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FOLIAR APPLICATION OF TRIFLOXYSTROBIN IN COMBINATION WITH TEBUCONAZOLE ENHANCES ANTIOXIDANT DEFENSE SYSTEM AND YIELD IN TOMATO (*LYCOPERSICON ESCULENTUM* MILL.)

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ABSTRACT

A field experiment was carried out to find out the extent of changes occurred in tomato in terms of antioxidant potential and yield in response to trifloxystrobin treatment combined with tebuconazole, a fungicide cum plant growth regulator in two different seasons. Exogenous application of *trifloxystrobin and tebuconazole* 75 WG was given at four different concentrations (200, 300, 400 and 600 g ha^{-1}) at 35-40 and 55-60 days after transplanting compared with Mancozeb @ 1000 g ha^{-1} and unsprayed control. Foliar spray of trifloxystrobin and tebuconazole showed an increase in proline and phenol content, chlorophyll stability index, catalase, peroxidase, super oxide dismutase and phenylalanine ammonia lyase activities. A significant difference was observed in both the seasons and trifloxystrobin and tebuconazole treatments. *Trifloxystrobin* and *tebuconazole* @ 600 g ha^{-1} was found optimum in both seasons as it caused higher levels of reactive oxygen scavenging enzymes, phenol, proline and lower levels of poly phenol oxidase (PPO) thereby, causing the inhibition of oxidative stress and maintenance of juvenile state of the plants.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.), the world's largest tropical vegetable crop is referred as protective food for its special nutritive value. The fruits contain higher amount of minerals, vitamins, essential amino acids, sugars and dietary fibres. Among the several factors limiting the cultivation of tomato, fungal diseases cause about 30 per cent losses in yield. Senescence associated with disease incidence was driven by active oxygen species (AOS) such as hydrogen peroxide (H_2O_2), superoxide radicals (O_2^-), singlet oxygen ($^1O_2$), hydroxyl radicals (OH), responsible for various stress induced changes in macromolecules and ultimately results in damage to cellular structures (Li *et al*., 2007) of plants. The AOS can attack polysaccharides, proteins and nucleic acids. Plants have evolved enzymatic protection mechanisms that efficiently scavenge AOS and prevent damaging effects of free radicals. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) are involved in the scavenging of AOS. Phenolics are also able to act as radical scavengers or radical-chain breakers, thus extinguishing strongly oxidative free radicals such as the hydroxyl radical yielding products with much lower oxidative capacities as compared to the parent compounds (Grossmann *et al*. 2002). The O^{2-} produced in the "Mehlar reaction" will be dismutated to O_2 and H_2O_2 by SOD. Peroxidase often regarded as antioxidant enzymes, catalyze the dehydrogenation of structurally diversed phenolic substrates by H_2O_2 (Shigeoka *et al*. 2002). Under adverse environmental situations, catalase removes the H_2O_2 produced (Foyer and Noctor, 2000). The maintenance of this enzyme prevents an increase in cytosolic H_2O_2, which can create toxic conditions in the plant cell leading to oxidative stress and cell death (Prochazkova *et al*., 2001). This can be completely prevented by application of fungicides.

The introduction of strobilurins, a new class of systemic fungicides and its evolution has gained new perspectives on the concept of disease control especially while considering the positive physiological effects on plants (Venancio *et al*. 2003). In many crops, the compound has been reported to prolong the retention of green tissue, increase chlorophyll content in leaves, enhance cyokinin and auxin content, inhibit ethylene biosynthesis, induce better N assimilation, increase CO_2 assimilation and thereby, harvest index. Strobilurins (Trifloxystrobin) along with triazole compounds (Tebuconazole) commercialized as *Trifloxystrobin and tebuconazole* are found to possess plant growth regulating properties and have been taken up for the present study. Therefore, the objective of the present experiment was to examine variations, if any, in the degree of antioxidant enzyme activity and phenol level in tomato plants sprayed with trifloxystrobin in combination with tebuconazole and its impact on yield.

MATERIALS AND METHODS

Tomato (*Lycopersicon esculentum*, Mill.) cv. Vijaya Hybrid was planted under normal climatic conditions during 2011 in the experimental field of Tamil Nadu Agricultural University, Coimbatore (11ºN; 77ºE; 426.7m MSL), India. The experiment was carried out in *Kharif* and *Rabi* seasons of Tamil Nadu. Tomato seedlings (30 days old) were transplanted to the field in 60 x 45 cm spacing pattern and a net of 88 plants was maintained in 24 m^2 plot (3 plants m^{-2}). The soil of the experimental field was well drained clay with texture having pH of 8.52 and EC of 0.85 dS m^{-1}, low in available N (193 kg ha^{-1}) medium in available P (14 kg ha^{-1}) and high in available K (345 kg ha^{-1}). The experiment was performed with four replications. The plants were irrigated once in five days. *Nativo* 75 WG (trifloxystrobin + tebuconazole), a proprietory product of Bayer CropScience was applied as foliar spray at 35-40 DAT and 55-60 DAT at different concentrations (Table 1), *Mancozeb* as check and unsprayed plot served as control.

Fresh leaf samples in duplicate were collected from all the four replications for the estimation of enzymes, CSI, proline and phenol content. The yield was arrived from twenty plants from each treatment and yield in tonnes per hectare was calculated (t ha^{-1}).

Proline content of leaves was estimated by the method described by Bates *et al.* (1973) and expressed in μg g^{-1} and total phenol content of leaf was estimated by the method of Mallick and Singh (1980) and expressed in mg g^{-1}. Chlorophyll stability index (CSI) was determined by adopting the method of Leopold *et al.* (1981) and expressed in percentage.

Catalase (CAT) was estimated according to Teranishi (1974). Peroxidase (POX) activity was determined based on Racusen and Foote (1965). Superoxide dismutase (SOD) was determined by nitroblue tetrazolium (NBT) method of Beau-Champ and Fridovich (1971) measuring the photoreduction of NBT at 560 nm. Polyphenol oxidase (PPO) was quantified by the method described by Bateman and Daly (1967). Phenylalanine ammonia lyase (PAL) activity was estimated by adopting the procedure of Brueske (1980).

The data collected on the different parameters were statistically analyzed as suggested by Gomez and Gomez (1992) and the critical difference (CD) was computed at five per cent probability.

RESULTS AND DISCUSSION

Proline content (mg g^{-1} fresh weight)

Proline, an iminoacid is believed to protect plant tissues against stress, by acting as nitrogen storage, osmoregulator and protectant of enzymes and cellular structure. From the results obtained, the present study indicated that combined treatment of trifloxystrobin and tebuconazole could significantly increase the proline content in tomato, irrespective of the stages and a maximum increment of 43.5 per cent in tomato was evident (Table 2). Among the fungicide concentrations, higher proline (201.1) at 45 DAT and 65 DAT (178.3) was obtained in T_6. The enhanced content of proline in tomato treated with fungicides may function as a singlet oxygen quencher that trap OH^- radicals and stabilize proteins endogenously (Matysik *et al.*, 2002). The higher proline content could be due to enhanced activity of ornithine aminotransferase (OAT) and pyrroline 5-carboxylate reductase (P5CR), the enzymes involved in proline biosynthesis as well as due to the inhibition of proline oxidase (Debnath, 2008). Also, the increase in proline content due to strobilurin combined with triazole might have occurred due to a transient rise in ABA content due to triazole treatment.

Total phenol content (mg g^{-1} fresh weight), PPO (Δ495 nm g^{-1} fresh weight min^{-1}) and PAL (μmol trans cinnamic acid mg^{-1} protein min^{-1}) activity

Phenolics are physiologically active secondary compounds produced by higher plants and are involved in the modulation of cell wall plasticity (Wallace and Fry, 1994). A significant increase in total phenols (31.91 percent) was observed in plants treated with trifloxystrobin (Table 2). The maximum phenol content (8.37) was observed at 45 DAT in T_5 followed by T_6 (7.80) and the lowest in T_1 (6.20). Although, the accumulation of phenols decreased at the stage of 65 DAT, T_5 recorded the higher phenol (6.78) and the lower in untreated control, T_1 (5.14). In the present study, PPO was decreased to an extent of 14.54 per cent in the treated plants (Fig.1). The decreased polyphenol oxidase activity in treated plants might have resulted in high phenol content, causing delayed senescence. The earlier findings of Jaleel *et al.* (2009) in turmeric and Lakshmanan *et al.* (2007) in *Plectranthus* also indicated a significant increase in total phenol content due to fungicide application. The elimination of ammonia from L-phenylalanine yielding trans-cinnamic acid by phenyl alanine ammonia lyase (PAL) is the first reaction in phenyl propanoid

Table 1: Treatment details

S.No.	Treatment	Formulation (g/ha)
T_1	Untreated control	-
T_2	*Nativo* 75 WG	200
T_3	*Nativo* 75 WG	300
T_4	*Nativo* 75 WG	400
T_5	*Nativo* 75 WG	600
T_6	Mancozeb	1000

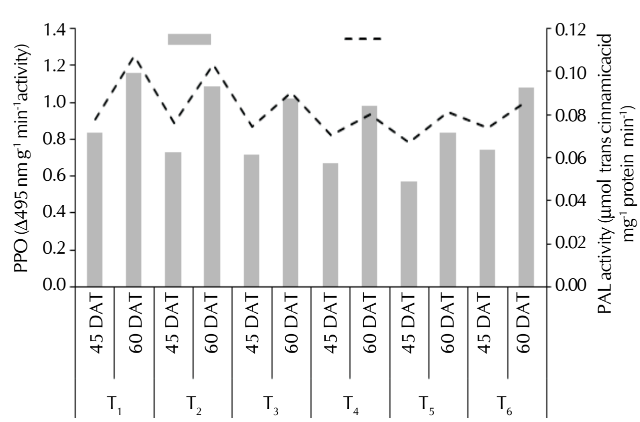

Figure 1: Effect of trifloxystrobin in combination with tebuconazole on PPO and PAL activity

Table 2: Effect of trifloxystrobin combined with tebuconazole on total phenol content and proline content

Treatment	Proline content ($\mu g\ g^{-1}$ fresh weight)						Total phenol content (mg g^{-1} fresh weight)					
	45 DAT			65 DAT			45 DAT			65 DAT		
	Kharif	*Rabi*	Mean	*Kharif*	*Rabi*	Mean	*Kharif*	*Rabi*	Mean	*Kharif*	*Rabi*	Mean
T_1	149.3	177.5	163.4	119.8	153.9	136.9	2.59	1.80	2.20	2.25	1.23	1.74
T_2	161.5	185.6	173.6	141.2	159.6	150.4	3.13	1.83	2.48	2.52	1.25	1.89
T_3	169.4	196.4	182.9	156.3	173.2	164.8	3.39	2.64	3.02	2.59	1.84	2.22
T_4	189.6	199.3	194.5	171.1	178.6	174.9	3.85	3.19	3.52	2.86	2.48	2.67
T_5	193.7	204.5	199.1	173.5	181.9	177.7	4.93	3.81	4.37	3.65	2.91	3.28
T_6	198.4	203.8	201.1	170.9	185.6	178.3	4.47	3.13	3.80	3.26	1.92	2.59
Mean	177.0	194.5	185.8	155.5	172.1	163.8	3.73	2.73	3.23	2.86	1.94	2.40
	T	S	T x S	T	S	T x S	T	S	T x S	T	S	T x S
SEd	0.020	0.012	0.028	0.015	0.009	0.021	0.090	0.052	0.127	0.056	0.032	0.079
CD (P:0.05)	0.041	0.024	0.058	0.030	0.017	0.043	0.183	0.106	0.259	0.114	0.066	0.161

Table 3: Effect of trifloxystrobin combined with tebuconazole on CSI and SOD activity

Treatment	Chlorophyll Stability index (%)						SOD activity (enzyme units mg $protein^{-1}$ min^{-1})					
	45 DAT			65 DAT			45 DAT			65 DAT		
	Kharif	*Rabi*	Mean	*Kharif*	*Rabi*	Mean	*Kharif*	*Rabi*	Mean	*Kharif*	*Rabi*	Mean
T_1	72.31	74.36	73.33	69.21	73.81	71.51	2.576	2.187	2.382	2.226	1.766	1.996
T_2	73.05	75.23	74.14	69.95	75.35	72.65	2.597	2.236	2.416	2.435	2.183	2.309
T_3	75.20	76.80	76.00	72.11	76.26	74.18	3.696	2.869	3.282	2.899	3.276	3.088
T_4	77.34	78.14	77.74	74.19	77.98	76.08	3.828	3.886	3.857	3.292	3.497	3.394
T_5	77.71	78.81	78.26	74.70	78.90	76.80	3.997	4.736	4.367	4.129	4.710	4.420
T_6	77.99	78.83	78.41	74.87	78.63	76.75	3.569	3.031	3.300	3.599	4.067	3.833
Mean	75.60	77.03	76.31	72.50	76.82	74.66	3.377	3.157	3.267	3.097	3.250	3.173
T	S	T x S	T	S	T x S	T	S	T x S	T	S	T x S	
SEd	0.538	0.311	0.761	0.331	0.191	0.468	0.026	0.015	0.037	0.031	0.018	0.044
CD (P:0.05)	1.094	0.632	NS	0.674	0.389	NS	0.053	0.031	0.075	0.063	0.036	0.089

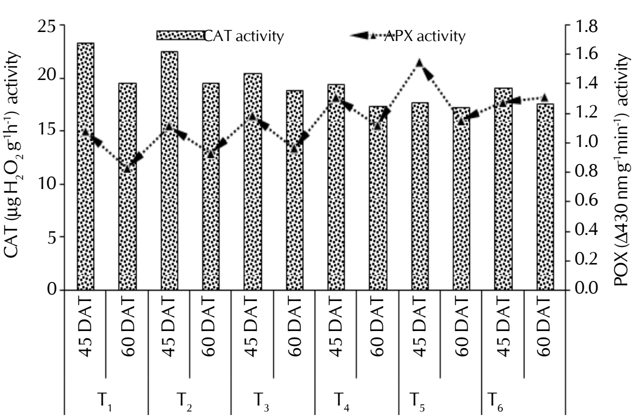

Figure 2: Effect of trifloxystrobin combined with tebuconazole on CAT and POX activity

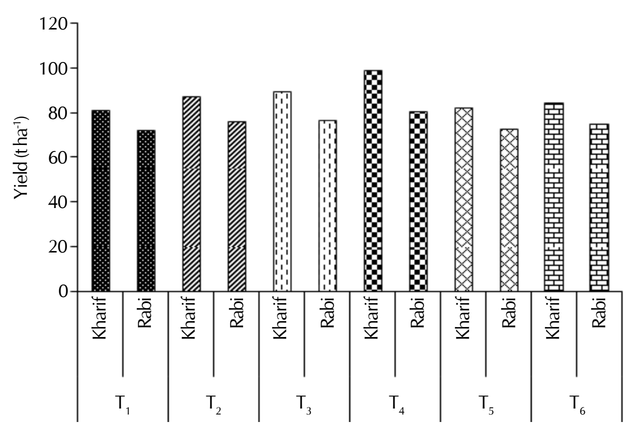

Figure 3: Effect of trifloxystrobin in combination with tebuconazole on tomato yield (t ha^{-1})

pathway. PAL activity was significantly increased by the application of strobilurin combined with triazole and an increase of 26.47 percent was observed in tomato (Fig.1). Similar findings were reported in grapes (Archana, 2009) due to azoxystrobin treatment, which supported the present findings and the enhancement of PAL activity due to fungicide application may be attributed to high phenol accumulation in plants.

Chlorophyll stability index (%)

In the present experiment, the strobilurin fungicide in combination with triazole could increase CSI to maximum of 13.94 per cent (Table 3). The increase in CSI, due to triazoles, might have occurred due to accelerated chlorophyll production and its integrity, chloroplast differentiation and enlarged chloroplast (Fletcher *et al.*, 2000). The inhibition of chlorophyll and protein loss, which are parameters of senescence progression by strobilurins was earlier reported by Grossmann and Retzlaff (1997).

Catalase (μg H_2O_2 g^{-1} min^{-1}) activity

Catalases (CAT) have a dismuting, disproportionating H_2O_2, a key product in inducing senescence and the increase in enzyme activity is correlated to decrease in membrane degradation. The catalase activity was significantly increased by the fungicide application (Fig. 2) compared to untreated control, at 45 DAT and 65 DAT. Among the treatments, T_5 was observed to register higher catalase activity (17.66) and T_1, the lower (23.24) at 45 DAT. Almost, a similar trend was observed at 65 DAT. The increase in catalase activity of tomato treated with trifloxystrobin mixed with tebuconazole could cause a significant decrease in membrane disruption and H_2O_2 mediated the induction of catalase enzyme synthesis. Similar findings were reported by Ye *et al*. (2000).

Peroxidase (Ä430 nm g^{-1} min^{-1}) activity

Peroxidases (POX) constitute a class of haem containing enzymes, ubiquitously present in prokaryotic and eukaryotic organisms and catalyse the dehydrogenation of structurally diversed phenolic and endolic substrates by H_2O_2, hereby protecting cells from the destructive nature of H_2O_2. In the present study, a significant increase was observed in plants treated with fungicide at 45 DAT and 65 DAT (Fig. 2), the maximum activity in T_5 followed by T_4 (1.310) and T_6 (1.274) at 45 DAT. At 65 DAT, higher peroxidase activity was noticed in T_6 (1.311) followed by T_5 (1.149). The cell membranes are protected by increased activity of POX as that of SOD and CAT due to fungicide application. The maintenance of this enzyme at higher level prevents an increase in cytosolic H_2O_2 that can induce toxic conditions in plant cell leading to oxidative stress and cell death (Foyer and Noctor, 2000).

Superoxide dismutase (enzyme units mg protein^{-1} min^{-1}) activity

Superoxidase dismutase (SOD) constitutes the first line of defense, within a cell, against active oxygen species and catalyses the disproportion of superoxide radicals, converting them to molecular oxygen and H_2O_2. In the fungicide treated plants, enhanced activity of SOD activity was observed in tomato (Table 3). The application of strobilurin along with triazole fungicide could promote the activity of SOD and the increment is related to delaying of leaf senescence (Wu and Tiedemann, 2001) and the results are in agreement with that observed in wheat (Zhang *et al*., 2010).

Yield (t ha^{-1})

Fungicides have been associated with yield increase due to the maintenance of the photosynthetic surface during filling in wheat (Pepler *et al.,* 2005) and strobilurins have the ability to prolong the duration of leaf area than triazoles (Bryson *et al*., 2000). *Trifloxystrobin and tebuconazole* 75 WG @ 400g/ha could significantly increase the economic yield to an extent of 18.00 per cent (Fig.3). Similar increase in yield by the application of fungicide was earlier reported by Mejia Arreaza and Herrandez (2001) in tomato, Choudhary *et al*. (2013) and Ingle *et al*. (2014). The increase in CSI, enhanced catalase, peroxidase and superoxide dismutase activity in trifloxystrobin treated plants might have delayed the membrane degradation and improved the longevity of the leaves in the present study. This led to the significant increase in yield.

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REFERENCES

Archana, 2009. Studies on the evaluation of azoxystrobin 23 SC against downy mildew and powdery mildew of grapevine. *M.Sc. (Ag) thesis submitted to Tamil Nadu Agricultural University.*

Bateman, D. F. and Daly, J. M. 1967. The respiratory pattern of *Rhizoctonia* infected bean hypocotyls in relation to lesion maturation. *Phytopathol*. **57:** 127-131.

Bates, L. S., Waldren, R. P. and Teare, I. D. 1973. Rapid determination of free proline for water stress studies. *Plant Soil*. **39:** 205-207.

Beau-Champ, C. and Fridovich, I. 1971. Superoxide dismutase: Improved assays and assay applicable to acrylamide gels. *Ann. Biochem*. **44:** 276-87.

Brueske, C. H. 1980. Phenylalanine ammonia lyase activity in tomato roots infected and resistant to the root-knot nematode, *Meloidogyne incognita*. *Physiol. Plant Pathol.* **16(3):** 409-414.

Bryson, R. J., Leandro, L. and Jones, D. R. 2000. The physiological effects of kresoxim-methyl on wheat leaf greenness and the implications for crop yield. In: The Proc. of the BCPC Conf., Brighton, UK. 13–16. British Crop Production Council, Hampshire, UK. pp. 739-749.

Choudhary, C. S., Jain, S. C. Kumar, R. and Chiudhary, J. S. 2013. Efficiency of different fungicides, biocides and botanical extract seed treatment for controlling seed-borne Coolectotrichum sp. *in chilli. The Bioscan*. **8(1):** 123-126

Debnath, M. 2008. Responses of *Bacopa monnieri* to salinity and drought stress *in vitro*. *J. Medicinal Plants Res.***11:** 347-351.

Fletcher, R. A., Gilley, A., Davis, T. D. and Sankhla, N. 2000. Triazole as plant growth regulators and stress protectants. *Hort. Rev*. **24:** 55-138.

Foyer, C. H. and Noctor, G. 2000. Oxygen processing in photosynthesis regulation and signaling. *New Phytol*. **146:** 359-388.

Gomez, K. A. and Gomez, A. A. 1992. Statistical procedure for agricultural research, New York, Wiley Inter-Science Publications.

Grossman, J., Hippeli, S. and Elstner, E. F. 2002. Plant's defence and its benefits for animals and medicine: role of phenolics and terpenoids in avoiding oxygen stress. *Plant Physiol. Biochem*. **40:** 471-478.

Grossmann, K. and Retzlaff, G. 1997. Bioregulatory effects of the fungicidal strobilurin kresoxim-methyl in wheat (*Triticum aestivum*), *J. Pestic. Sci*. **50:** 11-20.

Ingle, Y. V., Patil, C. U. and Thakurand Kalyani Ingle, K. D. 2014. Effect of fungicides and plant resistance activator on *Colletotrichum* leaf spot of soybean. *The Bioscan*. **9(3):** 1187-1190.

Jaleel, C. A., Zhao, C., Mohamed, S., Al Juburin, H. M., Moussa, H. R., Gomathinayagam, M. and Paneerselvam, R. 2009. Alterations in sucrose metabolizing enzyme activities and total phenol content of *Curcuma longa* L. as affected by different triazole compounds, *Front. Biol*. **4(4):** 419-423.

Lakshmanan, G. M. A., Jaleel, C. A., Gomathinayagam, M. and Panneerselvam, R. 2007. Changes in antioxidant potential and sink organ dry matter with pigment accumulation induced by hexaconazole in *Plectranthus forskholii* Briq, *Comptes Rendus Biologies*. **330:** 814-820.

Leopold, A. C., Musgrave, M. E. and Williams, K. M. 1981. Solute leakage resulting from leaf desiccation. *Plant Physiol.* **68:** 1222-1225.

Li, Z. Z., Niu, W., Qiao, X. W. and Ma, L. P. 2007. Anti-oxidant response of *Cucumis sativus* L. to fungicide. *Carbendazim*. *Biochem. Phy.* **89:** 54-59.

Mallick, C. P. and Singh, M. B. 1980. Plant Enzymology and Histo-Enzymology, *Kalyani Publishers,* New Delhi. p. 286.

Matysik, J., Alia, A., Bhalu, B. and Mohanty, P. 2002. Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. *Curr. Sci.* **821:** 525-532.

Mejia, A. and Hernandez, M. M. 2001. Evaluation of azoxystrobin on the early blight control (*Alternaria solani*) in tomatoes. *J. the Faculty of Agronomy, University of Zulia.* **18(2):** 106-116.

Pepler, S., Gooding, M. J., Ford, K. E. and Ellis, R. H. 2005. A temporal limit to the association between flag leaf life extension by fungicides and wheat yields. *European J. Agri.* **22(4):** 363-373.

Prochazkova, D., Sairam, R. K., Srivastava, G. C. and Singh, D. V. 2001. Oxidative stress and antioxidant activity as the basis of senescence in maize leaves. *Plant Sci.***161:** 765-771.

Racusen, D. and Foote, M. 1965. Protein synthesis in dark grown bean leaves, *Canadian J. Bot.* **43:** 817-824.

Shigeoka, S., Ishikawa, T., Tamoi, M., Miyagawa, Y., Takeda, T., Yabuta, Y. and Yoshimura, K. 2002. Regulation and function of ascorbate peroxidase isoenzymes, *J. Exp. Bot.* **43:** 1305-1319.

Teranishi, A. M., Tanaka, S., Osumi, S. and Fukuli, S. 1974. Catalase activity of hydrocarbon utilizing candida yeast. *Agric. Biol. Chem.* **38:** 1213-1216.

Venancio, W. S., Rodrigues, M. A. T., Begliomini, E. and Souza, N. L. D. 2003. Physiological effects of strobilurin fungicides on plants, *UEPG Exact Soil Sci., Agr. Sci. Eng. Ponta Grossa.* **9:** 59-68.

Wallace, G. and Fry, S. C. 1994. Phenolic compounds of the plant cell wall. *Int. Rev. Cytol.* **151:** 229-267.

Wu, Y. X. and Tiedemann, A. V. 2001. Physiological effects of azoxystrobin and epoxiconazole on senescence and the oxidative status of Wheat. *Pesticide Biochem. Physiol.* **71:** 1-10.

Ye, Z. Z., Rodriguez, R., Tran, A., Hoang, H., De Los Santtos, D., Brown, S. and Vellanoweth, R. L. 2000. The developmental transition to flowering repress ascorbate peroxidase activity and induces enzymatic lipid peroxidation in leaf tissue of *Arabidopsis thaliana, Plant Sci.* **158:** 115-127.

Zhang, Y. J., Zhang, X., Zhou, M. J., Chen, C. J., Wang, J. X., Wang, J. X. and Zhang, H. 2010. Effect of fungicides JS399-19, azoxystrobin, tebuconazole, and carbendazim on the physiological and biochemical indices and grain yield of winter wheat. Pesticide *Biochem. Physiol.* **98:** 151-157.