHARGAVA et al., 1976 ; JAMALUDDIN, 1977 ; SUBRAMONIAM and RAO, 1975). But their relative importance with the previously known fungi have not been assessed. MANDAL and DASGUPTA (1980) considered *A. niger* and *B. theobromae* along with *Rhizopus oryzae* WENT and PRINSEN GEERLINGS as the most serious rotters in West Bengal. Since the former two usually remain in

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mixed infection in nature attempts to control these diseases were deemed necessary.

Application of 1000 ppm of NAA a week before harvest was effective in checking the berry drop in Bangalore Blue grapes and the same three days before harvest in Anab-e-Shahi grapes reduced the postharvest decaying by 75 % (RAO and KOLOIGI, 1971). Spraying of grapes with either captan 0.2 % or DCNA 0.2 % four days prior to harvest could control the storage decay (SUBRAMANIAN et al., 1973). Postharvest dipping of grapes with captan 0.2 % for ten min was effective against *Aspergillus niger* and *Penicillium* sp. (TANDON et al., 1975). A few more fungicides were also found to be effective as postharvest dips but none could be recommended for their high dosage or unsuitability for being uneconomic or other reasons (TANDON et al., 1977). SO_2 fungigation is done to achieve control of *B. cinerea* in USA (NELSON and AHMEDULLAH, 1972). Survey of literature also showed that attempts have been made to control other fungi like *Botrytis cinerea* and *Rhizopus oryzae* in India and abroad but no attempt has been made to control either *B. theobromae* alone or in combination with *A. niger* causing spoilage of grapes in storage.

MATERIALS AND METHODS

Experiments were conducted in four different sets using Thomson's Seedless, Anab-e-Shahi and Kishmish because any single variety could not be obtained in the market throughout the season. Apparently unifected grapes obtained from the market were surface-sterilized by $HgCl_2$ (0.1 %) for 30 sec. followed by washing in sterile water. Such fruits were dipped in a conidial suspension (50×10^3 spores per ml) and blended mycelial mass (one 10 cm petriplate growth in 10 ml sterile water) of *A. niger* and *B. theobromae* respectively, for five min for inoculation. An interval of 24 hr was maintained between treatment and inoculation or vice versa in case of pre- or postinoculation treatments respectively. In all cases inoculated fruits were incubated in room temperature.

SCREENING OF THE TREATMENTS

After a certain period, effects of treatments were observed and analysed by using the variables of per cent infection, degree of softening, average infection index, superficial growth and/or sporulation of the pathogen. Any change in colour, flavour of visible residue left were also observed. Data were based on three replicates with ten fruits per replicate.

A six-point simplified infection index adopted from McKINNEY (1923) was used :

$$\text{Average infection index} = \frac{\text{Sum of all ratings}}{\text{Total number of replicates}}$$

Ratings were as follows :

- Average infection index in the six-point scale :

0. No infection, superficial fungal growth, softening nil, necrosis at the point of injury, no change in colour or flavour.

1. Infected area below 25 % of fruit surface, fungal growth nil, softening up to 0.2 cm around injury, no change in colour or flavour.

2. Infected area below 50 % of the fruit surface, superficial growth covering up to 0.2 % cm in diam, softening up to 0.3 - 0.4 cm around injury. Colour may change towards naples yellow to brown but without any disagreeable odour.

3. Infected area below 75 % of the fruit surface, superficial growth from 0.2 - 0.4 cm in diam, softening up to 0.4 - 0.6 cm around injury and slightly beyond. Colour changes towards brown and with some disagreeable odour.

4. Infected area below 90 %, superficial growth extensive but yet to cover fruit surface, softening 0.6 - 1.0 cm, colour brown and fermentative odour.

5. Infected area covering whole fruit, superficial growth covering entire surface, softening above 1.0 cm, colour brown to deep brown, fermentative and decomposing odour.

While calculating the average infection index, in case all the descriptions did not agree with any particular rating but fall somewhere between the consecutive ratings the midvalue between the two was considered the rating for the specimen.

Besides the average infection index following ratings were separately done :

- Growth and/or sporulation was rated on a four-point scale :

0. Nil or not evident
1. Up to 0.2 cm in diam
2. 0.2 - 0.4 cm in diam
3. Coverage of entire fruit surface

- Softening was rated on a four-point scale :

0. No softening
1. Over an area of 0.2 to 0.4 cm in diam
2. Over an area of 0.5 to 1.0 cm in diam
3. Exceeding 1 cm in diam.

RESULTS AND DISCUSSION

Nature of spoilage and loss - Six fungi viz. *A. niger*, *B. theobromae*, *Penicillium frequentans*, *Rhizopus stolonifer*, *R. oryzae* and *Cladosporium tenuissimum*, were commonly obtained on grapes in West Bengal during the survey (1977 and 1978). Variation was noted between localities representing humid and subhumid tracts, between wholesale markets and retail markets and among seasons as well as varieties. Irrespective of these variations, *A. niger* and *B. theobromae* were most frequently encountered. Loss of 6-60 % was obtained in different markets during March - September. Details of loss data of perishables will be published elsewhere. Since *A. niger* and *B. theobromae* may be carried from the field both pre - and post-inoculation treatments were tried.

Control of spoilage of grapes by *A. niger* - In preinoculation treatments (table 1) out of fifteen treatments including control, none were found to be effective in terms of reducing per cent infection, but in terms of average infection index, growth and/or sporulation, or degree of softening some chemicals viz, boric acid, sodium carbonate, macuprax, diflotan, allisan, allisan + captan mixture (1:1) showed some promise (table 1). Each of them had one or the other of certain undesirable effects such as

TABLE 1 - Preinoculation treatment of grape (variety Thompson seedless) against *Aspergillus niger*.

Treatment	Dose (a.i.%) or method	Average % infection	Average infection index	Growth and/or sporulation index	Degree of softening	Other observations
1	2	3	4	5	6	7
1. Potassium thiocyanate	0.02	93.3	4.0	3	2	Browning with fermentative odour due to yeast. Fungal growth not checked.
2. Dimethylamine	0.005	100	4.7	3	3	Browning but without any offensive odour. Fungal growth not checked.
3. Boric acid	5	86.7	3.3	2	1	Browning but without any bad odour. *Penicillium* growth not checked.
4. Sodium carbonate	5	100	3.5	3	1	Normal colour and flavour retained.
5. 2,4-D (Sodium Salt)	0.001	100	4.5	3	3	As with Dimethylamine
6. Cycocel	0.02	100	4.8	2	3	do
7. Dithane Z-78	0.05	100	4.9	3	3	Browning with fermentative odour due to yeast. Left some fungicidal residue.
8. Macuprax	0.05	86.7	3.2	1	1	Browning with slight green fungicidal residue. Yeast rotting not checked. Growth of others checked.
9. Allisan	0.05	86.7	1.7	0	1	Browning with bad odour. Yellow fungicidal residue.
10. Allisan + Captan (1:1)	0.04	86.7	2.0	0	2	Normal colour retained. Yellow fungicidal residue.
11. Brassicol	0.05	100	4.0	3	2	Colour turned fleshy with fermentative smell.
12. Difolatan	0.04	80	2.4	1	1	Normal colour nearly retained. No bad smell. Fungal growth not checked.
13. Linseed oil	10	100	3.8	2	2	Browning. No bad smell. Oil residue.
14. Chalmugra oil	5	-	3.6	1	3	Offensive oily smell. Colour nearly normal.
15. Control	-	100	5.0	3	3	Naples yellow to semibrown colour. Bad offensive smell of fermentation. Rot developed within 72 hours.

Table 2 - Postinoculation treatment of grape (variety Thompson seedless) against *Aspergillus niger*.

Treatments	Dose (a.i.%) or method	Average % infection	Average infection index	Growth and/or sporulation index	Degree of softening	Other observations
1	2	3	4	5	6	7
1. Aureofungin	0.01	100	4.8	3	3	Browning with fermentative odour.
2. Nystatin	0.02	100	1.0	0	1	Normal colour nearly retained without any bad odour.
3. Bavistin	0.05	13.3	0.3	0	0	Apparently normal including colour and flavour.
4. NF-48	0.1	20.0	0.5	0	0	Browning only at the point of inoculation. Slight fermentative smell.
5. Topsin M-70	0.02	100	2.3	1	2	Apparently normal colour. Fermentative smell.
6. Thiabendazole	0.2	100	2.8	1	2	Browning with bad smell of fermentation.
7. Benomyl	0.05	86.7	2.3	1	1	Partially browned fruit without any bad smell.
8. Calixin	0.02	100	4.5	2	3	Browning with bad smell of fermentation
9. Cina	0.04	100	4.9	2	3	Browning with highly irritating smell.
10. Flix mas	0.04	100	4.8	3	3	do
11. Hot water dip (45-46ºC for 5 min)		100	4.8	3	3	Browned fruits with fermentative odour.
12. Hot water dip (65ºC for 2 min)		100	4.4	1	2	Normal colour. Fermentative odour.
13. Control		100	5.0	3	3	Naples yellow to semibrown colour. Bad offensive fermentative smell, rot developed within 60 hrs.

browning, fermentative odour, visible fungicidal residue on surface etc. It appeared that preinoculation dipping was rendered at least partially useless during inoculation when fruits were dipped in spore suspension. Therefore unless a protective sticker strong enough to resist dipping or unless the method of inoculation is changed efficacy of fungicides in protection may not be perhaps assessed properly.

In postinoculation stage (table 2) out of thirteen treatments only three chemicals were found to be most promising in terms of all the variables used in the assay. NF-48 left undesirable effects like restricted browning and fermentative odour. Nystatin also could not reduce per cent infection but could reduce the intensity of infection, growth of pathogen, degree of softening and did not have any undesirable effect. On the other hand, bavistin had no known or observed undesirable effect deserved strong recommendation. Interstingly enough, benomyl and bavistin behaved differently, which suggested further investigation. Similar differences have also been made by other workers (MARSH, 1978).

Control of spoilage of grapes by *B. theobromae* - In preinoculation stage out of twenty-five treatments potassium metabisulphite was most effective except that the treated fruits showed tendency to get dried (table 3). Diphenylamine (both aquous & alcoholic) and macuprax were effective but left undesirable effects.

In postinoculation stage, out of eleven treatments only NF - 48 could reduce 100 % infection but it could not be recommended since it could not prevent fermentation by yeast (table 4). Thiabendazole and bavistin could reduce infection by 80 % and 73 % respectively but the latter permitted other pathogens to grow.

Control of spoilage against joint inoculation of *A. niger* and *B. theobromae* - In postinoculation stage out of twenty-two treatments, bavistin + potassium metabisulphite, NF-48 + potassium metabisulphite and bavistin + NF-48 showed 80 %, 80 % and 67 % reduction of infection respectively, of which bavistin + potassium metabisulphite (0.1 + 5 %) was the most desirable and worth recommendation.

TABLE 3 - **Preinoculation treatment of grape (variety - Kishmish) against** *Botryodiplodia theobromae.*

Treatments	Dose (a.i.%) or method	Average % infection	Average infection index	Growth and/or sporulation index	Degree of softening	Other observations
1	2	3	4	5	6	7
1. Potassium thiocyanate	0.2	100	4.5	3	3	Yeast growth not checked. Fermentative odour.
2. Potassium metabisulphite	5	6.7	0.1	0	0	Normal colour ; but desiccation took place.
3. Boric acid	5	93.3	2.1	1	0	Browning but growth of *Aspergillus* & *Penicillium* not checked.
4. Alcoholic diphenyl amine (pH 2.3)	2	0	0	0	1	Fruit stained. Whole fruit turned towards deep blue to black with irritating smell.
5. Dimethylamine (pH 2.0)	1	0	0	0	1	do
6. Dimethylamine (pH 9.5)	0.1	100	3.1	1	2	Nearly normal colour but growth of other fungi not checked.
7. Naphthylamine (pH 1.8)	0.1	100	2.1	1	2	Normal colour nearly retained. Bad smell. Fungal growth checked.
8. Alcoholic naphthylamine (pH 5.6)	2	86.7	2.0	0	3	do
9. 2.4-D (Sodium salt)	0.2	93.3	1.6	1	1	Browning but devoid of any offensive odour. Fungal growth not checked.
10. Maleic hydrazide	0.02	80.8	1.9	1	2	Normal colour nearly retained. No bad odour. Growth of *Aspergillus* & *Penicillium* not checked.
11. Cycocel	0.2	93.3	2.5	1	3	Normal colour nearly retained. A red staining zone attached to the pedicel *Aspergillus* & *Penicillium* growth not checked.
12. Dithane Z-78	0.1	100	4.6	3	4	Left fungicidal residue. Growth of *Aspergillus* not checked.
13. Macuprax	0.2	46.6	1.3	0	0	Fungicidal residue. Yeast rot not checked but no growth of saprophytic fungi.
14. Brassicol	0.01	86.7	0.7	0	0	Incipient infection. Colour slightly fleshy in nature. Saprophytic fungal growth less.
15. Sicarol	0.2	80.0	1.5	1	2	Colour nearly normal without any bad odour. Growth of saprophytic fungi not checked at all.
16. Difolatan	0.2	73.3	1.4	1	1	Left visible residue. Colour changed to brown and growth of saprophytic fungi not at all checked.
17. Allisan	0.1	56.6	1.2	0	1	Only incipient infection. Left yellow deposits. Slight growth of other fungi.
18. Allisan + Captan mixture (1:1)	0.1	93.3	1.6	1	2	Same as above excepting incipient infection.
19. Calotropis	0.4	86.7	2.8	1	2	Black point on fruit. Growth of *Aspergillus* not checked.
20. *Blatta orientalis*	0.4	100	3.2	1	2	Colour nearly normal but with fermentative smell. Growth of *Penicillium* not checked.
21. Tercium	0.4	100	4.7	3	3	Colour changed and fruits desiccation very quickly. Growth of *Aspergillus* not checked.
22. Ruta G	0.4	80	4.8	3	3	Growth of saprophytic fungi not at all checked.
23. Neem oil	1	93.3	4.3	3	3	Offensive smell of oil along with blackened brown colour. No saprophytic fungi grown.
24. Linseed oil	0.25	93.3	4.1	2	2	Retained normal colour without any bad smell.
25. Control	-	100	5.0	3	3	Browning along with growth on the surface. In some fruits fermentative odour easily reconognized. Rot developed within 4 days.

TABLEAU 4 - Postinoculation treatment of grape (variety - Kishmish) against *Botryodiplodia theobromae*.

Treatments	Dose (a.i.%) or method	Average % infection	Average infection index	Growth and/or sporulation index	Degree of softening	Other observations
1	2	3	4	5	6	7
1. Aureofungin	0.1	66.7	2.4	1	2	No fermentative odour. Saprophytic growth of *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp., etc.
2 Griseofulvin	0.0125	73.3	3.0	2	2	Nearly normal colour. Saprophytic growth of *Aspergillus* spp.. and yeast not checked.
3. Nystatin	0.2	93.3	3.6	2	2	Fruit turned brown but without any fermentative odour.
4. Calixin	0.1	86.7	4.5	3	3	Fruit stained. Growth of *Aspergillus* spp. not checked. Fruit emits fermentating odour.
5. Topsin M	0.1	100	0.3	0	0	Highly restricted incipient infection. Normal colour retained very sparse growth of *Aspergillus* spp.
6. Benomyl	0.1	100	2.9	1	2	Browning of fruit. Growth of *Aspergillus* spp. not checked.
7. Thiabendazole	0.25	20	0.8	0	0	Highly restricted incipient infection. *Aspergillus* spp. growth also less.
8. NF-48	0.1	0.0	0.2	0	0	Rotting by saprophytic fungi including yeast. Retained normal colour
9. Bavistin	0.05	26.7	0.5	0	0	Highly restricted incipient infection. Saprophytic growth of *Aspergillus* spp. also there.
10. Hot water treatment (52°C for 5 min)		60	2.4	1	2	Retained normal colour. No saprophytic fungi grown.
11. Control		100	5.0	3	3	Browning developed with or without growth of saprophytic fungi. Fermentative smell. Rot developed within 4 days.

Storage at low temperature - Surface-sterilized apparently unifected Anab-e-Shahi fruits were kept in the refrigerator at 3-5.5°C, at room temperature varying between 28.3°C-36.1°C and 61.2-68.4 p. c. R.H. The freeze-stored fruits did not rot up to at least 35 days and could be stored on shelf for at least another 10 days. While it is generally recommended to store grapes at 0.2°C, the finding indicated that higher temperature may not be harmful. Further, that the freeze-stored fruits could be kept in the open for at least 10 days would be valuable piece of information for shipping of grapes.

Economics of the suggested treatment - Assuming 25 % average loss, annual loss was computed to be 90,000 tons and Rs.80 crores ($ 160 m) in India. The cost of mixed treatment of bavistin + potassium metabisulphite has been computed @ Rs.1.000 ($ 0.2) per 10 kg and 20 % average loss can be saved that way.

SUBSTANCES USED IN THE EXPERIMENT

1. Allisan = 2,6 dichloro-4-nitroaniline
2. Aureofungin = Heptaene macrolide : manufactured by HAL, Pune, India.
3. Bavistin = 2- (methoxycarbamoyl) benzimidazole
4. Benomyl = 1 (butylcarbamoyl)-2 benzimidazole
5. Blatta orientalis = Homeopathic drug
6. Brassicol = pentachloronitrobenzence
7. Calixin = N-tridecyl-2,6-dimethylmorpholine
8. Calotropis = Homeopathic drug
9. Captan = N-(trichloromethylthio) tetrahydro-3a, 4, 7, 7a-isoindolinedione-1,3)
10. Cina = Homeopathic drug
11. Cycocel = (2-chloroethyl) trimethylammonium chloride
12. 2,4-D = Sodium salt of 2,4-dichlorophenoxyacetic acid
13. Difolatan = cis N- (1,1,2,2-tetrachloroethylthio) 4-cyclohexane-1,2 dicarboximide

TABLE 5 - Postinoculation treatment of grape (var. Anab-e-Shahi) against *A. niger* and *B. theobromae*.

Treatments	Dose (a.i.%) or method	Average % infection	Average infection index	Growth and/or sporulation index		Degree of softening	Other observations
1	2	3	4	5		6	7
				A.n.	B.t.		
1. Potassium metabisulphite	5	46.6	1.8	1	0	1	Slight tendency towards browning. Desiccation of fruits but without any fermentative odour.
2. Nystatin	0.1	100	3.9	3	0	3	Slight volatile fermentative odour.
3. Bavistin	0.1	86.7	1.5	1	0	2	Slight tendency towards browning with slight fermentative odour only at the point of the infection.
4. NF-48	0.2	66.7	1.7	2	0	2	Browning with slight fermentative odour.
5. Thiabendazole	0.5	100	4.2	3	0	3	do
6. Bavistin + 2,4-D	0.1+0.2	86.7	1.4	0	0	1	No bad smell in the fruit .
7. Bavistin + Macuprax	0.1+0.2	86.7	2.0	1	0	2	Sclerotial bodies formed on a few fruits. Left green deposits on surface.
8. Bavistin + Potassium metabisulphite	0.1+5	20.0	0.2	0	0	0	Extremely sound fruit without any bad odour.
9. Bavistin + Brassicol	0.1+0.1	100	3.5	1	0	3	Naples yellow to brown fruit, along with visible residue.
10. Bavistin + Allisan	0.1+ 0.1	100	3.0	1	0	2	Left visible yellow residue with fermentative odour.
11. Bavistin + Thiabendazole	0.1+0.25	80	1.0	0	0	1	Browning only around infected inoculations
12. Bavistin + Nystatin	0.1+0.25	80	2.3	1	0	2	Cracking of fruit with slight fermentative odour.
13. Bavistin + Topsin M	0.1+0.2	100	2.0	1	0	2	Slight fermentative odour.
14. Bavistin + NF-48	0.1+0.2	33.3	0.9	0	0	1	do but less than earlier treatment.
15. NF-48 + 2,4-D (Sodium salt)	0.2+0.2	100	2.3	0	0	2	do
16. NF-48 + Macuprax	0.2+0.2	86.7	4.6	3	0	3	Left green fungicidal deposits
17. NF-48 + Potassium metabisulphite	0.2+5	20	0.5	0	0	0	Partial browning without fermentative odour
18. NF-48 + Brassicol	0.2+0.1	73.3	2.9	1	1	2	Left visible residue, slight fermentative and cracking of the fruit.
19. NF-48 + Allisan	0.2+0.1	93.3	3.5	1	1	3	Left yellow deposits with slight fermentative odour.
20. NF-48 + Thiabendazole	0.2+0.25	93.3	2.6	1	1	3	Naples yellowing with slight fermentative odour
21. NF-48 + Topsin M	0.2+0.2	100	4.3	3	0	3	Slight fermentative odour.
22. Control	-	100	5.0	2	2	3	Naples yellow to brown along with fermentative odour. Rot developed within 60 hours. Rotting is also fast.

14. Dithane Z-78 = Zinc ethylene bisdithiocarbamate
15. Flix mas = Homeopathic drug
16. Macuprax = Basic copper sulphate (73 %) & ethylene bisdithiocarbamate complex of zinc, iron, copper and manganese (7 %), i.e. cupraneb
17. NF-48 = 2-3 (methoxycarbamoyl-2-thioureido)aniline
18. Nystatin = a polyene macrolide antibiotic
19. Ruta G = Homeopathic drug
20. Sicarol (pyracarbolid) = 2-methyl-5-6,dihydro-4-H-pyran-3-carboxylic acid anilide
21. Tercium = Homeopathic drug
22. Thiabendazole
23. Topsin M-70 = Dimethyl-4,4-0-phenylene bisthioallophanate.

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