Role of Iprovalicarb 5.5+Propineb 61.25 fungicide in the management of Blight diseases of Tomato vis-à-vis its effect on the physiology of Tomato plants

BHAWNA PANT, BANDANA BOSE, KAUSHIK BANERJEE, TANMAY KUMAR KOLEY, A. B. RAI, ARPITA SINGH AND SUJOY SAHA

J. Mycopathol, Res, 56(2) : 101-108, 2018;
ISSN 0971-3719

Department of Botany,
University of Calcutta,
Kolkata 700 019, India

J. Mycopathol. Res. ***56(2)*** *: 101-108, 2018;* (ISSN 0971-3719)

Role of Iprovalicarb 5.5+Propineb 61.25 fungicide in the management of Blight diseases of Tomato

BHAWNA PANT[1], BANDANA BOSE[1], KAUSHIK BANERJEE[2], TANMAY KUMAR KOLEY[3], A. B. RAI[3], ARPITA SINGH[3] AND SUJOY SAHA[3*]

[1]Seed Physiology Laboratory, Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi 221005, Uttar Pradesh
[2]ICAR-National Research Centre for Grapes(ICAR), Pune 412307, Maharashtra
[3]ICAR-Indian Institute of Vegetable Research(ICAR)Varanasi 221305 Uttar Pradesh

Received : 20.04.2018 Accepted : 30.04.2018 Published : 30.07.2018

Fungal pathogens, *Alternaria solani* and *Phytophthora infestans*, causing early and late blight of tomato respectively, are responsible for an economic loss to the crop. Iprovalicarb 6.25 + Propineb 61.5@ 2250 - 2500 g ha^{-1} provided a significant disease control as well as stimulated the synthesis of the pigments Chlorophyll a, Chlorophyll b and carotenoids of the tomato plants. There was an increased level of SOD, NR and proteins in the fungicide treated plants as well.The increased TSS, lycopene, total phenols and antioxidants content of the fruits signifies that the fungicide is not only responsible for successful disease management but improves the fruit quality too.

Key words: Tomato, early and late blight, physiology, fungicides, fruit quality

INTRODUCTION

Tomato (*Solanum lycopersicum*) is a vegetable crop which due to its acid sweet taste and unique flavour has gained a considerable importance in global market. The fruits are either consumed raw or cooked and used in large quantities to make soup, puree paste, ketchup and dehydrated powder. Moreover, tomato provides nutrient components like vitamins, carbohydrates, minerals, protein, water and roughages, essential for a balanced diet. Tomato also helps to forestall prostate cancer due to the presence of a tetraterpene 'lycopene' (Lee *et al.* 2011). In India, 17.39 million tonnes of tomatoes are produced from an area of about 0.79 million ha (Anon, 2014).

However, the productivity of tomato has always suffered due to the onslaught of several biotic factors. Among the major biotic constraints, *Alternaria solani* and *Phytophthora infestans,* causing early and late blight respectively, are responsible for a considerable economic loss to the crop. The dearth of effective resistance genes in tomato is a limiting factor in the efficacy of resistance breeding concerning the management of blight diseases and foliar application of fungicides is the major arsenal to combat the disease (Banerjee *et al.* 1998). Chemical management of tomato blight diseases utilizes several different classes of fungicides including multisite protectants such as dithiocarbamates, chlorothalonil, copper; penetrants such as dimethomorph, iprovalicarb, cymoxanil; systemics such as fosetyl-al, mefanoxam; QoI such as azoxystrobin, pyraclostrobin and QiI such as cyazofamid(Bacci *et al.*2007). Recently, two new fungicides, Tebuconazole 50% + Trifloxystrobin 25% and Fenamidone 10% + Mancozeb 50% have been reported to provide excellent control of blight diseases of tomato (Saha *et al.* 2013; Saha *et al.* 2014). However, repeated use of these fungicides has raised the major concern of resistance

*Corresponding author : sujoyta@gmail.com

development. To avoid this, it is mandatory to evaluate the optimum and effective dose of new fungicides which would render maximum mortality to the fungus and are profitable at the user's end. Considering this, a field trial is envisaged to evaluate the efficacy of the combination of Iprovalicarb 5.5+propineb 61.25-66.75WP against the diseases as well as to investigate the physiological and biochemical changes in the plants and fruits due to the application of the fungicide.

MATERIALS AND METHODS

Bioefficacy studies

The trial was conducted at the Research Farm of ICAR-Indian Institute of Vegetable Research at Varanasi, Uttar Pradesh, India for two consecutive *rabi*(dry) seasons (2010-11, 2011-12). Twenty eight days old seedlings of tomato var. Kashi Vishesh were transplanted in plots of size 5 m x 4 m. Standard package of practices were followed to raise the crop (Anon, 2015).Different sets of plants were maintained for each early and late blight diseases. Eight different treatments(Table 1) comprising of three combination doses of Iprovalicarb 5.5+propineb 61.25-66.75WP@ 2000, 2250 and 2500 g ha^{-1}, a combination dose of metalaxyl 8% WP +mancozeb 64% - 72WP @ 2000 g ha^{-1}, a solo dose of each Iprovalicarb 50 % WG@ 275 g ha^{-1},propineb70% WP @ 2187 g ha^{-1} and mancozeb 75WP@2000g ha^{-1} and an untreated control without any spray were laid down in a randomized block design with each treatment replicated three times. Fungicide application began with the visibility of initial disease symptoms i.e. 49 days and 59 days after transplanting in case of early and late blight, respectively and repeated once after 12 days. Ten plants from each replication excluding the border rows were taken at the beginning of each spray and scored for disease using 0-5 scale rating (1- 1-10%, 2– 11-20% ,3 – 31-50%, 4 –51-75% and 5 –76% and above area infected) of Pandey *et al.* (2003) in case of early blight and 0-9 scale rating (1- 0%, 2–1-3%, 3–4-10%, 4–11-20%, 5–21-50%, 6–51-75%, 7 – 76-90%, 8 -91- 97%, 9 - 100% area infected) of Hartman and Huang (1995) in case of late blight with some minor modifications. The per cent disease index (PDI) was calculated based on the observation using the formula of Wheeler (1969) where,

$$PDI = \frac{\text{Sum of numerical values}}{\text{(Number of leaves counted x maximum disease rating)}} X\ 100$$

The harvesting was done after fruit maturity and fruit yield was calculated in quintals/ha. All the data obtained was then statistically analyzed using the IRRISTAT programme developed by International Rice Research Institute, Philippines (Gomez *et al.* 1994).

Physiological studies
Leaf pigment content

Chlorophyll a, b and carotenoids were extracted from the leaves and estimated by the method of Arnon (1949).The fresh plant material was homogenized with 10 mL of 80% acetone at 4°C and centrifuged at 2500 rpm for 10 min at 4°C. This process was repeated until the residue became colourless. The extract was transferred to graduated tube and made up to 10 mL with 80% acetone and assayed immediately. Absorbance was measured at 645, 663 and 480 nm with a spectrophotometer (ELICO SL-177) against 80% acetone as blank. Chlorophyll content was calculated using the formula of Arnon(1949).

Chlorophyll a'= (0.0127) × (A.663) - (0.00269) × (A.645)
Chlorophyll 'b'= (0.0229) × (A.645) - (0.00468) × (A.663)
Carotenoid = (A.480) + (0.114 × A.663 - 0.638 × A645)

Protein estimation

Quantification of the protein content in leaves of tomato was made by using Bradford's (1976) method. The extraction of protein from plant was done in 0.2M Tris.Cl (pH-8) which was centrifuged at 10000 rpm at 4°C for 20 min. The supernatant was transferred in fresh tubes. To 300µL of protein solution (20µL protein extract + 280µL DW) 3mL of Bradford reagent was added. The absorbance was measured after 5 minute incubation at 37°C against a reagent blank (300µL buffer and 3ml of Bradford reagent) at 595 nm in colorimeter.

Superoxide dismutase (SOD) assay

SOD was measured according to Dhindsa *et al.* (1981) method. The 3.0 mL reaction mixture

contained: 0.1 mL of sodium carbonate (1.5 M), 1.5 mL of 100 mM potassium phosphate (pH=7.8), 0.2 mL of methionine (200 mM), 0.1mL Nitro Blue Tetrazolium (2.25mM), 0.1mL riboflavin (60ìM), 1mL of distilled water and 0.05mL enzyme extract. The unit of SOD activity was defined as the amount of enzyme that inhibits the Nitro Blue Tetrazolium photo reduction by 50 %. SOD activity values are given in units per mg of protein.

Nitrate reductase (NR)activity

NR was measured according to the method of Srivastava (1974).The chopped samples (0.5 g) were taken in a black paper covered test tubes containing 8 mL of phosphate buffer, 1 mL of 0.1 M potassium nitrateand 1 mL propanol (5%).The sample were incubated at 30^0C for 30 min in dark. The reaction was stopped by placing the test tubes in boiling water bath for 2 min. In separate test tubes1mL of enzyme extract, 1mLN-(1-Naphthyl)ethylene diaminedi hydrochloride (0.02%) and 1mL sulphonilamide (1%) were added.The intensity of the pink colour developed after some time was read on a spectrophotometer(ELICO SL-177) at 540 nm.

Biochemical studies

It was conducted in randomly harvested fresh fruits at fully ripened stage.

Estimation of total phenol and antioxidants:

For extraction of hydrophilic antioxidants,5 g of homogenized samples were extracted with 30mL of 80% ethanol twice. The homogenate was then centrifuged for 15 min at 10,000 g at 4^0C (Eppendorf, Westbury, U.S.A). The supernatant was then used as sample extract for estimation of total phenolics and antioxidant activity. Total phenolics were estimated spectrophotometrically using Foline Ciocalteu reagent (Singleton *et al*. 1999).An aliquot of 100ml ethanolic extract was mixed with 2.9ml of deionized water, followed by addition of 0.5 ml of Folin-ciocalteu reagent and 2 ml of 20% sodium carbonate solution. The mixture was allowed to stand for 90 min and absorption was measured at 760 nm against a reagent blank in U.V. vis spectrophotometer (Shimadzu UV 1601). The results was expressed as mg Gallic acid equivalent (GAE)/100g.

Antioxidant activity of plant extract was measured by CUPRAC (cupric ion reducing antioxidant capacity)assay according to the method of Apak *et al.* (2004).According to the protocol 100 µl of aliquot was mixed with 1 mL each of copper chloride solution (1.0 × 10"2 mol L"1), neocuproine alcoholic solution (7.5 × 10"3 mol L"1) and ammonium acetate(1 mol L"1, pH 7.0)buffer solution and 1 mL of water to make the final volume 4.1 mL. After 30 min, the absorbance was recorded at 450 nm against the reagent blank. Standard curve was prepared using different concentration of Trolox (100–2000 µM). The results were expressed as µmol TE/g fresh weight.

Total soluble solids

Total soluble solids (TSS) was measured using an Abbe refracto meter(Carl Zeiss, Jena Germany) and expressed in °Brix

Lycopene

Lycopene from tomato products was extracted with hexane: methanol: acetone (2:1:1), containing 2.5% BHT (butylated hydroxy toluene). Optical density of the hexane extract was measured spectrophotometrically at 502 nm against a hexane blank. Concentration of lycopene was calculated using the extinction coefficient (E%) of 3150 (Rao *et al.* 1998) and expressed as mg/100 g dry weight (dw).

RESULTS AND DISCUSSION

Bioefficacy studies

The two doses of Iprovalicarb 6.25 + Propineb 61.5@ 2250 g $ha^{-1}$and 2500 g ha^{-1} provided a significant disease control of early and late blight of tomato with significant increase in the yield over its solo doses as well as the untreated control(Table 2). The dose of Iprovalicarb 6.25 + Propineb 61.5 @ 2250 g ha^{-1} manifested a disease control of 73.44% and 79.28% for early blight and late blight of tomato, respectively with a corresponding yield increase of 55.78%. The higher dose of Iprovalicarb 6.25 + Propineb 61.5@ 2500 g ha^{-1}gave the best disease control of 74.66% and 81.66% for early and late blight of tomato with a yield increase of 57.65%. The disease control of early and late blight obtained from both these doses of the test fungicide were also significantly higher than the combination of Metalaxyl 8% WP +Mancozeb 64% @ 2000 g ha^{-1}. The results of the field trials are in accordance with the findings of Rahman *et al.* (2008) who reported an exceptional efficacy of

Table 1: Different fungicidal treatments for the management of early and late blight of tomato

Serial no	Treatments	Dose (g/Ha)
T1	Control	-
T2	Iprovalicarb 6.25 + Propineb 61.5	2000
T3	Iprovalicarb 6.25 + Propineb 61.5	2250
T4	Iprovalicarb 6.25 + Propineb 61.5	2500
T5	Iprovalicarb 50 % WG	275
T6	Propineb 70% WP	2187
T7	Metalaxyl 8%+ Mancozeb 64%	2000
T8	Mancozeb 75WP	2000

fungicide with moderate resistance risk(Cohen *et al*. 2007), there is always a chance of its reduced efficacy within a span of time. This perhaps necessitated the use of combination of the fungicide with other molecules having different mode of action and chemical group, as an ideal choice for effectively controlling the blight diseases of tomato. Propineb, a bisdithiocarbamate is a non-specific, multi-site fungicide with protective action against germinating conidia and works as an anti-sporulant on disease causing pathogens and hence was used in the combination. The combination product is a tool of anti- resistant

Table 2 : Effect of different treatments on early and late blight diseasesand fruit yield of tomato

Treatments	Dose (g/ha)	Early blight PDI				Late blight PDI				Yield (q/ha)			
		2010-11	2011-12	Mean PDI	Percent Disease Control	2010-11	2011-12	Mean PDI	Percent Disease Control	2010-11	2011-12	Mean	Percent yield increase
Untreated control	-	78.16	80.96	79.56	0.00	63.40	65.96	64.68	0.00	382.60	381.13	381.87	0.00
Iprovalicarb 5.5% + Propineb 61.25%WP	2000	29.46	30.70	30.08	62.19	17.70	17.93	17.82	72.45	400.43	399.00	399.72	17.85
Iprovalicarb 5.5%+ Propineb 61.25%WP	2250	20.23	22.03	21.13	73.44	12.10	14.70	13.40	79.28	438.60	436.70	437.65	55.78
Iprovalicarb 5.5% + Propineb 61.25%WP	2500	19.80	21.23	20.16	74.66	11.60	12.03	11.86	81.66	440.43	438.60	439.52	57.65
Iprovalicarb 50% WG	275	36.80	38.76	56.18	29.39	28.90	28.96	28.93	55.27	385.16	383.60	384.38	2.51
Propineb 70% WP	2187	39.10	40.96	40.03	49.69	36.80	37.73	37.27	42.38	385.96	385.20	385.58	3.71
Metalaxyl 8%+Mancozeb 64%	2000	27.80	29.20	28.41	64.29	23.10	24.76	23.93	63.00	392.66	390.40	391.53	9.66
Mancozeb 75WP	2000	39.23	41.30	40.27	49.38	30.30	31.86	31.08	51.95	390.73	391.26	390.99	9.12
CD (0.05%)		14.71	14.95	14.83		13.29	13.64			18.08	17.90	17.99	
SEm±		6.75	6.86	6.81		6.10	6.260			8.30	8.21	8.26	

Iprovalicarb 6.25 + Propineb 61.5 against *Phytophthora infestans.* Samoucha and Cohen (1986) reported the combination fungicide to be effective in controlling the late blight of potatoes. Iprovalicarb belongs to the newly developed group of carbamic acid isopropylesters with a highly active systemic function preventing the formation of sporangia and spores. After penetrating into the plant tissue it is transported acropetally thereby protecting the new sprouts as well but beinga strategy and may be recommended for the effective management of early and late blight of tomatoes.

Physiological studies

Table 3 presents the pigment content of the leaf of tomato plants collected from untreated diseased and fungicide treated diseased plants. With the treatment of fungicides, pigment content

Table 3: Effect of different treatments on the pigments of the leaves of tomato leaves

	Chlorophyll A ($mgg^{-1}FW$)				Chlorophyll B ($mgg^{-1}FW$)				Carotenoids ($mgg^{-1}FW$)			
Treatments	7 day after application	15day after application	Mean	Percent change	7 day after application	15day after application	Mean	Percent change	7 day after application	15day after application	Mean	Percent change
T1	0.78	0.65	0.72	0.0	0.88	1.03	0.96	0.00	0.81	0.96	0.89	0
T2	1.24	1.53	1.39	93.71	1.80	1.58	1.69	76.96	1.50	1.51	1.51	70.06
T3	1.27	1.56	1.42	97.90	1.95	1.82	1.89	97.38	1.65	1.63	1.64	85.31
T4	1.36	1.59	1.47	99.75	1.96	1.84	1.90	98.95	1.77	1.75	1.76	98.87
T5	1.27	1.58	1.43	99.30	1.22	1.66	1.44	50.79	1.43	1.48	1.46	64.41
T6	1.25	1.54	1.40	95.10	1.17	1.49	1.33	39.27	1.34	1.42	1.38	55.93
T7	1.17	1.41	1.29	80.42	1.04	1.46	1.25	30.89	1.14	1.33	1.24	39.55
T8	0.87	1.37	1.12	56.64	1.02	1.17	1.10	14.66	0.95	1.09	1.02	15.25
Cd 5%	0.10	0.16	_	_	0.10	0.08	_	_	0.18	0.11	_	_

Table 4 : Effect of different treatments on the enzymes and total protein of tomato leaves

	Superoxide dismutase (SOD) ($U\ g^{-1}FW\ min^{-1}$)				Nitrate reductase(NR) ($nmol\ NO_2 h^{-1} g^{-1} FW$)				Protein ($mg\ g^{-1}FW$)			
Treatm-ents	7 days after application	15 days after application	Mean	Percent change	7 days after application	15 days after application	Mean	Percent change	7 days after application	15 days after application	Mean	Percent change
T1	1.071	1.278	1.17	0	1620.93	1333.5	1477.22	0	170.94	510.03	340.49	0
T2	1.655	1.858	1.76	49.55	3182.21	2291.76	2736.99	85.28	275.34	806.21	540.78	58.82
T3	1.725	1.875	1.80	53.26	3205.77	2495.99	2850.88	92.99	304.72	818.04	561.39	64.87
T4	1.734	1.970	1.85	57.68	3295.23	2552.87	2924.05	97.94	332.91	831.39	582.15	70.97
T5	1.545	1.662	1.60	36.53	3136.67	2287.5	2712.09	83.59	249.14	791.02	520.09	52.74
T6	1.428	1.849	1.64	39.51	2361.98	2208.91	2285.45	54.71	235.52	774.95	505.24	48.38
T7	1.367	1.637	1.50	27.88	2318.31	2124.3	2221.31	50.37	227.81	763.35	495.59	45.55
T8	1.124	1.525	1.32	12.77	2224.34	1977.67	2101.01	42.23	213.97	649.51	431.74	26.79
Cd 5 %	0.04	0.09	-	-	9.17	12.11	-	-	8.41	10.24	-	-

(chlorophyll a, b and carotenoid) was found to increase as compared to non-treated diseased plants. Maximum pigment content was observed in T4 followed by either T5 (chlorophyll a) or T3 (chlorophyll b and carotenoid). In general chlorophyll a content was found to increase with increasing age in all the fungicide treated plants as compared to untreated control. Activity of SOD enzyme was found maximum with T4 treatment followed by T3 and T2 in both days i.e.7 and 15 days after application (Table 4). The diseased non treated (T1) set has shown the lowest activity of SOD in respect to all fungicide treated treatments, but, in all the cases the activity of SOD increased

Table 5: Effect of different treatments on the fruit quality of tomato

Treatments	TSS(°Brix)	Lycopene (mg/100 g dry weight)	Antioxidant (µmol TE/g fresh weight)	Total Phenol (mg GAE/100g)
T1	4.20	1.08	6.7	39.7
T2	4.90	1.28	7.4	41.1
T3	5.23	1.61	7.6	43.1
T4	5.57	1.70	7.6	47.1
T5	4.63	1.20a	5.6	40.0
T6	4.57	1.14	6.1	42.0
T7	4.07	1.74	6.4	38.5
T8	4.70	1.79	5.9	42.8

with the age of plants. Maximum activity of nitrate reductase enzyme, which is the first and the most important enzyme of nitrogen metabolism was observed in T4 followed by T3 and T2 treatments; all the fungicide treated sets showed better performance as compared to untreated control (T1). However, the activity of this enzyme was found to decline with increasing age of plants (Table 3). The total protein content was also highest in T4 followed by T3 and T2 as compared to untreated control (Table 4) and in this case a three-fold increment approximately was observed with increasing age of plant in almost all the treatments including the untreated control.

The results clearly brought to the fore that with the use of Iprovalicarb 6.25 + Propineb 61.5, the test fungicide , content/ level of the pigment was found higher which may be either due to enhanced synthesis or reduced degradation of the pigment systems. Feng *et al*. (2003) had noted that in cucumber, treatment of triadimef on fungicide improved the carotenoid content in leaves. Pepler *et al*. (2005) reported that treatments with low disease frequency and long leaf life duration resulted in elevated chlorophyll content. The same may happen in the present case, where in the disease affected sets the chlorophyll content was reduced due to the presence of pathogenic fungi *Alternaria solani* and *Phytophthora infestans.* On the contrary in the pesticide treated ones, where the disease frequency has been restricted, the status of the chlorophyll content in the leaves improved considerably. Further the scavenging enzyme super oxide dismutase activity was noted to increase in plants with fungicide treatments. This type of increment in the activity of SOD was shown by various workers in various crops. Mohamadi and Rajaei (2013) showed an increament in the activity of SOD, chlorophyll and proline contents in triadimefon treated tomato plants under drought stress. They suggested that chlorophyll content increased due to higher net photosynthetic rates. The same was observed in case of okra treated with triazole (Rebert *et al*.2013). Increased activity of enzyme nitrate reductase in T4 followed by T3 and T2 suggested that treatment of Iprovalicarb + Propineb, the test fungicides were effective in restricting the disease in the test plant as a result the protein content in the leaves of these treated sets were also found more as compared to diseased non treated plants (T1).This type of correlation between the enzyme nitrate reductase activity with protein content is a well-documented fact. Therefore this part of the study concluded that the used fungicides were able to restrict the disease via inducing the antioxidant system of the tested crop.

Biochemical studies

TSS is a key determinant of shelf life and quality of the fruit crop, whether it is for the fresh produce or for processing purposes. TSS value ranged from 4.07 to 5.57 Brix, the highest value being in T4. High TSS is desirable for processing industry as it is directly linked with yield of processed product. If high TSS is maintained due to application of fungicides, it is desirable for processing industry. However it is always prudent to be vigilant whether pesticide residue is present in processed product or not. According to Guimaraes *et al.* (2017) application of Pyraclostrobin + Metiram increased the TSS values of tomato as compared to untreated control and this is in confirmation to the present study. The combination of formulations such as Pyraclostrobin + Metiram, which belong to the strobilurin and dithiocarbamate classes of fungicides, has active principles showing antifungal activity with broad spectrum of action (Kozlowski

et al. 2009). In addition to acting directly on the pathogen, they stimulate physiological effects which are highly beneficial to the plants, resulting in increased photosynthesis and increased nitrate reductase enzyme activity, and reduced ethylene synthesis. All these factors contribute to the increase in fruit yield and quality (Fagan *et al.* 2010; Jadoski *et al*. 2015).Lycopene, total antioxidants and phenol content were also relatively higher in T4 as compared to untreated control.

Higher antioxidant in T4 might be due to better physiological activity of plant which produced fruits with better antioxidants (Table 5).

The current study proves that the two doses of Iprovalicarb 6.25 + Propineb 61.5@ 2250 g $ha^{-1}$and 2500 g ha^{-1} provided a significant disease control of early and late blight of tomato with corresponding increase in the yield. In addition to the disease control, it also stimulated the synthesis of the pigments chlorophyll a, chlorophyll b and carotenoids, along with an increased level of SOD, NR and proteins. This clearly brings out to the fore the beneficial role of the fungicide in accentuating the physiological parameters of the plant. The increased TSS and lycopene content of the fruits also bears a testimony of the fact that the fungicide is not only toxic to the pathogens but improves the fruit quality. Iprovalicarb 6.25 + Propineb 61.5@ 2250 - 2500 g ha^{-1} may be recommended for package of practices to the farmers in managing the disease

REFERENCES

Anon, 2014. *Indian Horticulture Database*- Pub: National Horti-culture Board, Ministry of Agriculture, Government of India, Gurgaon, pp.181.

Anon, 2015. *Scientific cultivation of Tomato*: Extension Folder No. 12/2015: ICAR-IIVR, Varanasi, 1-2.

Apak,R. Güçlü, K. Özyürek, M. and Karademir, S. E.2004. Novel Total Antioxidant Capacity Index for Dietary Polyphenols and Vitamins C and E, Using Their Cupric Ion Reducing Capability in the Presence of Neocuproine: CUPRAC Method. *Journal of Agricultural and Food Chemistry*, **52**:7970-7981.

Arnon,D.I.1949.Copper enzymes in isolated chloroplasts and polyphenol oxidase in *Beta vulgaris. Plant Physiol.,*24:1-15.

Bacci, L.Leader,A.and Kemmitt,G. 2007. *Eûcacy of Zoxamide and Mancozeb (Electis) against Late Blight and other diseases of tomato*. In: Tenth Workshop of an European Network for development of an Integrated Control Strategy of potato late blight Bologna Italy, PPO-Special Report no. **12**: 99 – 106.

Banerjee,M.K. Chhabra,M.L Saini,P.S. and Gandhi,S.K.1998. Responses of tomato cultivars to *Alternaria* blight. *Tests of Agrochemical and Cultivars,* **19**:50-51

Bradford, M.M.1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding, *Analytical Biochemistry,* **72**:248-254.

Cohen,Y.,Rubin,Hadad,E.T.,Gotlieb,D.Sierotzki,H.andGisi,U.2007.Sensitivity of *Phytophthora infestans* to mandipropamid and the effect of enforced selection pressure in the field. *Plant Pathology***,56**:836-842.

Dhindsa,R.A.Plumb-Dhindsa,P.andThorpe, T.A. 1981. Leaf senescence: correlated with increased permeability and lipid peroxidation, and decreased level of super oxide dismutase and catalase.*J. Exp. Bot.*, **126**:93-110.

Fagan, E. B. Dourado Neto,D.D.Vivian,R.Franco,R.B. Yeda,M.P.Massignam,L.F.de Oliveira, R.F.andMartins,K.V.2010. Efeito da aplicação de piraclostrobinana taxa fotossintética, respiração, atividade da enzimanitratoredutase e produtividade de grãos de soja. *Bragantia*,**69**: 771-777.

Feng,Z. Guo,A. Feng, Z. 2003. Amelioration of chilling stress by triadimefon incucumber seedling. *Plant Growth Regul.*,**39**: 277-283.

Gomez,K.A.Bartolome,V.I. Calinga,R.T. and Cosico, A.B.1994.*IRRISTAT User's Manual*. Version 3. International Rice Research Institute, Los Banos.

Guimarães,L.R.P, Nozaki, D. N., MouraM.F., Spadotti, D. M.A, MitutiT, Krause-Sakate, R. and AgenorPavan, M. 2017. Fungicide application can improve production of tomato coinfected with Begomovirus and Crinivirus.*Pesq. agropec. bras.,Brasília*, **52**:435-442.

Hartman,G.L.and Huang, Y.H.1995. Characterization of *Phytophthora infestans* isolates and development of late blight in Taiwan. *Plant Diseases,***79**:849-852.

Jadoski, C. J.R,Soratto, J. D.,Santos, R. P.,andRibeiro, C. M., E. 2015. Açãofisiológica da piraclostrobinanaassimilação de CO2 e enzimasantioxidantesemplantas de feijãocondicionadoemdiferentestensões de água no solo. *Irriga*, **20**:319-333.

Kozlowski,L.A.Simões,D.F.M.de Souza,C.D.and Trento,M.2009. Physiological effects of strobilurins F 500® in the growth and yield of bean. *RevistaAcadêmicaCiênciasAgrárias e Ambientais*, **7**:41-54.

Lee,S. T.Wong,P. F. Cheah,S. C.and Mustafa,M.R.2011. Alpha-tomatine induces apoptosis and inhibits nuclear factor-kappa B activation on human prostatic adenocarcinoma PC-3 cells. *PLosOne*,**6**: e 18915.

Mohamadi,N.and Rajaei,P. 2013.Effect of Triamidefon fungicide on some growth parameters and antioxidantenzymes activity in tomato (*Lycopersicon esculentum* Mill.) plant underdrought stress. *International Journal of Advanced Biological and Biomedical Research.*

Pandey,K.K.Pandey,P.K. Kalloo,G.and Banerjee, M.K. 2003.Resistance to early blight of tomato with respect to parameters of disease epidemics. *Journal of General Plant Pathology,***69**:364-371.

Peppler,S. Gooding,M.J. Ford,K.E.Ellis,R.H.andJones, S.A.2005. A temporal limit to the association between flag leaf life extension by fungicides and wheat yields. *European Journal of Agronomy*, **22**:405-416.

Rahman, M. M. Dey, T. K. Ali, M. A. Khalequzzaman,K. M. and Hussa,M. A 2008. Control Of Late Blight Disease Of Potato By Using New Fungicides.*Int. J. Sustain. Crop Prod*, **3**: 10-15.

Rao, A.V. Waseem,Z.andAgarwal,S.1998. Lycopene content of tomatoes and tomato products and their contribution to dietary lycopene. *Food Res. Int.*, **31**:737–741.

Saha, S. Loganathan,M. Rai, A.B.and Singh,B.2013. Evaluation of fungicides and biocontrol agents in the management of Early and Late blight of Tomato.*Pestology*, **37**:22-25.

Saha,S. AhammedShabeer, T.P. Jadhav,M.R. Loganathan, M.Banerjee,K.and Rai, A. B. 2014. Bioefficacy, residue dynamics and safety assessment of the combination fungicide trifloxystrobin25% + tebuconazole 50%-75 WG in managing early blight of tomato (*Lycopersicon esculentum* Mill). *Journal*

of Environmental Science and Health, Part B: Pesticides, Food Contaminants, and Agricultural Wastes, **49**:134-141.

Samoucha, Y.and Cohen,Y. 1986.Efficacy of systemic and contact fungicide mixtures in controlling late blight in potatoes. *Phytopathology*, **76**:855-859.

Singleton,V.L. Orthofer,R.andLamuela-Raventos,R.M.1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol.*, **299**:152-178.

Srivastava,H.S.1974. *In vivo* activity of nitrate reductase in maize seedling. *Indian J. Biochem. Biophys.*,**11**:230-232.

Wheeler,B.E.J.1969. *An Introduction to Plant Diseases*. John Wiley and Sons Ltd. London, pg-301.