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## Bioassay of some agricultural chemicals, human drugs and disinfectants on *Phytophthora melonis* Katsura causing fruit and vine rot of pointed gourd

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### ABSTRACT

Some antibiotics, insecticides, disinfectants and antiprotozoal drugs were tested on *Phytophthora melonis* Katsura causing fruit and vine rot of pointed gourd in aqueous environment. Antifungal antibiotics viz. validamycin, kasugamycin, griseofulvin, fluconazole and ketoconazole did not have any pronounced effect on mycelial growth, sporangia formation and its germination. None of the eight antibiotics used in this study prevented mycelial growth. No sporangia formation was recorded in five antibiotics viz. streptomycin sulphate + tetracycline hydrochloride, streptomycin sulphate, oxytetracycline hydrochloride, ofloxacin and norfloxacin at 100 ppm. The insecticide, Profenophos prevented sporangia formation and consequently its germination. Copper sulphate (CuSO<sub>4</sub>, 5H<sub>2</sub>O) had adverse effect on mycelial growth and sporangia formation. Potassium permanganate (KMnO<sub>4</sub>), Savlon, Dettol and tinidazole were not good inhibitor for *P. melonis*. Sporangia did not form in water containing 0.0125g/L chloroquin phosphate - an antiprotozoal drug.

**Keywords:** Antibiotic, antiprotozoal drug, bioassay study, disinfectant, fruit and vine rot, insecticide, pointed gourd, *Phytophthora melonis*

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### Introduction

Pointed gourd (*Trichosanthes dioica* Roxb.) is an important and widely accepted cucurbitaceous vegetable extensively cultivated in West Bengal and Bihar in particular. It is called *King of gourds* because of its higher nutrient content than other cucurbits. As the crop prefers warm condition major part of its growth phase passes through rainy season. Fruit and vine rot of pointed gourd incited by *Phytophthora melonis* Katsura appears every year in West Bengal (Saha *et al.* 2004) and causes severe damage. The disease causes rotting of internodal region of the vine, fruit rot and leaf blight. Devastation of the entire crop is a common phenomenon in rainy season. The disease is popularly known to the farmers

as 'Haja. Saha *et al.* (2004) studied the effect of different fungicides on the pathogen. Present investigation was targeted to study the effect of antibiotics, insecticide, disinfectants and antiprotozoal drugs on *P. melonis* in aqueous environment to explore their usefulness in plant disease management.

### Materials and Methods

If a small bit of naturally infected fruit or stem tissue of pointed gourd was put in a Petriplate in semi-submerged condition in water, the pathogen (*P. melonis*) produced profuse mycelia and sporangia in water. Zoospores were released through the opening at the top of the sporangia. The interesting phenomenon was that the sporangia proliferated 1-3 times at the same point. Growth of sporan-

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giophore may continue through the empty proliferated sporangia to produce new sporangia. Empty sporangia remain attached to the sporangiophore (Guharoy *et al.* 2006; Mondal *et al.* 2013).

Using the above character, effect of different chemicals in aqueous environment was studied following the method described by Saha *et al.* (2004). Small pieces of naturally infected fruit tissues were put in a Petriplate containing aqueous solution/suspension of chemical, in semi-submerged condition. Three replicates were maintained for each concentration of a chemical. Suitable control was maintained using water only. The Petriplates were kept in BOD at  $28 \pm 1^\circ \text{C}$  for 48-72h. Then the Petriplates were observed under a microscope to record the extent of mycelial growth in water/chemical solution beyond the area of fruit tissue. Simultaneously, the extent of sporangia formation and germination of sporangia were recorded. Presence of empty sporangia or proliferated sporangia indicated the germination of sporangia.

## Results and Discussion

### *Bioassay of antifungal antibiotics against P. melonis*

Among the five antifungal antibiotics tested, two were agricultural formulations and three were human drugs (Table 1). The agricultural preparations, Sheathmar 3 (validamycin) and Kasu B (kasugamycin) did not inhibit mycelial growth but hyphae become deformed at concentration of 0.1875 ml/L and 0.0625 ml/L or above, respectively. Sporangia formation

and its germination were inhibited by Sheathmar 3 at 0.1875 ml/L concentration whereas Kasu B did it at higher concentration. Three human drugs Griseovin (griseofulvin), Fluzon (fluconazole) and Nizral (ketoconazole) did not affect hyphal growth, sporangia formation and its germination.

### *Bioassay of antibacterial compounds, insecticides, disinfectants and antiprotozoal drugs against P. melonis*

Nine chemicals were included to study their antifungal activity (Table 2). Among the nine chemicals, four were agricultural pesticides (Privintal BV, Nemarin, Carina, Copper sulphate), three were general disinfectants (Potassium permanganate, Savlon, Dettol) and two were antiprotozoal drugs (Tini 300, Resochin). Among the agricultural pesticides Privintal BV is an antiviral-antibacterial-antifungal preparation (claimed by the manufacturing company) used by the farmers for controlling fruit rot and viral diseases whereas Nemarin (neem based insecticide) and Carina used for controlling insect. Copper sulphate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) is used to protect the perishable vegetables from post harvest decay. It is commonly used as herbicide and for colouring of harvested vegetables. Potassium permanganate ( $\text{KMnO}_4$ ) is a general disinfectant and widely used in pisciculture. Savlon and Dettol are the disinfectants and occasionally used by few farmers in crop fields for disease control. In bioassay study, the protozoa developed frequently in aqueous suspension of chemotoxicant. Antiprotozoal drugs were selected to study their effect on *P. melonis* and protozoa

grown in the suspension. Other chemicals were directly or indirectly used in agriculture and included in this study.

Privital BV and Nemarin did not appear to be a good inhibitor for *P. melonis* infecting pointed gourd (Table 2). The insecticide, Carina (profenophos) prevented sporangia formation and consequently its germination. There was no mycelial growth at 1ml/L concentration. In low concentration though mycelial growth was present, hyphae became deformed.

Copper sulphate had adverse effect on mycelial growth and sporangia formation. The effect was similar to copper fungicide Blitox (copper oxychloride) reported by earlier researchers (Saha *et al.* 2004; Khatua *et al.* 2013). The effect was more pronounced on sporangia formation and its germination than mycelial growth (Saha *et al.* 2004). Potassium permanganate, Savlon and Dettol were appeared as not good inhibitor for *P. melonis*.

The antiprotozoal drug tinidazole failed to inhibit mycelial growth at 0.125g/L or above concentration and did not inhibit sporangia formation and its germination upto 0.5g/L concentration. In case of chloroquin phosphate, poor and deformed hyphal growth recorded at 0.0125 g/L or above concentration. Sporangia did not form in water containing 0.0125g chloroquin phosphate in 1 litre. The chloroquin phosphate showed good antifungal activity. In case of two antiprotozoal drugs, there was no protozoal growth in the aqueous solution used for bioassay.

#### *Bioassay of antibacterial antibiotics against P. melonis*

Eight antibiotics were tested against *P. melonis* infecting pointed gourd. Krosin AG, a combined agricultural formulation of two antibiotics-streptomycin sulphate and tetracycline hydrochloride was used in the study. Other seven were human drugs (Table 3).

There was no sporangia formation in five antibiotics out of nine at a concentration of 100 ppm (Table 3). All the antibiotics, except Cifran, inhibited sporangial germination at 200 ppm. In spite of non-inhibited mycelial growth, hyphal deformity was noted in Krosin AG, Ambistryn-S, Paraxin and Tarivid. There was difference in the extent of activity of the same antibiotic (i.e. ofloxacin - Tarivid and Oflin) of different manufacturing concern. In four antibiotics - Roxeptin, Paraxin, Oflin and Cifran sporangia formed in 100 ppm. Small fragments of hyphae were found in aqueous solution of Ambistryn-S and Tarivid.

The chemicals used for the study were agricultural chemicals, human drugs and disinfectants. Human drugs and disinfectants are not usually applied to control plant diseases in field condition. In the present laboratory based experiment, efficacy of these chemicals were evaluated against mycelial growth, sporangia formation and sporangial germination of a fungal plant pathogen *Phytophthora melonis* - the causal agent of fruit and vine rot of pointed gourd. Some potent chemicals were identified as good inhibitor of the pathogen. Future study may throw light in respect

to incorporation of profenophos (insecticide), streptomycin sulphate, streptomycin sulphate + tetracycline hydrochloride (antibiotic) and chloroquin phosphate (antiprotozoal drug) in field management of fruit and vine rot disease of pointed gourd.

## Literature Cited

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**Table 1.**

Bioassay of antifungal antibiotics against *P. melonis* infecting pointed gourd plants in aqueous environment

Chemicals (Trade name)	Active ingredient and Formulation	Extent of mycelial growth			Sporangia formation and its germination	
		Growth pattern of the fungi at concentration of chemical – g/l or ml/l				
		No growth	Poor or deformed growth	Normal growth	Inhibition of sporangia formation	Inhibition of sporangial germination
Sheathmar 3	Validamycin 3% L	>3.0	0.1875-3.0	-	0.1875	0.1875
Kasu B	Kasugamycin 3% SL	>1.0	0.0625-1.0	-	1.0	0.125
Griseovin	Griseofulvin (Tablet)	NI at 0.5	-	0.125 – 0.5	NI at 0.5	NI at 0.5
Fluzon	Fluconazole (Tablet)	NI at 0.5	-	0.125 – 0.5	NI at 0.5	NI at 0.5
Nizral	Ketoconazole	NI at 0.5	-	0.125 – 0.5	NI at 0.5	NI at 0.5

NI = Not inhibited

**Table 2.**

Bioassay of antibacterial compounds, insecticides, disinfectants and antiprotozoal drugs against *P. melonis* infecting pointed gourd plant in aqueous environment

Chemicals (Trade name)	Active ingredient and Formulation	Extent of mycelial growth			Sporangia formation and its germination	
		Growth pattern of the fungi at concentration of chemical – g/l or ml/l				
		No growth	Poor or deformed growth	Normal growth	Inhibition of sporangia formation	Inhibition of sporangial ger- mination
Privintal BV	Polyformphos, water soluble	>1.0	0.5-1.0	0.0625-0.25	0.5	0.25
Nemarin	Azadirachtin Q, 15 % W/W EC	>3.0	1.5-3.0	0.1875-0.75	1.5	0.75
Carina	Profenophos 50% EC	1.0	0.0625-0.5		0.0625	0.0625
Copper sulphate	(CuSO <sub>4</sub> .5H <sub>2</sub> O)	1.0	0.125-0.5	0.0625	0.0625	0.0625
Potassium permanganate	KMnO <sub>4</sub>	1.0	1.0	0.0625-0.5	1.0	1.0
Savlon	Chlorhexidine gluconate 0.3% V/ V and Cetrimide 0.6% W/V	1.0	0.125-0.5	0.0625	0.125	0.125
Dettol	Chloroxylenol 4.8% W/V + Terpineol 9% V/V + Alcohol absolute 13.1% V/V	1.0	-	0.0625-0.5	1.0	0.0625
Tini 300	Tinidazole, tablet	1.0	-	0.125-0.5	NI at 0.5	NI at 0.5
Resochin	Chloroquin phosphate, tablet	0.2	0.0125-0.1		0.0125	0.0125

NI = Not inhibited

**Table 3.**

Bioassay of antibacterial compounds and antibiotics against *P. melonis* infecting pointed gourd plant, in aqueous environment

Antibiotics	Conc.(ppm)	Mycelial growth	Sporangia formation	Sporangial germination
Krosin AG (streptomycin sulphate 90% + Tetracycline hydrochloride 10%)	200	Poor deformed hyphal growth	Absent	Absent
	100	Deformed hyphal growth	Absent	Absent
	50	Good hyphal growth	Good number	Present
	25	Profuse hyphal growth	Profuse	Present
Ambistryn - S (Streptomycin sulphate)	200	Deformed hyphal growth	Absent	Absent
	100	Deformed hyphal growth*	Absent	Absent
Terramycin (Oxytetracycline hydrochloride)	200	Moderate hyphal growth	Absent	Absent
	100	Profuse hyphal growth	Absent	Absent
Roxeptin (Roxythromycin)	200	Huge hyphal growth	Present	Absent
	100	Huge hyphal growth	Present	Absent
Paraxin (Chloramphenicol)	200	Poor deformed hyphal growth	Absent	Absent
	100	Huge hyphal growth	Present	Absent
Tarivid (Ofloxacin)	200	Huge deformed hyphal growth*	Absent	Absent
	100	Huge hyphal growth	Absent	Absent
Oflin (Ofloxacin)	200	Moderate hyphal growth	Present	Absent
	100	Huge hyphal growth	Poor number	Absent
Cifran (Ciprofloxacin hydro- chloride)	200	Huge hyphal growth	Present	Present
	100	Moderate hyphal growth	Poor number	Present
Norflox 400 (Norfloxacin)	200	Moderate hyphal growth	Absent	Absent
	100	Poor hyphal growth	Absent	Absent

\* Hyphal fragments found in water