



Effect of Strobilurins in Combination with Triazoles on Biochemical Parameters of Chilli (*Capsicum annum* L.)

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Strobilurins are new class of fungicide with an unique mode of action, effective in altering the physiological and biochemical traits of crop plants. An experiment was conducted to study the influence of strobilurin compounds on nitrate reductase, indole acetic acid oxidase enzymes and total phenolics of chilli. Combined application of strobilurin with triazole compound in the form of *Nativo* (trifloxystrobin + tebuconazole) @ 400 g ha⁻¹ recorded significantly higher NRase activity and total phenolics content compared to control and other treatments. It is also observed that the combined application of pyraclostrobin with metiram as *Cabriotop* @ 1500 g ha⁻¹ recorded the maximum IAA oxidase activity.

Key words: Chilli, Strobilurins, NRase, IAA oxidase, Total phenolics.

Chilli, popularly known as 'wonder spice', is a major spice as well as vegetable crop grown in many countries. It gained its popularity through more than 400 varieties available all over the world with different pungency, size, shape and colour and usage. India is the largest producer and consumer of chilli with contribution about 36 per cent to the total world production. Chilli is cultivated on different soil types and under different climatic conditions. It is cultivated throughout the year in India and two crops are produced a year each in wet and dry seasons with a duration of four months. Chilli suffers from many diseases caused by fungi, bacteria and viruses. Among the fungal diseases powdery mildew, leaf spot and anthracnose are the most prevalent ones and are major constraint in chilli production in India causing heavy yield loss ranging from 14 to 20 per cent, due to severe defoliation and reduction in size and number of fruits per plant (Mathur *et al.*, 1972; Sivaprakasam *et al.*, 1976; Gohokar and Peshney, 1981).

Strobilurins, belong to the E-betamethoxy acrylate group of fungicides, are produced by several Basidiomycete fungi (Glaab and Kaiser, 1999) and the key attributes of strobilurins are preventative mode of action (Grossmann *et al.*, 1999) and wide range of activity against *Ascomycetes*, *Basidiomycetes* and *Oomycetes* (Grossmann and Retzlaff, 1997). In addition to the fungicidal effect of strobilurins on the metabolism of pathogenic fungi, positive influences on physiological traits and consequently, on yield have been recognized in cereals (Beck *et al.*, 2000). These changes in the metabolism and physiology have been studied intensively (Grossmann and Retzlaff, 1997; Habermeyer *et al.*, 1998; Bertelsen *et al.*, 2001; Oerke *et al.*, 2004) and are ultimately referred to as "the greening effect". This comprises

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of enhanced chlorophyll content, higher amount of protein, inhibition of ethylene biosynthesis, increased dry matter and delayed senescence. Hence, the use of strobilurins is an approach to increase and sustain the yield in crop plants (Beck *et al.*, 2000) in addition to manage the fungal diseases. With this background, the present experiment was conducted to assess the effect of strobilurins in combination with other group of fungicides (triazoles and dithio-carbomates) on certain biochemical traits of chilli crop. Since, the biochemical traits such as IAA oxidase, NRase and total phenolics have role in growth and development of crop plants, increasing their activity or content by the application of strobilurins would return positively influence the overall growth and yield.

Materials and Methods

A field trial was conducted to assess the physiological efficacy of different formulations of strobilurins on chilli crop during kharif 2014 in the Department of Crop Physiology, TNAU, Coimbatore. The experiment consisted of seven treatments with different concentrations of strobilurin formulations *viz.*, trifloxystrobin combined with tebuconazole as *Nativo* @ 300, 350 and 400 g ha⁻¹, pyraclostrobin combined with metiram as *Cabriotop* @ 1500 g ha⁻¹ and azoxystrobin as *Amistar* @ 500 ml ha⁻¹, along with carbendazim @ 500 g ha⁻¹ and untreated control. The trial was laid out using randomized block design with four replications. The treatments were imposed as foliar spray, two times at 45 DAT (days after transplanting) and 60 DAT. Observations on activities of nitrate reductase (NRase), IAA oxidase and total phenolics content were recorded on 30, 60, 90 and 120 DAT. NRase activity was estimated in fully expanded functional leaves following the method of Nicholas *et al.* (1976) and the enzyme activity was

expressed as $\mu\text{g NO}_2 \text{ g}^{-1} \text{ h}^{-1}$. IAA oxidase activity of the leaf sample was estimated by the method proposed by Parthasarathy *et al.* (1970) and the enzyme activity was expressed as μg of unoxidised auxin $\text{g}^{-1} \text{ h}^{-1}$. Total phenol content of leaf was estimated by the method of suggested by Mallick and Singh (1980) and expressed as mg g^{-1} fresh weight. The collected data were analyzed statistically using ANOVA as per the procedure of Gomez and Gomez (2010).

Results and Discussion

The results obtained from the present study revealed that there was a significant difference among the treatments for NRase, IAA oxidase activity and total phenolics content in chilli crop. NRase, a key enzyme in nitrate reduction pathway, is a substrate inducible enzyme that mediates conversion of nitrate to nitrite. The activation of NRase results, transitorily in increase of nitrite levels and may enhance plant growth when nitrogen assimilation is a level limiter.

Table 1. Effect of strobilurin fungicides on IAA oxidase activity (μg of unoxidised auxin $\text{g}^{-1} \text{ h}^{-1}$) of chilli

Treatments	Growth Stages			
	30 DAT	60 DAT	90 DAT	120 DAT
T1 - Untreated control	114.54	291.11	179.79	93.75
T2 - Nativo 75 WG @ 300 g ha ⁻¹	106.78	264.08	142.71	90.42
T3 - Nativo 75 WG @ 350 g ha ⁻¹	109.00	259.06	139.47	90.60
T4 - Nativo 75 WG @ 400 g ha ⁻¹	105.23	256.88	137.61	87.21
T5 - Carbendazim 50 WP @ 500 g ha ⁻¹	107.53	268.91	162.71	88.45
T6 - Cabriotop 60 WG @ 1500 g ha ⁻¹	104.09	232.75	135.43	78.47
T7 - Amistar 23 SC @ 500 ml ha ⁻¹	111.12	267.56	154.22	90.84
Mean	108.33	262.91	149.99	88.53
SEd	2.97	2.35	1.32	0.80
CD (P=0.05)	5.12	4.76	2.68	1.62

The different combinations of strobilurins exhibited a significant increase in nitrate reductase activity and the maximum increase of 33.43 per cent over untreated control was observed with the treatment T₄ which is the combination of trifloxystrobin and

tebuconazole applied as Nativo @ 400 g ha⁻¹ at 90 DAT (Fig.1). Koehle *et al.* (2003) reported that the nitrate assimilation in plants was increased due to the application of pyraclostrobin, which indirectly activated the nitrate reductase enzyme.

Table 2. Effect of strobilurin fungicides on total phenolics (mg. g^{-1}) at different growth stages of chilli

Treatments	Growth stages			
	30 DAT	60 DAT	90 DAT	120 DAT
T1 - Untreated control	2.85	3.48	4.04	1.19
T2 - Nativo 75 WG @ 300 g ha ⁻¹	2.85	4.29	5.05	1.69
T3 - Nativo 75 WG @ 350 g ha ⁻¹	2.83	4.47	5.20	1.80
T4 - Nativo 75 WG @ 400 g ha ⁻¹	2.72	4.70	5.64	2.37
T5 - Carbendazim 50 WP @ 500 g ha ⁻¹	2.69	3.86	4.51	1.47
T6 - Cabriotop 60 WG @ 1500 g ha ⁻¹	2.87	4.55	5.60	2.24
T7 - Amistar 23 SC @ 500 ml ha ⁻¹	2.98	3.91	4.86	1.64
Mean	2.83	4.18	5.00	1.77
SEd	0.025	0.039	0.046	0.016
CD (P=0.05)	NS	0.079	0.094	0.033

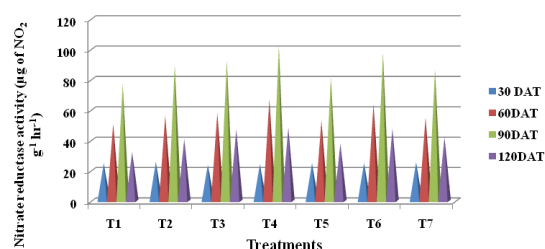
The higher yields in crops that result from the use of strobilurins can be attributed to the ability of strobilurins to increase NRase activity (Glaab and Kaiser, 1999). In the present study also, the application of strobilurin (trifloxystrobin) in combination with tebuconazole applied as Nativo irrespective of concentrations increased the NRase activity. Obviously, the increases in NRase activity due to the foliar application of strobilurins can have positive effect on nitrogen assimilation. Studies in wheat and barley have demonstrated that

strobilurin applications improved the grain nitrogen concentration, while helping to maintain leaf area (Ruske *et al.*, 2003). Leaf disks treated with kresoxim-methyl demonstrated increased levels of NRase activity and reduced its degradation (Glaab and Kaiser, 1999). Strobilurins treated corn showed the increased grain nitrogen concentration due to more rapid nitrogen accumulation and increased nitrogen harvest index (Ruske *et al.*, 2003).

IAA oxidase regulates the auxin level and consequently the apical dominance in plants. It

is favorable that low IAA oxidase activity during vegetative growth and high during reproductive stage for higher yield. The analysis of data on IAA oxidase activity indicated a significant enhancement in IAA oxidase activity due to the application of strobilurin fungicides in combination with azole group of fungicides at all the stages of observation. The maximum increase of 6.98 per cent was recorded in combined application of pyraclostrobin with metiram (T_6) as Cabriotop @ 1500 g ha⁻¹ over the untreated control at 60 DAT (Table 1). Similar findings were also reported by Koehle *et al.* (2003) in wheat due to the application of pyraclostrobin and paclobutrazol (Aly and Latif, 2011). The retardation of senescence by strobilurins had close relation with increase of IAA oxidase activity and decreasing levels of amino cyclopropane carboxylate (ACC) synthase, formation of ACC and ethylene.

Fig. 1. Effect of strobilurin fungicides on nitrate reductase activity



T₁ - Untreated control T₂ - Nativo 75 WG @ 300 g ha⁻¹
 T₃ - Nativo 75 WG @ 350 g ha⁻¹ T₄ - Nativo 75 WG @ 400 g ha⁻¹
 T₅ - Carbendazim 50 WP @ 500 g ha⁻¹
 T₆ - Cabriotop 60 WG @ 1500 g ha⁻¹
 T₇ - Amistar 23 SC @ 500 ml ha⁻¹

Phenols are physiologically active secondary compounds produced by higher plants and act as protective compounds against disease causing agents such as fungi, bacteria and viruses. Phenolic constituents of plants have an anti-oxidant activity and offer protection against oxidative damage (Evans *et al.*, 1997) and involved in the modulation of cell wall plasticity (Wallace and Fry, 1994). The total phenolics content significantly increased with the application of strobilurin compounds in combination with other group of fungicides in chilli and the maximum increase in total phenol content of 49.79 per cent over untreated control was observed in the treatment T₄ which is the combination of trifloxystrobin and tebuconazole applied as Nativo @ 400 g ha⁻¹ at 90 DAT (Table 2). Kishorekumar *et al.* (2006) reported that total phenolics content increased with the triazole treatment both in the shoot and tuber at all stages of growth of potato and in turmeric reported by Jaleel *et al.* (2009). Burrows and Boag (1992) indicated that application of paclobutrazol considerably enhanced the total phenolic content of leaves, terminal buds and alter the phloem to xylem ratio of the stem. These alterations could be important in restricting vegetative growth and enhancing flowering by altering assimilates partitioning and patterns of nutrient supply for the new growth.

From the results of this study, it is concluded that foliar application of strobilurin (trifloxystrobin) and triazole (tebuconazole) compounds as Nativo @ 400 g ha⁻¹ significantly increased activity of IAA oxidase and NRase compared to control and other fungicidal treatments. The increase in the activity of IAA oxidase favours higher levels of auxin, which in turn help in better growth and development. Also, the higher activity of NRase enzyme helps in better assimilation of nitrogen in plants. The higher phenolics content observed in the same treatment favoured lesser incidents of pathogens.

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