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Investigations on Phytotoxicity of Two New Fungicides, Azoxystrobin and Difenoconazole

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Abstract: Investigations were carried out on the non-target effects of two new fungicides, Amistar and Score (Syngenta, Mumbai, India) in terms of phytotoxicity. These fungicides are found to be generally non phytotoxic at or below the recommended dose for field application (2.2 μg (a.i.) mL^{-1}). At higher concentrations, the extent of phytotoxicity of Azoxystrobin and Difenoconazole varied with host genotype. Among the test plants, *Vigna catjung* was most sensitive to both the fungicides for all the studied parameters like seed germination, shoot elongation, root elongation, number of lateral roots initiated and may serve as good indicator of phytotoxicity of these fungicides. Both the fungicides at their different concentrations significantly decreased community respiration and gross primary productivity. However, the net primary productivity was significantly increased by Azoxystrobin treatment up to 0.0073 μg (a.i.) mL^{-1} and Difenoconazole up to 0.0014 μg (a.i.) mL^{-1} concentrations. Treatment of leaf tissue with Azoxystrobin resulted in electrolyte leakage as measured by increased electrical conductivity (EC). The increase in EC was pronounced with the increase in fungicide concentration and incubation period. The negative EC values obtained in the Difenoconazole treatment may be due to fast and efficient uptake of the fungicide from the ambient solution by the leaf tissue.

Key words: Amistar, score, phytotoxicity, primary productivity, community respiration, membrane stability

Introduction

The damage caused to the biological communities in the soil and aquatic environments due to agrochemical application is of serious concern. Plants are the main recipients of pesticides through direct application of pesticides or through the uptake from soil, water and air drift. Phytotoxicity of agrochemicals on crop plants such as ornamentals, vegetables, cereals, oil seeds and fruits may be caused by misuse or misapplication of chemicals and therefore, phytotoxicity is an essential element of biological evaluation (Sahni, 1983).

Fungicides reach aquatic systems by direct application, spray drift from ground or aerial spraying, atmospheric fall out, run-off from agricultural land, discharge of effluent from chemical factories and from sewage. As the progressive use of organic fungicides reached massive proportions in the past century, it becomes increasingly important to study their detrimental effects on aquatic forms of life.

A number of fungicides are being routinely used for crop protection but their phytotoxic effects have been often ignored (Vyas, 1993). Application of systemic fungicides as coatings on the seeds has been suggested as an effective method of controlling pathogenic fungi in contrast to conventional foliar application (Sithanantham, 1973). Fungicides used for seed treatment often interfere with germination

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and seedling growth (Mitra *et al*., 1970; Das and Chandrika, 1972; Rao and Rao, 1980; Rajamani *et al*., 1987). Knowledge on the effect of fungicides on the germination of seeds and the growth of seedlings is essential in choosing desirable fungicides for coating on seeds.

Phytoplanktons play a key role in freshwater ecosystem, as they are the primary producers in the food chain (Kumar and Singh, 2000). They are important in maintaining a proper equilibrium between biotic and abiotic components in an aquatic ecosystem. The most commonly measured functional attributes of an aquatic ecosystem are gross and net primary productivity (GPP and NPP) and community respiration (CR). These two matrices have been shown to be sensitive indicators of ecosystem stress (Jindal and Kaur, 2000). A reduction in GPP and NPP may be due to the impact of pesticides on algal growth and their pigmentation as reported by Anand and Veerappan (1980) and Tarar and Shewale (1984).

Amistar (azoxystrobin 25% EC) and Score (difenoconazole 25% EC) are the two broad spectrum, foliar, systemic fungicides, yet to be released to the farmers and planters in India by Syngenta India Limited, Mumbai. The objective of the present study was to investigate their non-target effects in terms of phytotoxicity at concentrations ranging from 0.44 - 22000 μg (a.i.) mL^{-1}. For both the fungicides, manufacturer's recommended dose for foliar spray is 2.2 μg (a.i.) mL^{-1}.

Materials and Methods

Test Plants

The test plants used include certain legumes (*Cicer arietinum* Linn., *Dolichos biflorus* Roxb., *Phaseolus aureus* Roxb., *Phaseolus mungo* Linn., *Vigna catjang* Walp), non-legumes (*Brassica juncea* Coss., *Capsicum frutescens* Linn., *Lycopersicum esculentum* Mill., *Sesamum indicum* Linn., *Trigonella foenumgraecum* Linn.) and cereals (*Pennesetum typhoides* Linn., *Sorghum vulgare* Pers., *Triticum aestivum* Lamk, *Zea mays* Linn.). All the seeds were obtained from the seed section of Tamil Nadu Agricultural University, Coimbatore. All sets of measurement were repeated by conducting a separate set of measurements on a separately executed experiment.

Seed Germination Assay

Seed germination assay was used to evaluate the phytotoxicity, if any, of the fungicides Azoxystrobin and Difenoconazole. Prior to treatment with fungicides, the seeds of the test plants were surface sterilized in 0.1% mercuric chloride for 1-2 min followed by repeated rinsing with distilled water. The surface sterilized seeds were then soaked overnight (12 h) in different concentrations of the fungicides.

One hundred treated seeds were spread in a petri dish (15 cm dia.), lined with moist filter paper and allowed to germinate. Germination was observed for seven days. Triplicate plates were maintained for each treatment. Alteration in germination percentage of the test seeds due to fungicide treatment was calculated as follows:

$$\text{Alteration in germination percentage} = [(\text{Number of seeds germinated in treatment} / \text{Number of seeds germinated in control}) \times 100] - 100$$

Parameters like shoot and root elongation besides the number of lateral roots initiated, were also noted.

Effect on Membrane Stability (Tripathi et al., 1982)

The effect of different fungicide concentrations on the membrane stability of plant tissue was studied using the third unfolded leaves of *Coccinea indica* L. The fresh leaves were washed in tap water followed by distilled water. Leaf discs of 1 cm diameter and uniform thickness were cut using a cork

borer. The discs were blotted and 10 pieces were floated in 250 mL of fungicide concentrations taken in 500 mL beakers and incubated at 25°C in dark with minimum agitation for 3, 6 and 12 h. After incubation, leaf segments were removed and the ambient solutions were used to measure the ion efflux by conductivity measurements using a Digitalmeter (CyberScan pH/Ion 510, Eutech Instruments, Singapore). Blanks with fungicide concentrations in which leaf discs were not incubated, were also used to account for any conductivity due to fungicide itself, which were tared from the treatment values.

Effect on Primary Production

Primary production was determined using the light and dark bottle method of Wallenweider (1975). Different concentrations of Azoxystrobin and Difenoconazole were prepared and 1mL was added into the initial oxygen bottle (IB), light bottle (LB) and dark bottle (DB) before the water sample is collected. One of the simple methods of estimating primary production is the determination of dissolved oxygen evolved during photosynthesis. The increase in dissolved oxygen of water as a result of photosynthesis is measured in a BOD bottle (light bottle) containing a sample of water under study. Simultaneously, the decrease in oxygen content in a darkened bottle was measured to estimate the respiration alone in the sample of water. From the data obtained thus, gross and net photosynthesis and the respiration of the community are calculated.

Three BOD bottles [two clear bottles i.e., initial oxygen bottle (IB) and light bottle (LB) and one dark bottle (DB)] were filled with the water sample collected from Singanallur pond, Coimbatore. The light bottle (LB) and dark bottle (DB) were suspended to a depth of ½ feet in the pond by means of a thread and the amount of oxygen in the sample was fixed in the initial oxygen bottle (IB) for the estimation of dissolved oxygen using a stirred oxygen electrode chamber (Orion Research, model 290A, USA). The samples (LB and DB) were allowed to remain in the location for 2 h so that photosynthesis and respiration take place. To maintain uniformity, the experiment was carried out every time between 10 am and 12 pm. After incubation time, the LB and DB samples were removed from the pond and the oxygen content in the sample bottles was fixed and the amount of dissolved oxygen was calculated. Three replicate bottles were maintained for each treatment.

Calculation

The primary productivity was calculated as follows: -

Oxygen content of IB mg L^{-1} = X
Oxygen content of DB mg L^{-1} = Y
Oxygen content of LB mg L^{-1} = Z

Then,

Community respiration (CP) = (x-y) mg/L/2h
Gross Primary Productivity (GPP) = (z-y) mg/L/2h
Net Primary Productivity (NPP) = (z-x) mg/L/2h

Results

Effect on Seed Germination

Azoxystrobin and Difenoconazole enhanced percent seed germination in *S. indicum* and *P. mungo* at all concentrations studied. In six other test plants (*C. frutescens*, *C. arietinum*, *L. esculentum*, *P. mungo*, *S. vulgare* and *Z. mays*), the former increased the percent seed germination up to the recommended dose (2.2 µg (a.i.) mL^{-1}) beyond which a steady decline in the percent seed germination

was noted. Similar observation was made with Difenoconazole also in the test plants *S. indicum*, *P. typhoides* and *S. vulgare*. In all other test plants, there was a gradual decrease in percent seed germination with increasing concentrations of the said fungicides. A total inhibition of seed germination was not noted in any of the test plants treated with Azoxystrobin. However, higher concentrations (4400 and 22000 μg (a.i.) mL^{-1}) of Difenoconazole resulted in 100% inhibition of seed germination in *D. biflorus*, *B. juncea*, *S. indicum* and *S. vulgare* (Table 1).

Effect on Shoot Elongation

Of the 14 test plants treated with Azoxystrobin, five viz., *C. arietinum*, *D. biflorus*, *S. indicum*, *T. foenumgraecum* and *T. aestivum* showed gradual increase in shoot length up to the recommended dose. Beyond the recommended dose (Table 2), the shoot elongation was progressively decreased with increasing concentrations of both the fungicides.

Effect on Root Elongation

Of the 14 test plants, only four viz., *V. catjung*, *S. indicum*, *P. typhoides* and *S. vulgare* showed progressive decrease in root length with increasing concentrations of Azoxystrobin (Table 3). In all the other test plants, the root length increased with the fungicide concentration to certain levels beyond which progressive decrease with concentration was noted. Difenoconazole treatment also promoted root elongation in certain plant species (*C. arietinum*, *D. biflorus*, *P. aureus*, *P. mungo*, *B. juncea*, *C. frutescens* and *T. foenumgraecum*) in the lower concentrations, but the changes were not as pronounced as in Azoxystrobin.

Effect on Lateral Root Initiation

Lower concentrations of Azoxystrobin promoted lateral root initiation in *D. biflorus*, *S. indicum*, *P. typhoides* and *T. aestjvum*. In other test plants, gradual decrease in the number of lateral roots was observed with increasing concentrations (Table 4). Among the Difenoconazole treated seedlings, *C. arietinum*, *D. biflorus*, *S. indicum*, *P. typhoides*, *S. vulgare* and *Z. mays* registered enhanced lateral root initiation at lower concentrations. In the other plants, the lateral root initiation was progressively decreased with increasing concentrations of Difenoconazole.

Lateral roots were not initiated within seven days of germination in certain test plants (*B. juncea*, *C. frutescens*, *L. esculentum* and *T. foenumgraecum*). So, it was not possible to study the effect of fungicides on lateral root initiation in these species.

Effect on Membrane Stability

The effect of Azoxystrobin and Difenoconazole on the membrane stability is expressed in terms of increases in electrolyte leakage over untreated control, from the leaf discs of *Coccinea indica* (Table 5). Blanks with fungicide concentrations in which leaf discs were not incubated, were also used to account for any conductivity due to fungicide itself, which were tared from the treatment values. Treatment of leaf tissue with Azoxystrobin resulted in electrolyte leakage as measured by increased electrical conductivity (EC). The increase in EC was pronounced with the increase in fungicide concentration and incubation period. However, in the case of Difenoconazole, the electrolyte leakage was not obvious even at higher fungicide concentrations and extended incubation periods. The EC of Difenoconazole treatment showed only negative values (because EC due to fungicide concentrations were tared from treatment values) due to fast and efficient uptake of the fungicide by the leaf tissue.

Effect on Primary Productivity

Table 6 shows that both the fungicides at their different concentrations significantly decreased community respiration and gross primary productivity. However, the net primary productivity was

Table 1. Effect of Amistar and Score on seed germination in various test plants (expressed as % change in germination)

Conc. (µg (a.i.)	Test plant													
mL^{-1})	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Amistar														
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.44	0	-2a	0	2c	0	-20a	0	11ab	5c	0	0	0	-4a	5c
1.10	0	-2a	0	14ab	-4a	-25a	0	16a	20ab	-4a	0	4a	-12a	5c
1.46	9ab	-6ab	0	14ab	-4a	-27ab	4.a	16a	25a	-4a	0	0	-12a	10bc
2.20	9ab	-6ab	0	14ab	-4a	-35bc	4a	16a	25a	-4a	-4 a	-4ab	-12a	15ab
4.40	13a	-6ab	0	14ab	-4a	-35bc	-4abc	6bc	25a	-8ab	-4a	-4ab	-12a	15ab
220	4ab	-6ab	-4ab	19a	-4a	-35bc	-8bcd	4bc	25a	-16bc	-6a	-4ab	-12a	20a
440	4ab	-8ab	-4ab	19a	-4a	-40cd	-10cd	2cd	25a	-16bc	-35b	-4ab	-12a	20a
220	4ab	-8ab	-8abc	19a	-4a	-40cd	-13cde	0	25a	-16bc	-40bc	-4ab	-12a	20a
440	4ab	-8ab	-10bc	9bc	-4a	-45d	-17de	-4de	25a	-20cd	-44c	-8bc	-12a	20a
2200	-9c	-8ab	-12bcd	8bc	-4a	-45d	-17de	-11e	25a	-28d	-46c	-16c	-12a	20a
4400	-9c	-12b	-16cd	-78d	-4a	-62.5e	-21e	-20f	12bc	-48e	-55d	-29d	-33b	-5d
22000	-18d	-12b	-20d	-95e	-52b	-65e	-21e	-25f	12bc	-60f	-64e	-35d	-83c	-10d
Score														
0.44	0	0	0	4c	0	0	-2b	0	0	0	8.8c	0	0	0
1.10	0	-2ab	0	9bc	0	0	-4b	0	5ab	0	20b	4.16a	0	-2.5a
1.46	0	-4ab	0	9bc	0	0	-15c	-6ab	7ab	0	26b	0	0	-5a
2.20	0	-4ab	0	14ab	0	0	-17cd	-6ab	12	0	24b	-4ab	0	-15b
4.40	9a	-4ab	-4ab	14ab	-4a	-5ab	-17cd	-6ab	12a	0	24b	-4ab	0	-25c
220	-2b	-4ab	-4ab	19a	-4a	-20c	-17cd	-11bc	12a	-8ab	28b	-4ab	0	-30c
440	-4b	-4ab	-4ab	19a	-4a	-20c	-17cd	-16c	7.5a	-8ab	42a	-4ab	0	-40d
220	-4b	-8ab	-4ab	19a	-4a	-40cd	-21cde	-25d	-70c	-8ab	-55d	-4ab	-4a	-40d
440	-13b	-8ab	-8ab	19a	-44b	-65d	-26de	-32d	-100d	-12b	-55d	-10bc	-4a	-40d
2200	-18c	-10b	-12b	14ab	-48b	-75e	-30e	-51e	-100d	-12b	-57d	-16c	-25b	-40d
4400	-59d	-16c	-80c	9bc	-66c	-77e	58a	-65f	-100d	-12b	-86e	-60d	-25b	-100e
22000	-72e	-100d	-96d	4c	-96d	-100f	-78f	-67f	-100d	-12b	-91e	-60d	-29b	-100e

Negative and positive values indicate respectively germination inhibition and germination promotion. I. *Cicer arietinum,* II. *Dolichos biflorus,* III. *Phaseolus aureus,* IV. *Phaseolus mungo,* V. *Vigna catjung,* VI. *Brassica juncea*, VII. *Capsicum frutescens,* VIII. *Lycopersicum esculentum,* IX. *Sesamum indicum,* X. *Trigonella foenumgraecum,* XI. *Pennisetum typhoides,* XII. *Sorghum vulgare,* XIII. *Tritcum aestivum,* XIV. *Zea mays,* In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

Comparison	SED	LSD (5%)	LSD (1%)
Concentration vs Test plant vs Fungicide means	4.31	8.46	1.12

Table 2: Effect of Amistar and Score on shoot length (cm / plant) of test seedlings

Conc. (µg.(a.i.)	Test plant (cm)													
mL^{-1})	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Amistar														
Control	2.8b	8.1f	13.1a	13.1a	13.1a	5.8a	4.0a	2.9b	5.9a	4.2i	3.9 a	9.6a	13.8e	15.1a
0.44	2.9a	9.5d	13.1a	13.1a	10.1b	5.1b	3.8a	3.1a	5.9a	4.9g	3.8 a	7.8b	14.0d	13.3b
1.10	2.8a	9.7c	13.2a	13.2a	8.7c	4.6c	3.6b	3.2a	5.9a	5.1f	3.8 a	6.4de	14.0d	10.2c
1.46	2.7b	10.0b	13.0b	13.1ab	8.6d	4.5d	3.6b	2.9bc	5.9a	5.8d	3.6b	6.5cd	14.3c	8.4d
2.20	2.6c	15.4a	13.0b	13.0b	8.1e	4.4d	3.2c	2.8c	5.9a	6.3a	3.6b	6.6c	14.7a	6.8e
4.40	2.6c	9.0e	12.7c	12.7c	8.0e	4.2e	3.0d	2.5d	6.0a	6.2b	3.5b	6.4e	14.5b	6.1f
220	2.5c	7.2g	12.2d	12.4d	8.1e	3.4f	2.4e	2.3d	4.8b	6.2b	3.4c	6.3e	14.3c	5.8g
440	2.2d	7.2g	12.7e	12.0e	7.9f	3.4f	2.1f	2.1e	4.3c	6.0c	3.3d	5.1f	13.4f	5.3h
220	2.1e	6.7h	11.0f	11.0 f	6.1g	3.4f	1.8g	1.5f	3.4d	5.5e	3.3e	4.3h	13.0g	4.8i
440	2.0e	6.3i	11.0f	10.4g	3.3h	2.9g	1.8gh	1.2g	2.1e	4.9fg	0.8f	4.5g	12.2h	4.3j
2200	2.0e	5.9j	9.6g	9.6h	1.8i	2.5h	1.7h	1.0h	1.5f	4.4h	0.6g	3.8i	11.5i	3.1k
4400	1.8f	4.0k	4.7h	5.4i	0	2.1i	0	0	0	4.2i	0.5g	2.9j	0	0
22000	1.8f	2.2l	1.5i	1.5j	0	1.4k	0	0	0	4.1i	0.1h	2.8j	0	0
Score														
Control	2.8a	8.1h	13.1a	13.1a	13.1a	5.8a	4.0a	2.9b	5.9a	4.2i	3.9a	9.6a	13.8e	15.1a
0.44	2.7a	8.6g	12.7b	12.4b	10.1b	5.5b	3.8a	3.1a	5.9a	4.9g	3.8a	7.8b	14.0d	13.3b
1.10	2.7a	8.7f	11.8c	11.8c	8.7c	5.4b	3.6b	3.2a	5.9a	5.1f	3.8a	6.4e	14.0d	10.2c
1.46	2.5b	9.3e	11.0d	10.7d	8.6d	5.0c	3.6b	2.9bc	5.9a	5.8d	3.6b	6.5cd	14.2c	8.4d
2.20	2.4bc	9.5d	10.5e	10.5e	8.1e	4.7d	3.2c	2.8c	5.9a	6.3a	3.6b	6.6c	14.7a	6.8e
4.40	2.1d	9.7c	9.9f	10.2f	8.1e	4.6e	3.0d	2.4d	6.0a	6.2b	3.5b	6.4de	14.5b	6.1f

Table 2: Continued

Conc (µg (a.i.) mL^{-1})	Test plant (cm)													
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
220	2.3c	11.1b	9.3g	9.3g	8.1e	4.5ef	2.4e	2.3d	4.8b	6.2b	3.4c	6.3e	14.4c	5.8g
440	2.3c	11.6a	7.7h	7.7h	7.9f	4.5f	2.1f	2.2e	4.3c	6.0c	3.3d	5.1f	13.4f	5.3h
220	2.4bc	6.1i	7.0i	7.0i	6.1g	3.9g	1.8g	1.5f	3.4d	5.5e	3.1e	4.3h	12.9g	4.8i
440	2.0d	6.0j	4.1j	3.8j	3.3h	3.4h	1.8gh	1.2g	2.1e	5.0fg	0.8f	4.5g	12.2h	4.3j
2200	2.0d	5.9j	2.9k	2.3k	1.8i	3.0i	1.7h	1.0h	1.5f	4.4h	0.6g	3.8i	11.5i	3.1k
4400	0	0.5k	2.6l	2.3k	0	2.5j	0	0	0	4.2i	0.5g	2.9j	0	0
22000	0	0	2.3m	2.1l	0	1.9j	0	0	0	4.1i	0.1h	2.8j	0	0

I. *Cicer arietinum*, II. *Dolichos biflorus*, III. *Phaseolus aureus*, V. *Phaseolus mungo*, V. *Vigna catjung*, VI. *Brassica juncea*, VII. *Capsicum frutescens*, VIII. *Lycopersicum esculentum*, IX. *Sesamum indicum*, X. *Trigonella foenumgraecum*, XI. *Pennisetum typhoides*, XII. *Sorghum vulgare*, XIII. *Tritcum aestivum*, XIV. *Zea mays*, '0' indicates inhibition of seed germination and hence no growth. In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

Comparison	SED	LSD (5%)	LSD (1%)
Concentration vs Test plant vs Fungicide means	0.061	0.119	0.156

Table 3: Effect of Amistar and Score on root length (cm.plant^{-1}) of test seedlings

Conc (µg (a.i.) mL^{-1})	Test plant (cm)													
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Amistar														
Control	6.7c	10.2e	9.2f	9.2g	13.5a	6.4d	4.6c	2.9g	5.6a	2.6h	14.9a	13.6a	11.0e	17.9e
0.44	6.8c	10.7c	10.7e	10.4f	11.5b	6.4d	4.6c	3.2f	5.5a	2.9g	14.9a	13.0b	12.2c	18.7d
1.10	6.8c	13.2a	11.2d	11.3d	9.0c	6.4d	4.6c	4.1e	4.6b	3.4f	14.9a	12.2c	12.6b	19.8b
1.46	6.8c	11.1b	11.2d	13.3b	7.9d	7.1b	4.9b	4.4d	4.5b	4.0e	13.0b	11.8d	13.1a	20.0b
2.20	6.8c	10.3d	11.3d	14.9a	6.5e	7.7a	5.1a	4.7c	4.4b	5.4b	12.1c	11.2e	13.2a	21.5a
4.40	6.8c	10.1d	13.2b	12.7c	5.6f	6.7c	4.8b	4.7c	4.2c	5.5ab	11.4d	10.1f	11.6d	19.1c
220	6.8c	9.8f	14.9a	11.2d	5.0g	5.8e	3.7d	4.9bc	2.9d	5.6a	10.7e	5.3g	10.7f	17.2f
440	9.4b	9.6f	11.8c	11.0e	5.1g	5.9e	3.4e	5.7a	2.6e	4.7c	8.9f	4.7h	9.6g	11.4g
220	10.3a	9.4g	9.0g	9.0h	4.7h	5.3f	3.1f	4.9b	2.4f	4.4d	8.3g	4.3i	5.8h	9.3h
440	4.8d	9.0h	8.4h	8.4i	4.4i	5.3f	2.9f	4.1e	2.1g	3.8e	7.7h	3.2j	4.3i	8.6i
2200	4.8d	7.4i	8.2i	8.2j	4.3j	3.2g	2.7g	2.2h	2.0g	2.0i	7.7h	3.1j	3.2j	7.6j
4400	4.7de	6.9j	5.7j	5.9k	4.2jk	2.6h	2.0h	1.8i	1.4h	0	5.8i	2.6k	3.2j	4.9l
22000	4.6e	5.2k	4.0k	4.01	4.0k	0.8i	1.2i	0.9j	0.9i	0	3.1j	0	3.1j	5.9k
Score														
0.44	6.6de	11.1a	10.7e	10.7f	11.1b	6.5f	4.9d	4.2b	5.2b	3.8h	14.6b	13.6a	10.7b	17.6b
1.10	6.5e	5.8c	11.2d	11.2e	9.2c	6.7e	5.1c	4.1b	4.9c	4.1g	14.5b	13.5a	9.5c	17.3c
1.46	6.7cd	5.6c	11.4d	11.4d	9.1c	7.0d	5.2b	3.9c	3.7d	4.3f	14.5b	13.2b	9.2d	16.8d
2.20	6.8c	5.1d	11.9c	11.9c	9.0d	7.2c	5.5a	3.4d	2.4e	4.5e	14.5b	12.0c	8.9e	16.3e
4.40	6.6de	4.6e	12.2b	12.5b	8.7e	8.8b	4.0f	2.2e	2.4ef	4.5e	12.2c	11.9c	8.8e	12.4f
220	7.9b	4.5e	13.8a	13.8a	7.6f	10.5a	3.9f	2.1f	2.4fg	4.5e	11.2d	11.9c	8.3f	10.0g
440	8.0b	3.9f	10.0f	12.1b	6.9g	6.9d	3.1g	2.0f	2.3fg	4.9d	10.0e	11.9c	8.2f	8.5h
220	8.1a	3.8f	8.6h	8.6h	6.6h	6.3g	2.3h	0.4g	2.2gh	5.1d	9.5f	10.7d	7.9g	6.9j
440	4.5f	3.8f	5.5i	5.1i	6.0i	6.0h	2.1i	0.2h	2.1h	5.6c	8.9g	8.5e	7.3h	6.7k
2200	3.2g	3.4g	3.9j	3.9j	4.6j	5.6i	1.8j	0.1hi	2.0h	5.7bc	8.4h	6.1f	7.2h	7.4i
4400	0	1.9h	3.7k	3.9j	3.0k	4.6j	2.0i	0	0	5.9b	8.5h	5.9g	0	0
22000	0	0	3.9j	3.9j	2.4l	2.4k	0.9k	0	0	6.1a	7.0i	4.3h	0	0

I. *Cicer arietinum*, II. *Dolichos biflorus*, III. *Phaseolus aureus*, VI. *Phaseolus mungo*, V. *Vigna catjung*, VI. *Brassica juncea*, VII. *Capsicum frutescens*, VIII. *Lycopersicum esculentum*, IX. *Sesamum indicum*, X. *Trigonella foenumgraecum*, XI. *Pennisetum typhoides*, XII. *Sorghum vulgare*, XIII. *Tritcum aestivum*, XIV. *Zea mays*, In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

Comparison	SED	LSD (5%)	LSD (1%)
Concentration vs Test plant vs Fungicide means	0.085	0.168	0.220

Table 4: Effect of Amistar and Score on the number of lateral root (Number / plant) initiation in the test seedlings

Conc. (µg (a.i.) mL^{-1})	Test plant									
	Cicer arietinum	*Dolichos biflorus*	*Phaseolus aureus*	*Phaseolus mungo*	*Vigna catjung*	*Sesamum indicum*	*Pennisetum typhoides*	*Sorghum vulgare*	*Triticum aestivum*	*Zea mays*
Amistar										
Control	14.862f	12.956g	11.085a	11.085a	23.080a	2.925f	13.400f	18.650c	4.923e	19.104a
0.44	14.622g	13.024g	10.745b	10.117b	20.776b	3.124e	13.914e	10.117b	4.994de	18.940b
1.10	14.545g	13.457f	9.000c	8.250c	11.125c	3.680d	14.842d	20.166a	5.260c	18.529c1.46
	16.777e	14.085d	8.711d	8.176cd	10.006d	3.861c	20.080b	17.533d	5.893b	18.037d

Table 4: Continued

Conc. (µg (a.i.) mL^{-1})	Test plant: *Cicer arietinum*	*Dolichos biflorus*	*Phaseolus aureus*	*Phaseolus mungo*	*Vigna catjung*	*Sesamum indicum*	*Pennisetum typhoides*	*Sorghum vulgare*	*Triticum aestivum*	*Zea mays*
2.20	19.160d	15.343c	8.250e	8.045d	9.739e	3.972bc	25.294a	15.695e	6.203a	17.530e
4.40	20.007c	15.709b	8.114ef	7.844e	9.641e	4.102ab	19.917c	15.498f	5.114cd	16.248f
220	25.250a	15.932a	8.045f	7.240f	9.083f	4.203a	13.166g	11.011g	5.031de	13.651g
440	23.071b	13.807e	8.000f	7.143fg	8.000g	4.234a	12.196h	9.443h	4.898e	10.366h
220	14.863f	12.955g	7.240g	7.083fg	7.045h	2.809f	11.916i	9.318hi	4.526f	9.932I
440	14.283h	12.526h	7.142g	7.007g	6.417i	2.651g	11.255j	9.170i	3.222g	9.120j
2200	13.130i	12.483h	7.083g	7.000g	6.375i	1.255h	10.000k	8.330j	2.884h	8.123k
4400	12.659k	10.005i	6.659h	6.114h	6.222j	1.009i	8.713 l	7.555k	2.765hi	6.780l
22000	12.863j	5.332j	4.000i	4.000i	6.173j	0.938i	6.166m	0	2.686i	4.537m
Score										
Control	14.842g	12.956b	11.085a	11.085a	23.080a	2.925a	13.400e	18.650c	4.924h	19.104c
0.44	15.123f	14.048a	10.876b	8.000b	21.874b	2.903a	14.515d	19.082b	5.104g	19.523b
1.10	15.363e	11.833c	6.900c	6.900c	19.375c	2.733b	16.142c	21.909a	5.626f	19.983a
1.46	16.642d	11.758cd	6.589d	6.473d	19.244c	2.527c	18.928b	19.142b	6.986a	20.096a
2.20	17.517c	11.649d	6.391e	6.391d	18.863d	2.115d	20.923a	18.223d	6.606b	20.122a
4.40	18.952b	9.313e	6.094f	6.143e	17.044e	2.400c	14.416d	17.890e	6.288c	20.010a
220	19.312a	8.857f	5.840g	5.840f	14.541f	2.071d	11.416f	16.611f	6.134d	18.802d
440	14.770g	5.956g	5.822g	5.833f	14.312g	2.056d	10.428g	14.110g	5.828e	18.264e
220	13.750h	2.938h	5.800g	5.800f	14.240g	1.896e	9.545h	13.789h	5.240g	17.166f
440	4.480i	2.390i	5.333h	4.773g	10.100h	1.262f	8.118i	13.130i	5.116g	17.104f
2200	2.666j	2.008j	5.200h	3.125h	6.500i	1.100g	7.400j	10.149j	4.936h	16.524g
4400	0	1.463k	5.194h	2.426i	5.855j	0	0	8.227k	0	0
22000	0	0	3.125i	2.201j	5.000k	0	0	0	0	0

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

Comparison	SED	LSD (5%)	LSD (1%)
Concentration vs Test plant vs Fungicide means	0.077	0.151	0.199

Table 5: Electrolyte leakage* (mScm^{-1}) from the leaf discs of *Coccinea indica* Linn. as influenced by different concentrations of the fungicides Amistar and Score

	Incubation period (h)					
	Amistar			Score		
Conc. (µg (a.i.) mL^{-1})	3	6	12	3	6	12
0.44	0.003bc	0.017de	0.047fg	-0.007a	-0.032a	-0.153b
1.10	0.010bc	0.040de	0.060efg	-0.007a	-0.077ab	-0.160b
1.46	0.010bc	0.050de	0.083def	-0.010a	-0.110bc	-0.160b
2.20	0.010bc	0.083cd	0.100c-f	-0.020a	-0.120bc	-0.207bc
4.40	0.013bc	0.130bc	0.120b-f	-0.020a	-0.133bcd	-0.267cd
22.00	0.013bc	0.137bc	0.137a-e	-0.023a	-0.160cde	-0.280c-e
44.00	0.017bc	0.140bc	0.160a-d	-0.027a	-0.21de	-0.307de
220	0.020bc	0.170b	0.170abc	-0.030a	-0.230e	-0.350e
440	0.05abc	0.187b	0.187ab	-0.030a	-0.230e	-0.437f
2200	0.087ab	0.200b	0.190ab	-0.037a	-0.367f	-0.557g
4400	0.0117a	0.273a	0.193ab	-0.057a	-0.510g	-0.590g
22000	0.082ab	0.290a	0.217a	-0.190b	-0.703h	-0.730h*

Measured as electrical conductivity (EC) of the ambient solution. The EC due to fungicide concentrations were tared, In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

Comparison	SED	LSD (5%)	LSD (1%)
Concentration vs Inucation period vs Fungicide	0.037	0.073	0.097

significantly increased by Azoxystrobin treatment up to 0.0073 µg (a.i.) mL^{-1}and Difenoconazole up to 0.0014 µg (a.i.) mL^{-1} concentrations. Higher concentrations, however, were detrimental for net primary productivity.

Table 6: Effect of Amistar and Score on community respiration and primary productivity (mg.O_2 L h) in a pond ecosystem

	Amistar			Score		
Conc. (µg (a.i.) mL^{-1})	Community respiration	Gross primary productivity	Net primary productivity	Community respiration	Gross primary productivity	Net primary productivity
Control	6.21a	12.87a	6.63c	6.21a	12.86a	6.65b
0.0014	5.58b	12.83a	7.25b	3.23c	10.55b	7.38a
0.0037	5.03c	12.65a	7.61a	3.27b	7.75c	4.81c
0.0049	3.33d	11.06b	7.73a	3.16d	7.24cd	4.26d
0.0073	3.08e	10.81b	7.72a	3.16d	6.76de	3.75e
0.0147	3.01f	9.41bc	6.39d	3.01e	6.65e	3.49f
0.0733	2.39g	8.45d	6.06e	2.97f	6.21ef	2.97g
0.1467	2.32h	7.09e	4.77f	2.93g	5.69fg	2.68h
0.7333	1.69i	6.18f	4.48g	2.75h	5.44g	2.42i
1.4667	1.58j	5.18g	3.27h	1.80i	3.17h	1.44j
7.3333	1.07k	3.5h	2.48i	1.72j	2.31i	0.51k
14.6667	1.08k	3.00i	1.91j	1.54k	1.83ij	0.29l
73.3333	0.790l	2.44j	1.65k	1.43l	1.61j	0.18l

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

Community Respiration	SED	LSD (5%)	LSD (1%)
Concentration vs Fungicide means	0.019	0.038	0.051
GPP	SED	LSD (5%)	LSD (1%)
Concentrtion vs Fungicide means	0.494	0..93	1.323
NPP	SED	LSD (5%)	LSD (1%)
Concentration vs Fungicide means	0.155	0.311	0.414

Discussion

One of the easiest and most favoured methods of assessing phytotoxicity is the seed germination assay (Wong and Keturi, 1990). A number of earlier workers have recommended the usefulness of seed germination assay for ecotoxicology studies (Ratsch, 1983; Wang and Williams, 1988). Ratsch (1983) concluded that inhibition of root elongation was a valid and sensitive indicator of environmental toxicity. Miller *et al*. (1985) and Thomas *et al*. (1986) used root elongation of five plant species as part of a test battery for hazard assessment of toxic waste sites.

The present study indicated that the fungicides were generally non phytotoxic at or below the recommended dose for field application (2.2 µg (a.i.) mL^{-1}). At higher concentrations, however, both Azoxystrobin and Difenoconazole exhibited concentration dependant phytotoxicity. The systemic fungicide Carboxin and Oxycarboxin were phytotoxic to soybean seedlings at 100 µg mL^{-1} when incorporated in soil (Gray and Sinclair, 1970). The extent of phytotoxicity of Azoxystrobin and Difenoconazole also varied with host genotype. Among the test plants, *V. catjung* was more sensitive to both the fungicides for all the studied parameters and may serve as good indicator of phytotoxicity of these fungicides.

A notable observation in the present study was the stimulatory effect of Azoxystrobin and Difenoconazole, at or below the recommended dose, in terms of percent germination, shoot and root elongation and lateral root initiation in a number of test plants. Schulz (1888) first introduced the hypothesis that all poisonous substances are stimulatory in small quantities and has since become the basis of Arndt-Schulz rule (Luckey, 1959).

Only Azoxystrobin affected the membrane stability of plant tissue where the extent of damage was concentration dependent. The other fungicide Difenoconazole had no adverse effect on the integrity of membrane. The negative EC values obtained in the Difenoconazole treatment may be due to fast and efficient uptake of the fungicide from the ambient solution by the leaf tissue. Carbendazim, another systemic fungicide belonging to azole group also prevented the leakage of electrolytes

and disorganization of cell organelles at lower concentrations (5 and 20 μg mL^{-1}). However, at 100 μg mL^{-1} concentration, it s timulated the loss of electrolytes from the wheat leaves (Tripathi *et al*., 1982).

In aquatic bodies, the biocides have produced unwanted changes in the biota and also upset primary productivity (Saha and Singh, 1981; Somashekar and Sreenath, 1983). Gross and net primary productivity (GPP and NPP) and community respiration (CR) are the most sensitive indicators of ecosystem stress. In the present study, CR was significantly reduced at all concentrations studied whereas the productivity was enhanced at lower concentrations. Similar reports are available on other fungicides like Brassicol, Bavistin, Fytolan, MBC, Difolatan and Hexacap, which are known to be lethal to some nitrogen fixing blue green algae only at higher concentrations (Gangawane and Saler, 1979; Anand and Veerappan, 1980). A gradual reduction in GPP and NPP was noticed with endosulfan, monocrotophos and cumin-L pesticides at low concentration (0.25 ppm) while a noticeable reduction was observed at higher concentrations (beyond 50 ppm) of all the three pesticides (Ravindra and Zohra, 2000).

The present study confirmed that when the two fungicides, Amistar and Score are used at the manufacturer's recommended doses, there will not be any non-target effects in terms of phytotoxicity.

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