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# *In Vitro* Effect of Fungicides, Plant Extracts and Smoke on Conidial Germination of *Fusarium oxysporum* Root Rot Pathogen of *Piper betle*

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**Five fungicides such as rovral, bavistin, cupravit, dithane M-45 and thiovit were tested against conidial germination of *Fusarium oxysporum*. Dithane M-45 was the most effective against the fungus. Rests of the fungicides were more or less effective in the inhibition of conidial germination after 5~30 minutes immersion in 500~2500 ppm concentration. Five plant extracts (leaf extracts of *Ocimum sanctum, Lantana camera, Calotropis procera, Azadirachta indica* and *Vinca rosea*) found to be more or less effective against the fungus. 'Dhup' somke have good inhibitory effect on conidial germination of *F. oxysporum*.**

**KEYWORDS: Fungicides, *Fusarium oxysporum*, Plant extracts, *Piper betle*, Root rot, Smoke**

Betelvine (*Piper betle* L.) is a perennial dioecious creeper and important cash crop of Bangladesh. This crop suffers several diseases in Bangladesh. Root rot disease of betelvine caused by *Fusarium oxysporum* Schlecht.emend. Snyd. & Hans. is a serious soil borne fungal problem in the cultivation of betelvine every year in Bangladesh. *F. oxysporum* is a common soilborne fungus, well represented in every type of soil throughout world (Burgess, 1981). It includes a large diversity of strains, all of which are successful saprophytes, i.e. able to grow and survive for long periods on organic matter in soil and in the rhizosphere of many plant species (Garrett, 1970). Many strains are parasitic but nonpathogenic, i.e., able to invade to some extent plant tissues without inducing symptoms, and some of them are pathogenic and induce either root rot or tracheomycoses. The wilt-inducing strains of *F. oxysporum* cause severe damage on a wide range of economically important crops (Snyder and Hansen, 1949). They exist in many specialized forms and are grouped into formae speciales and physiological races depending on their pathogenicity towards particular plant species or cultivars (Armstrong and Armstrong, 1981). *Fusarium moniliforme* Sheldon and *F. oxysporum* cause root, stem, and crown rots of asparagus (*Asparagus officinalis* L.) (Cohen and Heald, 1941; Johnston *et al.*, 1979). Soil borne pathogens are known to persist in field soils long after a susceptible crop has been removed (Haware and Nene, 1982). It is also known that many *Fusarium* spp. persist in soil by means of chlamydospores (Park, 1965). The soil borne pathogen infects root and reduces stand persistence (Graham *et al.*, 1979; Hason and Allison, 1951; Houston *et al.*, 1960). In natural stand, the infected stems wilt and eventually the entire plant is killed. A distinctive characteristic of the disease is dark black discoloration of the root and stem, which corresponds to external symptom severity of betelvine. Several workers have reviewed fungal diseases of "betelvine" from time to time (Dastur, 1935; Mehrotra, 1961; Saksena, 1977; Maiti and Sen, 1979). Mehtotra (1981) and Balasubrahmanyam *et al.* (1981) presented good review on the types of fungal diseases of betelvine along with other aspect of betelvine diseases. The microbial succession on leaves and stems has been the subject matter for investigations since 1955 (Last, 1955; Ruinen, 1965; Preece, 1963; Hallam and Juniper, 1971; Preece and Dickinson, 1971; Sharma and Mukerji, 1972, 1973, 1976; Dickinson and Preece, 1976). The systemic fungicide benomyl has been shown to be effective against both fusaria associated with asparagus (Manning and Vardaro, 1977) and other seed borne fungi (Harman and Nash, 1978). Alam *et al.* (2002) used different plant extracts for the inhibition of *F. oxysporum* f. sp. *vasinfectum* and other fungi and got good results. To solve this problem, we compared the efficacy of several fungicides, plant extracts and smoke in this experiment.

## Materials and Methods

**Organism used.** *F. oxysporum* was isolated from root tissue of infected *Piper betle* and cultured on PDA. Conidia were taken from 10 days old culture on PDA and conidial suspension ($10^5$ conidia/m*l*) was made separately in different concentrations of active ingredients (500, 1000, 1500, 2000 and 2500 ppm) of selected fungicides namely rovral (eprodion), bavistin (methyl-2-benzimidazole car-

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bamate), cupravit (copper oxychloride), dithane M-45 (manganous ethylene bisdithiocarbamate + zinc sulfate) and thiovit (sulphur fungicides). The suspension of fungicides with conidia were taken in sterilized watch glass and kept at 25±2°C for 5~30 minutes. A drop of fungicidal treated conidial suspension was taken on separate slide at 5 minutes interval and kept in moisture chamber at 25± 2°C for 24 hours of incubation. Then a drop of lactophenol cotton blue was put on conidial suspension on the slides. The slides were examined under the microscope of high power (×400) for recording the percentage of conidial germination.

**Extraction of plant.** Extraction of leaf tissues of *Ocimum sanctum*, *Lantana camera*, *Calotropis procera*, *Azadirachta indica* and *Vinca rosea* in alcohol was done following the method described by Mahadevan and Sridhar (1982). The volume (10 m*l*) of the extracts were evaporated on a steam bath to dryness and 1.25 m*l* of sterilized distilled water was added for five grams of tissues and the extracts were used as fungicides.

**Germination of conidia.** Conidia from the culture on PDA plates were taken and conidial suspensions ($10^5$ conidia/ml) were made separately with different plant extracts. These suspensions (1.25 m*l*) were taken in small sterilized Petri dishes (65 mm) and were kept at 25±2°C for 5~30 minutes. After that, a drop of treated conidial suspension from different plant extracts were taken on separate slides at 5 minutes interval and were kept at 25±2°C in a moisture chamber for 24 hours of incubation. Then a drop of lactophenol cotton blue was put on the conidial suspension on the slides. The slides were examined under the microscope of high power (×400) for recording the percentage of conidial germination of *F. oxysporum*. Application of smoke in *in vitro* was done by according to the methods of Alam *et al.* (1999).

Statistical analysis of data given as percentage was carried out from angular transformed values and performed using Microsoft Excel software. LSD were determined, whenever, the calculated 'F' value were significant at 5% level (Snedecor and Cochran, 1980).

## Results and Discussion

Out of five fungicides tested, dithane M-45 was the most effective against *F. oxysporum* when the fungus was immersed for 5~30 minutes at 500~2500 ppm concentrations. Hundred per cent conidial germination inhibition occurred after treating the fungicide in all cases of immersion and concentrations. Rests of the fungicides (rovral, bavistin, cupravit and thiovit) have more or less inhibitory effect on conidial germination (39, 16, 26 and 43%) of *F. oxysporum* at the concentration of 2500 ppm after immersion duration of 30 minutes. Except dithane M-45, with the increase of concentration and immersion period, the inhibitory effect of conidial germination of this fungus also decreased (Table 1). Correlation values 0.999 to 0.995, –0.999 to 0.998, –0.999 to 0.997 and 0.999 to 0.993 in case of rovral, bavistin, cupravit and thiovit, respectively, indicate highly negative correlation between immersion period and conidial germination. Correlation values 0.993, –0.999, –0.990 and 0.998 for rovral, bavistin, cupravit and thiovit, respectively, also indicate highly negative correlation between concentrations and conidial germination. Calculated 'F' value is greater than table value in all the cases of rovral, bavistin, cupravit and thiovit. It indicated that there was significant difference role on conidial germination of *F. oxysporum* of the concentrations of each fungicide and immersion period of conidia in all cases. Alam *et al.* (2002) tested the fungicides viz., cupravit, thiovit, dithan M-45, bavistin, rovral and suncozeb against conidial germination of *Colletotrichum gloeosporioides* and found that dithane M-45, rovral, suncozeb

**Table 1.** Effect of different concentrations of fungicide on the inhibition of conidial germination of *Fusarium oxysporum* after 8 hours of incubation at 25±2°C

| Name of fungicide[a] | Concentration (ppm) | Percentage of conidial germination in different incubation period (minutes)[a] | | | | | |
|---|---|---|---|---|---|---|---|
| | | 5[b] | 10 | 15 | 20 | 25 | 30 |
| Rovral | 500 | 91 | 88 | 85 | 81 | 75 | 69 |
| | 1000 | 88 | 84 | 79 | 75 | 69 | 63 |
| | 1500 | 83 | 78 | 72 | 66 | 62 | 57 |
| | 2000 | 79 | 73 | 68 | 62 | 56 | 51 |
| | 2500 | 70 | 64 | 57 | 51 | 44 | 39 |
| Bavistin | 500 | 68 | 64 | 58 | 52 | 45 | 40 |
| | 1000 | 63 | 58 | 52 | 46 | 40 | 35 |
| | 1500 | 57 | 52 | 46 | 39 | 34 | 28 |
| | 2000 | 51 | 45 | 40 | 34 | 28 | 23 |
| | 2500 | 44 | 38 | 33 | 27 | 22 | 16 |
| Cupravit | 500 | 75 | 71 | 66 | 60 | 55 | 49 |
| | 1000 | 71 | 65 | 59 | 53 | 48 | 42 |
| | 1500 | 67 | 61 | 56 | 52 | 46 | 41 |
| | 2000 | 61 | 55 | 49 | 44 | 38 | 32 |
| | 2500 | 54 | 49 | 43 | 37 | 31 | 26 |
| Dithane M-45 | 500 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1000 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1500 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 2000 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 2500 | 0 | 0 | 0 | 0 | 0 | 0 |
| Thiovit | 500 | 94 | 91 | 87 | 82 | 76 | 70 |
| | 1000 | 90 | 85 | 79 | 74 | 68 | 63 |
| | 1500 | 85 | 78 | 72 | 68 | 63 | 57 |
| | 2000 | 79 | 73 | 67 | 62 | 57 | 51 |
| | 2500 | 72 | 66 | 61 | 55 | 49 | 43 |

[a]Mean of three replications.
[b]Period of incubation.

and thiovit were most effective ones. Cupravit and bavistin were less effective against conidial germination of the fungus. Hossain *et al.* (2001) reported the efficacy of different fungicides in controlling purple blotch of onion seed-crop and observed that combined application of rovral 50wp 0.2% + redomil MZ-72 0.2% gave the best control of purple blotch and maximum seed yield of onion followed by individual application of rovral 50wp 0.2% and score 250EC 0.05% when sprayed at an interval of 15 days. Alam *et al.* (2000) reported the effect of fungicides on the inhibition of *Bipolaris sorokiniana* and found bavistin, dithane M-45 and tilt were the most effective fungicides. They stated that at 500 to 2500 ppm and 1/10 to 1/1000 m*l* concentrations were most effective after 5 to 30 minutes immersion. Alam *et al.* (1999) reported the growth inhibition (*in vitro*) of chilli fruit rot pathogen *Alternaria tenuis* and found that redomil, dithane M-45, cupravit, bavistin and rovral proved to be the most effective against *A. tenuis* when immersed for 5 to 30 minutes at 500 to 2500 ppm concentrations. Cochrane (1958) reported that there was no useful universal fungicide. A fungicide, which is lethal or highly toxic to a particular fungus, may be totally ineffective against another fungus even at higher concentrations. Present finding shows that bavistin, cupravit and thiovit were less effective and dithane M-45 was highly effective against *F. oxysporum*. The results of the present investigation are in the same opinion with the comments of Cochrane (1958).

Different concentrations of five plant extracts were tested against the conidial germination inhibition of this fungus. All of the plant extracts and their concentrations inhibited more or less conidial germination of *F. oxysporum*. Conidial germination of *F. oxysporum* was occurred at 20, 13, 9, 15 and 17% by using *O. sanctum*, *L. camera*, *C. procera*, *A. indica* and *V. rosea* extracts respectively after immersion 30 minutes at 2.5% concentration in all cases (Table 2). In every case, conidial germination decreased with the increase of concentrations and immersion period. Correlation (r3) values 0.998 to 0.983, –0.999 to 0.998, –0.999 to 0.996, –0.999 to 0.998 and –0.999 to 0.998 for *O. sanctum*, *L. camera*, *C. procera*, *A. indica* and *V. rosea*, respectively, indicate highly negative correlation between immersion period and conidial germination. Correlation (r4) values 0.969, –0.986, –0.962, –0.999 and 0.999 for *O. sanctum*, *L. camera*, *C. procera*, *A. indica* and *V. rosea*, respectively, also indicate highly negative correlation between concentration of extracts and conidial germination. Calculated 'F' value is greater than table value in all the cases of plant extracts. It indicated that there was significant difference role of concentrations of each tested plant extracts and immersion period on conidial germination of *F. oxysporum*. Alam *et al.* (2002) considered ten plant extracts as fungicides and found *T. eracta* leaf and *A. indica* bark extracts were most effective in inhibiting

**Table 2.** Effect of different concentrations of plant extracts on conidial germination of *Fusarium oxysporum* after 8 hours of incubation at 25±2°C

| Name of plant | Concentrations (%) | Percentage of conidial germination in different immersion period (minutes)[a] | | | | | |
|---|---|---|---|---|---|---|---|
| | | 5[b] | 10 | 15 | 20 | 25 | 30 |
| *Ocimum sanctum* (Leaf) | 1 | 59 | 53 | 47 | 39 | 35 | 28 |
| | 1.5 | 54 | 48 | 40 | 35 | 27 | 22 |
| | 2 | 49 | 43 | 38 | 31 | 27 | 23 |
| | 2.5 | 46 | 40 | 35 | 29 | 24 | 20 |
| *Lantana camera* | 1 | 50 | 45 | 39 | 33 | 27 | 22 |
| | 1.5 | 46 | 41 | 37 | 31 | 26 | 22 |
| | 2 | 41 | 37 | 32 | 26 | 21 | 17 |
| | 2.5 | 37 | 32 | 27 | 22 | 17 | 13 |
| *Calotropis procera* | 1 | 45 | 39 | 34 | 30 | 26 | 23 |
| | 1.5 | 42 | 38 | 34 | 30 | 26 | 22 |
| | 2 | 38 | 32 | 27 | 21 | 16 | 11 |
| | 2.5 | 32 | 27 | 21 | 17 | 13 | 9 |
| *Azadirachta indica* | 1 | 51 | 47 | 41 | 35 | 30 | 26 |
| | 1.5 | 47 | 41 | 36 | 32 | 27 | 22 |
| | 2 | 43 | 39 | 34 | 28 | 23 | 18 |
| | 2.5 | 40 | 35 | 28 | 24 | 19 | 15 |
| *Vinca rasea* | 1 | 63 | 57 | 52 | 46 | 42 | 36 |
| | 1.5 | 58 | 52 | 45 | 39 | 35 | 29 |
| | 2 | 52 | 47 | 41 | 35 | 28 | 22 |
| | 2.5 | 46 | 40 | 34 | 26 | 21 | 17 |

[a]Mean of three replications.
[b]Period of immersion.

conidial germination of *Colletotrichum gloeosporioides* after 5~30 minutes of immersion in 5 : 1.25 (w/v) concentration. Alam *et al.* (2002) investigated on inhibition of spore/conidial germination of four fungi namely *B. sorokiniana*, *F. oxysporum* f. sp. *vasinfectum*, *R. artocarpi* and *Botryodiplodia theobromae* by using the extracts of different parts of *V. rosea* and *A. indica*. *V. rosea* root extracts inhibited 100% spore germination of *B. sorokiniana* and *R. artocarpi* when it was immersed from 5~30 minutes at 5 : 1.25 (w/v) concentration. *A. indica* (leaf, root and seed) extracts showed good (100%) inhibition results on *B. sorokiniana*, and *R. artocarpi*. Alam *et al.* (1999) reported the antifungal effects of leaf and root extracts of *V. rosea* and leaf, root and seed extracts of *A. indica* against chilli fruit rot pathogen *A. tenuis*. Singh *et al.* (1993) reported the antifungal activities of leaf extracts against *B. theobromae*, *F. oxysporum*, *Helminthosporium spiciferum*, *Curvularia lunata*, *Aspergillus flavus* and *Trichothecium roseum*. They used some medicinal plants such as, *C. procera*, *Vitex negundo*, *L. camara*, *A. indica*, *Ficus religiosa*, *O. sanctum*, *Thuja orientalis*, *Argemone mexicana*, *Achyranthes aspera*, *Datura fastuosa* and *Ricinus communis* and observed good control against these pathogens. Of the 11 leaf extracts, those of *A. indica* and *O. sanctum* were most

**Table 3.** Effect of different smokes on conidial germination of *Fusarium oxysporum* grown in PDA mediun previously exposed to rice straw, wheat straw, tobacco leaf and dhup burnt for different duration of time

| Treatment | Percentage of conidial germination after exposing smokes for different period (minute)[a] | | | |
|---|---|---|---|---|
| | 5[b] | 10 | 15 | 20 |
| Rice straw | 13 | 6 | 0 | 0 |
| Wheat straw | 21 | 8 | 0 | 0 |
| Tobacco leaf | 87 | 83 | 71 | 60 |
| Dhup | 11 | 4 | 0 | 0 |

[a]Mean of three replications.
[b]Period of incubation.

effective in controlling the fungi. The present study indicates the presence of some antifungal compounds in leaf of tested plants.

Smoke of rice straw, wheat straw, tobacco leaf and 'dhup' were effective for the inhibition of conidial ger mination of *F. oxysporum*. Hundred percent inhibition of conidia germination occurred when the fungus was smoked using rice straw, wheat straw and 'dhup' for 15 minutes in a smoke chamber. Among the used smoke for the inhibition of this fungus, 'dhup' smoke showed better results. Effect of tobacco leaf smoke was less effective on controlling the fungus (Table 3). Correlation value –0.987 to –0.946 indicate that there is highly significant negative relationships between smoke exposed period and conidial germination. Calculated 'F' value of treatment and exposed period were greater than table value. This result indicates significant role of smoke from different source and their exposure period on the inhibition of conidial germination. Alam *et al.* (2002) stated that smoke of rice straw, wheat straw, tobacco leaf and dhup had a great antifungal effect against *B. sorokiniana*, *F. oxysporum* f. sp. *vasinfectum*, *R. artocarpi* and *B. theobromae*. Alam *et al.* (1999) reported that when smoke of rice straw and dhup exposed for 5 to 15 minutes on chilli fruit rot pathogen *A. tenuis* inoculated medium, the growth of the pathogen was totally inhibited. They also observed tobacco leaf smoke was ineffective against *A. tenuis* as fungitoxicide.

It is noticable that the application of tested fungicides at low dose and mentioned indigenous plant extracts and smoke will reduce the severity of root rot disease of *P. betle* in field.

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