# *In Vitro* Efficacy of Some Plant Extracts and Fungicides Against the Wilting of *Dalbergia sissoo* Caused by *Fusarium Solani* f. *dalbergiae*

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## Abstract

Seven plant extracts and seven fungicides were tested to control the wilting disease of *Dalbergia sissoo* caused by *Fusarium solani* f. *dalbergiae*. Plant extracts of *Vitex negundo* (Nishinda), *Azadirachta indica* (Neem), *Ocimum basilicum* (Tulsi), *Tagetes patula* (Gandha), *Polygonum hydropiper* (Bishkantali), *Adhatoda vasica* (Basok) and *Centella asitica* (Thankuni) were tested in 100%, 50% and 25% concentration in controlling the vegetative growth of the fungus. Basilieum inhibited the highest amount, about 56% growth of the culture. *A. vasica* and *V. negundo* were the second and third in controlling about 43% and about 42% of the vegetative growth. *C. asiatica* controled 40% while *A. indica* and *T. patula* controled 39% and 37%. *P. hydropiper* was the least effective antifungal that inhibited about 32% growth of the tested fungus.

Synthetic fungicides namely Ridomil Gold (Metalaxyl-M & Mancozeb), Thiovit (Sulphur), Sunvit (Copper Oxychloride), Dithane M-45 (Mancozeb), Avistin (Carbendazim), Bavistin (Carbendazim) and Forastin (Carbendazim) were screened out to test their efficacies in 500 ppm, 250 ppm and 125 ppm concentrations. Bavistin was the most effective of all in digesting the growth of the culture by 73%, 65% and 64% in three concentrations respectively. Forastin was the second in destroying about 66%, 63% and 58% while Avistin ranked the third in digesting 59%, 52% and 41% mycelial growth in three fugicidal concentrations. Diathene M-45, Ridomil Gold, Sunvit and Thiovit inhibited 30%, 28%, 27% and 24% respectively.

## সারসংক্ষেপ

শিশু গাছের উইল্টিং রোগের জন্য দায়ী ফিউজেরিয়াম সোলানী নামক ছত্রাক দমনের উদ্দেশ্যে সাতটি গাছের নির্যাস ও রাসায়নিক ছত্রাকনাশকের কার্যকারীতা পরীক্ষা করা হয়। *Vitex negundo* (নিশিন্দা), *Azadirachta indica* (নিম), *Ocimum basilicum* (তুলসী), *Tagetes patula* (গাঁদা), *Polygonum hydropiper* (বিষকাঁঠালী), *Adhatoda vasica* (বাসক) এবং *Centella asiatica* (থানকুনি) গাছের ১০০%, ৫০% ও ২৫% গাঢ়ত্বে এসব উদ্ভিজ্জ নির্যাসের ছত্রাক-বিধ্বংসী ক্ষমতার মূল্যায়ণ করা হয়। পরীক্ষাধীন ছত্রাকের ৫৬% দৈহিক বৃদ্ধি বন্ধ করে তুলসী সর্বাপেক্ষা বেশি কার্যকরী প্রাকৃতিক ছত্রাকনাশক হিসেবে প্রমাণিত হয়েছে। এ কাজে ছত্রাকের প্রায় ৪৩% ও প্রায় ৪২% বৃদ্ধি ধ্বংস করে বাসক ও নিশিন্দার কার্যকারীতার অবস্থান ছিল যথাক্রমে দ্বিতীয় ও তৃতীয়। থানকুনির নির্যাস ছত্রাকের ৪০% এবং নিম ও গাঁদা যথাক্রমে ৩৯% ও ৩৭% বৃদ্ধি ধ্বংস করেছে। ছত্রাক ধ্বংসের কাজে বিষকাঁঠালীর কার্যক্ষমতা ছিল সর্বনিম্ন; এটি ছত্রাকের প্রায় ৩২% দৈহিক বৃদ্ধি বন্ধ করতে সক্ষম হয়েছে।

রিডোমিল গোল্ড (মেটালেক্সিল-এম এন্ড ম্যানকোজেব), থিওভিট (সালফার), সালভিট (কপার অক্সিক্লোরাইড), ডায়থেন এম-৪৫ (মানকোজেব), এভিস্টিন (কার্বনডেজিম), ব্যাভিস্টিন (কার্বনডেজিম) ও ফোরাসটিল (কার্বনডেজিম) নামক সংশ্লেষীয় ছত্রাকনাশকের ৫০০ পিপিএম, ২৫০ পিপিএম ও ১২৫ পিপিএম ঘনত্বে এদের কার্যকারীতা পরীক্ষা করা হয়। উক্ত তিনটি ঘনত্বে ছত্রাকের প্রায় ৭৩%, ৬৫% ও ৬৪% মৈহিক বৃদ্ধি ধ্বংস করে তিনটি ভিন্ন গাঢ়ত্বে ব্যাভিস্টিন সর্বাধিক সফলতা দেখিয়েছে। এ কাজে দ্বিতীয় স্থানে থাকা ফোরাসটিন-এর পারঙ্গমতা ছিল প্রায় ৬৬%, ৬৩% ও ৫৮% এবং এভিস্টিলের স্থান ছিল তৃতীয় যা ছত্রাকের প্রায় ৫৯%, ৫২% ও ৪১% মৈহিক বৃদ্ধি ধ্বংস করেছে। ডায়থেন এম-৪৫, রিডোমিল গোল্ড, সালভিট ও থিওভিট ছত্রাকের বৃদ্ধি রোধে যথাক্রমে ৩০%, ২৮%, ২৭% ও ২৪% কার্যকরী।

**Keywords** : Antifungal, *Dalbergia sissoo*, fungicides, *Fusarium solani*, f. *dalbergiae*, mycelial growths

## Introduction

During 1990s, a serious disease threatening the survival of *Dalbergia sissoo* Roxb. was observed in several SAARC countries including Bangladesh. The plant was widely used for growing plantations of community and agro-forestry in Bangladesh. The disease was studied critically in the Bangladesh Forest Research Institute (BFRI), Chittagong. The causal fungus was identified as *Fusarium solani* f. *dalbergiae*, and some management practices for the disease were suggested (Basak 1994, Basak *et al.* 2003, Basak 2006, Basak and Basak 2011). The objective of the research was to develop ways of prevention or curation of the disease.

Integrated Pest Management (IPM) that utilizes all suitable techniques and methods in a compatible manner and maintains the population at levels below causing economic injury, was tried. Among the several components of IPM e.g., appropriate silvicultural practices, crop rotation, quarantine, biological control, biotechnology of developing host-plant resistance and use of pesticides, use of plant extracts, biological agents and fungicides, were applied.

Green plants possess a vast reservoir of chemicals which are effective against different diseases. Only 10% of these have been tested against human diseases but less against diseases of plants (Nityananda 1977). The earliest mention of poisonous plants is found in Wrik Veda during second millennium B.C. (Hoddy 1991). Recently, search for antifungal natural compounds has become intense due to increasing concern about pollutive effects of synthetic fungicides on environment (Alice and Rao 1987). Many pathogens are becoming resistant against those chemicals due to their consistent usages. Due to low phytotoxicity, cost-effectiveness, systemicity, biodegradability and capacity to stimulate host metabolism, antifungal compounds of plants are advantageous over synthetic fungicides (Beye 1978). Tewari (1998) listed some promising plant extracts which have proved antifungal toxicities. Anwar *et al.* (1994) reported antifungal activity of 23 plant extracts. There are works on antimicrobial studies of essential oils from plants (Chowdhury *et al.* 2003). Bhowmik and Chowdhury (1982) reported that leaf extract of *Azadirachta indica* had the highest inhibition of mycelial growth of *Alternaria alternata*. In Bangladesh, some plant extracts showed effective in controlling selected fungal pathogens of jute, rice and chickpea (Miah *et al.* 1990, Basher and Rai 1991).

Chemical pesticides are poisons and can control many plant diseases, though their misuse is destroying our environment. With the application of first commercial pesticide in 1867 that contained arsenical compound followed by Bordeaux mixture in 1885, lime

sulphur in 1880, lead arsenate in 1892, Thiram in 1931, DDT in 1939 and 2, 4-D in 1942, much reliance was placed on chemicals to control pests and diseases from 1940s by replacing all other control practices. This led to the misuse of pesticides and the emergence of environmental, health, economic and new pest and disease problems. Consequently, Integrated Pest Management (IPM) was developed to reduce the usages of pesticides. There is no extensive work on the antifungal activity of plant extracts and fungicides against the wilt fungus of *D. sissoo*. So, the present work was conducted to find out the procedures and impacts of the evaluated herbicides and fungicides on inhibiting the growth the fungal mycelium.

## Materials and Methods

The mycelial growth inhibition test of the fungus, *Fusarium solani f. dalbergiae* was conducted in 2004-05 in the Silviculture Genetics Division of Bangladesh Forest Research Institute, Chittagong. Fresh leaves of *Azadirachta indica, Vitex negundo, Adhatoda vasica, Ocimum basilicum, Centella asiatica, Polygonum hydropiper* and *Tagetes patula* were collected, cleaned, washed with water, dried over tissue paper and cut into 1-2 cm long pieces. Twenty-five grams of each sample was kept for 24 hours, inside separate conical flasks containing 50 ml sterile water maintaining a materials-water ratio of 1:2 (W/V). Then, they were crushed in a mortar and pastle. Extracts were first sieved through several layers of cheese cloth and finally through filter papers. Filtrates were sterilized for 15 minutes at 121°C under 15 PSI. Potato Dextrose Agar (PDA) medium was prepared, sterilized for 20 minutes at 40-45°C, and poured into Petridishes. Concentrated extracts of 10 ml having 100% concentration, were poured into 50, 55 and 57.5 ml PDA medium. Thus, 60 ml poisoned PDA for each concentration of each extract was prepared. The poisoned-foods were plated aseptically into 90 mm Petridishes at the rate of 20 ml in each for three replications of each concentration. Against each concentration, three replications of control Petridishes contained PDA and sterile water only. From seven days old culture, 9 mm diameter mycelial disc was cut by an agar cutter; excess agar was removed by a sterile scalpel, and the disc was placed inverted onto a food medium for helping the fungus to grow without delay. The plates were left at 27-30°C. Data on average diameter of inhibited as well as control growth, were recorded after 5 days of incubation.

The synthetic fungicides, namely Ridomil Gold, Thiovit, Sumvit, Dithane M-45, Avistin, Bavistin and Forastin were diluted into 500, 250 and 125 ppm and marked properly [The preparation of three concentration profiles for each of the fungicides: 1 g Bavistin + 100 ml water = 1% solution, i.e., 10,000 ppm. 1 ml solution from 10,000 ppm plus 9 ml water=1000 ppm. 10 ml from 1000 ppm solution+10 ml water=500 ppm. 10 ml from 500 ppm solution+10 ml water = 250 ppm. 10 ml from 250 ppm solution+10 ml water = 125 ppm]. One millilitre of the prepared solution of any strength was placed at the centre of a sterile Petridish and 15 ml hot PDA was added to that. By agitating the plates for mixing up of the fungicidal solution and PDA gel, the plates were left to solidification. Control plates contained three replications of PDA and sterile water against each concentration. The remaining procedures were similar to those used in case of the plant extracts. The inhibition percentage was calculated.

## Results

The results of the percent inhibition of mycelial growth of *F. solani* f. *dalbergiae* due to antifungal effect of plant extracts were presented in the Table 1. The findings differed greatly depending on the materials

and doses of the plant extracts. *O. basilicum* inhibited the highest amount of 56.36, 45.45 and 35.76% growth in 100, 50 and 25% concentrations respectively. *A. vasica* was the second in case of destruction of 43.04, 41.82 and 29.71% mycelia at 100, 50 and 25% concentrations respectively. *P. hydropiper* was less efficient in inhibiting the fungus, nearly 32% in every concentration. The antifungal performances of *V. negundo* ranked the third and *C. asiatica* the fourth. *A. indica* and *T. patula* were similar in their capabilities.

The result of efficacies of the synthetic fungicides is given in the Table 2. Bavistin proved superior to others in inhibiting the test fungus by 72.73, 64.85 and 64.25% in three concentrations followed by Forastin (66.0, 62.8 and 57.69%). In controlling the fungus, *F. solani f. dalbergiæ*, Avistin ranked the third (59.4%, 51.53% and 41.22%). The range of inhibition of fungal growth by the remaining synthetic fungicides was recorded much lower, 24-30% than those of the Bavistin, Forastin and Avistin (about 41-73%).

## Discussion

Among the plant extracts, *O. basilicum, A. vasica, V. negundo and C. asiatica* showed a very promising result by digesting about 50% vegetative growth of the test fungus within

**Table 1.** *In vitro* efficacy of herbicides in controlling growth of *Fusarium solani f. dalbergiæ*

| Names of the plant extracts | Mean inhibition of mycelial growth (%) | | |
|---|---|---|---|
| | 100% conc. | 50% conc. | 25% conc. |
| ***Vitex negundo*** (Nishinda) | 41.82 | 40.00 | 37.58 |
| ***Azadirachta indica*** (Neem) | 38.80 | 36.98 | 25.45 |
| ***Ocimum basilicum*** (Tulsi) | 56.36 | 45.45 | 35.76 |
| ***Tagetes patula*** (Gandha) | 36.76 | 35.16 | 29.09 |
| ***Polygonum hydropiper*** (Bishkantali) | 32.12 | 32.13 | 31.53 |
| ***Adhatoda vasica*** (Basok) | 43.04 | 41.82 | 29.71 |
| ***Centella asiatica*** (Thankuni) | 40.00 | 35.76 | 34.55 |

**Table 2.** *In vitro* efficacy of synthetic fungicides in controlling *Fusarium solani f. dalbergiæ*

| Fungicides | Mean inhibition of mycelial growth (%) | | |
|---|---|---|---|
| | 500 ppm | 250 ppm | 125 ppm |
| ***Ridomil Gold*** (Metalaxyl-M & Mancozeb) | 27.89 | 23.04 | 19.40 |
| ***Thiovit*** (Sulpher) | 23.73 | 21.81 | 16.04 |
| ***Forastin*** (Carbendazim) | 66.04 | 62.83 | 57.69 |
| ***Bavistin*** (Carbendazim) | 72.73 | 64.85 | 64.25 |
| ***Dithane M-45*** (Mancozeb) | 30.31 | 18.18 | 13.95 |
| ***Sunvit*** (Copper Oxychloride) | 26.67 | 16.98 | 09.09 |
| ***Avistin*** (Carbendazim) | 59.40 | 51.53 | 41.22 |

five days. The above plant extracts contained antimicrobial substances like unsaturated lactones, cyanogenic glycosides, sulphur compounds, phenols, phenolic glycosides and saponins (Singh 1984). Plant extracts of *O. basilicum* inhibited 22 fungi (Dube *et al.* 1989). Plant extracts of *Vitex negundo* was found phytotoxic against *Helminthosporium oryzae* (Grainage *et al.* 1985) and in the present study, it ihhibited 38-42% mycelial growth of the test fungi. The leaf extracts of *Polygonum hydropiper* and *Azadirachta indica* showed 60% and 58% inhibition of mycelial growth of *F. solani* (Basak and Paul 1999). The results indicated that the doses of the plant extracts should be higher than those applied. Autoclaving might have destroyed the antifungal properties of the essential oils of the extracts to some extents and perhaps not by heating alone and storage for a few days. Pesticides of microbes and plants are compatible with IPM programme, and Neem products, at present are being applied as a pesticide.

Due to systemic in nature Bavistin, Forastin and Avistin showed higher results over the others for three concentrations in killing a substantative quantity of the mycelial growth of *F. solani* f. *dalbergiae* within five days. Bavistin inhibited both mycelial growth and spore germination in *in vitro* conditions. It is worthwhile to note that fungicides could be used prior to sowing of seeds, in the seedling stage and also in the plantations after the onset of symptoms of diseases. Harsh (1993) treated seeds of *D. sissoo* with Topsin-M (Thiophanate methyl) and Bavistin (Carbendazim) which provided an adequate control of damping-off in seedlings caused by *Fusarium* spp. Sinha (1975) observed reduction of wilt disease by Bavistin when applied as soil drench at 2000 ppm, 10 days before inoculation of pigeonpea with *F. udum*. Solarization alone or in combination with Bavistin and Captan proved very effective (Kaushik *et al.* 2002). Chakravarty and Misra (1986) found positive results of using VAM in decreasing wilting of *D. sissoo*. In a greenhouse trial, pre-inoculation of VAM fungi and *Ganoderma lennis* against *F. solani* and *F. oxysporum*, increased growth of the plant and reduced serverity of wilting of *D. sissoo*. Organic substrates with high C/N ratio, suppressed *F. solani* when soil was amended with bean straw and saw dust; casualty was minimum (Kaushik *et al.* 1993). Further work may be conducted on application of potassium fertilizer in extra doses, solarization and host-plant resistance.

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## References

Alice, D. and Rao, A. V. 1987. Antifungal effect of plant extract on *Drechslera oryzae* in rice. *International Rice Research Newsletter* 12 (2): 28.

Anwar, M. N.; Singh, P.; Begum, J. and Chowdhury, J. U. 1994. Antifungal activity of some selected plant extracts on phytopathogenic fungi. *Bangladesh Journal of Life Sciences* 6 (2): 23-26.

Basak, A. B. and Paul, P. 1999. Effect of some plant extracts on fruit rot fungal pathogens of chilli. *The Chittagong University Journal of Sciences* 23 (1): 129-135.

Basak, A. C. 1994. Wilting of sissoo (*Dalbergia sissoo* Roxb.) in strip plantations in Bangladesh. *Bangladesh Journal of Forest Sciences* 23 (1): 67-68.

Basak, A. C. 2006. Mortality of sissoo (*Dalbergia sissoo* Roxb.) in Bangladesh and its Management. *PhD Thesis*. Jahangirnagar University, Savar, Dhaka. 240 pp.

Basak, A. C.; Baksha, M. W. and Khair, A. 2003. Symptom and extent of damage caused by wilting of sissoo (*Dalbergia sissoo* Roxb.) in Bangladesh. *In*: Baksha, M. W. (ed.). *Proceedings of the Roundtable Meeting on the Mortality of Sissoo (Dalbergia sissoo) and Top-dying of Sundri (Heritiera fomes) in Bangladesh*. BFRI, Chittagong. PP. 39-42.

Basak, A. C. and Basak, S. R. 2011. Biological control of *Fusarium solani* sp. *dalbergiae*, the wilt pathogen of *Dalbergia sissoo* by *Trichoderma viridae & T. harzianum*. *Journal of Tropical Forest Sciences* 23(4): 460-466.

Basher, M. A. and Rai, B. 1991. Antifungal activity of extracts of some plant parts against *Fusarium oxysporum s.sp. ciceri*. *Bangladesh Journal of Botany* 20 (2): 219-222.

Beye, F. 1978. Insecticides from the vegetables kingdom. *Plant Research Development* 7: 13-31.

Bhowmik, B. N. and Chowdhury, B. K. 1982. Antifungal activity of leaf extracts of medicinal plants on *Alternaria alternata*. *Indian Botanical Report* 1 (2): 164-165.

Chakravarty, P. and Mishra, R. R. 1986. The influence of VA Mycorrhiza on the wilting of *Albizia procera and Dalbergia sissoo*. *European Journal of Forest Pathology* 16 (2): 91-97.

Chowdhury, J. U.; Yusuf, M.; Begum, J.; Sultana, S. A. and Husain, M. M. 2003. Composition and fungitoxic properties of the essential oil of *Lippia javanica leaves*. *Indian Perfumer* 47 (4): 385-388.

Dube, S.; Upadhay, P. D. and Tripathi, S. C. 1989. Antifungal, physico-chemical and insect-repelling activity of the essential oil of *Ocimum basilicum*. *Canadian Journal of Botany* 67: 2085-2087.

Grainge, M.; Ahmed, S.; Wallace, C.; Mitchell and Hylin, J. W. 1985. *Plant Species Reportedly Possessing Pest Control Properties*- AN-EWC/UH Data BAS, pp. 249.

Harsh, N. S. K. 1993. Fungicidal control of damping-off in seedlings of *Albizia lebbek, Dalbergia sissoo and Leucaena leucocephala caused by Fusarium spp*. *Commonwealth Forestry Review* 72 (1): 53-56.

Hoddy, E. 1991. *Nature's Bitter Boon: The Neem Tree-a Subsitute for Pesticides*. Development and Cooperation. No. 5. pp. 29-30.

Kaushik, J. C.; Singh, A. and Nair, K. S. S. 1993. Effect of soil texture and moisture on seedling root rot of *D. sissoo* and its control. *In*: Sharma, J. K. and Varma, R. V. (eds.). *Impact of Diseases and Insect Pests in Tropical Forests*. Proceedings of IUFRO Symposium, Peechi, Kerala, India, 23-26 Nov. 1993. pp. 209-216.

Kaushik, J. C.; Sanjay-Arya.; Gupta, P. P. and Arya, S. 2002. Management of damping-off disease in forest nurseries by soil solarization and bio-control agents. *Forage Research* 28(1):29-31.

Miah, M. A. T.; Ahmed, H. U.; Sharma, N. R.; Ali, A. and Miah, S. A. 1990. Antifungal activity of some plant extracts. *Bangladesh Journal of Botany* 19 (1): 5-10.

Nityanand. 1977. Research on Indigenous Drugs. *Science and Culture* 43:17-21.

Singh, R. S. 1984. *Introduction to Principles of Plant Pathology*. 3rd edition, Oxford and IBH Publication Company, New Delhi. 534 pp.

Sinha, A. K. 1975. Control of *Fusarium* Wilt on Pigeon Pea with Bavistin. *Current Science* 44: 700

Tewari, S. N. 1998. Botanical Toxicants as Potential Component in the Strategy of IPM. *In*: Upadhyay, R. K., Mukherji, K. G., Chamola, B. P. and Dubey, O. P. (eds.). *Integrated Pest and Disease Management*, New Delhi. pp. 617-637.