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Effect of systematic acquired resistance inducing compound Benzothiadiazole (Bion) on powdery mildew and *Colletotrichum* leaf diseases of rubber

Edwin Prem E, Sadanand K. Mushrif, Srinivas P and Kuruvilla Jacob C
Rubber Research Institute of India, Kottayam, Kerala- 686 009.

Abstract

Management of rubber (Hevea brasiliensis) diseases are mainly confined to the use of systemic and contact fungicides. Benzothiadiazole, a systemic resistance inducing compound was evaluated for the expression of three defence related enzymes viz. peroxidase, polyphenol oxidase and catalase activity. In vivo studies for the management of powdery mildew (Oidium heveae) and Colletotrichum (Colletotrichum acutatum and C. gloeosporioides) leaf diseases under taken in the nursery and juvenile field plants clearly indicated that the bezothiadiazole was able to activate the enzymes studied. Benzothiadiazole when sprayed at copper brown leaf stage yielded better protection against O. heveae. Nursery and field evaluation of benzothiadiazole revealed its effectiveness in protecting H. brasiliensis against powdery mildew and colletotrichum leaf diseases and it was comparable to the recommended fungicides. The effectiveness was observed to be enhanced by the application of benzothiadiazole in combination with fungicides.

Key words: *Oidium, Colletotrichum, Hevea,* benzothiadiazole, systemic acquired resistance, peroxidase, polyphenol oxidase, catalase, disease control

Introduction

Powdery mildew (*Oidium heveae*) and Colletotrichum leaf disease (*Colletotrichum gloeosporioides and C. acutatum*) cause considerable damage to the foliage of nursery, young and mature plants of rubber (*Hevea brasiliensis*). Powdery mildew disease (PMD) assumes epidemic proportions during refoliation phase, leading to severe defoliation. The resultant poor canopy and vigour of trees reduce yield (Radziah *et al.,* 1992; Jacob *et al.,* 1992; Mondal and Jacob, 2002). *Colletotrichum* infects the new flushes of young rubber plants of age 1 – 4 years causing severe deformation and defoliation of leaves. This results in growth retardation and prolongation of the immaturity period of rubber plants (Manju *et al.,* 1999). The use of systemic and contact fungicides has been the main strategy for controlling the diseases. Fortnightly application of mancozeb (0.2%), carbendazim (0.05%) or Bordeaux mixture (1%) is recommended for colletotrichum leaf disease (CLD) control (Edathil *et al.,* 2000). Protective applicaton of sulphur fungicide either as dust or wettable powder has been in practice for the control of PMD. Use of systemic dust formulation of tridemorph (Edathil *et al.,* 1988), carbendazim (Jacob *et al.,* 1996) and hexaconazole (Prem *et al.,* 2002) were found to be effective against *Oidium.*

A new strategy for crop protection involves the induction of systemic acquired resistance (SAR) in plants, which activates the plant's own defence mechanisms leading to an increased plant resistance against diseases. Resistance to disease can be induced systemically in a number of plant species by biological and chemical means (Hammerschmidt and Kuc, 1995). Few endophytic bacteria are capable of inducing SAR in plants (Van Peer and Schippers, 1989). The most commonly used biological method is inoculation of a leaf with a local lesion –causing pathogen. Some chemical agents are known which appear to mimic the systemic effects of localized infection and they include 2,6-dichloroisonicotinic acid (INA), salicylic acid (Kessmann *et al.,* 1994)

and benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH) (Lawton *et al.*, 1996 and Kessmann, 1996). Frey and Carver (1998) reported the induction of SAR in pea to powdery mildew by exogenous application of salicylic acid. In tobacco and *Arabidopsis* benzothiadiazole induced systemic acquired resistance has been reported (Lawton *et al.*, 1996). Benzothiadiazole reduced the lesion development by *Alternaria* in cotton (Brock *et al.*1994 and Colson-Hanks and Deverall, 2000). The present study was aimed to evaluate benzothiadiazole (Bion) for the induction of systemic acquired resistance and management of powdery mildew and colletotrichum leaf diseases in rubber.

Materials and Mthod

Bio-chemical studies: Budded plants of RRIM 600 grown in poly bags were used to determine the induction of bio-chemical changes in the plants due to the spraying of two chemicals viz. salicylic acid and benzothiadiazole. Control plants were maintained and sprayed with sterile water. Leaf samples were collected at 24h, 48h, 72h and 96h after spraying and the activity of peroxidase, polyphenol oxidase and catalase were determined spectrophotometrically.

Estimation of peroxidase: Peroxidase activity was estimated using guaiacol as substrate (Putter, 1974). One gram of leaf sample was ground in a pre-cooled mortar and pestle by adding 3 ml of 0.1 M phosphate buffer (pH 7.0). The homogenate was centrifuged at 18,000 rpm at 5^{0}C for 15 minutes. The supernatant collected served as the enzyme source. From the enzyme extract 0.1 ml was drawn into a separate test tube and 3.0 ml of phosphate buffer solution, 0.05 ml guaiacol solution and 0.03 ml hydrogen peroxide were added. The peroxidase activity was determined spectrophotometrically (Shimadzu, UV-1601, Japan) at 436 nm. The enzyme activity was expressed in units per litre of the extract.

Estimation of polyphenol oxidase: Polyphenol oxidase activity was measured according to Sridhar *et al* (1969). The reaction mixture contained 2ml catechol, 0.5ml phosphate buffer and0.5ml enzyme extract. Polyphenol oxidase activity were assayed by determining the absorbance increase at 470nm and expressed as unit change in absorbance (ΔA/minute/mg protein)

Estimation of catalase: Catalase activity was measured according to Luck (1974). Pipetted 2.5ml 0.1M phosphate buffer into cuvette and added 0.1ml hydrogen peroxide and 0.05ml enzyme extract. The reaction was closely monitored by recording changes in absorbance at 240nm, for 75 seconds at 15 seconds interval starting from the first reading recorded 15 seconds after the addition of hydrogen peroxide. A cuvette containing tissue extract and buffer was used to adjust the absorbance to zero. The enzyme activity was expressed in units per mg protein, where one enzyme unit was defined as the change in absorbance per minute caused by enzyme reaction.

Evaluation of benzothiadiazole against Oidium heveae

Influence of leaf stages and concentration of benzothiadiazole on powdery mildew intensity: The experiment was conducted in the RRII Farm, Kottayam using the budwood nursery plants of the *Oidium* susceptible clone PB 5/51. Benzothiadiazole was sprayed on the different stages of leaves viz. copper brown, light green and mature leaves. Each stage of leaves was sprayed at a concentration of 0.05%, 0.1%, 0.25%, 0.5%, and 1%. Two rounds of spraying were undertaken at an interval of 3 days. Observation on the powdery mildew disease intensity was assessed after 15days. Scoring was done on a 0-5 scale based on the intensity of spotting and deformity of leaves.

0 = no disease

1 = 1-10% of leaf area infected (very light)

2 = 11-20% leaf area infected (light)
3 = 21-40% leaf area infected (moderate)
4 = 41-75% leaf area infected (severe)
5 = >75% area infected and leaf fall (very severe)

The percent disease intensity (PDI) was calculated (Horsfall and Huberger 1942) using the following formula

$$PDI = \frac{\text{Sum of numerical ratings}}{\text{No. of plants observed} \times \text{maximum disease grade}} \times 100$$

Nursery evaluation: Two experiments were carried out in the nursery at Central Experiment Station (CES) of the RRII and RRII Farm, Kottayam to evaluate the efficacy of benzothiadiazole using the budded plants of clone RRII 105 and RRIM 600 respectively. In the first location, benzothiadiazole (0.05%) was compared with recommended fungicides wettable sulphur (0.2%) and carbendazim (0.05%). A chelated zinc (0.05%) formulation was also applied. Control plants were maintained without spraying. At the second location, seven treatments were imposed. Benzothiadiazole (0.05%), carbendazim (0.05%), wettable sulphur (0.2%) and difenconazole (0.025%) were sprayed individually. Further, a combination of benzothiadiazole (0.05%) + carbendazim (0.05%) and benzothiadiazole (0.05%) + hexaconazole (0.02%) were sprayed to assess the cumulative effect of these treatments. A control plot was maintained for comparison. Both the nursery trials were conducted in completely randomised design with 15 replications. Observation on the disease intensity was recorded as described earlier. Percentage disease index (PDI) was calculated and analysed statistically.

Field evaluation: A field experiment was undertaken at TR & T estate, Mundakayam on juvenile (First year) plants of clone RRII 105 to evaluate the performance of benzothiadiazole against PMD. Seven treatments were applied. Benzothiadiazole at two concentrations (0.25 and 0.1%), carbendazim (0.05%), and difenconazole (0.025%) were sprayed individually. Combined effect of benzothiadiazole (0.05%) + carbendazim (0.05%) and benzothiadiazole (0.05%) + hexaconazole (0.02%) were also evaluated. An unsprayed control was also maintained. The experiment was laid out in a randomised block design with four replications each comprising of 25 plantlets. Fungicides were applied at weekly intervals using a knap-sack sprayer. Observations on the disease intensity were assessed as described earlier. Percentage disease index (PDI) was calculated and analysed statistically.

Evaluation of benzothiadiazole against Colletotrichum

Nursery evaluation: Nursery trials were undertaken at CES, Chethackal and RRII Farm, Kottayam to evaluate the efficacy of benzothiadiazole against CLD using the budded plants of clone RRII 105 and RRIM 600 respectively. In CES, benzothiadiazole (0.05%) was compared with recommended fungicides carbendazim (0.05%) and mancozeb (0.2%). In RRII Farm, six treatments were imposed. Benzothiadiazole (0.05%), carbendazim (0.05%), and difenconazole (0.025%) were sprayed individually. A combination of benzothiadiazole (0.05%) + carbendazim (0.05%) and benzothiadiazole (0.05%) + hexaconazole (0.02%) were sprayed to assess the combined effect. Control plots were maintained for comparison at both locations. The trials were conducted with a completely randomised design with 15 replications. The disease intensity was assessed after each round of spraying by grading diseased leaves on a 0 – 5 scale based on the percentage leaf area infected. Percentage disease index (PDI) was calculated and analysed statistically.

Field evaluation: A field experiment was undertaken at TR & T estate, Mundakayam using the first year plants of clone RRII 105 to evaluate the field performance of benzothiadiazole to control CLD. Seven treatments were applied. Benzothiadiazole at

two concentrations (0.25 and0.1%), carbendazim (0.05%), and mancozeb (0.2%) were sprayed individually. In addition, combined effect of benzothiadiazole (0.05%) + carbendazim (0.05%) and benzothiadiazole (0.05%) + hexaconazole (0.02%) were evaluated. Unsprayed control was also maintained. The experiment was laid out in a randomised block design with four replications, each replication comprising of 25 plants. Fungicides were applied at weekly intervals using a knap-sack sprayer. Observations on disease intensity was assessed, as described earlier and analysed statistically.

Results and Dscussion

Studies on the induction of defence related enzymes in the treated clone RRIM 600 showed increased peroxidase activity. The activity increased with time after application upto 72 hours. Induction of salicylic acid was slightly higher than benzothiadiazole (Fig .1). The application of benzothiadiazole and salicylic acid showed a higher induction of polyphenol oxidase activity at 24 h after treatment. A slight decline was noticed at 48 h. At 72 hours after treatment benzothiadiazole recorded an increasing trend in the polyphenol oxidase activity. Sharp increase in the catalase activity was observed with salicylic acid after 24 h but it declined at 48 h and 72 h. In the case of benzothiadiazole catalase activity increased at 24 h and slightly decreased at 48 h but increased later at 72 h.

Peroxidase activity of tissues has been reported to be well correlated with the expression of disease resistance in different crops (Smith and Hammerschmidt 1988; Angelini *et al.,* 1993; Jite and Tressa 1999). The expression of resistance is often accompanied by the activation of phenol oxidising enzymes such as peroxidase and polyphenol oxidase (Goodman and Novacky 1994). In the present study increased activity of peroxidase was observed on the treated leaves of RRIM 600. Cools and Ishii (2002) showed that in cucumber peroxidase was directly induced by benzothiadiazole and its expression was further enhanced upon elicitation with fungal pathogen. Increase in polyphenol oxidase activity may contribute to defence through the production of oxidized forms of quinines, which can inactivate pectinolytic enzymes produced by the pathogen (Leatham *et al.,* 1980). Application of benzothiadiazole showed increased production of polyphenol oxidase in the treated plants in the present study. Gradual increase in the catalase activity was observed with the application of benzothiadiazole on RRIM 600 plants. Changes in the catalase activity as a result of fungal infection have been reported in various host-pathogen combinations and were related to diseases resistance (Vir and Grewal, 1974; Lebela *et al.,* 2001; Ronald, 2001). Fric and Fuchs (1970) observed marked increase in catalase activity of resistant wheat leaves infected with *Puccinia graminis tritici.* Mushrif *et al.* (2004) reported that the activity of peroxidase was more in clone RRIM 600 inoculated with *Colletotrichum* spp.

Application of benzothiadiazole on various stages of leaves indicated better protection against *Heveae* when the copper brown leaves were sprayed (Fig.2). The lowest concentration of benzothiadiazole (0.05%) when applied at copper brown stage recorded less than 30% disease intensity. Colson-Hanks and Deverall (2000) reported that the wettable granule formulation (35µg/ml) of benzothiadiazole applied on cotyledons reduced lesion formation by *Alternaria macrospora* in the successive leaves on cotton.

In the evaluation against powdery mildew disease, benzothiadiazole recorded lowest disease intensity (14.75%) on the budded nursery plants of RRII 105.(Table 1). Carbendazim, chelated Zinc and wettable sulphur were on par in their efficacy. However, in the budded plants of RRIM 600, benzothiadiazole was on par with other fungicides (Table 2). Combination of benzothiadiazole along with carbendazim recorded the lowest disease (14.8%). In the field evaluation, benzothiadiazole (0.25% and 0.1%)

performance was on par with all other treatments (Table 3). But, the combination of benzothiadiazole+carbendazim recorded minimum disease intensity (11.34%).

Nursery evaluation of benzothiadiazole (0.05%) recorded lowest disease intensity (3.57%) in the clone RRII 105 against colletotrichum leaf disease (Table 4). However, it was on par with recommended fungicides viz. mancozeb and carbendazim. Similar observation was recorded in the nursery trial (Table 5) with the clone RRIM 600 also. Combined application of benzothiadiazole (0.05%) and a triazole fungicide hexaconazole (0.02%) recorded lowest disease (16.0%). In the field study (Table 6), individual application of benzothiadiazole (0.1% and 0.25%) were on par in their effectiveness with mancozeb (0.2%) and carbendazim (0.05%). However, when applied in combination with mancozeb disease intensity was much lower.

Benzothiadiazole is translocated systemically in plants and can take the place of salicylic acid in the natural SAR signal pathway, inducing the same spectrum of resistance (Oostendorp *et al.*, 2001; Kunz *et al.*, 1997). Chemicals that have been shown to mimic more closely the mode of action of SA are 2,6-dichloroisonicotinic acid and benzothiadiazole. Crops where they showed best results under field conditions include tobacco, tomato, and vegetables for protection against a broad spectrum of pathogens. (Oostendorp *et al.*, 2001). Treatment of benzothiadiazole on the first leaves reduced the susceptibility to powdery mildew caused by *Uromyces viciae-fabae* and leaf spot pathogen *Mycosphaerella pinodes* on pea (Dann and Deveerall, 2000).

In the present study it was evident that the benzothiadiazole could induce the activity of peroxidase, polyphenol oxidase and catalase. Such triggering of enzymes related to systemic resistance could protect plants from the subsequent invasion of pathogen. It was evident from the nursery and field studies that, benzothiadiazole is comparable to recommended fungicides in the control of powdery mildew and colletotricum leaf disease of rubber. However, the effectiveness could be further enhanced by the application of benzothiadiazole in combination with fungicides.

Table 1. Effect of benzothiadiazole on the powdery mildew disease intensity in the nursery plants of clone RRII 105

Fungicides	Concentration (%)	Disease intensity (%)
Carbendazim	0.05%	19.39
Chelated Zinc	0.05%	19.29
Flowable sulphur	0.2%	19.92
Benzothiadiazole	0.05%	14.75
Wettable sulphur	0.2%	19.92
Control-unsprayed	--	22.47
CD(P≤0.05)		3.47

Table 2. Effect of benzothiadiazole on the powdery mildew disease intensity in the nursery plants of clone RRIM 600

Fungicides	Concentration(%)	Disease intensity (%)
Benzothiadiazole	0.05%	19.6
Carbendazim	0.05%	16.5
Difenconazole	0.025%	15.1
Carbendazim+benzothiadiazole	0.05%+0.05%	14.8

Hexaconazole+benzothiadiazole	0.02%+0.05%	16.8
Wettable sulphur	. 0.2%	17.0
Control-unsprayed	--	29.4
CD (P≤ 0.05)		6.1

Table 3. Efficacy of benzothiadiazole on the powdery mildew disease intensity in the first year plants of clone RRII 105

Fungicides	Concentration(%)	Disease intensity (%)
Carbendazim	0.05%	14.3
Difenconazole	0.025%	14.2
Benzothiadiazole	0.25%	13.0
Benzothiadiazole	0.1%	13.7
Carbendazim+benzothiadiazole	0.05%+0.1%	11.3
Difenconazole+benzothiadiazole	0.025%+0.1%	12.3
Control-unsprayed	--	24.7
CD (P≤ 0.05)		4.2

Table 4 Effect of benzothiadiazole on the colletotrichum leaf disease intensity in the nursery plants of RRII 105

Fungicides	Concentration	Disease intensity (%)
Carbendazim	0.05%	4.71
Mancozeb	0.2%	5.51
Chelated Zinc	0.05%	13.92
Benzothiadiazole	0.05%	3.57
Control-unsprayed	--	25.89
CD (P≤ 0.05		6.07

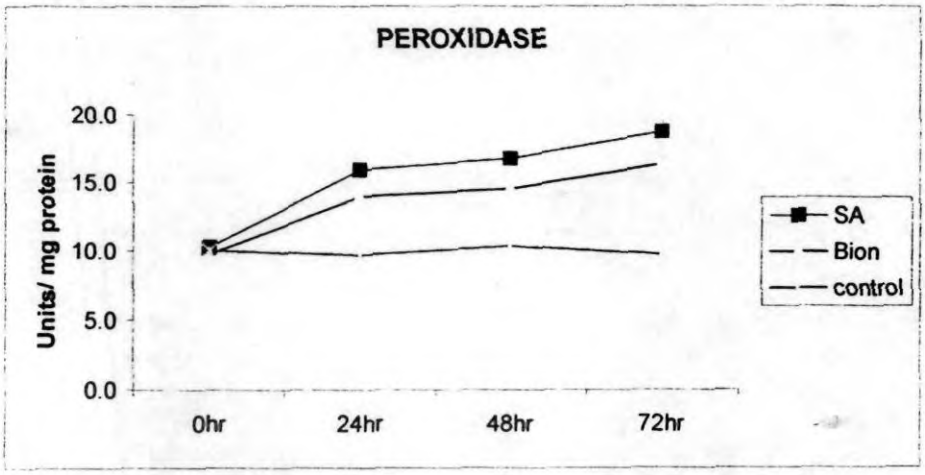
Table 5 Effect of benzothiadiazole on the colletotrichum leaf disease intensity in the nursery plants of RRIM 600

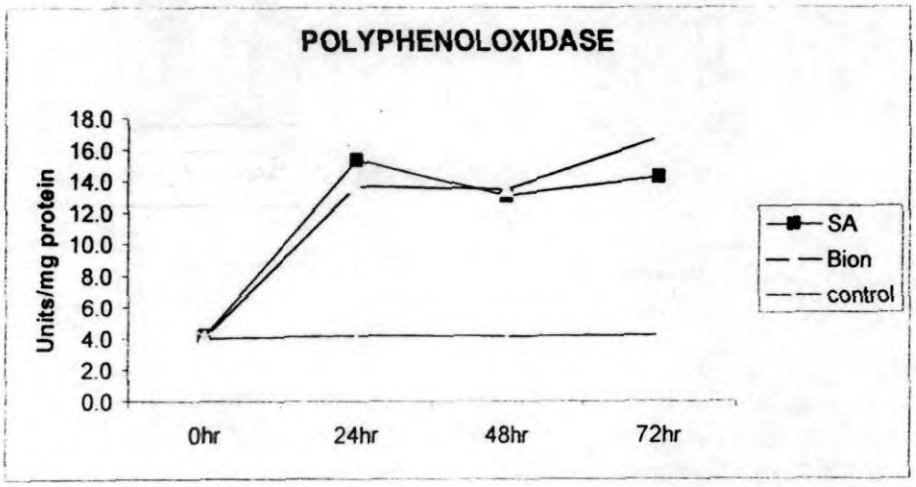
Fungicides	Concentration	Disease intensity (%)
Benzothiadiazole	0.05%	18.1
Carbendazim	0.05%	17.5
Difenconazole	0.025%	16.8
Carbendazim+benzothiadiazole	0.05%+0.05%	17.8
Hexaconazole+benzothiadiazole	0.02%+0.05%	16.0
Control - unsprayed	--	25.10
CD (P≤ 0.05)		6.73

Tablel 6. Efficacy of benzothiadiazole on the colletotrichum leaf disease intensity in the first year plants of RRII 105

Fungicides	Concentration	Disease intensity (%)
Mancozeb	0.2%	12.1
Carbendazim	0.05%	14.0
Benzothiadiazole	0.25%	16.2
Benzothiadiazole	0.1%	19.1
Mancozeb +benzothiadiazole	0.2%+0.1%	9.3
Carbendazim +benzothiadiazole	0.05%+0.1%	11.1
Control – unsprayed	--	29.3
CD (P≤ 0.05)		4.8

Fig. 1 Changes in peroxidase, polyphenol oxidase and catalase activities in the leaves of the clone RRIM 600 treated with benzothiadiazole





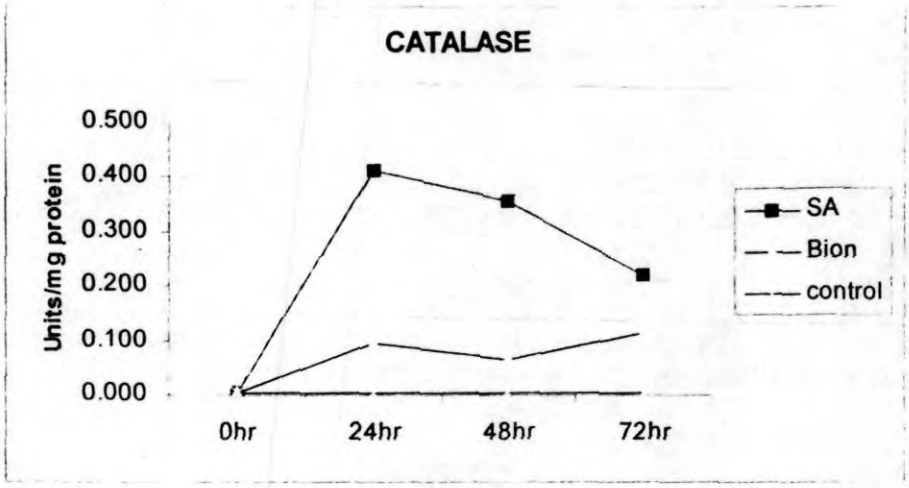
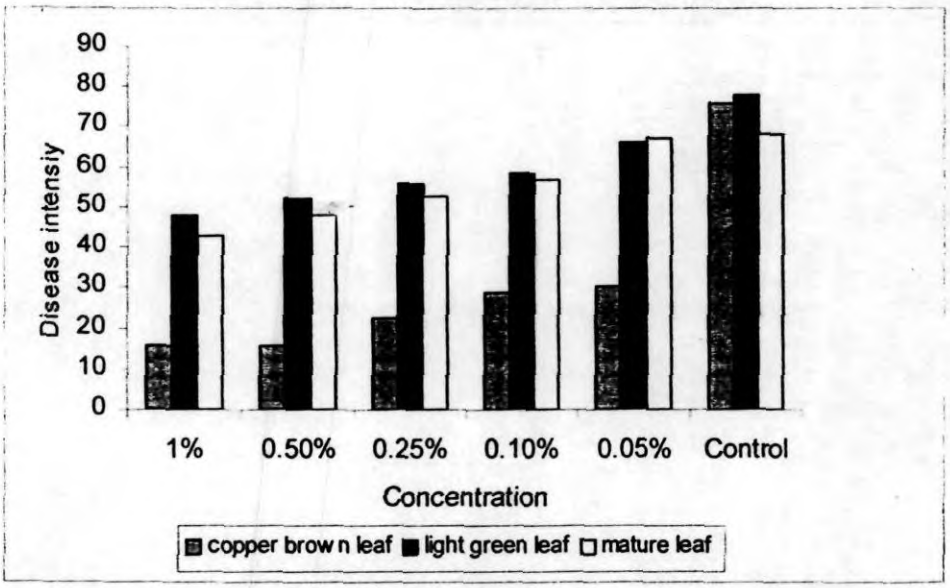


Fig. 2 Effect of leaf stages and concentration of benzothiadiazole on the powdery mildew disease intensity in clone PB 5/51



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