

Effect of Micronutrients on the Control of Blue Mold Caused by *Penicillium expansum* in ‘Red Delicious’ Apples

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Abstract: Blue mold of apple (*Penicillium expansum* Link) is a serious post-harvest disease affecting apples worldwide, including in India. In the present study, twenty-three (23) isolates of *P. expansum* were obtained from infected apple fruits collected from different fruit markets across Maharashtra. These isolates were tested for their sensitivity to Thiophanate Methyl on Potato Dextrose Agar (PDA) medium, and the Minimum Inhibitory Concentration (MIC) was determined using the food poisoning technique. Six micronutrients, viz., boron acetate, cobalt acetate, copper acetate, iron sulphate, magnesium sulphate, and zinc sulphate, were evaluated for their effectiveness in controlling *P. expansum*, both individually and in combination with Thiophanate Methyl. In vitro studies revealed that copper acetate showed the highest Percent Control Efficiency (PCE) of 55.00 at 50 µg/ml and 69.23 at 100 µg/ml concentrations. In contrast, magnesium sulphate exhibited the lowest PCE (42.56) at 50 µg/ml, while zinc sulphate showed moderate effectiveness at 100 µg/ml, followed by other micronutrients when compared to Thiophanate Methyl. Overall, PCE values increased with higher concentrations of micronutrients (100 µg/ml) compared to 50 µg/ml. Among them, copper acetate and iron sulphate exhibited significant PCE values of 60.24 and 70.15, respectively, followed by zinc sulphate, boron acetate, magnesium sulphate, and cobalt acetate in decreasing order of effectiveness. In vivo studies demonstrated that boron acetate was most effective at 50 µg/ml (PCE 57.70), while cobalt acetate showed the lowest PCE (47.28). At 100 µg/ml, magnesium sulphate achieved a high PCE value of 68.32. Overall, cobalt acetate consistently recorded the lowest PCE values (53.66 and 63.57 at 50 µg/ml and 100 µg/ml, respectively), whereas the other micronutrients ranged between 58.65–61.50 and 69.22–72.52 at respective concentrations.

Keywords: Apple, Blue mold, *Penicillium expansum*, Thiophanate Methyl, Micronutrients

I. INTRODUCTION

Apple (*Pyrus malus* L.) is susceptible to blue mold caused by *Penicillium expansum*. It is also affected by several other fungal and bacterial pathogens, including *Podosphaera leucotricha*, *Erwinia amylovora*, *Schizothyrium pomi*, *Gymnosporangium clavipes*, *Gymnosporangium globosum*, *Peltaster fructicola*, *Leptodontium elatius*, *Geastrum polystigmatis*, *Venturia inaequalis*, *Monilinia fructicola*, *Botrytis cinerea*, *Alternaria alternata*, *Aspergillus fumigatus*, *A. flavus*, *Phytophthora cactorum*, *Sclerotinia fructigena*, *Rhizopus stolonifer*, *Mucor piriformis*, and *Penicillium expansum*. Among these, *P. expansum* is recognized as one of the most destructive post-harvest pathogens of apples, causing significant economic losses due to blue mold decay. Effective management of this disease is therefore crucial for maintaining fruit quality during post-harvest storage. In the present study, various micronutrients, viz., boron acetate, cobalt acetate, copper acetate, iron sulphate, magnesium sulphate, and zinc sulphate, were evaluated for their potential to control blue mold of apple. Furthermore, recent reports have indicated the emergence of resistance in *P. expansum* to certain fungicides, particularly Thiophanate Methyl [3,6,11,8,5], highlighting the need for alternative management approaches such as micronutrient-based treatments.



II. MATERIALS AND METHODS

During the 2024–2025 survey, infected apple fruits were collected from various fruit markets across Maharashtra. A total of six micronutrients were selected for evaluation. Twenty-three (23) isolates of *Penicillium expansum* obtained from apple fruits were tested for their response to Thiophanate Methyl both on potato dextrose agar (PDA) plates and directly on apple fruits. The selected micronutrients, namely boron acetate, cobalt acetate, copper acetate, iron sulphate, magnesium sulphate, and zinc sulphate, were incorporated into 2X PDA medium, each at equal concentrations, and poured into Petri plates. The food poisoning technique ^[7] was employed to assess the effect of these micronutrients on *P. expansum*. Thiophanate Methyl concentrations were adjusted to 50 and 100 µg/ml, both independently and in combination with the micronutrients, to determine their synergistic effect. After solidification, the plates were inoculated at the centre with a resistant mutant strain (Pe-EMS-10) and incubated at 27 ± 2 °C. Colony growth was recorded after 8 days, and the Percentage Control Efficacy (PCE) was calculated. For *in vivo* studies, apple fruits were treated with the above-mentioned micronutrients (50 and 100 µg/ml) in combination with Thiophanate Methyl to evaluate the combined efficacy. The treated fruits were inoculated with the resistant mutant Pe-EMS-10, wrapped in tissue paper, and incubated at 27 ± 2 °C for 15 days. The radial growth of *P. expansum* lesions was measured, and PCE values were calculated using the standard formula ^[1].

III. RESULTS AND DISCUSSION

In *in vitro* experiments, copper acetate exhibited the highest Percentage Control Efficacy (PCE) at both 50 µg/ml (55.00) and 100 µg/ml (69.23) concentrations. In contrast, magnesium sulphate showed the lowest PCE value (42.56) at 50 µg/ml, while zinc sulphate recorded a PCE of 51.25 at 100 µg/ml. At 50 µg/ml concentration, the PCE values of cobalt acetate, zinc sulphate, iron sulphate, and boron acetate ranged between 43.67 and 52.57. At 100 µg/ml, magnesium sulphate, cobalt acetate, boron acetate, and iron sulphate exhibited PCE values between 53.15 and 65.42. In comparison, Thiophanate Methyl alone recorded a PCE of 57.94.

When the micronutrients were combined with Thiophanate Methyl, a marked increase in PCE was observed for all treatments against *Penicillium expansum*. The 100 µg/ml concentration consistently produced higher PCE values than 50 µg/ml. Among the combinations, copper acetate and zinc sulphate proved most effective, showing PCE values of 60.24 and 68.25, respectively, followed by iron sulphate, boron acetate, magnesium sulphate, and cobalt acetate in descending order (Table 1).

In *in vivo* studies, six micronutrients were evaluated for their efficacy against *Penicillium expansum*. At 50 µg/ml concentration, boron acetate exhibited the highest Percentage Control Efficacy (PCE) of 57.70, followed by iron sulphate, magnesium sulphate, zinc sulphate, copper acetate, and cobalt acetate. At 100 µg/ml concentration, magnesium sulphate showed a significant PCE value of 68.32, followed in order by boron acetate, iron sulphate, zinc sulphate, copper acetate, and cobalt acetate.

When Thiophanate Methyl was combined with the micronutrients, the PCE values increased notably compared to those obtained with micronutrients alone. Iron sulphate displayed the highest PCE values at both concentrations (63.50 and 74.25), whereas cobalt acetate recorded the lowest (53.60 and 63.57) at 50 µg/ml and 100 µg/ml, respectively. Whereas copper acetate, zinc sulphate, boron acetate, and magnesium sulphate showed intermediate PCE values ranging from 58.65 to 61.50 at 50 µg/ml and from 69.22 to 72.52 at 100 µg/ml (Table 2).

Similar findings were previously reported by Gangawane and Reddy (1987), who demonstrated that certain micronutrients, when combined with Thiophanate Methyl, could reduce resistance in *Aspergillus flavus*. Dekker (1981) also suggested that the use of fungicide mixtures significantly delays the development of resistance in pathogens. In the present investigation, agrochemicals other than fungicides were also found effective in managing Thiophanate Methyl-resistant *Penicillium expansum* mutants. Srivastava and Tiwari (1997) reported that pre-harvest fungicidal sprays effectively controlled storage diseases in onions. Similarly, Suryawanshi and Deokar (2001) studied the influence of fungicides on the growth and sporulation of fungal pathogens responsible for fruit rot in chilli.

The use of potassium tetraborate was also shown to inhibit conidial germination of *Colletotrichum gloeosporioides*, thereby aiding in the management of post-harvest rot in mango ^[12]. Application of $K_2B_4O_7$ reduced the incidence of



anthracnose in harvested fruits. Borate compounds have also been reported effective against gray mold in table grapes caused by *Botrytis cinerea* and blue mold in apple fruits caused by *P. expansum* [9,10].

Table 1: PCE of Thiophanate Methyl individually and in mixture with micronutrients against resistant mutant (Pe-EMS10) *Penicillium expansum* on agar plate (*In vitro*).

Sr. No.	Micronutrients (µg/ml)	PCE individual	PCE mixture with Thiophanate Methyl
1.	Boron acetate		
	50	52.57	56.66
	100	63.12	68.32
2.	Cobalt acetate		
	50	43.67	44.66
	100	54.32	55.47
3.	Copper acetate		
	50	55.00	60.24
	100	69.23	61.39
4.	Iron sulphate		
	50	52.00	54.33
	100	65.42	70.15
5.	Magnesium sulphate		
	50	42.56	46.66
	100	53.15	58.03
6.	Zinc sulphate		
	50	46.00	54.00
	100	51.25	68.25
7.	Thiophanate Methyl	57.94	---
	(µg/ml)		
	SE	2.365	2.812
	CD at 0.05	4.587	5.175
	0.01	5.415	6.190

Table 2: PCE of Thiophanate Methyl individually and in mixture with micronutrients against resistant mutant (Pe-EMS10) of *Penicillium expansum* on apple (*In vivo*).

Sr. No.	Micronutrients (µg/ml)	PCE individual	PCE mixture with Thiophanate Methyl
1.	Boron acetate		
	50	57.70	61.50
	100	66.32	71.22
2.	Cobalt acetate		
	50	47.28	53.60
	100	57.37	63.57
3.	Copper acetate		
	50	51.25	58.65
	100	60.39	69.22
4.	Iron sulphate		



	50	57.25	63.50
	100	64.52	74.25
5.	Magnesium sulphate		
	50	56.00	60.50
	100	68.32	72.52
6.	Zinc sulphate		
	50	52.00	58.50
	100	63.42	69.82
7.	Thiophanate Methyl	73.15	---
	(µg/ml)		
	SE	2.356	6.680
	CD at P= 0.05	4.568	7.358

IV. CONCLUSION

The present study demonstrated that selected micronutrients, when used individually and in combination with Thiophanate Methyl, can play a significant role in the management of blue mold caused by *Penicillium expansum* in 'Red Delicious' apples. Among the six micronutrients evaluated, viz., boron acetate, cobalt acetate, copper acetate, iron sulphate, magnesium sulphate, and zinc sulphate, copper acetate and zinc sulphate exhibited the most promising results under *in vitro* conditions, showing the highest Percentage Control Efficacy (PCE) at both 50 µg/ml and 100 µg/ml concentrations. Under *in vivo* conditions, magnesium sulphate and boron acetate were comparatively more effective, indicating that the response of *P. expansum* may vary depending on the host environment and application conditions. When micronutrients were combined with Thiophanate Methyl, a marked increase in PCE was recorded across all treatments, suggesting a synergistic interaction that enhances the fungicidal efficacy and potentially reduces the risk of resistance development in *P. expansum*. Iron sulphate, in particular, showed the highest PCE in combination treatments, followed by zinc sulphate, copper acetate, magnesium sulphate, and boron acetate, while cobalt acetate exhibited relatively lower effectiveness. The results confirm that the combination of micronutrients with conventional fungicides such as Thiophanate Methyl can improve disease management efficacy compared to individual applications.

These findings are in agreement with earlier reports that certain micronutrients can influence fungal growth, sporulation, and resistance mechanisms. The use of such micronutrients as fungicide enhancers offers a sustainable and eco-friendly approach to post-harvest disease management. Moreover, their inclusion may contribute to reduced chemical residues, minimized fungicide dosage, and delayed resistance buildup in pathogen populations.

Therefore, the integration of specific micronutrients, particularly copper acetate, zinc sulphate, and iron sulphate, with Thiophanate Methyl could serve as an effective strategy for controlling blue mold in apples during storage and marketing. Further studies on the physiological and biochemical mechanisms underlying micronutrient fungicide interactions, as well as their field-scale validation, are recommended to optimize their use in integrated disease management programs.

REFERENCES

- [1]. Cohen E. (1989). Evaluation of fenpropimorph and flutriafol for control of sour rot, blue and green mold in lemon fruits. *Plant Disease*, 73: 807-809.
- [2]. Dekker J. (1981). Counter measures for avoiding fungicide resistant in crop protection. (Dekker, J. and S. G. Georgopoulos, Eds.). *CAPD Wageningen, Netherlands*, 128-138.
- [3]. Gangawane L.V. (1981). Fungicide resistance in crop protection. *Pesticides*, 15(11): 12-16.
- [4]. Gangawane L.V. and Reddy B. R. C. (1987). Distribution and survival of toxigenic strain of *Aspergillus flavus* resistant to fungicides in Marathwada. In: *Progress in Venum and Toxin Research* (P. Gpoalkrishnakone and C.K. Ten Eds.). *National University of Singapore Publications*, 658-388.



- [5]. Latorre B. Enfermedades de las Plantas Cultivadas. (2004). Sexta Ed. Ediciones Universidad Catolica de Chile. Santiago, Chile, 638.
- [6]. Loomis W. D. and Durst R. W. (1992). Chemistry and biology of boron. *Bio Factors*, 3: 229-239.
- [7]. Nene Y. L. and Thapliyal P. N. (1990). Fungicide in plant disease control. *Oxford and IBH Publication Company*, New Delhi, 212-349.
- [8]. Ogawa J. M. and H. English. (1991). Diseases of Temperate Zone Tree Fruit and Nut Crops. Publication 3345. *Div. of Agric. and Nat. Res. University of California. Oakland, California, USA*, 461.
- [9]. Qin G. Z., Tian S. P., Chan Z. L. and Li B. Q. (2007). Crucial role of antioxidant proteins and hydrolytic enzymes in pathogenicity of *Penicillium expansum*. *Mol. Cell Proteomics*, 6: 425-438.
- [10]. Qin G. Z., Zong Y. Y., Chen Q. L., Hua D. L. and Tian S. P. (2010). Inhibitory effect of boron against *Botrytis cinerea* on table grapes and its possible mechanisms of action. *Int. J. Food Microbiol*, 138: 145-150.
- [11]. Rolshausen P. E. and Gubler W. D. (2005). Use of boron for the control of Eutypa dieback of grapevines. *Plant Disease*, 89: 734-738.
- [12]. Shi X. Q., Li B.Q., Qin G. Z. and Tian S. P. (2011). Antifungal activity and possible mode of action of borate against *Colletotrichum gloeosporioides* on mango. *Plant Disease*, 95: 63-69.
- [13]. Srivastava P. K. and Thiwari B. K. (1997). Effect of pre-harvest fungicidal spray on the control of storage diseases of onion. *National Horticulture Research and Development Foundation Newsletter*, 17: 4-6.
- [14]. Suryawanshi A. V. and Deokar C. D. (2001). Effect of fungicides on growth and sporulation of fungal pathogen causing fruit rot of chilli. *Madras Agricultural Journal*, 88(1-3): 181-182

