*PHOMA*S – CAN THESE FUNGI BE USED AS BIOCONTROL AGENTS AND SOURCES OF SECONDARY METABOLITES? (A REVIEW)

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Introduction

Phoma is a genus containing more than 2000 described species. This genus traditionally refers to simple stem inhabiting pycnidial fungus with small hyaline, unicellular pycnidiospores (Sutton, 1980). *Phoma*s cause various serious diseases to plants as well as to humans (Rai 1989, 2000). Besides these harmful aspects, certain *Phoma* species also contains antibiotic potential and economically useful secondary metabolites.
As widespread plant pathogens around the world occur on a broad range of plant species, e.g. *P. glomerata* has been reported on grape, potato, wheat, pear, mango, rice and many other crops. Some important example include *Phoma lingam* that causes the very serious blackleg disease in canola. It is also found on other cruciferous crops such as cabbages, cauliflower and summer rape. *P. medicaginis* var. *medicaginis* is the causal agent of spring black stem of alfalfa. *P. pinodella* causes foot rot in peas and black stem in clovers. All of these pathogens can overwinter in crop debris and are often seedborne. Development of disease symptoms caused by these *Phoma* spp. will often be enhanced in wet and cool conditions. Within this genus large pathogenic variations are shown which often complicates the control of *Phoma*, especially in legumes.

The present review paper is aimed to discuss the role played by *Phoma* species as biocontrol agents and their potential for the production of secondary metabolites.

*Phoma*s as biocontrol agents in weed control

Weeds are serious problems not only for agricultural and forestry fields, but also responsible for several major problems to human and animal health around the world including India. Synthetic chemical herbicides have been the mainstay for weed control practices since the end of World War II and no doubt are responsible for much of the unparalleled increased crop productivity that has occurred during this period. The high costs involved in developing and registering chemical herbicides and recent trends in environmental awareness have prompted researchers to investigate alternative systems of weed control. Ideally, such a system would control target weeds at or near the same levels as that achieved with chemical herbicides while not poisoning a threat to either the environment or non-target organisms at the same time (Pandey et al., 2001).
The science and technology of weed control by using plant pathogens more especially fungal pathogens as an effective alternatives to chemical herbicides have gained significant attention and momentum in 1970. Century old concept in weed control and plant disease epidemiology were successfully put to text and few economically important weeds were controlled by fungal pathogens used under classical and mycoherbicidal strategies. With the advancement in the knowledge biorationals and integrated management strategies have also came into foray. Biological, technological and economical perspectives of various strategies have been extensively reviewed in several publications (Auld, 1990; Charudattan, 1991, 1996; Abbas and Duke, 1995, 1997; Boyette and Abbas, 1995; Hasija et al., 1994; Hoagland, 1990; 1999, 2001; Pandey 1999, 2000; Pandey et al., 1996a,b, 1997, 2001; Saxena and Pandey 2000; Saxena et al., 2001).

As a matter of fact, *Phoma* spp. could be the novel agents for many weed problems. They have both mycoherbicidal and biorational properties. Looking to the number of known species, species associated with weeds are very less. This might be because of ignorance of weed pathogens. Mycologists as well as plant pathologists in the past have given attention to economically important plant diseases. Therefore, urgent attention towards herbicidal potential of *Phoma*s is needed.

It is surprising that despite of excellent phytopathogenic potential shown by various species or varieties of the genus, their mycoherbicidal potential has been ignored significantly. There are only few scanty attempts have been made to evaluate them as mycoherbicides. Heiny (1990, 1994) isolated a highly host specific strain of *Phoma proboscis* from diseased parts of field bindweed (*Convolvulus arvensis*). Heiny and Templeton (1991) have

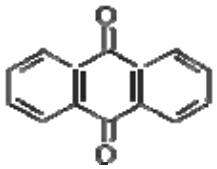
reported very high mycoherbicidal effect when the agent applied to the seedling of the weed and atmospheric temperature ranged from 16-28^{o}C and more than 9 hrs dew period. Heiny (1994) has extensively evaluated the compatibility of synthetic herbicides for integration with the mycoherbicidal agent.

Rajak et al. (1990) isolated a strain of *P. herbarum* from diseased leaves of *Parthenium hysterophorus* L. collected from Central India. The fungus caused more than 90% inhibition in seed germination, seedling mortality and leaf damage followed by reduction in height of *Parthenium* (Pandey et al., 1991). Pandey and Pandey (2000) recovered three strains of *P. herbarum* (LC#32,37,39) from diseased leaves and stem of *Lantana camara.* All the three strains incite severe infection in the weed, especially at seedlings stage (Pandey, 2000). Pandey (2002) also isolated a strain of *P. herbarum* FGCC#70 from disease seedlings of an invasive weed, *Hyptis suaveolens* (L.) Poit. He recorded very high mycoherbicidal potential when seedlings were treated. Several other species viz. *Phoma campanulata* on *Cassia fistula* (Rajak and Rai, 1982), *P. exigua* on *Sesamum indicum* (Singh and Agarwal 1973), *P. eupyrena* on *Achyrenthus aspera* (Khanna and Chandra, 1977), *P. glomerata* on *Crotalaria juncea* (Pathak and Chauhan, 1976), and *Parthenium hysterophrous* (Padmbai, 1976*), P. lantanae* on *Lantana camara* (Singh and Agarwal, 1974), *P. palmarum* on *Calotropis procera* (Khanna and Chandra, 1977; Kamal and Singh, 1979), *P. tridocis* on *Tridex procumbens* (Wehmeyer, 1964), *P. herbarum* var. *ipomoeae* (Kamal and Singh, 1979), and *P. euphorbiae* on *Euphorbia hirta* (Rangaswami et al., 1970) have been reported for various parts of India.

Production of anthraquinone pigments by *Phoma* species

There are many fungi which produce anthraquinones (Figure 1A) as secondary metabolites. Fungal anthraquinones as polyketide-derived secondary metabolites occur widely in many genera of fungi. Compared with the commercially available hydroxyanthraquinones most possess an additional methyl substitution in position three, e.g. emodin and this allows a study of the effect of such a group on the dyeing properties of dyestuffs derived from them. A fungal anthraquinone was cynodontin (Figure 1B) produced in sufficient purity to allow it to be transformed using a simple chemical step to a dye product and this was compared with a commercially available close analogue (Hobson et al., 1997).

A/ B/



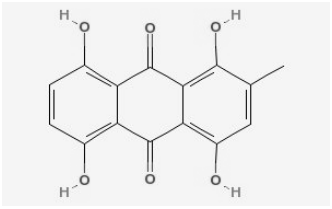


Figure 1. A/ Chemical structure of anthraquinone (anthracene-9,10-dione) and B/ cynodontin (1,4,5,8-tetra hydroxy-3-methylanthraquinone)

The chemical synthesis of anthraquinones require the use of strong acids at high temperature and heavy metal catalysts, as a consequence of which environmentally hazardous effluents and byproducts are produced. With increasing awareness of the environment degradation by industry the disposal of industrial effluent is becoming more costly and strictly regulated. A return to dyes extracted from molluscs, insects, fungi or plants grown in their environment is not the intention.

Phoma exigua Desm. varieties produces pigments. Bick and Rhee (1966) have reported that *P. exigua* var. *foveata (=P. foveata)* contains many anthraquinone pigments, such as pachybasin (Figure 2), chrysophanol (Figure 3), emodin (Figure 4) and phomarin. In acid condition this complex of pigments becomes yellow, and in alkaline conditions red. This character is based on ammonia test described by Logan and Khan (1969). On malt-agar, *P. exigua* var. *foveata* gives pinkish colour after exposure to ammonia. This is due to reaction of diffusible anthraquinones and their reaction with ammonia. In old cultures, anthraquinone pigments crystallize out as yellow-green crystals. Tichelaar (1974), found that the fungicide thiophanate-methyl accelerates and increases the crystallization process of the pigments. Both *P. exigua* var. *exigua* and *P. exigua* var. *inoxydabilis* produce cytochalasin B, which are also known as “phomine” (Bousquet and Barbier, 1972; Scott et al., 1975).

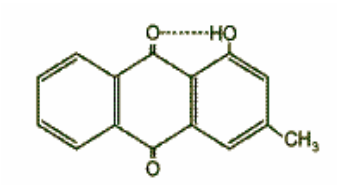


Figure 2. Pachybasin (1-hydroxy-3-methylanthraquinone)

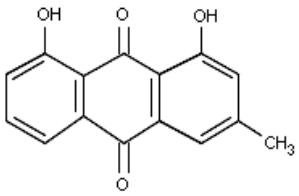


Figure 3. Chrysophanol (1,8-dihydroxy-3-methylanthraquinone)

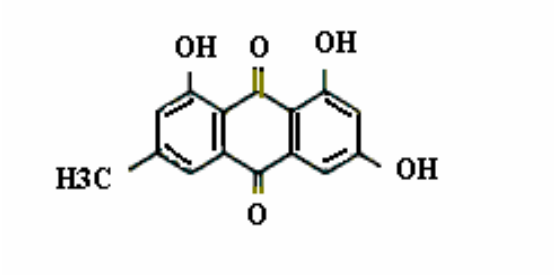


Figure 4. Emodin (1,6,8-trihydroxy-3-methyl-anthracene-9,10-dione)

Some isolates of *P. exigua* var. *foveata* (=*P. foveata*) produce antibiotic substance ('E' metabolite) similar to isolates of the ubiquitous *P. exigua* var. *exigua* (Boerema and Höweler, 1967). It is a colourless substance and can easily be demonstrated in cultures by sodium hydroxide test. On application of a drop of sodium hydroxide at the margin of colonies on malt agar oxidation takes place and pigment alpha (∝) converts into pigment beta (β). Pigment alpha (∝) is red-purple at pH <10.5 and blue-green at pH >12.5. Pigment beta (β) is yellow at pH <3.5 and red at pH >5.5.

Recently, three known anthraquinones have been isolated and identified by Borges and Pupo (2006), 1,7-dihydroxy-3-methyl-9,10-anthraquinone (Figure 5), 1,6-dihydroxy-3-methyl-9,10-anthraquinone (Figure 6) and 1-hydroxy-3-methyl-9,10-anthraquinone (Figure7), one new anthraquinone (1,7-dihydroxy-3-hydroxymethyl-9,10-anthraquinone), and two new hexahydroanthra-quinone derivatives, dendryols E and F (Figure 8), were isolated from the culture of the endophytic fungus *Phoma sorghina*, found in association with *Tithonia diversifolia* (Asteraceae). Their structures were identified on the basis of spectroscopic data, mainly 1D and 2D NMR.

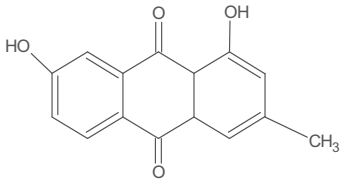


Figure 5. 1,7-Dihydroxy-3-methyl-methyl-9,10-anthraquinone

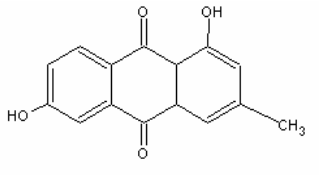


Figure 6. 1,6-Dihydroxy-3-9,10-anthraquinone

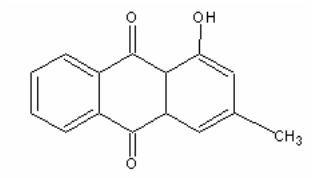


Figure 7. 1-Hydroxy-3-methyl-9,10-anthraquinone

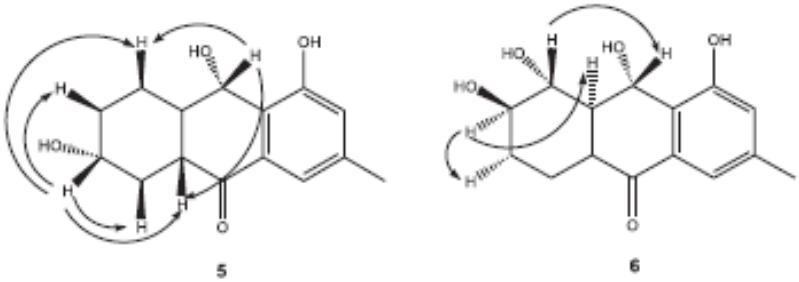


Figure 8. Antraquinone derivates produced by the endophytic fungus *P. sorghina*, the main correlation observed in the NOE different experiments for dendryol E and F (5-6) (after Borges and Pupo, 2006).

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