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Effect of Botanical Formulation of *Polygonum Minus* (P-40) on Control of *Alternaria Solani*

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Abstract

An ecofriendly botanical formulation "Polymin 40 EC" was developed from the chloroform extract of *P. minus*. The effect of various concentrations of P-40 on seed infection, germination and vigour of tomato and 2 per cent P-40 was found to increase the germination and vigour but reduced the seed infection to a significant extent. Two percent P-40 was found to be the optimum concentration for plant disease control (tomato – *Alternaria solani*) under pot culture conditions. Application of P-40 increased the activity of peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL) and phenol content of the crop plants.

Keywords: Polymin, botanical formulation, plant disease control, Alternaria solani

Introduction

In agriculture, the crop loss due to plant pathogens has become a major concern. 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. During the past few years, there is a growing trend all over the world to shift from synthetic to natural products including medicinal plants. The neglected and little known botanicals should be considered now to cure the plant diseases, which create challenging problems in agriculture and pose real economic and environmental threats.

The success of green revolution has been based solely on high input agriculture and excessive utilization of chemicals. This era of production and productivity did improve availability of food grains but affected the agro ecosystem adversely. Besides pollution of the environment, there has been development of resistance in pests and diseases. 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.

Materials and Methods

Formulation of botanical fungicide

The chloroform leaf extract of *P. minus* were used for preparation of the product. The leaves were washed and ground with chloroform (1:5). The ground material was filtered several times through cheese cloth and the supernatant solution was collected. The clear solution was condensed in vacuum and the material was reduced to 100 ml. The partially purified material was used for further studies.

The partially purified chloroform leaf extract of *P. minus* was used to develop emulsifiable concentrates. The condensed material was considered as 100 per cent concentration. Using these materials, different emulsifiable concentrates were prepared (30 and 40EC). The formulation was developed by using recommended quantities of emulsifying agent (Unitox 30X and Unitox 60Y), stabilizing agent (Epichlorohydrin) and solvent (Cyclohexanone). The combined formulation was named as 'Polymin'.

Effect of botanical formulation of *P. minus* (P-40) on seed infection, seed germination and seedling vigour

Disease incidence (Blotter method): The tomato seeds (PKM1) showing natural infections were used to find out the efficacy of various concentrations (0.5, 1.0, 1.5 and 2.0%) of botanical formulation of *P. minus* (P-40). The seeds (100 seeds) were soaked in each treatment with botanical formulation of *P. minus* (P-40) 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 ± 2°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: T_1- 0.5 % P-40, T_2- 1.0 % P-40, T_3- 1.5 % P-40, T_4- 2.0 % P-40, T_5- Mancozeb (0.2 %), T_6- Biocontrol agent (*T. viride*), T_7- Uninoculated control.

Germination (%): The tomato seeds (PKM1) were soaked in botanical formulation of *P. minus* (P-40) 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) [1] and kept in germination room maintained at 25 ± 2°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* (P-40) was used for evaluating the per cent germination.

Root length (cm) and Shoot length (cm): on fourteenth day, ten normal seedlings per replication from roll towel medium were carefully removed at random from each treatment. The root length was measured from the base to the top of the primary root and the shoot length was measured from the base of the shoot to tip of primary leaf and the mean value was calculated and expressed in cm.

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Vigour index (VI): The Vigour Index (VI) was compared [2] 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 P-40 on *A. solani* under pot culture conditions

Per cent disease index and per cent disease incidence were calculated from the observations recorded under pot culture condition.

The treatments include: T_1- 0.5 % P-40, T_2- 1.0 % P-40, T_3- 1.5 % P-40, T_4- 2.0 % P-40, T_5- Mancozeb (0.2 %), T_6- Biocontrol agent (*T. viride*), T_7- Uninoculated control and T_8- Inoculated control.

Method of inoculation of *A. solani* : Fourty five days old tomato plants (PKM1) were inoculated with *A. solani* using the following method. The concentration of the conidia was at 7 x 10^6 spore/ml.

Pin prick method of inoculation: The leaves were injured with sterilized pin and the mycelial disc of pathogen was placed over the injured leaf portion and covered with moist cotton and incubated inside the moist chamber.

The plants were sprayed frequently with water for 2 days. After 48 h, the plants were sprayed with different concentration of P-40 (0.5, 1.0, 1.5 and 2.0 %). Second and third spraying was taken up at 15 days interval. The per cent disease index of *A. solani* was scored at 15 days after inoculation in intensity scale of 0-5 (Table 1).

Per cent disease incidence (PDI) was calculated based on the following formula [3].

$$PDI = \frac{\text{Sum of all numerical grades}}{\text{Total number of leaves Counted x Maximum Grade}} \times 100$$

Biochemical changes induced by plant extracts in crop plants

Forty seven days old **t**omato (PKM1) plants were inoculated with P-40 at the concentration of 0.5, 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.

Preparation of plant extracts: Tomato leaves collected from 3 plants/replicate were snap frozen and homogenized immediately with liquid nitrogen. The homogenated sample was macerated at 4°C with appropriate buffer solutions, and the homogenate, which was centrifuged for 20 minutes at 1000 rpm, was used for further studies. For estimation of the total phenols, fresh tomato leaves (1.0g) were extracted with 80% methanol, and the supernatant was evaporated to dryness and finally redissolved in distilled water.

Assay of peroxidase [4]:The reaction mixture consisted 1.5 ml of 0.05 M pyrogallol, 0.5 ml of enzyme extract and 0.5 ml of 1 per cent H_2O_2. 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 [5]: The reaction mixture consisted of 200µl of enzyme extract and 1.5 ml of 0.1 M sodium phosphate buffer. To start the reaction, 200µl 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 [6]: 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 asµg catechol per g of sample.

Assay of phenyl ammonia lyase [7]: 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 co-efficient of 9630 M-1. Enzyme activity was expressed as nmol trans cinnamic acid per min per g of sample.

Results

The seed infection by *A. solani* was reduced by 99.50 per cent in 2.0 per cent P-40 treated seeds (Table 2). The treatments which received Mancozeb (0.2 %) and *Trichoderma viride* recorded 89.32 and 90.17 per cent reduced seed infection over the control. The germination per cent was increased by 14.32 per cent in T_4 (2.0% P-40) when compared to the control.

The effect of various concentrations of P-40 on vigour of tomato seedlings is presented in Table 2. The maximum shoot (12.90 cm) and root length (9.12 cm) were observed in T_4 which received 2.0 per cent P-40 treatment. The 2.0 per cent P-40 treated plants recorded 76.71

Disease score (Score)	Disease severity
0	No infection
1	0.1 to 5 % leaf area affected
2	5.1 to 15 % leaf area affected
3	15.1 to 30 % leaf area affected
4	30.1 to 50 % leaf area affected
5	50.1 to 100 % leaf area affected

Table 1: Description of the disease score and Per cent Disease Incidence (PDI).

Treatment	Seed infection (%)*	Germination (%)*	Shoot length (cm)*	Root length (cm)*	Vigour index (VI)*
T_1	10.00	82.60	10.23	8.96	792.55
T_2	9.50	85.50	11.20	9.51	885.35
T_3	1.50	87.80	12.50	9.52	966.68
T_4	0.44	91.00	12.90	9.12	1001.91
T_5	9.50	83.00	7.60	7.15	612.13
T_6	8.75	84.00	7.90	7.20	634.20
T_7	89.00	79.60	7.30	6.98	568.34

*Mean of four replications (5 plants/replication)

Table 2: Effect of botanical formulation of *P. minus* (P-40) on seed infection, seed germination and vigour of tomato – *A. solani.*

Treatments	Days after inoculation (change in absorbance/min/g of leaf tissue)*				
	0	2	4	6	10
T_1	1.58	1.83	2.18	2.21	2.20
T_2	1.59	1.89	2.09	2.20	2.15
T_3	1.63	2.16	2.28	2.28	2.30
T_4	1.61	2.25	2.31	2.42	2.40
T_5	1.60	1.73	1.70	1.85	1.78
T_6	1.61	1.69	1.70	1.79	1.74
T_7	1.62	1.69	1.69	1.72	1.72
T_8	1.63	1.80	1.71	1.58	1.60
SEd	0.1182	0.1389	0.1452	0.1489	0.1475
CD (0.05)	0.2506	0.2945	0.3077	0.3157	0.3127

*Mean of three replications

Table 3: Changes in the activities of peroxidase due to application of P-40 and challenge inoculation with *A. solani.*

Treatments	Days after inoculation (change in absorbance/min/g of leaf tissue)*				
	0	2	4	6	10
T_1	1.63	2.25	2.51	2.67	2.70
T_2	1.61	2.00	2.48	2.57	2.69
T_3	1.60	2.05	2.56	2.70	2.70
T_4	1.65	2.27	2.65	2.72	2.75
T_5	1.54	1.63	1.70	1.72	1.80
T_6	1.53	1.61	1.68	1.70	1.78
T_7	1.59	1.65	1.66	1.68	1.69
T_8	1.35	1.79	1.94	1.51	1.38
SEd	0.1150	0.1413	0.1607	0.1630	0.1654
CD (0.05)	0.2438	0.2996	0.3407	0.3456	0.3507

*Mean of three replications

Table 4: Changes in the activities of polyphenol oxidase due to application of P-40 and challenge inoculation with *A. solani.*

Treatments	Days after inoculation (change in absorbance/min/g of leaf tissue)*				
	0	2	4	6	10
T_1	45.17	47.04	47.66	47.46	46.94
T_2	44.86	46.31	47.14	47.66	47.04
T_3	44.95	46.94	47.08	48.29	47.46
T_4	45.48	47.56	48.70	48.60	47.66
T_5	44.55	45.90	46.52	46.52	46.10
T_6	44.49	46.09	46.73	46.20	46.04
T_7	44.86	45.28	45.29	45.13	45.11
T_8	45.17	45.52	45.78	44.69	44.38
SEd	3.3026	3.4078	3.4489	3.4604	3.4308
CD (0.05)	7.0012	7.2243	7.3115	7.3357	7.2730

*Mean of three replications

Table 5: Changes in the activities of phenylalanine ammonia lyase due to application of P-40 and challenge inoculation with *A. solani.*

Treatments	Days after inoculation (µg of catechol/g of leaf tissue)*				
	0	2	4	6	10
T_1	107.5	119.7	128.5	144.5	139.5
T_2	105.6	148.3	165.2	182.5	176.9
T_3	107.3	149.2	163.5	182.5	179.5
T_4	109.1	149.6	165.5	186.5	180.2
T_5	107.5	119.7	128.5	144.5	139.5
T_6	106.9	118.7	127.9	143.8	137.9
T_7	104.3	115.9	117.1	119.6	120.5
T_8	104.2	117.8	130.1	121.8	107.4
SEd	7.8307	9.6055	10.4388	11.1161	11.3214
CD (0.05)	16.6005	20.3630	22.1296	23.5653	24.0007

*Mean of three replications

Table 6: Changes in the content of phenol due to application of P-40 and challenge inoculation with *A. solani.*

and 30.66 per cent increased shoot and root length over the control. The vigour index was also maximum with T_4 treatment (2.0 % P-40-1001.91). The treatments which received Mancozeb (T_5) and *T. viride* (T_6) recorded 7.70 and 11.59 per cent increased vigour index over control.

The changes in activities of various enzymes were monitored on 0, 2, 4, 6 and 10 days after challenge inoculation of *A. solani*. The PO activity reached maximum at 6 days after inoculation with 2.0 per cent P-40 treated plants (2.40 OD min/g fresh tissue) (Table 3) and then declined thereafter. Though the PO activities increased in inoculated control, the values were significantly lower than the plants treated with various concentrations of P-40, Mancozeb (0.2%) and *T. viride*. In inoculated control, the activity reached maximum (1.80 OD min/g) on second day of inoculation and then declined. The PO activities in treatments T_1, T_2, T_3, T_5 and T_6 which received 0.50, 1.0, and 1.5 per cent Polymin 40 EC, Mancozeb (0.2%) and *T. viride* were lower than 2.0 per cent P-40 treated plants.

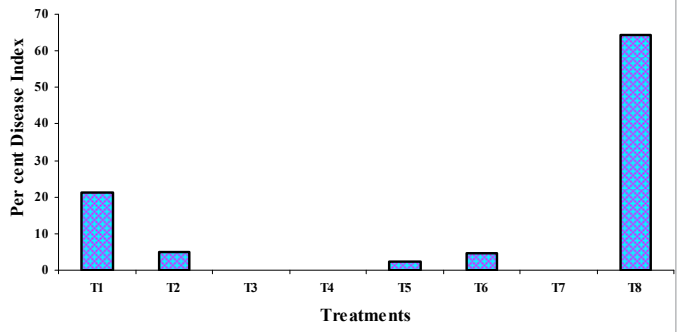

Figure 1: Effect of botanical formulation of *P. minus* (P-40) on early blight of tomato under glass house conditions.

Similarly, the PPO activity increased in plants treated with P-40 when compared to inoculated control (Table 4). P-40 at 2.0 per cent concentration significantly increased the activity of PPO to maximum level (2.72 OD min/g) on sixth day of inoculation. Among the treatments, maximum PAL activity was observed (48.70 OD min/g) with T_4 (2.0 % P-40) on fourth day of inoculation (Table 5). The concentration of phenol was significantly higher in plants treated with 2.0 per cent P-40 (186.5µg of catechol/g of leaf tissue) than all other treatments on sixth day of challenge inoculation (Table 6). In all the treatments, maximum phenol content reached on sixth day after inoculation. However, all the treatments retained the content of phenol without much reduction even on tenth day, but the phenol content reduced drastically in inoculated control.

Effect of P-40 on early blight of tomato under glass house conditions

The per cent disease index was calculated using the disease score card for *A. solani*. It has been observed that, the per cent disease index of the Polymin treated plants was significantly lesser when compared to the control. The treatment T_4 which received 2 per cent P-40 recorded 99.9 per cent reduced PDI when compared to control (T_8). This was followed by the treatment T_3 (1.5 % P-40) which recorded 94.57 per cent reduced PDI, whereas the treatment T_5 and T_6 which received mancozeb (0.2 %) and *T. viride* recorded 96.43 and 92.86 per cent reduced PDI respectively (Figure 1).

Discussion

The botanical formulation, Polymin 40 EC at various concentrations (0.5, 1.0, 1.5 and 2.0 per cent) was tested under pot culture conditions in controlling *A. solani*. The P-40 at 2.0 per cent effectively controlled the pathogens under pot culture conditions and was considered as the optimum concentration.

Ramanathan A [8] have reported that biotic and abiotic inducers play an important role in activating the defense genes in plants. Induction of defense proteins makes the plant resistant to pathogen invasion [9]. 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 [10].

Spraying of P-40 enhanced the accumulation of defense enzymes and total phenol content on challenge inoculation with *A. solani* in tomato. Similar results were reported by [11] in grapes due to the application of botanical formulation - Wanis. Enhancement of PO and PPO catalyzes the biosynthesis of lignin and other oxidative phenols, which are considered as the mechanical barrier and results in disease resistance of plants.

Plant phenolics are well known antifungal, antibacterial and antiviral compounds and increase the physical and mechanical strength of the host cell wall. Lignin is a phenolic polymer which is difficult to be breached by pathogens and has been implicated in plant defense against pests and diseases. Such rapid defense reactions at the site of fungal entry delays the infection process and allows sufficient time for the host to buildup other defense reactions to restrict pathogen growth.

It has been concluded that the botanical formulation "Polymin" increased the germination percentage by reducing the seed infection by *A. solani* in tomato. It also showed potential inhibitory effect on the selected plant pathogen under pot culture conditions. Polymin showed high level of thermo and storage stability. The botanical formulation is capable of inducing the resistance in tomato plants through enhancement of defense compounds (PO, PPO, PAL and phenol). Polymin showed the potential for managing the major disease in tomato. The future thrust and follow-up research efforts may aim to study the effect of Polymin on plant disease control under field conditions by and this will provide an opportunity for eco-friendly disease management on a variety of crops.

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