VOL. 2, NO. 3, MAY 2007 ISSN 1990-6145
Journal of Agricultural and Biological Science

www.arpnjournals.com

EFFICACY OF AZOXYSTROBIN ON *COLLETOTRICHUM GLOEOSPORIODES* PENZ GROWTH AND ON CONTROLLING MANGO ANTHRACNOSE

S. Sundravadana[1], D. Alice[1], S. Kuttalam[1] and R. Samiyappan[1]
[1]Department of Plant Pathology, Centre for Plant Protection Studies, Tamil Nadu Agriculture University, Coimbatore, India
E-mail: sundravadana@rediffmail.com

ABSTRACT

Anthracnose caused by *Colletotrichum gloeosporioides* is one of the most damaging disease causing reduction of flower set and yield losses in mango. Application of fungicide was one of the approaches to control the disease. In this study, the efficacy of azoxystrobin, one of the strobilurin class fungicides, was evaluated both *in vitro* and *in vivo* conditions. In *in vitro* tests, azoxystrobin completely inhibited mycelial growth of *C. gloeosporioides*. In field experiment, azoxystrobin at 1, 2 and 4 ml/l significantly suppressed the development of both panicle and leaf anthracnose. Mango trees treated with azoxystrobin produced more fruits compared to control and showed no phytotoxicity. The reduction of anthracnose incidence and yield increased curve obtain, shows flattening between the range 2.0 and 4.0 ml/l rates, hence the optimum rate of azoxystrobin was fixed to be at 2.0 ml/l for the control of anthracnose disease. This systemic fungicide azoxystrobin was useful in managing this destructive disease of mango in field.

Keywords: mango, disease, anthracnose, azoxystrobin, colletotrichum gloeosporioides, phytotoxicity.

INTRODUCTION

India stands first in global mango production (52%). However, the productivity of mango is affected by various diseases. Among the diseases, mango anthracnose caused by *Colletotrichum gloeosporioides* is the most serious disease (Ploetz, 1999). It affects both vegetative and reproductive structures. Initial infection starts from leaves and spreads to flowers causing blossom blight, which destroys inflorescence (flower panicles) leading to considerable reduction in fruit set and yield loss. Much of attention and efforts on anthracnose control has concentrated mostly on the use of fungicides. Using of organic sulphur (Dithio-carbamates) fungicides like zineb, maneb and heterocyclic nitrogen compounds - captan gave adequate control against anthracnose (Ruehl and Ledin, 1960) however these fungicides have shown phytotoxic effect to flowers (McMillan, 1972). Organic sulphur group fungicide mancozeb was found to be effective in controlling anthracnose, but cannot be used because of ethylene produced as a by-product. Moreover *C. gloeosporioides* developed resistant to benomyl (0.1%) a benzimidazoles systemic fungicides- for controlling of pre and post- harvest development of anthracnose (Akthar *et al.*, 1998; Dodd *et al.*, 1991). Assessment of these reports, focus the evaluation of new fungicides for controlling mango anthracnose and enhanced its production.

Strobilurins are the leading systemic fungicide, developed from naturally occurring antifungal compounds found in wood-decaying mushroom fungus like *Strobilurus tenacellus*. It has broad-spectrum activity against the four major groups of plant pathogenic fungi including Ascomycota, Basidiomycota, Deuteoromycetes and Oomycetes. Azoxystrobin (Methyl *E*)-2-{2-[6-(2cyanophenoxy) pyrimidin-4-yloxy] phenyl}-3-methoxyacrylate) is one of the strobilurin class fungicide. There are many reports on efficacy of azoxystrobin against plant diseases such as gray mold (*Botrytis cinerea*) of fruits and vegetables, leaf spot (*Cercospora beticola*), powdery mildew (*Erysiphe betae*) of sugar beet, black spot (*Guignardia citricarpa*) of citrus, post-harvest rot (*Colletotrichum gloeosporioides*) of avocado (Slawecki *et al* .,2002 ;Anesiadis *et al* .,2003 ; Miles *et al* .,2004)

Hence the present study was carried out to evaluate the efficacy of azoxystrobin on mycelial growth of *C. gloeosporioides* under *in vitro* condition and in controlling, mango anthracnose (panicle and leaf anthracnose). Its effect on the fruit yield and its phytotoxic action were also evaluated.

MATERIALS AND METHODS

Pathogen and fungicide

The pathogen *C. gloeosporioides* was isolated from infected mango leaves and flowers. Isolation was made by cutting a small section of anthracnose infected portion, which was surface sterilized with 0.1% $HgCl_2$ solution and rinsing in sterilized distilled water. It was then placed on PDA (Potato Dextrose Agar) medium, in sterilized Petri plates and incubated at 28 ± 2°C. The pure culture was maintained in PDA slants.

In vitro experiments

Efficacy of azoxystrobin in solid media

The pathogen *C. gloeosporioides* was maintained on PDA medium for five days. Mycelial plugs of half cm diameter were cut using cork borer from the *C. gloeosporioides* colony. The plugs were transferred to PDA plates amended with azoxystrobin at 0.25, 0.5, 1.0, 2.0 and 4.0 ppm concentrations with three replications. These plates were incubated at 28 ± 2°C. After incubation the colony diameters of *C. gloeosporioides* grown on poisoned medium were compared with that of control and difference was recorded as the percent of inhibition of mycelial growth.

$$M_i = \frac{M_c - M_t}{M_c} \times 100$$

Where

M_i = Inhibition of mycelial growth
M_c = Colony diameter of control set
M_t = Colony diameter of target fungi on poisoned medium.

Efficacy of azoxystrobin in liquid medium

100ml of Potato Dextrose Broth (PDB) amended with different concentrations (0.25, 0.5, 1.0, 2.0 and 4.0 ppm) of azoxystrobin was added to 250ml of Erlenmeyer flask and autoclaved for 20 minutes. Half cm diameter of mycelium were cut from margin of the fungal colony and transferred to the flask which containing 100ml sterilized PDB. After 7 days incubated culture was harvested, oven dried and weighted.

Field experiment

Experiment design and treatments

Field experiment was conducted at Tamil Nadu Agricultural University, India in a mango orchard with 15-20 years old trees. 21 trees with uniform flowering were chosen and treatments assigned (each with three replications) employing completely randomized design. A treatment was azoxystrobin with five doses of 0.25, 0.5, 1.0, 2.0 and 4.0 ml/l and control (without fungicide). Three sprays were given, first at initial appearance of symptoms followed by two sprays at 15 days intervals.

Disease and yield assessment

Disease severity assessments were made regularly at 7 and 15 days intervals from inflorescence and leaves, respectively selected at random in four directions of trees. The assessment carried out using the scale and Percent Disease Index (PDI) was worked out.

Scale-Anthracnose-Inflorescence
(Jamadar and Desai 1997)

Scale	Description
0	No infection observed
1	1-10 %
3	10.1-15.0 %
5	15.1-25 %
7	25.1-50 %
9	More than 50 %

Scale-Anthracnose-leaves
(Suharban *et al*., 1985)

Grade	Disease intensity (%)	Description
0	0	No spots
1	1-20	1-5 spots
2	21-40	6-10 spots
3	41-60	11-15 spots
4	61-80	16-25 spots
5	>80	>25 spots

PDI was computed using McKinney's (1923) formula:

$$PDI = \frac{\text{Sum of numerical rating x Maximum disease grade}}{\text{Total number of leaves observed x 100}}$$

Mango fruit yield calculated as kg /tree.

Phytotoxicity

An observation for the phytotoxicity effect of azoxystrobin was made in the trees after each spray in the field trials. The leaves and panicles were regularly examined for injury of leaf tip, leaf surface, wilting, vein clearing, necrosis, epinasty and hyponasty.

Rating	Phytotoxicity (%)
0	No phytotoxicity
1	1-10
2	11-20
3	21-30
4	31-40
5	41-50
6	51-60
7	61-70
8	71-80
9	81-90
10	91-100

RESULTS

***In vitro* efficacy of azoxystrobin**

The efficacy of azoxystrobin against mango anthracnose pathogen *C. gloeosporioides* was tested under *in vitro* conditions. Azoxystrobin significantly reduced mycelial growth both in solid and liquid medium. Azoxystrobin at 0.25 and 0.5 ppm slightly inhibited the mycelial growth whereas 1 and above ppm completely inhibited the mycelial growth of *C. gloeosporioides*. In control, 89 mm and 946.43 mg of mycelial growth were observed in solid and liquid medium, respectively (Figure-1).

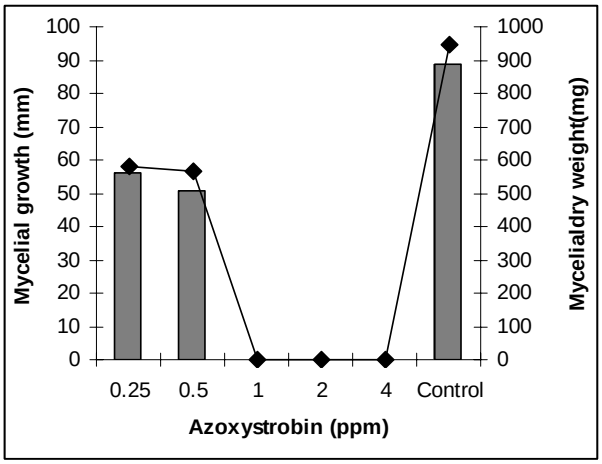


Figure-1. *In vitro* efficacy of azoxystrobin on growth of *C. gloeosporioides*.

***In vivo* efficacy of azoxystrobin**

Disease control

Bioefficacy of azoxystrobin against mango anthracnose was tested under field conditions. Spraying of azoxystrobin significantly reduced the panicle and leaves anthracnose.

Panicle anthracnose or blossom blight was observed at 7 days intervals flowering to pea stage, control trees showed higher disease incidence (27.73 PDI) with typical symptoms like blighted flowers, elongated dark gray or black spots of stalk, aborted flowers (mustard and pepper stage) and mummified fruit (pea stage), whereas azoxystrobin treated panicle did not show any anthracnose symptoms and was completely free from panicle anthracnose (Table-1).

Table-1. Efficacy of azoxystrobin on mango panicle anthracnose.

Azoxystrobin dose (ml /l)	**Disease Index (PDI)** (%)							**Disease reduction over control** (%)
	Before spray	**7 DAS**	**14 DAS**	**21 DAS**	**28 DAS**	**35 DAS**	**42 DAS**	
0.25	0.00[a] (0.50)	0.00[a] (0.50)	0.00[a] (0.50)	0.00[a] (0.50)	0.00[a] (0.50)	0.00[a] (0.50)	0.00[a] (0.50)	100.00
0.50	0.00[a] (0.50)	0.00[a] (0.50)	0.00[a] (0.50)	0.00[a] (0.50)	0.00[a] (0.50)	0.00[a] (0.50)	0.00[a] (0.50)	100.00
1.0	0.00[a] (0.50)	0.00[a] (0.50)	0.00[a] (0.50)	0.00[a] (0.50)	0.00[a] (0.50)	0.00[a] (0.50)	0.00[a] (0.50)	100.00
2.0	0.00[a] (0.50)	0.00[a] (0.50)	0.00[a] (0.50)	0.00[a] (0.50)	0.00[a] (0.50)	0.00[a] (0.50)	0.00[a] (0.50)	100.00
4.0	0.00[a] (0.50)	0.00[a] (0.50)	0.00[a] (0.50)	0.00[a] (0.50)	0.00[a] (0.50)	0.00[a] (0.50)	0.00[a] (0.50)	100.00
Control	0.00[a] (0.50)	0.00[a] (0.50)	7.33[b] (15.65)	9.43[b] (17.97)	13.60[b] (21.63)	25.00[b] (29.99)	27.73[b] (31.77)	

DAS-Days after Spray *Mean of three replications.
In a column, means followed by a common letter are not significantly different at the 5 % level by DMRT
Values in parentheses are arcsine-transformed value.

Leaf anthracnose was observed at 15 days intervals from flowering to harvest. Azoxystrobin treated trees showed lesser leaf anthracnose than control. The reduction of leaf anthracnose varied between the doses of azoxystrobin. Among the doses, 0.25 and 0.5 ml/l slightly reduced the leaf anthracnose i.e. 28.72 and 27.52 PDI, respectively where as other high doses 1, 2 and 4.0 ml per liter significantly suppressed the leaf anthracnose i.e. 18.61, 17.02 and 16.31 PDI respectively. So, the efficacy of azoxystrobin increased with increase in the concentrations (Table-2).

Yield

Due to higher panicle and leaf anthracnose, controlled trees produced less number of fruits (13.8 kg/tree), whereas azoxystrobin treated trees produced maximum number of mango fruits (more than 40 kg/tree) which was found to be statistically significant (Table-2).

Table-2. Efficacy of azoxystrobin on mango leaf anthracnose.

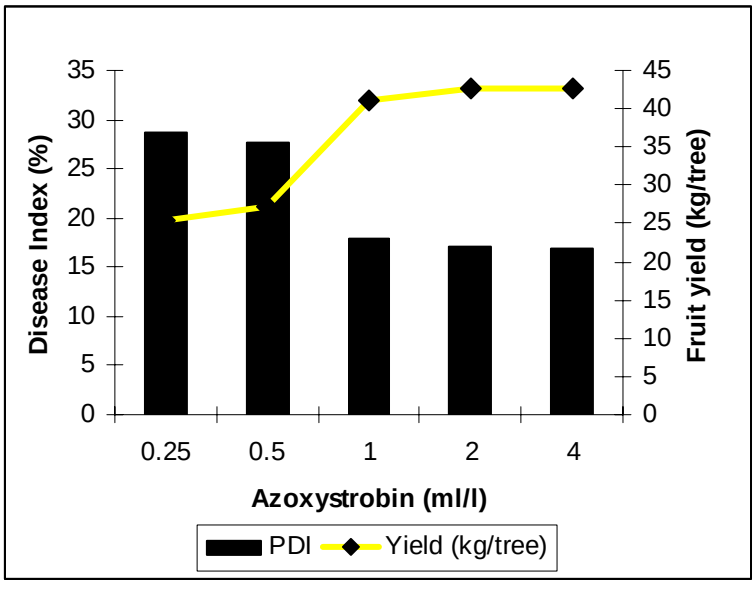
Azoxystrobin dose (ml /l)	Disease Index (PDI) (%)								Disease reduction over control (%)	Fruit Yield (kg /tree)
	Before spray	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	105 DAS		
0.25	10.43[a] (18.48)	11.32[b] (19.46)	14.51[d] (22.25)	17.34[d] (24.57)	20.13[d] (26.51)	23.3[d] (28.81)	26.44[c] (30.90)	28.72[d] (32.39)	46.5	25.5
0.50	10.65[a] (18.61)	10.85[a] (19.01)	14.25[cd] (22.04)	16.28[c] (23.75)	18.55[c] (25.34)	20.41[c] (26.78)	25.17[b] (30.06)	27.58[c] (31.66)	48.62	27.3
1.0	10.61[a] 18.57	13.25[d] (21.19)	13.62[b] (21.50)	14.00[b] (21.92)	14.23[b] (21.89)	15.11[b] (22.75)	15.58[a] (23.13)	17.85[b] (24.50)	65.39	41.2
2.0	10.23[a] 18.16	12.62[c] (20.64)	12.75[a] (20.75)	12.62[a] (20.74)	12.95[a] (20.77)	13.52[a] (21.42)	15.54[a] (23.09)	17.02[a] (24.34)	68.29	42.6
4.0	10.65[a] 15.61	12.47[c] (20.50)	12.74[a] (20.74)	12.31[a] (20.47)	12.56[a] (20.42)	13.43[a] (21.34)	15.53[a] (23.09)	16.31[a] (23.78)	69.62	43.9
Control	10.61[a] (18.57)	15.6[e] (23.14)	22.35[e] (28.15)	25.38[e] (30.23)	39.86[e] (39.12)	42.78[e] (40.84)	49.23[d] (44.56)	53.68[e] (47.11)		13.8

DAS-Days After Spray *Mean of three replications.
In a column, means followed by a common letter are not significantly different at the 5 % level by DMRT
Values in parentheses are arcsine-transformed value.

Fungicide responses to rate of application

The fungicide rate of application experiment was useful in determining the optimum rate of azoxystrobin for anthracnose control. The lower concentrations 0.25 and 0.5 ml/l had shown higher infecting rates, more disease than the higher concentrations of 1.0, 2.0 and 4.0 ml/l. The response of azoxystrobin fungicide to rates applied is illustrated graphically. Although the leaf anthracnose incidence continuous to decline in 1.0, 2 .0 and 4.0 ml/l the optimum rate was arrived at 2.0 ml/l by considering the flattening of the disease curve between rates (Figure-2). Similarly optimum yield was also achieved at 1.0 ml/l where yield response continuously to climb with increasing rates.

Figure-2. The effect of rate of application of azoxystrobin on disease severity (PDI) and fruit yield (kg/tree).



Phytotoxicity

Azoxystrobin at all concentrations did not produce any phytotoxic effect, injury of leaf tip, leaf surface, wilting, vein clearing, necrosis, epinasty or hyponasty on mango trees.

DISCUSSIONS

Azoxystrobin is one among the strobilurin class of systemic fungicides. Inhibition of mitochondrial respiration is an important mode of action for inhibiting pathogen growth that is ability to inhibit electron transfer in fungal mitochondria by binding at a specific site on cytochrome b. The result of this activity is the cessation of normal energy production (ATP production) within the cell, which results in cell death. Evidence of this effect on fungi can be observed in spore mortality, mycelial collapse and inhibitors of sporulation or disruption of other vital stages of fungal development (Harrison and Tedford, 2002). The present study has shown 100 % inhibition of mycelial growth of *C. gloeosporioides,* the causal agent of mango anthracnose by azoxystrobin. Inhibitory activity of azoxystrobin has been reported in several studies; for example Hsiang *et al.* (2004) found that, azoxystrobin (1.0–100 µg a.i /ml) eliminated urediospore germination of *Puccinia hemerocallidis*. Mycelial growth and sporulation of *Alternaria alternata* (Reuveni and Sheglov,2002), *Botryosphaeria parva* and *Phomopsis* sp (Everett *et al.*, 2005) also inhibited by azoxystrobin. Azoxystrobin inhibited the conidia to germinate and form appressoria of *Guiganardia bidwellii* (Hoffman and Wilcox, 2003).

Spraying of azoxystrobin has limited the anthracnose development. The disease incidence reduced was greater when azoxystrobin sprayed at 1, 2 and 4 ml/l. Treating trees with these concentrations provided 100 and more than 60 % reduction of panicle and leaf anthracnose compared to untreated trees for which disease incidences were 27.73 and 53.68 PDI, respectively. This controlling effect was mainly due to translaminar and systemic movement of azoxystrobin, inside the tissues, azoxystrobin is widely distributed from the application side by diffusion (Vincelli, 2002). Azoxystrobin at said concentration not only suppressed development of anthracnose incidence as well as increased fruits per trees. Several other studies demonstrated the efficacy of azoxystrobin in reducing disease severity. For examples, Reuveni (2001) reported that azoxystrobin effectively controlled downy mildew (*Plasmopara viticola*) and powdery mildew (*Uncinula necator*) of grapevines as against control. Azoxystrobin (100 g a.i /ha) controlled black sigatako leaf spot (*Mycosphaerella fijiensis*) of banana (Perez *et al*., 2002). Azoxystrobin gave effective control of smoulder (*Botrytis narcissicola)* of narcissus (O'Neil *et al*., 2004) and leather rot (*Phytophthora cactorum*) of strawberry (Rebollar-Alviter *et al.,* 2005). The fungicide rate of application experiment was useful for determining the optimum rate of fungicide application. Prior to commencement of this study no work had been undertaken on optimum rates of applications of fungicides for anthracnose control. The result of the experiments in this study indicated responses to rates between 0.25 to 4ml/l. The product was subsequently registered for use at 2ml/l. Moreover, all the doses of azoxystrobin had not caused any phytotoxic effect to the mango trees. The phytotoxic observation was very much important because azoxystrobin causes blossom abnormality and chronic toxicity on apple trees (Lange, 2004).

The efficacy of strobilurin fungicides, in particular azoxystrobin against mycelial growth of *C. gloeosporioides* and incidence of mango anthracnose respectively under *in vitro* and *in vivo* conditions was well illustrated in this study. Thus azoxystrobin completely inhibited the mycelial growth of *C. gloeosporioides* and provided more than 60 % disease reduction as well as more than 40kg fruits per tree with no phytotoxic effect.

REFERENCES

Akthar, K.P., Khan, I., Khar, I.A. and Khan, S.M.1998. Studies on the incident and pathogenesis of *Colletotrichum gloeosporioides* Penz. causing anthracnose of mango and chemical control. Pakistan Journal of Phytopathology, 10: 42-44.

Anesiadis, T., Karaoglanidis, R.and Klonari, K.T. 2003. Protective, curative and eradicant activity of the strobilurin fungicide azoxystrobin against *Cercospora beticol*a and *Erysiphae betae*. Journal of Phytopathology, 151: 647-651.

Dodd, J.C., Bugante, R., Koomen, I., Jeferies, P.and Jeger, M.J. 1991. Pre and post harvest control of mango anthracnose in the Philippines. Plant Pathology, 40: 576-583.

Everett, K.R., Owen, S.G. and Cutting, J.G.M. 2005. Testing efficacy of fungicides against post harvest pathogens of avocado (P*ersea americana* cv. Hass). NewZealand Journal of Plant Protection, 58: 89-95.

Harrison, S. and Tedford, E. 2002. Quadris, a novel fungicide for disease control in rice. In: Proceeding of the temperate rice conference, B. IRRI, India, pp. 289-294.

Hoffman, L. E. and Wilcox, W. F. 2003. Factors influencing the efficacy of myclobutanil and azoxystrobin for control of grape black rot**.** Plant Disease, 87: 273-281.

Hsiang, T., Cooks, S., and Zhao, Y. 2004. Studies on biology and control of daylily rust in Canada. The Daylily Journal, 59: 47-57.

Jamadar, M. and Desai, S.A. 1997. Bioefficacy of dimethomorph against downy mildew of grapevine. Advances of Agriculture Research in India ,4: 81-85.

Lange, J. 2004. A study of apple trees exposed to the fungicide azoxystrobin and maximum interaction with

surfactants. International Journal of Environmental Studies, 61: 173-187.

McKinney, H.H. 1923. A new system of grading plant diseases. Journal of Agricultural Research, 26: 195-218.

McMillan,R.T. 1972. Enhancement of anthracnose control of mangoes by combining copper with Nu-film-17. Florida State. Horticultural Society, 85:268-271.

Miles, A.K., Willingham, S.L. and.Cooke, A.W. 2004. Field evaluation of strobilurins and a plant activator for the control of citrus black spot. Australian Plant Pathology, 33: 371-378.

O'Neil, T.M., Hanks, G.R. and Wilson, D.W. 2004. Control of smoulder (*Botrytis narcissicola*) in narcissus with fungicides . Annals of Applied Biology, 145: 129-137.

Perez, L., Hernandez, A., Hernandez, l. and Perz, M. 2002. Effect of trioxystrobin and azoxystrobin on the control of black sigatoka (*Mycosphaerella fijiensis* Morelet) on banana and plantain. Crop Protection, 21:17-23.

Ploetz, R. 1999. Anthracnose: The most important disease in much of the mango producing world. The News Letter of the Plant Pathology, 3: 1-6.

Rebollar-Alviter, A., Madden, L. V. and Ellis, M. A. 2005. Efficacy of azoxystrobin, pyraclostrobin, potassium phosphite and mefenoxam for control of strawberry leather rot caused by *Phytophthora cactorum*. Online. Plant Health Progress doi:10.1094/PHP-2005-0107-01-RS

Reuveni, M. 2001. Activity of trifloxystrobin against powdery and downy mildew diseases of grapevines. Canadian Journal of Plant Pathology, 23: 52-59.

Reuveni, M. and Sheglov, D. 2002. Effect of azoxystrobin, polyoxin B (polar) and trioxystrobin on germination and growth of *Alternaria alternata* and decay in red delicious apple fruit. Crop Protection, 21: 951-955.

Ruehl, G.D. and Ledin,R.D. 1960. Florida Agric. Expt Statn. Bull. pp.174.

Sanders, G.M., Korsten, L. and Wehner, F.C. 2000. Survey of Fungicide Sensitivity in *Colletotrichum gloeosporioides* from different avocado and mango production areas in South Africa European Journal of Plant Pathology, 106:745-752.

Slawecki, R.A., Ryan, E.P. and Young, D.H. 2002. Novel fungitoxicity assays for inhibition of germination associated adhesion of *Botrytis cinerea* and *Puccinia recondita* spores. Applied Environmental Microbiology, 68: 597-601.

Suharban, M., Philip, S. and Thomas, Y. 1985. Fungicidal control of leaf spot disease of mango seedlings. South Indian Horticulture, 33: 125-126.

Vincelli, P. 2002. Q_oI (Strobilurin) Fungicides: Benefits and Risks. The Plant Health Instructor. doi: 10.1094/PHI-I-2002-0809-02.