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Role of antagonistic microbe *Pseudomonas fluorescens* on *Peronospora arborescens* infecting *Papaver somniferum*

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Abstract
The present study was attempted to find out the efficacy of commercially formulated *Pseudomonas fluorescens* in the form of foliar spray on *Peronospora arborescens* causing downy mildew disease in opium poppy. The seed dipped in bioagents and metalaxyl solution for 30 minutes was sown in their respective triplicate plots for field study. 30 days old plants were sprayed with *P. arborescens* spore collected from infected plants of opium poppy and subsequently on the next day by *Pseudomonas fluorecsens* and metalaxyl spray in their respective plots. The second spray was done 15 days after first spray. The leaf sample was collected from healthy, infected and treated plots for disease rating and biochemical analysis. The result revealed that *Pseudomonas fluorescens* was found effective treatments for management of downy mildew in opium poppy due to rapid cumulative antagonistic effect and antibiotic action against the pathogen.

Keywords: *Pseudomonas fluorescens*, metalaxyl, *Peronospora arborescens*, downy mildew biochemical changes

Introduction
Opium poppy (*Papaver somniferum* L.) is one of the most important renewable resources for pharmaceutical alkaloids. Among the opium producing countries India is one of the largest producer of licit opium, which meets the national and international demand. In recent year the global trends show that the consumption of opium alkaloids and its derivatives are growing. Morphine which is the main and narcotic component of opium showed tenfold demand in last two decades. Its utilization has increased from 5 – 8 metric tons from 1981 – 1994 to 46 metric tons (Shukla and Singh, 2003). Since thebaine is used as base component for manufacture of semi synthetic morphine analogues i.e. oxycodone, oxymorphine, buprenorphine etc and having no narcotic effect are some novel merits which increase its global demand. However, the poppy crop is seriously affected by downy mildew disease caused by the fungus, *Peronospora arborescens*. The pathogen attacks the plant at seedling stage and produces two kinds of symptoms, Viz. systemic and topical. The plants with systemic infection die prematurely thereby causing severe loss in terms of seeds and alkaloids (Sattar *et al.* 1995). The disease can be controlled by fungicides, but fungicides are undesirable due to their cost and particularly, their failure to achieve sustainable productivity. The biopesticides are usually inherently less toxic then conventional fungicides (Suseela, 2000; Singh, 1999) [10, 8]. They generally affect only the target pathogenic organism, in contrast to broad spectrum, conventional fungicides that may affect organisms as different as bird, insects and mammals. They are effective in very small quantities and often decompose quickly.

Materials and Methods
Pathogen
The pathogen *Peronospora arborescens* was collected from the infected leaves of *Papaver somniferum* and maintained on natural host.

Field Experiment
Field experiment was conducted in sandy loam soil the plot size 2.4 X 4.0 m^2 adopting a standard spacing 30 X 10 cm for opium poppy sowing. Farm yard manures (FYM) 10 tones / ha with enriched 2% *P. fluorescens* was mixed in the soil before sowing. The seed (@5 g/kg seed) was soaked in *P. fluorescens* (2%) and metalaxyl mz 35 SD solutions for 30 minutes. Triplicate plots in randomized block design were maintained for each treatments (healthy, infected; biocontrol and metalaxyl). 30 days old opium poppy plants were sprayed with *Peronospora arborescens 7-12 X10^4* /ml spore.

The first biocontrol and metalaxyl spray were done after 24 hrs. The leaves were collected on the 10 days after first spray for disease rating and biochemical analysis. The second spray of biocontrol and metalaxyl were done 15 days after first spray; again the leaves were collected on 10 day after second spray. According to the infected area seen on leaves, the disease intensity was measured (James and Shih, 1973) [2] as per method of Thakur (1973) by using four infection grades Such as VL= Very light 1-10% disease incidence), L Light (11-20% disease incidence), M= Moderate (21-30% disease incidence), and H= heavy (more the 31% disease incidence) ranging from very light to heavy on the basis of disease incidence. The data was recorded from the leaves of randomly selected 20 plants. The number of capsule per plants, seed yield and husk yield (mg) per plant were recorded at the maturity of plants.

Biochemical analysis

The fresh leaf samples of opium poppy were harvested randomly at 150 DAS from all the treatments for the spectrophotometric assay of total chlorophyll and carotenoids (Lichtenthaler and Buschmann, 2001) [3]. Morphine content in opium poppy was analyzed by the method of Pride and Stern (1954) [5]. The total phenol content was estimated by the method of Bray and Thorpe (1954) [1].

Results and Discussion

The present study has been conducted to find out the antifungal effect of *Pseudomonas fluorescens* and metalaxyl against *Perenospora arborescens*; the causal agent of downy mildew disease of Papaver somniferum and the effect on biochemical changes and yield of opium poppy. The results of experiment showed that the highest percent disease intensity was recorded in plant inoculated with an a *Peronospora arborescens* followed by plant treated with *P. fluoroscens*, metalaxyl and healthy plants. It is might be the growth and sporulation of the pathogen is suppressed either the activity of antifungal compounds produced by the *P. fluorescens* or the hyper parasitism of the pathogen by the rapid defense exerted in the treated plants at the site of fungal entry that delayed the infection process (Ramkumar *et al*, 2012) [6]. Spray of metalaxyl was found highly effective because matalaxyl penetrate in the host epidermal cells and inhibit the growth of the pathogen. Trigiano *et al* (2012) [12] reported that metalaxyl provides protective and therapeutic action against *Peronospora tabacini* infecting tobacco plants. The rapid acropetal translocation of the fungicide to the foliage quickly suppressed sporulation of the pathogen.

The maximum reduction in the photosynthetic pigments was seen in the infected plants and least in treated and healthy plants. This reduction was observed due to toxic compound produced by the pathogen (Muthuchelion, *et al* 1990) [4]. These toxic compounds initiated the chlorosis and necrosiscondition inleaves and reduce the chlorophyll and carotenoid contents. *P. fluorocens* and metalaxyl suppressed the chlorophylase activity or they produced organic acids for growth and development of host. Toxic compounds are inhibited with antibiotic compounds produced by *P. fluorescens* (Srivastava and Sharma, 1990) [9]. The treated plants exhibited an increase amount of total phenol conten over the infected plants. This might be due to the enhancement of systemic resistance in the host plants due to the treatment because the phenolic compounds are fungitoxic nature and act as adoptive mechanism in the host against the fungal infection (Ramkumar *et al*, 2012) [6]. The yield and quality characters of opium *viz;* number of capsules. Seed yield, husk yield per plant and morphine content were found to enhance by spray of microbe and metalaxyl. This might be attributed to the control of disease or due to production of photosynthetic pigments which allow the plants to withstand even the pathogenesis.

Table 1: Effect of foliar spray of *P. fluorescens* and metalaxyl on the disease intensity and biochemical changes in *Papaver somniferum* disease caused by *Peronospora arborescens*

Parameters	Stage	Healthy plants	Infected plants	Treatments with *P. fluorescens*	Treatments with metalaxyl
Disease intensity (%)	I	7.4±0.27	46.48±4.8	25.44±1.46	21.98±3.42
	II	9.7±0.34	56.89±3.74	36.54±3.16	33.41±2.67
Total chlorophyll contents(mg/g)	I	1.6±0.3	0.8±0.1	1.3±0.4	1.2±0.6
	II	1.8±0.1	1.2±0.2	1.6±0.5	1.4±0.7
Carotenoid contents (µg/g)	I	146.14±2.8	87.78±3.01	111.48±2.41	109.15±1.76
	II	166.23±1.6	109.59±3.1	121.17±2.1	135.48±3.4
Total phenol content (mg/g dw)	I	3.01±0.3	2.98±0.4	3.21±0.3	3.79±0.2
	II	4.44±0.2	3.89±0..1	4.05±0.3	3.95±0.12
Morphine (%)		12.55±0.01	10.85±2.1	11.68±1.47	11.96±1.05
Number of capsule/ plants	-	2.8±0.2	1.6±1.02	2.6±0.2	2.3±0.16
Seed yield /Plant(mg)	-	188.3±15.18	85.45±10.26	145.23±13.49	156.62±16.21
Husk yield/Plant(mg)		108.15±9.25	58.49±7.59	94.36±7.24	104.24±8.64

Values are mean ± SEM. Statistically significant at P=0.05

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