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# Studies on Ergot Disease of *Cumbu (Pennisetum typhoides)*

*by*

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**Introduction**: Bhide and Hedge (1957) reported the occurrence of ergot disease on *cumbu* caused by *Claviceps microcephala* (Wallr.) Tul. from Maharashtra State. With the introduction of high yielding varieties like HB1 in various parts of the country which are incidently highly susceptible to the ergot disease, the pathogen has gained a strong foothold and became a menace to *cumbu* cultivation. Dawkhar and Sulaiman (1965) reported that the disease is severe in cool humid weather in Poona. Shanmugasundaram *et al* (1967) reported the prevalence of the disease in late sown *cumbu* crop in Tamil Nadu. The incidence of the disease was noticed in many places in the Tamil Nadu since 1967 and in view of the poisonous nature of the ergotised grains, detailed studies of this disease were undertaken in this laboratory and the results are presented in this paper.

**Materials and Methods**: The causal organism, *C. microcephala* was isolated by single spore method from sugary exudations oozing out from the infected grains of HB 1 *cumbu* variety. A special medium (sucrose 10.0%, monopotassium phosphate 0.1%, magnesium sulphate 0.025% and asparagin 0.1%) which was found to be suitable for the cultivation of *Claviceps purpurea* by Kirchhoff (1929) was used for the isolation and maintenance of the pathogen. The pathogen was also obtained from the sclerotia formed in the *cumbu* earheads. The sclerotia were surface sterilized with mercuric chloride (1:1000) for about a minute, washed in several changes of sterile water and planted on Kirchhoff's medium. Profuse mycelial growth of the pathogen developed from the sclerotia within six days.

**Results**: I. *Pathogenicity*: The pathogenicity tests were conducted using both the naturally occurring conidia from the honey dew exudations and the conidia from the artificial agar medium. It was also observed that the germination percentage of naturally occurring conidia was found to be more than that of artificial conidia. The conidial suspensions were sprayed on the inflorescence 2 – 3 days after emergence. The spray-inoculated inflorescences were covered with polythene bags to provide high humidity condition for successful infection. Sterile water sprayed inflorescences were maintained to serve as control.

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On the fourth day of inoculation, a dull red or white coloured, sticky, viscous fluid started oozing out from the spikelets. Exudations became conspicuous by about the 5th day in many of the spikelets. The exudation was found to be produced continuously and drip down on the leaves and on the ground. After four days of the appearance of exudation, the initiation of sclerotial formation was noticed in the place of ovary. The sclerotia were found to increase in size gradually and attained their full growth within about 10 - 12 days of the appearance of the exudation. The immature sclerotia were gryish white and became hard and violet coloured black on drying. The matured sclerotia were slightly curved in the middle with broad base and tapering ends. Surface of the sclerotia was usually smooth but sometimes cracks were noticed. Sclerotia were 3 to 8 mm in length and 0.3 to 1.5 mm in breadth.

The intensity of the disease incidence with the conidia from honey dew (natural) and the conidia from culture media (artificial) was assessed and the results are presented in Table 1.

TABLE 1. *Relative virulence of natural and artificial conidia on HB cumbu*

| Inoculum | Number of earheads inoculated | Number of earheads infected | Percentage of infection | Intensity of spikelets infection |
|---|---|---|---|---|
| Conidia (Natural) | 20 | 20 | 100 | About 75% of spikelets infected in each earhead |
| Conidia (Artificial) | 20 | 9 | 45 | Not more than about 3% of spikelets infected in each earhead |
| Control (Sterile water) | 20 | 0 | 0 | No infection |

The results indicated that the naturally occurring conidia are highly virulent than those produced in arificial culture in producing infection.

II. *Effect of humidity on the incidence of the disease*: Under field conditions severe incidence of the disease was generally observed in cool and humid weather. To assess the role of humidity on the disease incidence an experiment was conducted under pot culture conditions. Twenty earheads in each treatment after spray-inoculation with conidial suspension were covered with polythene covers in one set while they were left uncovered after inoculation. The experiment was conducted twice during the season once during the winter month December and again during February when the temperatures are fairly high. The percentage of infected earheads as well as the number of

spikelets infected in each earhead were recorded and the results are presented in Table 2.

TABLE 2.. *Effect of humidity on the incidence of the disease*

| Treatment | Number of earheads infected | Percentage of infection | Intensity of spikelets infection |
|---|---|---|---|
| *December 1967* | | | |
| Polythene covered earheads | 20 | 100 | 90% of the spikelets infected |
| Uncovered control | 16 | 80 | 90% of the spikelets infected |
| *February 1968* | | | |
| Polythene covered earheads | 18 | 90 | 80% of the spikelets infected |
| Uncovered control | 6 | 30 | Only few spikelets infected |

It is seen from the results above that under high humid weather which prevailed during the month of December, 1967 the effect of covering the earheads with polythene was not marked. But under dry weather conditions which prevailed during February 1968, the polythene covered earheads showed remarkably high incidence of the disease than the uncovered earheads. The covered earheads have shown 90% infection against only 30% infection in the uncovered earheads. Thus the presence of high humidity for producing severe disease incidence is quite evident.

III. *Susceptible stage of the inflorescence*: To determine the optimum stage at which infection takes place during the development of the earhead from the time of emergence of the inflorescence,an experiment was conducted using highly susceptible HB 1 *cumbu* variety. Inflorescences of different ages from the just emerged to seven days old were spray inoculated and the number of infected inflorescences and the intensity of spikelet infection in each inflorescence were recorded. The data are presented in Table 3.

The results revealed that infection could take place at any time from the time of emergence of the inflorescence upto a period of six days of its development. But the most vulnerable period appears to be upto 2–3 days of emergence of the inflorescence only.

IV. *Mode of infection*: Three days old inflorescence were spray inoculated and the inoculated earheads were removed after 24, 48, 72 and 96 hours. The spikelets were dissected out under a dissecting microscope. The ovary was separated, stained with cotton blue and observed under the microscope. The earheads examined after 24 hours showed the presence of little growth of mycelium at the base of the ovary. After 48 hours of inoculation,

mycelial growth was found all over the ovary wall. After 72 hours ovaries were found to be completely invaded by the pathogen both externally and internally. The colour of the ovary was changed in the infected spikelets to light red from normal dull white colour. After 96 hours, there was abundant production of conidia all over the wall. The mycelial growth was noticed on the style after 48 hours of inoculation in a few cases only. Rarely the mycelium was found spreading from the style on to the ovary.

TABLE 3. *Susceptible stage of the inflorescence during earhead development*

| Stage of inoculation | Number of earheads infection | Percentage of infection | Intensity of spikelet infection |
|---|---|---|---|
| Partially emerged inflorescence (No stigmas were protruding out) | 25 | 100 | About 30% of the spikelets took infection. The bottom most ones remained healthy. Emergence of anthers was prevented. |
| One day old (Inflorescence fully emerged. Stigmas just protruding out) | 25 | 100 | About 50% of the spikelets took infection. |
| Two days old (Stigmas partially emerged) | 25 | 100 | More than 75% of the spikelets took infection. |
| Three days old (Stigmas fully emerged) | 25 | 100 | More than 75% of the spikelets took infection. |
| Four days old (Stigmas started withering) | 18 | 72 | Only 25% of the spikelets took infection. |
| Five days old (Anthers emerged out at the top. Top stigmas completely withered and the bottom most started withering) | 7 | 28 | Only about 5% of the spikelets took infection. In majority of the cases only bottom spikelets were infected. |
| Six days old (Anthers completely emerged out) | 2 | 8 | Only few spikelets took infection at the bottom of the earhead. |
| Seven days old | 0 | 0 | No infection |

(25 earheads were inoculated in each treatment)

V. *Transmission of the disease :* a) *Through seed:* Since the pathogen was found to infect the earheads, the possibility of transmission of the disease through seed was assessed. HB 1 *cumbu* seeds were treated with a heavy spore suspension of the pathogen and dried in the shade for 24 hours. Then the seeds were sown in sterilized soil and the plants were grown upto maturity under pot culture conditions. As soon as the inflorescence emerged, it was

covered with polythene covers to eliminate external contamination of spikelets by air-borne spores. Observations recorded on the incidence of the disease revealed that there was complete absence of the disease in all the plants raised from infected seeds indicating thereby that the disease is not seed-borne.

b) *Through soil:* The surgary exudations containing numerous conidia were found to drip down on the ground from affected *cumbu* earheads. Hence the possibility of soil borne infection of the disease was assessed. A heavy spore suspension of the pathogen was added to the sterilized soil in pots and the surface sterilized HB 1 *cumbu* seeds were sown. The earheads were covered when they were in boot leaf stage to prevent air-borne infection. No plants were found to be infected indicating that the disease is not transmitted through soil.

VI *Host range:* With a view to asses whether any of the grass hosts found around the *cumbu* fields could act as collateral hosts for this pathogen, 17 graminaceous hosts were tested for their susceptibility to the pathogen. The following grass hosts were grown in pots in the pot culture house and the inflorescences were spray inoculated when they were 2–3 days. old: *Pennisetum polystachyon, P. longistylis, P. orientale, P. squamulatum, P. massaicum, P. villosum, P. hohenackeri, P. rupelli, Appluda varia, Cenchrus ciliaris, C. setigerous, Paspalum dilatatum, Digitaria sp. Urochlova sp. Setaria holstii, S. sphacelata, Panicum maximum.* The inoculated plants were under observations for the honey dew exudations for a period of one month.

Among the different grass hosts tested only two species of *Pennisetum viz., P. squamulatum* (13 %) and *P. massaicum* (10 %) were found to be susceptible to the pathogen. The sclerotial production was noticed in *P. massaicum* while in *P. squamulatum* only honey dew exudation was noticed.

VII. *Cross inoculation studies:* The incidence of sugary diseases was on the increase with the introduction of high yielding varieties of *sorghum* like CSH 1. The incidence of ergot disease on HB 1 *cumbu* was also occurring on large scale. With a view to determine whether the pathogens from these two hosts are cross inoculable, experiments were conducted. *Sphacelia sorghi* was brought into pure culture by single spore isolation from sugary exudations on *sorghum* earheads and a heavy conidial suspension was sprayed on HB 1 *cumbu* inflorescences 3–4 days old. CSH 1 *sorghum* inflorescences were inoculated with conidial suspensions of *C.microcephala.*

The pathogen from *cumbu, C. microcephala* was not able to pass on to *sorghum* while the pathogen from *sorghum (S. sorghi)* was able to infect *cumbu* producing 20 % infection. The pathogen from the infected *cumbu*

earheads sprayed with *S. sorghi* was reisolated and found to be identical with *S. sorghi*. The isolated pathogen was reinoculated on CSH 1 and was found to give successful infection of the spikelets. The results thus clearly indicated that *S. sorghi* from *sorghum* is cross inoculable on *cumbu* while *C. microcephala* from *cumbu* is specific to its own host only.

VIII. *Varietal resistance studies:* The popular varieties of *cumbu* grown in this State *viz.*, CO 3, CO 4, CO 5, X-3 and HB 1 and the parents of HB 1 hybrid variety *viz.*, 23-A (Female parent) and BII 3-B (Male parent) were tested for their susceptibility to the pathogen under pot culture conditions. The inflorescences were spray inoculated 3 days after their emergence. The percentage of infected earheads in each variety was determined. The results are presented in Table 5.

TABLE 5. *Relative resistance of cumbu varieties to the ergot disease*

| *Cumbu* variety | Number of earheads inoculated | Number of earheads infected | Percentage of infection | Intensity of spikelet infection in each earhead |
|---|---|---|---|---|
| CO 3 | 50 | 27 | 54 | More than 50% of the spikelets infected |
| CO 4 | 50 | 33 | 66 | More than 50% of the spikelets infected |
| CO 5 | 50 | 45 | 90 | More than 75% of the spikelets infected |
| X-3 | 50 | 37 | 74 | ,, 50% ,, |
| HB 1 | 50 | 50 | 100 | ,, 75% ,, |
| 23-A | 50 | 28 | 56 | Only few spikelets infected |
| BII. 23-B | 50 | 32 | 64 | About 50% of the spikelets infected |

All the varieties tested were found to be susceptible to the disease in varying degrees of intensity. Among the varieties tested HB 1 and CO 5 were found to be highly susceptible to the disease.

IX. *Fungicidal control:* Seven different fungicides belonging to various groups were tested against the pathogen both in the laboratory as well as on the host plants. Six fungicides *viz.*, 1. Duter (triphenyl stannous hydroxide), 2. Dithane M 45 (Manganese zinc ethylene bisdithio carbamate), 3. Dithane Z 78 (Zinc ethylene bisdithio carbamate), 4. Ziram (Zinc dimethyl dithiocarbamate), 5. Cosan (Wettable sulphur), 6. Miltox (Copper oxychloride and zineb) and 7. Fytolan (Copper oxychloride) were tested.

The efficacy of the various proprietory fungicides against the pathogen was first assessed under laboratory conditions by slide germination tests. The results are presented in Table 6.

TABLE 6. *Efficacy of various fungicides in inhibiting the spore germination*

| Fungicide | Percentage of spore germination | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 5 ppm | 10 ppm | 20 ppm | 30 ppm | 40 ppm | 50 ppm | 60 ppm | 80 ppm | 100 ppm |
| Duter | 61.3 | 25.3 | 6.3 | 0 | 0 | 0 | 0 | 0 | 0 |
| Dithane M 45 | 64.1 | 54 4 | 52.9 | 51.8 | 36 4 | 30.7 | 19.1 | 13.4 | 0 |
| Dithane Z. 78 | 73.5 | 70.5 | 62.6 | 61.7 | 58 0 | 57.9 | 58.3 | 61.8 | 44.4 |
| Ziram | 3 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Miltox | 74.0 | 64.8 | 49.7 | 43.2 | 28.4 | 26.1 | 21.2 | 0 | 0 |
| Fytolan | 56 5 | 45.2 | 42.6 | 35.5 | 32.9 | 32.9 | 33.3 | 25.4 | 18.3 |
| Cosan | 25.6 | 11.8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Control (Distilled water) | 75.0 | — | — | — | — | — | — | — | — |

Some of the fungicides *viz.*, Ziram, Cosan and Duter were found to inhibit the spore germination even at low concentrations. Miltox and Dithane M. 45 were effective at 80 and 100 ppm concentration respectively while Dithane Z. 78 and Fytolan were found to be effective only to a small extent.

To assess the efficacy of fungicides as protectants under green house conditions, the fungicides were first sprayed on the earheads on the second day of their emergence. After 24 hours the fungicide treated earheads were inoculated with heavy spore suspension of the pathogen. The number of earheads infected in each treatment was counted and the data are presented Table 7.

TABLE 7. *Efficacy of various fungicides as pre-inoculation*

| Fungicide | Percentage of earhead in (Transformed values) | | | |
|---|---|---|---|---|
| | Rep. I | Rep. II | Rep. III | Average for transformed values |
| Duter | 48.45 | 41.55 | 56.79 | 48.93 |
| Dithane M. 45 | 56.79 | 56.79 | 50.77 | 54.78 |
| Dithane Z 78 | 52 53 | 68 03 | 57.42 | 59.32 |
| Ziram | 56.79 | 45.00 | 50.77 | 50.83 |
| Miltox | 52.53 | 49.02 | 45.00 | 48.85 |
| Fytolan | 54 94 | 41.55 | 57.42 | 51 30 |
| Cosan | 45.00 | 45.00 | 49.02 | 46.34 |
| Control | 90.00 | 90.00 | 90.00 | 90.00 |

(p=0 01%) S. E. : 3.70 C. D. : 11.09

Conclusion: 7, 5, 1, 4, 6, 2, 3, 8

All the fungicides were found to be statistically on a par and superior to untreated control in controlling the disease.

These results reveal that although there was considerable reduction in the disease intensity in the fungicide treated plants in comparison with untreated control, still the disease incidence is as high as 46% even in the case of best fungicide. This indicates that these fungicides can only reduce the severity of the disease and cannot afford any effective protection against the disease.

All the seven fungicides were also tried as post-inoculation sprays. The emerging inflorescences, 2–3 days old, were first spray inoculated with the pathogen. After 48 hours, various fungicides were sprayed and the percentage of infected plants was recorded. The results are presented in Table 8.

TABLE 8. *Efficacy of various fungicides as post-inoculation sprays*

| Fungicide | Percentage of earhead infection (Transformed values) | | | |
|---|---|---|---|---|
| | Rep. I | Rep. II | Rep. III | Average transformed values |
| Duter | 50.77 | 45.00 | 56.79 | 50.85 |
| Dithane M. 45 | 50.77 | 45.00 | 50.77 | 48.85 |
| Dithane Z. 78 | 63.44 | 45 00 | 50.77 | 53.07 |
| Ziram | 63.44 | 71.56 | 45 00 | 60.00 |
| Miltox | 45.00 | 71.56 | 90.00 | 68.85 |
| Fytolan | 90.00 | 50.77 | 63.44 | 68.07 |
| Cosan | 56.79 | 71.56 | 50.77 | 59.71 |
| Control | 90.00 | 90.00 | 90.00 | 90.00 |

The data were statistically analysed and found to be not significant. These results, however, indicated that the disease incidence in general is high compared to pre-inoculation treatments indicating thereby its superiority over post-inoculation treatment.

**Discussion:** The incidence of ergot disease on *cumbu* was not known in the Tamil Nadu before the introduction of high yielding hybrid variety of HB 1. The symptoms observed in the infected HB 1 *cumbu* variety in the present investigation were quite similar to those reported by Bhide and Hedge (1957) and Dawkhar and Sulaiman (1965) from Maharashtra. The pathogenicity tests conducted with both natural and artificial conidia revealed higher percentage of infection of earheads sprayed with conidia present in the honey dew. It was observed that the germination of naturally occurring conidia in the honey dew was high compared to the conidia produced in agar cultures. The reduced percentage of infection with conidia from the agar

culture may perhaps be due to their poor germination in comparison with conidia from the honey dew. It is probable that the naturally occurring conidia may derive certain stimulatory nutrients from the honey dew of the infected spikelets, which are not readily available in the agar medium. The nature of these substances, however still remains to be investigated. Kirchhoff (1929) and Tanda (1965) also obtained similar results in their inoculation studies with *Claviceps purpurea* on rye, with conidia obtained from diseased earheads and artificial culture media.

The covering of earheads with polythene bags after inoculation was found to yield higher percentage of infection than the uncovered ones. This is obviously due to the creation of high humidity condition congenial for infection. Inoculation experiments carried out during different periods in the season have clearly proved the importance of the presence of high humidity conditions for infection irrespective of the season. High humidity was reported to be congenial for the disease development (Shanmugasundaram *et al.*, 1967). Tanda (1965) reported that period of incubation was shortened in humid weather and secretion of the honey dew continued for 13 days in case of rye attacked by *Claviceps purpurea*.

The earhead of HB 1 *cumbu* was found to be highly susceptible to the pathogen from the day of emergence from boot leaf to the 4th day of emergence, the day when the stigmas were found to wither. In other words, only young ovaries are susceptible to the pathogen. Marudarajan *et al.* (1950) observed that infection was poor in the unopened spikelets and fertilised spikelets of rye when inoculated with *Claviceps purpurea*. Futrell and Webster (1965) reported that inoculation of fertilized florets of *sorghum* with *sorghum* ergot organism did not result in infection. This finding has considerable practical importance in the control of the disease in that the fungicidal protection should be given only at the vulnerable stage of inflorescence and not later in the development of the earhead.

Studies on the mode of entry of the pathogen have revealed that the pathogen could enter the ovary through its walls on any part. Profuse mycelial growth was observed at the base of the ovary. The mycelial growth at the stylar region was rare and scanty which was also found to be not spreading. Therefore, the chances of infection occurring through the stylar region appears to be remote. Ramakrishnan (1953), who studied the mode of entry of ergot pathogen (*C. microcephala*) on *Pennisetum* hybrids, observed that though the spores germinated on the style and stigma, they did not proceed further and infect other parts. But the spores germinated on the ovary wall penetrated and completely occupied the ovary within 96 hours.

The disease was found to be neither seed-borne nor soil-borne. Hence the collateral graminaceous hosts which may probably provide primary inoculum were tested for their susceptibility. The results showed that only two grasses *viz.*, *Pennisetum squamulatum* and *P. massaicum* among the 17 tried were found to be infected by the pathogen. The pathogen has been reported to occur on various grasses like *Phragmites communis*, *Poa annua*, *Pennisetum hohenackeri* and *Echinoclova crusgalli* (Stager, 1908; Thirumalachar, 1945 and Thirumalachar and Mishra, 1953). The cross inoculation studies conducted with *Sphacelia sorghi* from *sorghum* and *Claviceps microcephala* from *cumbu* revealed that the former was able to pass on to *cumbu* while the latter was unable to infect *sorghum*.

All the popularly cultivated varieties of *cumbu* were found to be susceptible to the pathogen indicating that there is no prospect of tackling this disease by growing resistant varieties at this stage. An intensive screening of all the available materials including wild types for resistance has to be taken up with a view to select resistant material, if any.

The efficacy of the various fungicides in the control of the disease was assessed both under laboratory and pot culture conditions. The laboratory studies revealed that a number of fungicides are effective against the pathogen even at very low concentrations. But the pot culture studies showed that none of the fungicides tested was effective in controlling the disease. The intensity of the disease was, however, reduced compared to control. As the inflorescence upto four days after emergence was found to be the most vulnerable stage for infection, the fungicidal spray should be given to protect the inflorescence during that period. Any sprays given after this period are not likely to prove useful.

**Summary**: The ergot disease of *cumbu* caused by *Claviceps microcephala* was studied in detail. The conidia occurring naturally in the honey dew were found to be more virulent than the conidia produced in the artificial culture medium. The earheads were found to be susceptible from the time of their emergence upto 6 days but maximum infection occurred only upto 4 days of their emergence. The presence of high humidity was found to be an important factor in obtaining high disease incidence. The mode of entry of pathogen was found to be largely through the walls of the ovary and the mycelial spread from style on to the ovary was noticed in a few cases only. The disease was found to be neither seed-borne nor soil-borne. Among the various grass hosts tried two grasses *viz.*, *Pennisetum squamulatum* and *P massaicum* were found to be infected by the pathogen. *Claviceps microcephala* was not found to infect *sorghum* while *Sphacelia sorghi* was found to be cross pathogenic to *cumbu*. All the popular varieties of *cumbu* were found to be susceptible to the disease.

A number of fungicides was found to be effective in arresting spore germination in the laboratory. But the green house studies showed that they were able to reduce the intensity of infection to some extent only and not yielded effective control of the disease. Prophylactic application was found to be more beneficial than post-inoculation sprays of the fungicides.

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