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Study on effect of Polymin 40 EC against the occurrence of *Aspergillus niger* in maize

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Abstract
Plant diseases and pests cause major economic loss in agriculture, in addition to the poor quality of the agricultural products. Occurrence of *Aspergillus niger* in maize crop causes post harvest losses and deterioration of the maize grain quality. In the present study, the effect of Polymin 40 EC – a newly developed botanical formulation was evaluated for its effect on *Aspergillus niger* in maize crop under *in vivo* conditions. Different concentrations of Polymin 40 EC was tested for its effect on seed infection, germination and vigour of maize. The study revealed that, Polymin 40 EC at 2% concentration was effective in increasing the germination and vigour of maize seedlings, in addition to decrease in seed infection. Experiments conducted under pot culture conditions revealed that, application of 2% Polymin 40 EC increased the activity of plant defense enzymes like peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL) and phenol content of maize plant. The incidence of *Aspergillus niger* was found to be controlled effectively in maize crop through application of 2% Polymin 40 EC.

Keywords: Polymin, Maize, *Aspergillus niger*

Introduction
Use of botanicals in plant disease management is gaining attention and it is regarded as safe to the environment. Maize remains as one of the important cereal crops and the incidence of *Aspergillus niger* causes major post harvest loss. Traditionally various plant extracts were used in agriculture to control important diseases of crop plants. The advantage of botanicals over chemicals is reduced residual effect on the environment and reduced risk of resistance and resurgence development in pathogens. The chloroform extract of *Polygonum minus* Huds was found to possess antimicrobial activity against various plant pathogens (Parimala devi and Marimuthu, 2020) [10]. A new botanical formulation "Polymin 40 EC" was prepared and evaluated for its effectiveness in controlling the incidence of *Aspergillus niger* in maize crop.

Materials and Methods
Disease incidence (Blotter method)
The maize seeds (COHM5) with natural infections were used for the study to find out the effect of different concentrations (0.50, 1.0, 1.5 and 2.0%) of Polymin 40EC. The seeds (100 seeds) were soaked in Polymin 40EC for 2 h and replicated four times. Seeds soaked in distilled water were maintained as control. Twenty five seeds of each treatment were placed on moist blotters (ISTA, 1993) [5] in petriplate and incubated (20 ± 2°C) for 24 h (12 h of natural UV light and 12 h darkness). On eighth day of treatment the seeds were examined for growth of seed borne pathogens. The seed infection was expressed in percentage.
The treatments include:
T_1- 0.50 % botanical formulation of *P. minus* (Polymin 40EC)
T_2- 1.0 % botanical formulation of *P. minus* (Polymin 40EC)
T_3- 1.5 % botanical formulation of *P. minus* (Polymin 40EC)
T_4- 2.0 % botanical formulation of *P. minus* (Polymin 40EC)
T_5- Mancozeb (0.2%)
T_6- Biocontrol agent (*T. viride*)
T_7- Uninoculated control

Germination (%)
Maize seeds were soaked in Polymin 40 EC for a period 18 h and then shade dried. Hundred seeds were uniformly placed on standard germination paper roll-towel medium (ROLL towel medium, ISTA, 1993) [5] with four replications and kept in germination room maintained at 25 ± 2°C and 90 ± 2 per cent relative humidity. After 14 days, the seedlings were evaluated as total number of normal seedlings and germination as percentage. Seven different concentration of *P. minus* (Polymin 40EC) was used for evaluating the per cent germination.

Root length (cm)
Ten normal seedlings per replication from roll towel medium were carefully removed at random from each treatment at fourteenth day 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 were 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) [1] 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 *A. niger* under pot culture conditions
Polymin 40EC at different concentrations *viz.*, 0.50, 1.0, 1.5 and 2.0% were tested for its activity under glass house conditions. Percent disease index and percent disease incidence was calculated from the observations recorded.
The treatments include:
T_1- 0.50 % botanical formulation of *P. minus* (Polymin 40EC)
T_2- 1.0 % botanical formulation of *P. minus* (Polymin 40EC)
T_3- 1.5 % botanical formulation of *P. minus* (Polymin 40EC)
T_4- 2.0 % botanical formulation of *P.minus* (Polymin 40EC)
T_5- Mancozeb (0.2%)
T_6- Biocontrol agent (*T. viride*)
T_7- Uninoculated control
T_8- Inoculated control

Method of inoculation
Maize plants (45 days old) were inoculated with spores of *A. niger* at a concentration of 7 x 10^6 spore/ml. The plants were sprayed with different concentration of Polymin 40EC *viz.*, 0.50, 1.0, 1.5, and 2.0% after 24 h. At 15 days interval, second and third spraying was done.

Biochemical changes in crop plants
Maize plants were inoculated with Polymin 40EC at different concentration *viz.*, 0.50, 1.0, 1.5 and 2.0 per cent. Distilled water spraying was given for control plants.
The pathogen was inoculated 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 changes in plant defense enzymes. Three replications were maintained for each treatment.

Peroxidase (Puttur, 1974) [11]
The reaction mixture consists of 1.5 ml of 0.05 M pyrogallol, 0.5 ml of enzyme extract and 0.5 ml 1 per cent H_2O_2. Changes in absorbance at 420 nm were recorded at 30 seconds interval for 3 min. Changes in the absorbance per min per g of sample was expressed as the enzyme activity.

Polyphenoloxidase (Mayer *et al.*, 1965) [9]
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.

Total phenol (Spies, 1955) [15]
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.

Phenyl ammonia lyase (Zucker, 1965) [16]
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
Maize seeds treated with 2.0 % Polymin 40 EC (T_4) recorded no seed infection by *A. niger ie*., 100 per cent less than the untreated control (Table 1). The germination of maize seedlings was 92.50 per cent in T_4 (2.0% per cent Polymin 40EC) which was 14.91 per cent increase over the control.
Vigour of maize seedlings was increased by 72.36 per cent over the control in T_4 (2.0% Polymin 40EC). The maximum shoot (19.00 cm) and root length (24.35 cm) was observed in T_4 (2.0% Polymin 40EC) followed by 18.40 cm and 22.00 cm in T_3, 17.50 cm and 20.60 cm in T_2 (Table 2 and Plate 1). Control recorded vigour index of 1163.23 in maize seedlings which was 28.49 per cent lower than the best performing treatment T_4.

Biochemical changes
The biochemical changes were assessed in maize plants treated with various concentrations of Polymin 40EC *viz.*, 0.50, 1.0, 1.5 and 2.0% on challenge inoculation with *A. niger*. The activities of peroxidase (PO), polyphenoloxidase (PPO), phenylalanine ammonia lyase (PAL) and phenol content were monitored up to 10 days after challenge inoculation. From the study it was observed that the enzyme activities were higher in treatments which received various concentrations of Polymin 40EC, Mancozeb (0.2%) and *T. viride*.
The maximum PO activity was recorded in 2.0 per cent Polymin 40EC treated maize plants (3.501 OD min/g) on 6 days after challenge inoculation followed by T_3 (3.434 OD min/g) (Table 3). The untreated control recorded 0.631 OD min/g of PO activity on 6 days after challenge inoculation and this was lower than all other treatments.
The treatment T_4 (2.0% Polymin 40EC) recorded the maximum PPO activity (2.502 OD min/g) on 4 days after inoculation (Table 4). The plants treated with various concentrations of Polymin 40EC, Mancozeb (0.2%) and *T. viride* recorded maximum PPO activity on fourth day after challenge inoculation and were significantly higher than the inoculated control (1.216 OD min/g) (Plate 2).
The PAL activity was maximum on 6 days after inoculation in all the treatments (Table 5). Maximum activity of PAL was recorded by the treatment T_4 (2.0% Polymin 40EC – 7.108 OD min/g) on sixth day after inoculation. The treatments T_5 (Mancozeb 0.2%) and T_6 (*T. viride*) recorded the PAL activity

of 6.920 and 6.896 OD min/g respectively on sixth day after inoculation which were higher than that of the inoculated control T_8 (2.896 OD min/g). The untreated control (T_7) recorded the lowest PAL activity on all the days of observation. Maize plants sprayed with Polymin 40EC recorded higher phenol content on six days after challenge inoculation (Table 6). The plants sprayed with 2.0 per cent Polymin 40EC (T_4) recorded maximum phenol content (539.60 µg of catechol/g) on 6 days after challenge inoculation followed by T_3 (526.50 µg of catechol/g) and T_2 (443.60 µg of catechol/g).

Discussion

Polymin 40 EC at 2 per cent was found to reduce the infection by *A. Niger*. It also increased the vigour of maize plants (72.36%) over the control. The role of botanicals against fungal plant pathogens and increasing the seedling vigour were reported by many workers (Groundnut-Bansal and Saboti, 1990, paddy - Shetty and Shetty, 1987) [2, 14]. The studies of Kumbhar *et al*. (1999) [7] revealed that *Phaseolus aconitifolius* seeds treated with *O. canum* and *B. juncea* formulations increased the germination percentage and vigour of seedlings. Promotion in the activity of seed enzymes due to the application of Polymin may be reason for increase in germination percentage and vigour of seedlings.

Under pot culture conditions Polymin 40EC (2%), effectively controlled the pathogens and was found to be the optimum concentration. According to Bowers and Locke, (2000) [3], pepper, clove and cassia at 5 per cent concentration controlled *Fusarium oxysporum* f.sp. *chrysanthemi* by 100 per cent under field conditions.

Ramanathan *et al*., (2000) [13] reported that induction of defense proteins makes the plant resistant to invasion by pathogens. Application of Polymin 40EC was found to induce the defense compounds (PO, PPO, PAL and phenol) significantly in maize plants when compared over the control. Polymin 40EC application enhanced the accumulation of defense enzymes and total phenol content in maize. Rajeswari (2002) [12] reported similar results in grapes due to the application of Wanis (a botanical formulation). It is known that 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. The studies of Kagale (2001) [6] revealed that foliar application of *Datura metel* and *Zizyphus jujuba* enhanced the PO and PPO activity on challenge inoculation with *Rhizoctonia solani*.

Phenylalanine ammonia lyase (PAL) plays an important role in the biosynthesis of phytoalexins. Phenylalanine ammonia lyase is the first enzyme of phenyl propanoid metabolism in higher plants and plays a significant role in regulating the phenolics accumulation (Massala *et al*., 1980; Glazzener, 1982) [8, 4]. Polymin 40EC containing the antimicrobial compounds led to increased biosynthesis of plant defense enzymes and phenols. The increase in these compounds was responsible for increased disease resistance in plants.

The mechanism of these plant defense compounds responsible for plant resistance are well documented by many workers and is as follows; Peroxidase enzymes - polymerization of proteins and lignin or suberin precursors into plant cell wall, thus constructing a physical barrier that could prevent pathogen penetration of cell walls and movement through vessels. Phenylalanine ammonia lyase (PAL) - catalyzes the de-amination of L-phenylalanine to trans-cinnamic acid, which is the first step in the biosynthesis of large class of plant natural products, including lignin monomers as well as certain classes of phytoalexins. It is the key enzyme in inducing synthesis of salicylic acid (SA), which induces systemic resistance in many plants.

Plant phenolics is known to increase the physical and mechanical strength of the host cell wall. Lignin is a phenolic polymer which is difficult to be degraded by pathogens and plays a main role in plant defense against pests and diseases. The present study proves the antifungal activity of newly developed botanical formulation “Polymin 40 EC” against Aspergillus niger in maize. Detailed study on the identification of lead molecule responsible for this antifungal activity and application of the lead molecule in most pure form will serve as a better plant protection measure in crop plants.

Table 1: Effect of botanical formulation of *P. minus* (Polymin 40EC) on seed infection and seed germination of maize – *A. niger*

Treatment	Seed infection		Seed germination	
	Infection (%)*	Reduction over control	Germination (%)*	Increase over control
T_1 (0.50% Polymin 40EC)	15.00	83.15	83.50	3.73
T_2 (1.00% Polymin 40EC)	11.50	87.08	86.30	7.20
T_3 (1.50% Polymin 40EC)	2.50	97.09	88.00	9.32
T_4 (2.00% Polymin 40EC)	0.00	100.00	92.50	14.91
T_5 (Mancozeb 0.2%)	10.75	87.92	83.00	3.11
T_6 (*T. viride*)	9.80	88.98	82.50	2.48
T_7 (Control)	89.00		80.50	

*Mean of four replications (5 plants/replication)

Table 2: Effect of botanical formulation of *P. minus* (Polymin 40EC) on vigour of maize seedlings –*A. niger*

Treatment	Shoot length (cm)*	Increase over control	Root length (cm)*	Increase over control	Vigour index (VI)*	Increase over control
T_1 (0.50% Polymin 40EC)	16.30	30.40	19.50	18.90	1494.65	28.49
T_2 (1.00% Polymin 40EC)	17.50	40.00	20.60	25.61	1644.02	41.33
T_3 (1.50% Polymin 40EC)	18.40	47.29	22.00	34.15	1777.60	52.81
T_4 (2.00% Polymin 40EC)	19.00	52.00	24.35	48.48	2004.94	72.36
T_5 (Mancozeb 0.2%)	15.50	24.00	19.00	15.85	1431.75	23.08
T_6 (*T. viride*)	15.25	22.00	19.00	17.38	1412.81	21.46
T_7 (Control)	12.50		16.40		1163.23	

*Mean of four replications (5 plants/replication)

Table 3: Changes in the activities of peroxidase due to application of Polymin 40EC and challenge inoculation with *A. niger*

Treatments	Days after inoculation (change in absorbance/min/g of leaf tissue)*				
	0	2	4	6	10
T_1 (0.50% Polymin 40EC)	0.778	1.426	2.938	3.336	2.901
T_2 (1.00% Polymin 40EC)	0.780	1.449	2.970	3.385	2.923
T_3 (1.50% Polymin 40EC)	0.782	1.501	3.196	3.434	3.185
T_4 (2.00% Polymin 40EC)	0.785	1.526	3.234	3.501	3.261
T_5 (Mancozeb 0.2%)	0.792	1.432	2.961	3.319	2.914
T_6 (*T.viride*)	0.775	1.429	2.959	3.312	2.907
T_7 (Uninoculad control)	0.663	0.682	0.685	0.631	0.621
T_8 (Inoculated control)	0.769	0.998	1.231	1.220	1.105
SEd	0.0563	0.0981	0.1972	0.2182	0.1949
CD (0.05)	0.1194	0.2080	0.4181	0.4625	0.4132

*Mean of three replications

Table 4: Changes in the activities of polyphenol oxidase due to application of Polymin 40EC and challenge inoculation with *A. niger*

Treatments	Days after inoculation (change in absorbance/min/g of leaf tissue)*				
	0	2	4	6	10
T_1 (0.50% Polymin 40EC)	0.831	1.392	2.236	2.102	1.921
T_2 (1.00% Polymin 40EC)	0.829	1.398	2.279	2.110	2.020
T_3 (1.50% Polymin 40EC)	0.843	1.462	2.461	2.336	2.198
T_4 (2.00% Polymin 40EC)	0.851	1.498	2.502	2.372	2.228
T_5 (Mancozeb 0.2%)	0.830	1.387	2.276	2.103	2.019
T_6 (*T.viride*)	0.826	1.369	2.272	2.100	2.010
T_7 (Uninoculad control)	0.712	0.726	0.721	0.712	0.701
T_8 (Inoculated control)	0.812	0.962	1.216	1.212	1.002
SEd	0.0601	0.0955	0.1534	0.1441	0.1354
CD (0.05)	0.1274	0.2025	0.3251	0.3055	0.2871

*Mean of three replications

Table 5: Changes in the activities of phenylalanine ammonia lyase due to application of Polymin 40EC and challenge inoculation with *A. niger*

Treatments	Days after inoculation (change in absorbance/min/g of leaf tissue)*				
	0	2	4	6	10
T_1 (0.50% Polymin 40EC)	2.863	3.613	4.861	6.934	5.908
T_2 (1.00% Polymin 40EC)	2.886	3.678	4.894	6.963	5.981
T_3 (1.50% Polymin 40EC)	2.996	3.863	5.224	7.086	6.324
T_4 (2.00% Polymin 40EC)	3.042	3.996	5.321	7.108	6.412
T_5 (Mancozeb 0.2%)	2.856	3.569	4.787	6.920	5.960
T_6 (*T.viride*)	2.857	3.542	4.769	6.896	5.891
T_7 (Uninoculad control)	2.653	2.710	2.692	2.650	2.590
T_8 (Inoculated control)	2.821	2.930	3.019	2.896	2.480
SEd	0.2112	0.2581	0.3339	0.4561	0.3982
CD (0.05)	0.4477	0.5471	0.7079	0.9668	0.8441

*Mean of three replications

Table 6: Changes in the content of phenol due to application of Polymin 40EC and challenge inoculation with *A. niger*

Treatments	Days after inoculation (µg of catechol/g of leaf tissue)*				
	0	2	4	6	10
T_1 (0.50% Polymin 40EC)	252.00	334.66	378.00	412.50	390.75
T_2 (1.00% Polymin 40EC)	260.00	356.70	386.50	443.60	421.50
T_3 (1.50% Polymin 40EC)	295.50	392.50	421.00	526.50	506.00
T_4 (2.00% Polymin 40EC)	298.06	396.00	432.75	539.60	515.06
T_5 (Mancozeb 0.2%)	248.60	330.50	366.50	410.60	389.50
T_6 (*T.viride*)	245.40	329.00	357.80	402.50	376.50
T_7 (Uninoculad control)	232.16	235.60	233.10	231.20	230.00
T_8 (Inoculated control)	249.50	311.00	366.50	341.50	252.00
SEd	19.1877	24.9172	27.3451	31.1334	29.1811
CD (0.05)	40.6767	52.8229	56.9697	66.0007	61.8620

*Mean of three replications

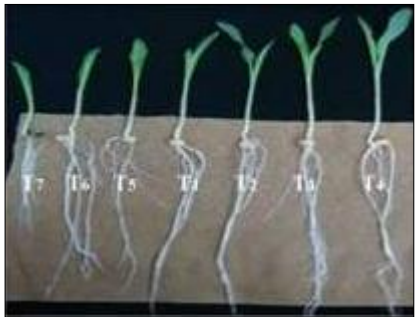

Plate 1: Effect of Polymin 40 EC on seed infection, germination and vigour of maize plants

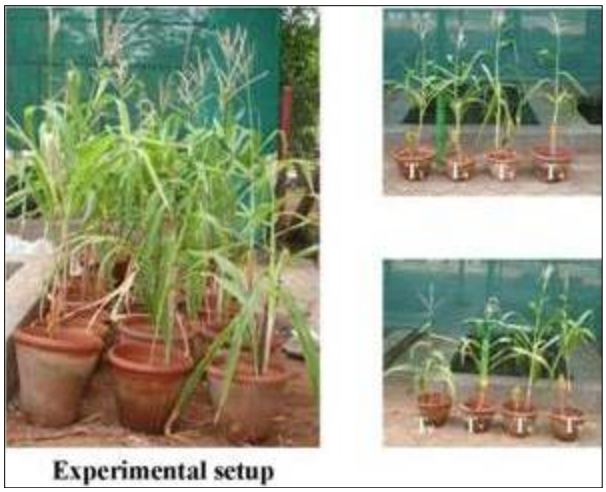

Plate 2: Effect of Polymin 40 EC on plant disease control under pot culture condition

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