

Original Research Article

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Controlling the Incidence of *Fusarium oxysporum* f.sp. *lycopersici* in Tomato by Application of the Newly Formulated Botanical Fungicide (Polymin 40 EC)

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ABSTRACT

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The chloroform extract of *Polygonum minus* Huds. was found to exhibit inhibitory effect against *Fusarium oxysporum* f.sp. *lycopersici* under laboratory conditions. Hence a new botanical fungicide (Polymin 40 EC) was formulated from the chloroform extract of *Polygonum minus* Huds. Polymin 40 EC to control the pathogen under *in vivo* conditions. Various concentrations of Polymin 40 EC were evaluated for their performance on seed infection, germination and vigour of tomato and it was found that Polymin 40 EC at 2% concentration increased the germination and vigour with the significant reduction in seed infection. Application of Polymin 40 EC increased the activity of plant defense enzymes such as peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL) and phenol content of tomato plant. Application of two percent Polymin 40 EC was found to be effective in controlling the incidence of *Fusarium oxysporum* f.sp. *lycopersici* in tomato plant.

Introduction

Plants have provided man with all his needs in terms of shelter, clothing, food, flavours, fragrances and not the least, medicines. Much of the wealth of a country resides in its plant inheritance, whether the plants are endemic, naturalized or recent introduction (Mitchell and Ahmad, 2005). India has an extensive area of forest enriched with plant diversity. In agriculture, the crop loss due to plant

pathogens has become a major concern nowadays. Increased usage of different chemical based products to control these pathogens have resulted in problems like residual effect of chemicals in agri-based products, increased resistance for chemicals in target pathogens and environmental pollution. Crude extracts of some well known medicinal plants are used to control some of the plant pathogens. Use of botanicals in plant disease management assumes special significance by

being an ecofriendly and cost effective strategy, which can be used in integration with other strategies for a greater levels of protection with sustained crop yields. With this background, the present research is formulated to explore the antimicrobial activity of *Polygonum minus* against *Fusarium oxysporum* fsp. *Lycopersici*.

Materials and Methods

Disease incidence (Blotter method)

The tomato seeds (PKM1) showing natural infections were used to find out the efficacy of various concentrations (0.50, 1.0, 1.5 and 2.0%) of botanical formulation of *P. minus* (Polymin 40EC). The seeds (100 seeds) were soaked in each treatment with botanical formulation of *P. minus* (Polymin 40EC) for 2 h and replicated four times. A control was maintained by soaking the seeds in distilled water. Twenty five seeds of each treatment were placed on moist blotters (ISTA, 1993) in petriplate and incubated ($20 \pm 2^\circ\text{C}$) for 12 h of alternate natural UV light and 12 h darkness. The seeds were examined for growth of seed borne pathogens on eighth day of treatment. The seed infection was expressed in percentage.

The treatments include:

T₁- 0.50 % botanical formulation of *P. minus* (Polymin 40EC)

T₂- 1.0 % botanical formulation of *P. minus* (Polymin 40EC)

T₃- 1.5 % botanical formulation of *P. minus* (Polymin 40EC)

T₄- 2.0 % botanical formulation of *P. minus* (Polymin 40EC)

T₅- Mancozeb (0.2%)

T₆- Biocontrol agent (*T. viride*)

T₇- Uninoculated control

Germination (%)

The tomato seeds (PKM1) seeds were soaked in botanical formulation of *P. minus* (Polymin 40EC) for 18 h and then dried under shade. Four replicates of 100 seeds were uniformly placed on standard germination paper roll-towel medium (ROLL towel medium, ISTA, 1993) and kept in germination room maintained at $25 \pm 2^\circ\text{C}$ and 90 ± 2 per cent relative humidity. After the test period of 14 days, the seedlings were evaluated as total number of normal seedlings and germination as percentage. Similarly different concentration of *P. minus* (Polymin 40EC) was used for evaluating the per cent germination.

Root length (cm)

On fourteenth day, ten normal seedlings per replication from roll towel medium were carefully removed at random from each treatment and the root length was measured from the base to the top of the primary root and the mean value was calculated and expressed in cm.

Shoot length (cm)

Ten normal seedlings from roll towel medium were carefully removed at random from each treatment and the shoot length was measured from the base of the shoot to tip of primary leaf on the fourteenth day and the mean value was expressed in cm.

Vigour index (VI)

The Vigour Index (VI) was compared (Abdul-Baki and Anderson, 1973) adopting the following formula and expressed as whole number.

VI = Germination (%) x Mean total length of seedling in cm (mean length of shoot and root).

Evaluation of the effect of Polymin 40EC on *F. oxysporum* f.sp. *lycopersici* under pot culture conditions

Different concentrations of Polymin 40EC (0.50, 1.0, 1.5 and 2.0%) were prepared to test the efficacy on different fungal pathogens under glass house conditions. Percent disease index and percent disease incidence was calculated from the observations recorded under pot culture condition.

The treatments include:

T₁- 0.50 % botanical formulation of *P. minus* (Polymin 40EC)

T₂- 1.0 % botanical formulation of *P. minus* (Polymin 40EC)

T₃- 1.5 % botanical formulation of *P. minus* (Polymin 40EC)

T₄- 2.0 % botanical formulation of *P. minus* (Polymin 40EC)

T₅- Mancozeb (0.2%)

T₆- Biocontrol agent (*T. viride*)

T₇- Uninoculated control

T₈- Inoculated control

Method of inoculation of *F. oxysporum* f.sp. *lycopersici*

Forty five days old plants of tomato (PKM1) were inoculated with spores *F. oxysporum* f.sp. *lycopersici* at a concentration of 7×10^6 spore/ml. After 24 h, the plants were sprayed with different concentration of Polymin 40EC

(0.50, 1.0, 1.5, and 2.0%). Second and third spraying was taken up in 15 days interval.

Biochemical changes induced by plant extracts in crop plants

Tomato (PKM1) plants were inoculated with Polymin 40EC at the concentration of 0.50, 1.0, 1.5 and 2.0 per cent. Control plants were sprayed with distilled water. Three replicates of each treatment were maintained. The sprayed plants were inoculated with the pathogen 48 h after spraying and the plant samples were collected at specific time intervals viz., 0, 48, 96, 144 and 240 h after inoculation, for studying the induced changes.

Assay of peroxidase (Puttur, 1974)

The reaction mixture consisted 1.5 ml of 0.05 M pyrogallol, 0.5 ml of enzyme extract and 0.5 ml 1 per cent H₂O₂. The changes in absorbance at 420 nm were recorded at 30 seconds interval for 3 min. The enzyme activity was expressed as changes in the absorbance per min per g of sample.

Assay of polyphenoloxidase (Mayer *et al.*, 1965)

The reaction mixture consisted of 200 µl of enzyme extract and 1.5 ml of 0.1 M sodium phosphate buffer. To start the reaction, 200 µl of 0.01 M catechol was added and the activity was expressed as changes in absorbance at 495 nm per min per g of sample.

Estimation of total phenol (Spies, 1955)

A sample quantity of 0.1 ml was added to 2.8 ml of water and 0.25 ml of Folin Ciocalteau reagent and the solution was kept at 25°C. After 3 min, 1 ml of 20 per cent sodium carbonate was added. The absorbance of developed blue colour was measured using spectrophotometer at 650 nm. Catechol was

used as the standard. The amount of phenolics was expressed as μg catechol per g of sample.

Assay of phenyl ammonia lyase (Zucker, 1965)

The reaction mixture containing 0.4 ml of enzyme extract was incubated with 0.5 ml of 0.1 M borate buffer and 0.5 ml of 12 mM L-phenylalanine in the same buffer for 30 min at 30°C. The amount of trans-cinnamic acid formed was calculated using its extinction coefficient of 9630 M⁻¹. Enzyme activity was expressed as nmol trans cinnamic acid per min per g of sample.

Results and Discussion

Tomato seeds treated with 2.0 per cent Polymin 40 EC (T₄) recorded 99.34 per cent reduced seed infection by *F. oxysporum* f.sp. *lycopersici* than the untreated control (Table 1). The treatments T₁ (0.50 per cent Polymin 40EC), T₂ (1.0 per cent Polymin 40EC), T₃ (1.5 per cent Polymin 40EC), T₅ (Mancozeb 0.2 %) and T₆ (*T. viride*) recorded 88.58, 90.76, 96.73, 91.30 and 91.84 per cent reduced seed infection over the control respectively.

The germination of tomato seedlings was 92.50 per cent in T₄ (2.0% per cent Polymin 40EC) which was 11.98 per cent increase over the control.

Vigour of tomato seedlings was increased by 75.28 per cent over the control in T₄ (2.0% Polymin 40EC) (Table 2). The maximum shoot (12.82) and root length (9.50) was observed in T₄ (2.0% Polymin 40EC) followed by 12.40 and 9.48 cm in T₃ (1.5% Polymin 40EC), 11.08 and 9.47 cm in T₂ (1.0% Polymin 40EC). Enzyme activities like PO, PPO, PAL and phenol content were constantly measured in tomato plants on 0, 2, 4, 6 and 10 days after challenge inoculation with *F.*

oxysporum f.sp. *lycopersici*. The maximum PO activity was recorded in 2 per cent Polymin 40EC treated tomato plants (3.943 OD min/g) on 6 days after challenge inoculation (Fig. 1). The untreated control recorded 1.612 OD min/g of PO activity on 6 days after challenge inoculation and this was lower than all other treatments.

Maximum PPO activity was observed in T₄ (2.0% Polymin 40EC) on 4 days after inoculation (Fig. 1). The PPO activity was lower in inoculated control (1.310 OD min/g) when compared to plants treated with various concentrations of Polymin 40EC, Mancozeb (0.2%) and *T. viride* on fourth day after challenge inoculation. The PAL activity was maximum in 2.0 per cent Polymin 40EC treated plants (8.985 OD min/g) on 6 days after inoculation than healthy and inoculated control (Fig. 1).

In all the treatments, maximum phenol content reached on sixth day after inoculation. The phenol content was significantly higher in T₄ (2.0% Polymin 40EC – 555.06 μg of catechol /g) than the control (Fig. 1). The Mancozeb (0.2%) and *T. viride* treated plants recorded the phenol content of 426.56 μg of catechol /g and 422.00 μg of catechol /g on 6 days after challenge inoculation.

The botanical formulation at 2 per cent was found to reduce the seed infection by *F. oxysporum* f.sp. *lycopersici* and also increased the vigour of tomato (75.28%). The beneficial effect of botanical formulations in inhibiting the fungal pathogens and increasing the seedling vigour has been reported in several studies. Dongzhi (2004) have reported that the medicinal plants like *Aloe vera* contained natural plant growth regulators and it promoted shoot and root length of turnips.

Induction of defense proteins makes the plant resistant to pathogen invasion.

Table.1 Effect of botanical formulation of *P. minus* (Polymin 40EC) on seed infection and seed germination of tomato –*F. oxysporum* f.sp. *lycopersici*

Treatment	Seed infection		Seed germination	
	Infection (%)*	Reduction over control	Germination (%)*	Increase over control
T₁ (0.50% Polymin 40EC)	10.50	88.58	86.50	4.70
T₂ (1.00% Polymin 40EC)	8.50	90.76	88.90	6.30
T₃ (1.50% Polymin 40EC)	3.00	96.73	90.60	9.68
T₄ (2.00% Polymin 40EC)	0.60	99.34	92.50	11.98
T₅ (Mancozeb 0.2%)	8.00	91.30	85.00	2.90
T₆ (<i>T. viride</i>)	7.50	91.84	84.90	2.78
T₇ (Control)	92.00		82.60	

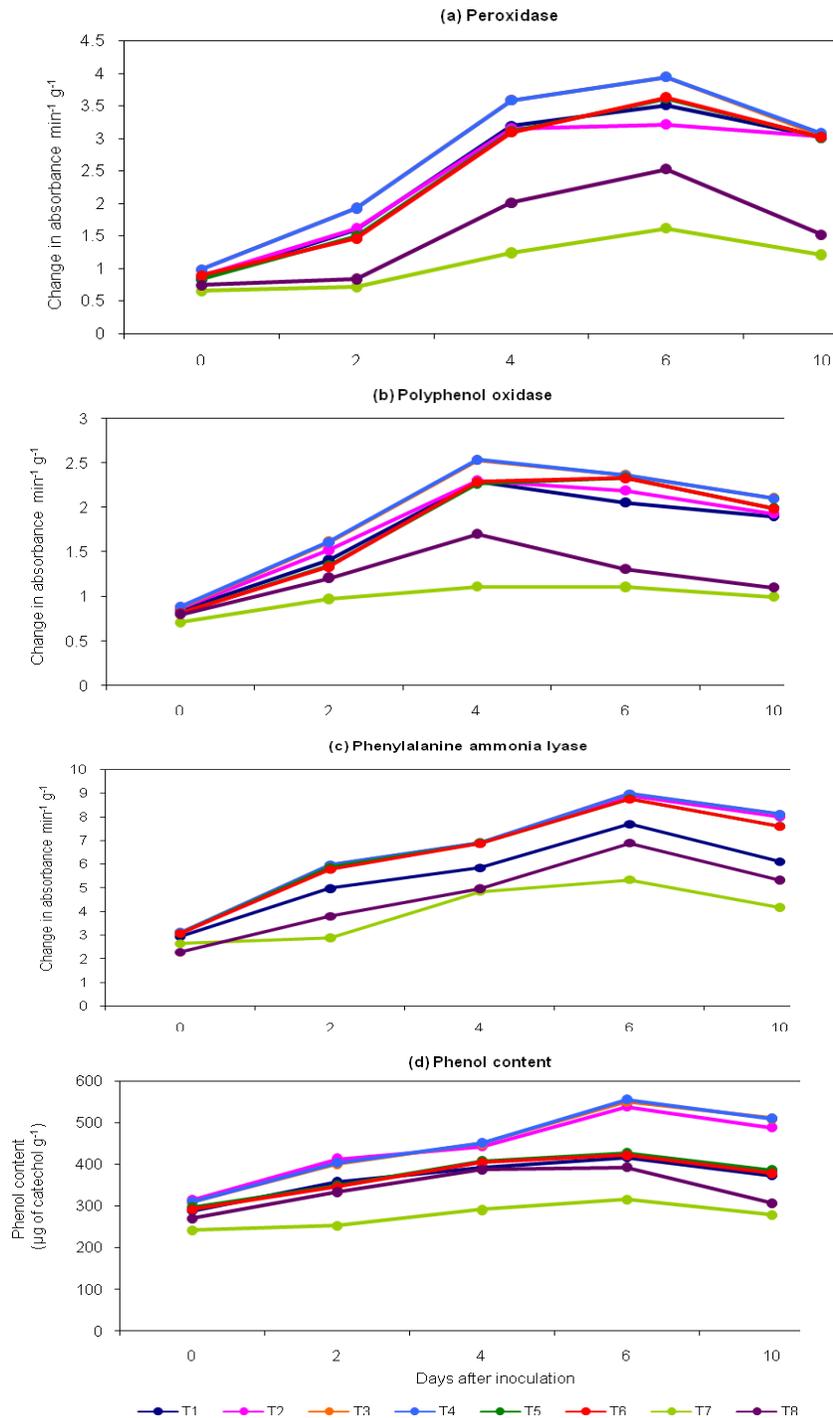
*Mean of four replications (5 plants/replication)

Table.2 Effect of botanical formulation of *P. minus* (Polymin 40EC) on vigour of tomato seedlings –*F. oxysporum* f.sp. *lycopersici*

Treatment	Shoot length (cm)*	Increase over control	Root length (cm)*	Increase over control	Vigour index (VI)*	Increase over control
T₁ (0.50% Polymin 40EC)	10.39	44.10	8.93	26.67	835.59	41.88
T₂ (1.00% Polymin 40EC)	11.08	53.67	9.47	34.33	913.45	55.10
T₃ (1.50% Polymin 40EC)	12.40	71.98	9.48	34.47	991.16	68.30
T₄ (2.00% Polymin 40EC)	12.82	77.80	9.50	34.75	1032.30	75.28
T₅ (Mancozeb 0.2%)	7.59	5.27	7.13	1.13	625.60	6.22
T₆ (<i>T. viride</i>)	7.63	5.83	7.18	1.84	628.68	6.74
T₇ (Control)	7.21		7.05		588.94	

*Mean of four replications (5 plants/replication)

Fig.1 Changes in enzyme activity and phenol content by application of Polymin 40EC in tomato challenged with *F. oxysporum f.sp. lycopersici*



According to Ramanathan *et al.*, (2000) the induction of defense proteins makes the plant resistant to pathogen invasion and the results

of the present study revealed that the tomato plants applied with P-40 significantly induced the defense compounds (PO, PPO, PAL and

phenol) compared to unsprayed control. The resistance of plants induced against the pathogens, due to the application of botanicals has been widely reported (Straub *et al.*, 1986).

Spraying of P-40 enhanced the accumulation of defense enzymes and total phenol content on challenge inoculation with *F. oxysporum* f.sp. *lycopersici* in tomato which is in accordance with the results were reported by Rajeswari (2002) in grapes due to the application Wanis (a botanical formulation).

In conclusion, the “Polymin” exhibited high level of thermo and storage stability. On application it increased the germination percentage of tomato by reducing the seed infection by *F. oxysporum* f.sp. *lycopersici*. Under pot culture conditions, it potentially induced the resistance in tomato plants against *F. oxysporum* f.sp. *lycopersici* through enhancement of defense compounds (PO, PPO, PAL and phenol). With these findings, it is evident that the “Polymin” has the potential of controlling the incidence of *F. oxysporum* f.sp. *lycopersici* in tomato. In future, detailed study on the effect of Polymin on plant disease control under field conditions will provide an opportunity for crop disease management in an environmentally friendly manner.

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