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# Responses of triazole type of plant growth regulators on fungal growth

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Responses of triazole derivatives, namely LAB 150978, BAS 110.. W and BAS 111..W were analysed on growth and metabolism of fungi isolated from banana. *Aspergillus flavus, Botryodiplodia theobromae, Curvularia fallax, Fusarium oxysporum, Fusarium moniliformae* and *Penicillium citrinum* were taken as test materials. Present investigation showed that BAS 111..W produced better response in causing inhibition of growth (dry weight) and spore germination than BAS 110.. W and LAB 150978. Low concentration of this chemical was effective in all the cases except *B. theobromae*. From $ED_{50}$ value the degree of toxicity was as follows : BAS 111.. W> BAS 110.. W> LAB 150978 > control.

**Key words :** Plant growth retardants (triazoles), fungal growth, spore germination, $ED_{50}$

## INTRODUCTION

Triazole responses on fungal growth have been studied by a number of workers (Fletcher *et. al.*, 1986; Buchenauer and Rohner, 1981; Pepin, *et al.*; 1990). The plant growth regulating properties of sterol inhibiting fungicides have been reviewed by Fletcher (1985). Several triazoles such as propiconazole, etaconazole, penconazole, diclobutrazole, BAS 45406 F, fusilazole etc. are used as fungicides. Fungicidal properties of triazole have also been demonstrated by Gupta *et al.* (1991, 1994). Many have already been or are being developed for use as agricultural fungicides (Ammermann *et al.*, 1990). According to Rademacher *et al.* (1987) triazoles presumably retard growth by inhibiting oxidative reaction in the biosynthesis of GA. Evidence accumulates in support of inhibition of sterol and ethylene biosynthesis by triazoles add further possible explanation for the growth regulating effect (Grossmann *et al.* 1989). Responses of triazole derivatives, namely LAB 150978, BAS 110..W and Bas 111..W were analysed on growth and spore germination of fungi isolated from banana which were stored in high relative humidity. Dose response relationship has been studied between triazole derivatives and fungal growth under *in vitro* in order to determine the effective concentration of triazole to inhibit fungal growth and metabolism (Sadhu, 1994 ; Creemers *et al.*, 1996).

## MATERIALS AND METHODS

Fruits of banana (*Musa acuminata* L. cv. giant governor) were collected from local market of Burdwan and identified by Banana Research Station, Chinsurah, W.B., India.

### *Isolation and purification*

Banana bunches were washed off with tap water. Then it was surface sterilised with 70% alcohol. Blocks of infected and the intermediate zone (between healthy and infected) of coat and pulp tissues were removed and transferred to malt agar (MA) slant. It was then incubated at 30 ± 1°C in dark for growth of individual colonies. Each colony was transferred to (MA) slant aseptically.

Single spore culture of the pathogen(s) was done according to the method of Smith (1960). In another experiment few bananas were kept in high RH (relative humidity) atmosphere after surface sterilization to sporulate fungi on the surface. A pure culture obtained by touching the spores with a fine gauge inoculating needle and stabbed into

sterile medium, then streaking into slant.

### *Identification*

After isolation 5 pure cultures were obtained and identified by International Mycological Institute, Kew, England. These were *Aspergillus flavus* Link (IMI No. 342479), *Penicillium citrinum* Thom (IMI No. 342480), *Curvularia fallax* Boedijn (IMI No. 342481), *Fusarium oxysporum* (Smith) Synder and Hansen (IMI No. 342482) and *Fusarium moniliformae* Sheldon (IMI No. 342482a). *Botryodiplodia theobromae* Pat. was identified comparing with the authentic culture (I.A.R.I. 2208).

### *Pathogenecity tests*

Pathogenecity tests were carried out with two isolates, *Curvularia fallax* and *Penicillium citrinum. Fusarium oxysporum, Fusarium moniliformae, Aspergillus flavus* and *Botryodiplodia theobromae* were previously reported to be pathogenic to banana (*M. acuminata* L.). Pathogenecity tests carried out with spores from 6 day old culture resulted in the development of typical symptom 6-8 days after inoculation. Reisolation from induced lesion established the identity with the original isolate.

### *Dry weight determination*

LAB 150978*, BAS 110..W** and BAS 111..W*** were mixed to prepare different concentrations (0, 100, 300, 500 mg/ml) in the liquid Czapek Dox broth (CMI Plant Pathologist Pocket Book, 1974) in 250 ml Erlenmeyer flask. Inoculum (5 mm) disc were transferred to liquid Czapek Dox broth and grown for 20 days at 30 ± 1°C in the dark. Twenty day old mycelial mats were harvested and repeatedly washed with distilled water. It was then oven dried at 80°C for 24 h. The mycelial dry weight was taken of each triazole treated as well as untreated control.

### *Spore germination assay*

Fungicidal efficacy of the triazoles were assayed by spore germination technique on glass slide following Pan and Sen (1976). Slant culture containing the fungus was flooded with sterilised distilled water with periodic scrapping of the surface of the culture. The spore mycelial suspension was then passed through double layer of cheese cloth. The approximate spore concentration of the suspension was determined using a haemocytometer under a microscope.

Different concentration (100, 300, 500 µg/ml) of LAB 150978, BAS 110..W and BAS 111..W were prepared with 2% sterile malt solution. Then equal volume of triazole malt solution and spore suspension of each fungi were taken on sterilised glass slide. It was mixed throughly and incubated for 20 h within a moist beljar. Similarly an untreated sterile malt solution mixed with spore suspension has been treated as control. The percentage of germination was calculated from the total number of spores under a microscope.

### *$ED_{50}$ determination*

$ED_{50}$ was determined according to the method followed by Martin and Edgington (1977). The concentration of triazole (µg/ml) required to cause 50% inhibition of mycelial growth ($ED_{50}$) was determined from a plot of per cent inhibition (probit scale) versus the concentration of triazole in the medium (log 10 scale). The inhibition per cent was calculated from the following formula :

Inhibition percentage = $\frac{a-b}{a} \times 100$, where a = average of mycelial growth in control after 20 days and b = average of mycelial growth of the treatment after 20 days.

## RESULTS

In all the fungi increase in triazole concentration decreased the growth as well as spore germination. In this study BAS 111..W was shown to be highly effective at 100 mg/ml in controlling growth of the test-fungi (dry weight basis) except *Botryodiplodia theobromae* (Table 1). None of the triazoles was effective against *B. theobromae* even at the highest concentration of 500 µg/ml. LAB 150978 has more inhibitory response in case of *A. flavus* and *F. oxysporum*. Spore germination inhibition was also found better in case of *A. flavus*.

* LAB 150978 = 1-(4-trifluromethyl)-2-(1,2,4-triazolyl-[1]-3-(5-methyl-1,3-dioxan-5-yl)-propen-3-ol.

** BAS 110..W = 1-(2,4-dichlorophenyl)-2-methoxyl-1-methyl-2-(1H-1,2,4-triazol-1-yl)-ethaxol.

*** BAS 111..W = 1-phenoxy-3-(1H-1,2,4-triazol-1-yl)-4 hydroxy-5, 5-dimethyl hexane.

**Table 1 :** Effect of triazole derivatives on the growth (mg. dry wt.) of fungal cultures after 20 days of inoculation (values are mean ± S.E.)

| Triazole derivatives | Concentration ( µg/ml ) | Fungi (mg. dry wt.) | | | | | |
|---|---|---|---|---|---|---|---|
| | | *A. flavus* | *B. theobromae* | *C. fallax* | *F. oxysporum* | *F. moniliformae* | *P. citrinum* |
| Control | 0 | 332.00 ±2.11 | 441.00 ±3.99 | 432.00 ±3.80 | 280.00 ±1.80 | 171.00 ±1.05 | 402.00 ±3.57 |
| LAB 150978 | 100 | 295.00 ±1.81 | 416.00 ±3.42 | 425.00 ±3.67 | 168.00 ±0.92 | 168.00 ±0.89 | 382.00 ±3.20 |
| | 300 | 213.00 ±1.60 | 412.00 ±3.26 | 402.30 ±3.42 | 142.00 ±0.82 | 142.00 ±0.98 | 266.50 ±1.62 |
| | 500 | 61.00 ±0.42 | 372.00 ±2.92 | 390.00 ±3.22 | 94.50 ±0.72 | 103.00 ±0.95 | 207.00 ±1.42 |
| BAS 11O..W | 100 | 290.30 ±1.80 | 397.00 ±3.49 | 94.00 ±0.53 | 167.00 ±0.86 | 161.00 ±0.82 | 281.00 ±1.72 |
| | 300 | 220.00 ±1.71 | 380.00 ±3.27 | 58.00 ±0.22 | 135.00 ±0.79 | 144.00 ±0.80 | 255.50 ±1.58 |
| | 500 | 182.40 ±1.10 | 374.00 ±3.10 | 10.00 ±0.07 | 18.00 ±0.08 | 95.00 ±0.60 | 146.60 ±0.83 |
| BAS 111..W | 100 | 28.50 ±0.18 | 388.00 ±3.30 | 21.20 ±0.09 | 62.00 ±0.21 | 50.00 ±0.16 | 29.00 ±0.17 |
| | 300 | 21.66 ±0.09 | 371.80 ±2.88 | 5.00 ±0.001 | 54.00 ±0.16 | 39.30 ±0.11 | 15.00 ±0.002 |
| | 500 | 2.00 ±0.001 | 355.00 ±2.23 | 0 | 20.50 ±0.002 | 20.50 ±0.001 | 0 |

**Table 2 :** Effect of triazole derivatives on the spore germination (%) of fungal cultures after 20 days of inoculation (Values are mean ± S.E.)

| Triazole derivatives | Concentration ( µg/ml ) | Fungi (% of spore germination) | | | | | |
|---|---|---|---|---|---|---|---|
| | | *A. flavus* | *B. theobromae* | *C. fallax* | *F. oxysporum* | *F. moniliformae* | *P. citrinum* |
| Control | 0 | 71.02 ±0.52 | 68.20 ±0.31 | 65.30 ±0.38 | 53.30 ±0.36 | 51.30 ±0.40 | 62.50 ±0.42 |
| LAB 150978 | 100 | 48.30 ±0.26 | 56.28 ±0.38 | 62.00 ±0.40 | 48.20 ±0.25 | 42.00 ±0.22 | 57.80 ±0.39 |
| | 300 | 37.00 ±0.21 | 32.00 ±0.20 | 48.50 ±0.25 | 34.00 ±0.19 | 35.50 ±0.21 | 43.00 ±0.28 |
| | 500 | 22.00 ±0.23 | 28.70 ±0.21 | 43.20 ±0.24 | 24.48 ±0.10 | 24.20 ±0.16 | 32.00 ±0.23 |
| BAS 110..W | 100 | 65.78 ±0.42 | 61.90 ±0.44 | 13.00 ±0.09 | 45.00 ±0.25 | 46.00 ±0.27 | 53.00 ±0.40 |
| | 300 | 58.00 ±0.40 | 35.00 ±0.21 | 6.00 ±0.01 | 30.00 ±0.12 | 33.00 ±0.18 | 43.70 ±0.27 |
| | 500 | 36.30 ±0.21 | 20.00 ±0.12 | 0 | 2.00 ±0.01 | 10.50 ±0.05 | 31.20 ±0.17 |
| BAS 111..W | 100 | 7.10 ±0.03 | 40.00 ±0.22 | 4.00 ±0.01 | 12.00 ±0.10 | 13.00 ±0.10 | 8.2 ±0.02 |
| | 300 | 3.00 ±0.01 | 23.52 ±0.13 | 0 | 7.00 ±0.02 | 5.00 ±0.01 | 0 |
| | 500 | 0 | 14.28 ±0.10 | 0 | 0 | 0 | 0 |

BAS 110..W responsed better in dry weight decrease in case of *C. fallax, F. oxysporum* and *P. citrinum* at 500 µg/m1. Complete inhibition of spore germination was also found in case of *C. fallax* and *F. oxysporum* at 500 µg/m1 (Table 2).

BAS 111..W has strong inhibitory response in relation to dry weight as well as spore germination. Dry weight decrease as well as spore germination inhibition found at 100 µg/m1 in all the cultures except *B. theobromae*. Complete inhibition of growth was found in all the cultures except *B. theobromae*.

All the fungi tested were highly sensitive to the triazoles with differences in the degree of sensitivity and the $ED_{50}$ of three different triazoles were compared (Table 3). In LAB 150978, $ED_{50}$ (50% growth inhibition as against control) of *P. citrinum* was 500 µg/ml. In *B. theobromae, C. fallax* and *F. moniliformae* $ED_{50}$ of LAB 150978 was more than 500 µg/ml. In BAS 110..W, $ED_{50}$ value of *A. flavus* and *F. moniliformae* was 500 µg/ m1 but less than 100 µg/ml in case of *C. fallax*. In BAS 111..W, the $ED_{50}$ value of *A. flavus, C. fallax, F. oxysporum, F. moniliformae* and *P. citrinum* was less than 100 µg/m1 but in *B. theobromae* it was more than 500 µg/m1. BAS 111..W has lower $ED_{50}$ value in most of the cultures than BAS 110..W and LAB 150978.

**Table 3 :** Fungitoxic activity of triazole derivatives

| Fungi | $ED_{50}$ (µg/ml) | | |
|---|---|---|---|
| | LAB 150978 | BAS 110..W | BAS 111..W |
| *A. flavus* | 335 | 500 | < 100 |
| *B. theobromae* | > 500 | > 500 | > 500 |
| *C. fallax* | > 500 | < 100 | < 100 |
| *F. oxysporum* | 240 | 200 | < 100 |
| *F. moniliformae* | > 500 | 500 | < 100 |
| *P. citrinum* | 500 | 340 | < 100 |

## DISCUSSION

The antifungal property of these chemicals in controlling fungal growth is evident from this study (Lyr, 1987 ; Mourichon and Bengnon, 1982 ; Sadhu and Gupta, 1994). Such triazoles effectively prevented the growth of fruit borne fungi and also secondary infections. Antifungal responses of triazoles, therefore, might primarily be attributed to inhibition of fungal growth as well as spore germination. Present investigation showed that BAS 111..W produced better response in causing growth inhibition (dry weight) and spore germination than BAS 110..W and LAB 150978. Low concentration of this chemical was effective in all the cases except *B. theobromae* (Sadhu, 1994). Such plant growth retardants known to inhibit isoprenoid synthesis in plants and generally used as plant growth regulator are also fungitoxic in nature indicating their potentiality as a fungicide (Rademacher *et al.*, 1987; Fletcher and Hofstra, 1988; Grossmann, 1990). Their fungitoxicity therefore might be due to their ability to inhibit ergosterol synthesis. Inhibitory effect of triazole might also be due to inhibition of oxidative demethylation reaction and blockage of ergosterol biosynthesis in fungi (Ragsdale, 1977; Fletcher, 1985). All the three compounds tested have in common a chlorophenyl and a 1,2,4-triazole group and hence their potency either as fungicides or as plant growth regulators appears to depend on the steric configuration of the substituents on the carbon chain (Fletcher *et al.*, 1986).

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