



PERFORMANCE APPRAISAL OF ZOAXAMIDE IN COMBINATION WITH CYMOXANIL AND MANCOZEB IN COMBATING THE BLIGHT DISEASES OF TOMATO

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ABSTRACT

Early and late blight are the two major biological constraints that hamper the yield and quality of tomato. Twelve different treatments, comprising of solo and combination doses of zoxamide with cymoxanil and mancozeb were evaluated against these diseases for two consecutive *rabi* seasons. The pooled data revealed that in these two seasons, the combination of zoxamide 33% + cymoxanil 33% WP at the dose of 600 g ha⁻¹ gave best control of both early and late blight diseases as compared to untreated check, manifesting 68.7 and 77.5% control, respectively. Similarly, the combination of zoxamide 8.3% + mancozeb 66.7% WP @ 2500 g ha⁻¹ gave best control of both the diseases as compared to untreated check manifesting a control of 62.5 and 65.3%, respectively. None of the doses of zoxamide 8.3% + mancozeb 66.7% WP and zoxamide 33% + cymoxanil 33% WP was phytotoxic to tomato plants.

Key words: Blight disease, resistance, synergistic effect, tomato, zoxamide

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, 18.73 million tonnes of tomatoes are produced from an area of about 0.88 million ha (Anon, 2014). However, crop production is affected by many biotic and abiotic constraints. Among the major biotic constraints, *Alternaria solani* and *Phytophthora infestans*, causing early blight and late blight, respectively, are responsible for a considerable economic loss to the crop. *A. solani* can cause yield loss upto 78% (Datar and Mayee, 1985) while *P. infestans* is responsible for complete crop loss under favourable conditions (Nowciki *et al.*, 2012).

The dearth of effective resistance genes in tomato is a limiting factor in developing an effective resistance breeding programme concerning the management of blight diseases; therefore, the foliar application of fungicides is a major arsenal to combat the disease (Banerjee *et al.*, 1998). Chemical

management of tomato blight diseases involves several classes of fungicides which include multi-site protectants (such as dithiocarbamates, chlorothalonil, copper, etc.), penetrants (such as dimethomorph, iprovalicarb, cymoxanil) systemics (such as fosetyl-al, mefenoxam), quinone outside inhibitor (QoI) [such as azoxystrobin, pyraclostrobin] and quinone inside inhibitor (Qil) [such as cyazofamid] (Bacci *et al.*, 2007). Recently, two new fungicides, tebuconazole 50% + trifloxystrobin 25% and fenamidone 10% + mancozeb 50% reportedly gave excellent control of blight diseases in tomato (Saha *et al.*, 2013, 2014). However, repeated use of these fungicides has raised the concern of resistance 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 was envisaged to evaluate the efficacy of the combination of zoxamide, a low-medium risk fungicide against the blight diseases of tomato. Zoxamide is highly active against a wide range of oomycetes disease (Olson *et al.*, 2002; Smith *et al.*, 2002; Kemmitt and Green, 2002) and its mode of action by inhibition of β -tubulin assembly in mitosis is unique amongst compounds currently registered to control this important group of pathogens (Egan *et al.*, 1998; Young and Slawecki, 2001). Mancozeb is a dithiocarbamate fungicide having a multisite contact activity, whereas cymoxanil is a cyanoacet-amideoxime, whose mode of action is unknown. Thus, a combination of zoxamide 33% + cymoxanil 33% WP and zoxamide 8.3% + mancozeb 66.7% WP were evaluated for their efficacy against the late and early blight diseases of tomato.

MATERIALS AND METHODS

The trial was conducted at the Research Farm, ICAR-Indian Institute of Vegetable Research, Varanasi, Uttar Pradesh (India) for two consecutive *rabi* seasons (2012-13, 2013-14). Twenty eight days old seedlings of tomato var. 'Kashi Vishesh' were transplanted in plots of size 5 x 4 m. Standard package of practices were followed to raise the crop (Anon, 2015). Four different sets of plants were maintained for two combinations of each fungicide for both early and late blight diseases. Fourteen different treatments comprising of 3 combination doses of zoxamide 33% and cymoxanil 33% WP @ 400, 500 and 600 g ha⁻¹, 4 combination doses of zoxamide 8.3% and mancozeb 66.7% WP @ 1750, 2000, 2250 and 2500 g ha⁻¹, a combination dose of cymoxanil 8% WP and mancozeb 64% WP @ 1500 g ha⁻¹, two solo doses of zoxamide 80% WP @ 240 g ha⁻¹ each, a solo dose of each cymoxanil 50% WP @ 1200 g ha⁻¹, and mancozeb 75% WP @ 2000 g ha⁻¹ and two different untreated controls without any spray for each of the two fungicide combinations were laid down in a randomized block design with each treatment replicated three times. Fungicide application was started no sooner the initial disease symptoms became visible (i.e. 49 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 = 98-100% area infected) of Hartman and Huang (1995) in case of late blight. The percent disease index (PDI) was calculated based on the observation using the formula of Wheeler (1969) where,

$$\text{PDI} = \frac{\text{Sum of numerical values}}{\text{Number of leaves counted} \times \text{maximum disease rating}} \times 100$$

The tomato plants were treated with proposed recommended dose and double dose of test chemicals and all the other field conditions were kept constant. Observations on leaf injury, wilting, vein clearing, necrosis, epinasty and hyponasty were recorded on ten randomly selected tomato plants from each plot at 0, 1, 3, 7 and 10 days after application (DAA). The harvesting was done at fruit

maturity and fruit yield calculated. All the data obtained was analyzed using IRRISTAT programme developed by International Rice Research Institute, Philippines (Gomez *et al.*, 1994).

RESULTS AND DISCUSSION

The two doses of zoxamide + cymoxanil [Z + C] @ 500 g ha⁻¹ and 600 g ha⁻¹ gave a significant disease control of early blight and late blight of tomato with significant increase in yield over its solo doses as well as the untreated control (Table 1). The dose of Z + C @ 500 g ha⁻¹ manifested a disease control of 68.1 and 76.9% for early blight and late blight of tomato, respectively, in both the seasons. This dose recorded highest yield increase of 81.6%. The higher dose of Z + C @ 600 g ha⁻¹ gave best disease control of 68.7 and 77.5% for early blight and late blight of tomato with a yield increase of 95.4%. The disease control of early and late blights obtained from both these doses of Z + C were also significantly higher than the combination of cyamoxanil + mancozeb @ 1500 g ha⁻¹.

In case of the combination of zoxamide and mancozeb (Z + M), the two higher doses @ 2250 g ha⁻¹ and 2500 g ha⁻¹ recorded a significantly higher control of early blight and late blight of tomato along with increased yield as compared to its solo doses and untreated control. The dose of Z + M @ 2250 g ha⁻¹ recorded early blight and late blight disease control of 61.9 and 64.2%, respectively, and increased the yield by 101.5%. The highest dose of Z + M @ 2500 g ha⁻¹ manifested early blight and late blight disease control of 62.5 and 65.1%, respectively (Table 2).

The observations on phytotoxicity on leaves of tomato such as leaf injury, necrosis, wilting, epinasty and hyponasty were also recorded. No phytotoxicity symptoms were developed on leaves upto 10 days of spray in any of the combinations of Z + C and Z + M. This indicated that the two test combinations are not phytotoxic to tomatoes upto the mentioned doses. The results obtained helped to elucidate the point that combination doses of Z + C and Z + M surpassed the solo doses of the same fungicides. The homologous component in both these combinations was benzamide fungicide, zoxamide, which arrests nuclear division and destroys microtubule cytoskeleton of oomycete organisms (Young and Slawewski, 2007). A scanning electron microscopy conducted on the effect of zoxamide on *P. infestans* revealed that it acts after germination, inhibits further development of hyphae and prevents the production of sporangiophores and sporangia (Cooke *et al.*, 2001). Unlike other benzimidazoles, zoxamide is the only fungicide which acts both against true fungi and oomycetes (Shaw, 1983; Young, 2007), thereby making it a potential product for control of oomycetous

Table 1: Effect of zoxamide 33% + cymoxanil 33% WP on early and late blight disease and yield of tomato under field conditions

Treatments	Dose (g ha ⁻¹)	Early blight PDI after application (DAA)				Late blight PDI after application (DAA)				Yield (q ha ⁻¹)			Yield incre- ase (%)
		2012- 2013	2013- 2014	Mean PDI	Disease control (%)	2012- 2013	2013- 2014	Mean PDI	Disease control (%)	2012- 2013	2013- 2014	Mean	
Zoxamide 33% + cymoxanil 33% WP	400	18.20	20.00	19.1	48.4	13.16	14.75	13.9	49.4	236.5	232.5	234.5	73.3
	500	10.00	13.65	11.8	68.1	6.27	7.00	6.6	76.9	250.5	240.8	245.6	81.6
	600	9.76	13.40	11.6	68.7	5.41	7.00	6.2	77.5	259.0	250.6	254.8	95.4
Cymoxanil 50% WP	1200	18.86	20.50	19.7	46.8	15.63	16.20	15.9	42.2	221.5	217.8	219.6	55.0
Zoxamide 80% WP	240	17.80	20.00	18.9	48.9	14.33	15.75	15.0	45.4	237.4	231.0	234.2	73.1
Cymoxanil 8% + mancozeb 64% WP	1500	20.90	22.50	21.7	41.4	15.30	16.95	16.1	41.4	225.0	217.6	221.3	63.6
Untreated control	--	35.75	38.30	37.0	0	24.33	30.76	27.5	0	138.5	132.1	135.3	0
CD _{0.05}	--	2.18	2.74			3.82	2.71			11.98	12.42		

Table 2: Effect of zoxamide 8.3% + mancozeb 66.7% WP on early and late blight of tomato under field conditions

Treatments	Dose (g ha ⁻¹)	Early blight PDI after application (DAA)				Late blight PDI after application (DAA)				Yield (q ha ⁻¹)			
		2012- 13	2013- 14	Mean PDI	Disease control (%)	2012- 13	2013- 14	Mean PDI	Disease control (%)	2012- 13	2013- 14	Mean	Yield increase (%)
Zoxamide	1750	19.00	24.46	21.7	40.06	18.00	19.50	18.8	34.19	215.0	212.6	213.8	63.63
8.3% +	2000	15.80	20.38	18.1	50.10	11.65	15.20	13.4	52.86	230.5	237.3	233.9	75.73
mancozeb	2250	12.10	15.50	13.8	61.93	8.25	12.12	10.2	64.23	268.0	268.5	268.2	101.54
66.7% WP	2500	11.96	15.25	13.6	62.46	8.00	11.85	9.9	65.15	277.3	281.3	279.3	109.84
Zoxamide 80% WP	240	17.75	20.48	19.1	47.26	14.45	15.80	15.1	46.89	230.1	228.5	229.3	72.28
Mancozeb 75% WP	2000	22.66	23.00	22.8	37.02	19.50	17.00	18.3	35.94	200.4	199.8	200.1	50.33
Untreated control	--	35.00	37.50	36.2	(-)	25.48	31.50	28.5	(-)	136.4	129.8	133.1	(-)
CD _{0.05}	--	3.78	1.49			3.06	3.47			23.11	17.50		

Note: Observations were recorded at 1, 3, 7 and 10 days after the spray of fungicides

pathogens. Moreover, this non-systemic fungicide strongly binds to cuticular waxes and is highly resistant to wash-off (Bradshaw and Schepers, 2001). Zoxamide also had good post-harvest disease control when applied soon after inoculation (Miller *et al.*, 2006). However, zoxamide in its solo application confronted two reported cases of resistance, that is against *P. capsici* (Bi *et al.*, 2011), and *Pythium sylvaticum* (Martinez *et al.*, 2005). Moreover, some benzimidazole-resistant field isolates of *Botrytis cinerea* and *Venturia inaequalis* exhibiting a cross-resistance to zoxamide also have been detected (Cai *et al.*, 2015; Young, 2007). This perhaps necessitated the use of combination of fungicides zoxamide with cymoxanil and mancozeb, having different mode of action and chemical group, as an ideal choice for effectively controlling the tomato blight diseases.

The water dispersible granule formulation of 83 g kg⁻¹ zoxamide and 667 g kg⁻¹ mancozeb was tested against *A. solani* and *P. infestans*, at multilocations in Europe and the fungicide @ 2 kg ha⁻¹ was reported superior to 2 g ha⁻¹ of mancozeb in most of the locations (Bacci *et al.*, 2007), thereby exhibiting synergy when zoxamide is formulated with mancozeb. In this combination, mancozeb kills the spores before germination, whereas zoxamide inhibits their subsequent development. In other combination, cymoxanil inhibited the sporangial germination and mycelial growth (Zoogas and Davidse, 1987) and provided protective and curative effect.

Conclusion: Zoxamide + cymoxanil @ 500-600 g ha⁻¹ and zoxamide + mancozeb @ 2250-2500 g ha⁻¹ as a foliar spray manifested significantly higher disease control of early and late blights over its solo doses, increased the yield and were devoid of any phytotoxic effects on tomato. Thus, these combinations at above doses may be recommended for the management of both early and late blights of tomato.

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REFERENCES

Anon. 2014. *Indian Horticulture Database-2014*. National Horticulture Board, Ministry of Agriculture, Government of India, Gurgaon, India.

- Anon. 2015. *Scientific cultivation of Tomato: Extension Folder No. 12/2015*. ICAR-IIVR, Varanasi, India.
- Bacci, L., Leader, A. and Kemmitt, G. 2007. Efficacy of zoxamide and mancozeb (Electis) against late blight and other diseases of tomato. pp. 99-106. **In:** *10th Workshop of an European Network for Development of an Integrated Control Strategy of potato Late Blight*. PPO-Special Report No. 12. Bologna, Italy.
- 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.
- Bi, Y., Cui, X., Lu, X., Cai, M., Liu, X. and Hao, J.J. 2011. Baseline sensitivity of natural population and resistance of mutants in *Phytophthora capsici* to zoxamide. *Phytopathology*, **101**: 1104–1111.
- Bradshaw, N.J. and Schepers, H.T.A.M. 2001. Experiences with RH-117281 (zoxamide) – A new fungicide for the control of potato blight. pp. 173-183. **In:** *Proceedings of the 5th Workshop on the European Network for Development of an Integrated Control Strategy of Potato Late Blight*. (Eds. C.E. Westerdijk and H.T.A.M. Schepers). PAV-Special Report No. 7. Edinburgh, Scotland.
- Cai M., Lin D., Chen L., Bi Y., Xiao L. and Liu X. 2015. M233I Mutation in the β -tubulin of *Botrytis cinerea* confers resistance to zoxamide. *Science Reporter*, **5**: 16881.
- Cooke, L.R., Carlisle, D.J., Wilson D.G. and McCall, R.D. 2001. Sensitivity of European isolates of *Phytophthora infestans* to zoxamide (RH-7281). pp. 39-48. **In:** *6th Workshop of an European Network for Development of an Integrated Control Strategy of Potato Late Blight* PPO-Special Report No. 8, Edinburgh, Scotland.
- Datar, V.V. and Mayee, C.D. 1985. Chemical management of early blight of tomato. *Journal of Maharashtra Agricultural University*, **10**: 278-280.
- Egan, A.R., Michelotti, E.L., Young, D.H., Wilson, W.J. and Mattiotta H. 1998. RH-7281: A novel fungicide for control of downy mildew and late blight. pp. 335-342. **In:** *Proceedings of Brighton Crop Protection Conference, Pest and Diseases*, British Crop Protection Council, Farnham, Surrey, UK.
- 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. Philippines.
- 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.
- Kemmitt, G.M. and Green, E. 2002. Zoxamide a novel fungicide for use against *Plasmopara viticola* in grape vines. **In:** *4th International Grape Powdery and Downy Mildew Conference*, Napa, CA, Oct 10-13.
- 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**(4): e 18915.
- Martinez, C., Lévesque, C.A., Bélanger, R.R. and Tweddell, R.J. 2005. Evaluation of fungicides for the control of carrot cavity spot. *Pest Management Science*, **61**: 767-771.
- Miller, J.S., Olsen, N., Woodell, L., Porter, L.D. and Clayson, S. 2006. Post-harvest applications of zoxamide and phosphite for control of potato tuber rots caused by oomycetes at harvest. *American Journal of Potato Research*, **83**: 269-278.
- Nowciki, M., Foolad, M.R., Nowakowska, M. and Kozik, E.U. 2012. Potato and tomato late blight caused by *Phytophthora infestans*: An overview of pathology and resistance breeding. *Plant Disease*, **96**: 4-17.
- Olson, B.D., Kemmitt, G., Ehr, R.J. and Edmonds, J. 2002. Control of foliar late blight with a premix formulation of zoxamide and mancozeb. **In:** *APS 2002 Annual Meeting*, Milwaukee, WI. (*Phytopathology* **92**: S61 Publication No. P_2002-0441-AMA).

- 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.
- Saha, S., Ahammed Shabeer, T.P., Jadhav, M.R., Loganathan, M., Banerjee, K. and Rai, A.B. 2014. Bioefficacy, residue dynamics and safety assessment of the combination fungicide trifloxystrobin 25% + 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.
- 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**(6): 22-25.
- Shaw, D.S. 1983. The cytogenetics and genetics of *Phytophthora*. pp. 81-94. **In:** *Phytophthora: Its Biology, Taxonomy, Ecology, and Pathology* (eds. D.C. Erwin, S. Bartnicki-Garcia, and P.H. Tsao), *American Phytopathological Society*, St. Paul, Minnesota, USA.
- Smith, R.L., Duttie, A.E, Olson, A.G., McFadden, A. and Kemmit, G.M. 2002. Control of oomycete diseases of vegetable crops with Gavel or zoxium fungicides. **In:** *APS 2002 Annual Meeting*, Milwaukee, WI. (*Phytopathology*, **92**: S77 Publication No. P_2002-0563-AMA).
- Wheeler, B.E.J. 1969. *An Introduction to Plant Diseases*. John Wiley and Sons Ltd, London, UK.
- Young, D.H. 2007. Zoxamide, an antitubulin fungicide for control of oomycete pathogens pp. 3 **In:** *Modern Crop Protection Compounds*, **16**: 3. (eds. W. Kramer, U. Schirmer), Pub:Wiley-VCH Verlag GmbH &Co. KGaA, Weinheim, Germany.
- Young, D.H. and Slawecki R.A. 2001. Mode of action of zoxamide (RH-7281), a new oomycete fungicide. *Pesticide Biochemistry and Physiology*. **69**: 100-111.
- Young, D.H. and Slawecki, R.A., 2007. Modern fungicides and antifungal compounds. pp. 125-131. **In:** *14th International Reinhardsbrunn Symposium* (eds. H. Dehne *et al.*), The British Crop Protection Council, Friedrichroda, Thuringia, Germany.
- Zoogas, B.N. and Davidse, L.C. 1987. Studies on the mechanism of action of cymoxanil in *Phytophthora infestans*. *Pesticide Biochemistry and Physiology*, **29**(2): 89-96.