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Effect of Passage on the Development of Thiophanate Methyl Resistant in *Penicillium expansum* Causing Blue Mold of Pear

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Abstract

Blue mold of Pear caused by *Penicillium expansum*, causes substantial losses on stored pears worldwide. Effect of Continuous passage of the sensitive isolate Pe-10 on thiophanate methyl increased thiophanate methyl resistance. But culturing of the pathogen on thiophanate methyl alternately or in a mixture with Polyram, and Dithane Z-78, the growth was completely reduced while the Kocide and acrobat growth was not reduced compared with the first passage and passage 2nd, 3rd, 4th and 5th growth was decreased. The use of thiophanate methyl in a mixture with Polyram, Dithane Z-78, Kocide, and acrobat reduced the growth of resistance significantly. Dithane Z-78 appeared to be more productively than others in mixtures. In *vivo,* it was observed that the use of thiophanate methyl with polyram, Dithane Z-78, Kocide, and acrobat decreased disease incidence. Dithane Z-78 was most effective than other fungicides. When the thiophanate methyl was applied in combination with Polyram, Dithane Z-78, Kocide, and acrobat, there was a significant reduction in the disease resistance, indicating a decrease in the resistance in the pathogen at the 5th passage. Here, acrobat was most effective.

***Key words*:** Blue mold, Pear, *Penicillium expansum*, thiophanate methyl resistant, Fungicide

Fungicides have significantly influenced fungal plant disease control since the late 19th century. Pear is one of the most important species among the temperate fruit crops. The potential exists for the fungicide application programme to influence the development of disease resistance. the fungicide application program is a critical factor in the development of disease resistance. A strategic and well-managed approach that considers the principles of resistance management is essential to preserve the efficacy of fungicides and ensure sustainable disease control in agriculture. This prompted scientists to investigate the impact of passage on agar media using thiophanate methyl alone, in combination with different fungicides (including acrobat, Polyram, Kocide and Dithane Z-78), or in an alternating fashion, in response to this observation. Moreover, a number of researchers have recorded instances of fungi that exhibit resistance to systemic fungicides [1-4].

The study focused on pear fruit infections that manifest after harvest. We gathered the diseased fruits from all over. *Rhizopus stolonifer*, *Botrytis cinerea*, *Alternaria* sp., *Aspergillus niger*, and *Aspergillus flavus* were the five illnesses that were documented. *Penicillium expansum* caused fruit scabs in more types and locations than any other fungus. Penicillium expansum is primarily known for causing blue mold, which is a postharvest decay in a variety of fruits, particularly apples and pears. Blue mold typically appears as circular lesions with a velvety blue-green mold growth on the surface of infected fruits [5]. This fungicide was subjected to extensive research since thiophanate methyl is suggested for the treatment of pear diseases. It has broad-spectrum activity against various fungal pathogens, making it effective for the control of certain diseases in pears and other crops. The fungicide works by inhibiting fungal cell division and disrupting the formation of microtubules [6]. Although fungicides are increasingly used in disease control, there has been a dearth of reports on fungicide resistance in plant pathogens across various crops. Therefore, this research aimed to determine whether *Penicillium expansum* may become resistant to thiophanate methyl.

MATERIALS AND METHODS

The effect of continuous and alternate treatment of a fungicide with two different modes of action, or a combination of the two, on the development of resistance in sensitive *Penicillium expansum* isolates was studied on agar plates and fruits after the minimum inhibitory concentration (MIC) of thiophanate methyl was determined against 23 isolates.

Agar plates were cultivated with a sub-lethal dosage of thiophanate methyl (752.8µg/ml) in each passage to examine the effect of passage *in vitro* on the sensitive isolate. As a control, we used the plates that did not contain fungicide. In the middle of each plate was a 5 mm diameter disc containing 10 days of culture from the previous passage of the same strain. At the 10-day mark, we recorded the linear increase in every sample. A rise in thiophanate methyl resistance was defined as an increase in the percentage of isolates growing at each passage. Up until the fifth passage, researchers examined how

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resistance evolved. Additionally, thiophanate methyl was alternately passed with acrobat, Polyram, Dithane Z-78, and Kocide combined with the same fungicide.

The pear was the subject of *in vivo* investigations. This was accomplished by following a method of fruit wrapping that used tissue paper. The fruits were submerged in a thiophanate methyl solution singly or combined with other fungicides. The sensitive isolate was introduced to them by inoculation. For the second passage, inoculums were derived from the infected fruit re-isolated during the first. This process was maintained until the fifth passing.

RESULTS AND DISCUSSION

In vitro studies

This investigation used the sensitive isolate Pe-10. For 5 successive passages, Pe-10 was grown on potato dextrose agar medium with lethal doses (LD) of fungicides (i.e., 752.8g/ml) singly and alternatively with other fungicides. It was observed that continuous culturing of the *Penicillium expansum* for five successive passages on thiophanate methyl increased the growth of the *P. expansum.* The growth increased with the increase of the passage. Thiophanate methyl was altered with Polyram, Dithane Z-78, Kocide, and acrobat. When the *P. expansum* was inoculated alternately with Polyram, the growth increased to the second passage, whereas passage 3rd, 4th and 5th growth was in decreasing order. In the case of Dithane, Z-78 growth was completely decreased in all passages. Similar results were indicated in Kocide. In the case of an acrobat, the second passage increased the growth compared with the 1st passage, and in 3rd, 4th and 5th passages, growth decreased in order. Results were indicated when *P. expansum* was inoculated on thiophanate methyl in a polyram, dithane Z-78, kocide, and acrobat mixture (Table 1).

Table 1 Effect of continuous and alternate passage and passage on the development of thiophanate methyl resistance in *Penicillium expansum* on agar plate

S. No.	Fungicides (µg/ml)	Passage number				
		I	II	III	IV	V
1	Thiophanate methyl continuous(752.8µg/ml)	12.15	22.12	25.00	40.33	45.84
2	Thiophanate methyl altered polyram	12.15	18.30	14.00	12.00	09.52
3	Thiophanate methyl altered Dithane	12.15	11.00	07.00	04.00	02.16
4	Thiophanate methyl altered Kocide	12.15	19.25	15.00	15.00	09.25
5	Tthiophanate methyl altered Acrobat	12.15	15.00	12.00	10.25	08.00

a = Linear growth as % of control,
* = Significance at p=0.05 and p* =0.01 with Wilcoxon's (1939) sum rank
Test results are compared to that of the first passage

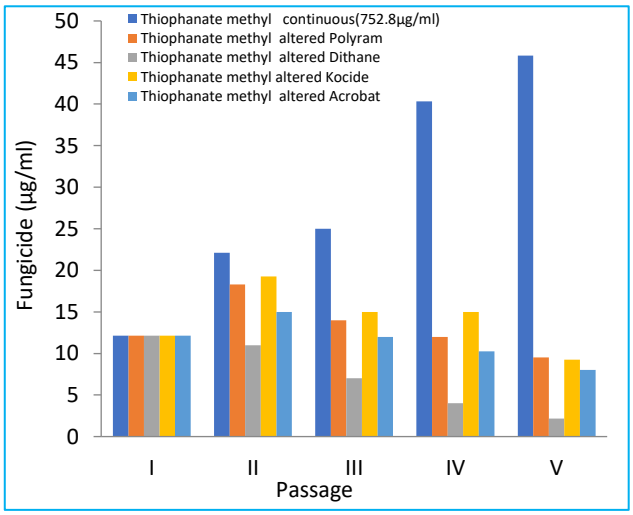

Fig 1 Effect of continuous and alternate passage and passage on the development of thiophanate methyl resistance in *P. expansum* on the agar plate

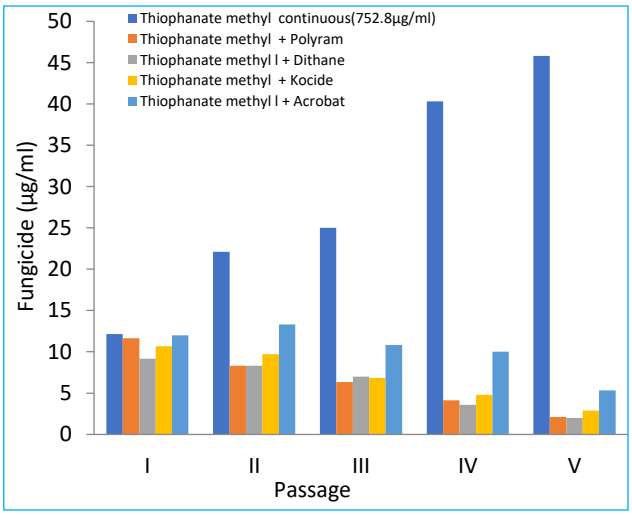

Fig 2 Effect of continuous individual passage and passage on mixed fungicide on the development of thiophanate methyl resistance in *P. expansum* on an agar plate

Table 2 Effect of continuous individual passage and passage on mixed fungicide on the development of thiophanate methyl resistance in *Penicillium expansum* on agar plate

S. No.	Fungicides (µg/ml)	Passage number				
		I	II	III	IV	V
1	Thiophanate methyl continuous (752.8µg/ml)	12.15	22.12	25.00	40.33	45.84
2	Thiophanate methyl + Polyram	11.66	08.30	06.32	04.12	02.11
3	Thiophanate methyl + Dithane	09.16	08.30	07.00	03.60	02.00
4	Thiophanate methyl + Kocide	10.67	09.72	06.84	04.8	02.90
5	Thiophanate methyl + Acrobat	12.00	13.33	10.84	10.00	05.33

a = Linear growth as % of control,
* = Significance at p=0.05 and p* =0.01 with Wilcoxon's (1939) sum rank
Test results are compared to that of the first passage

The use of thiophanate methyl continuous growth was increased in all passage. It was a mixture of Polyram, Dithane Z-78, and Kocide with thiophanate methyl; the growth of *Penicillium expansum* was inhibitory effective or decreased the growth of the mold pathogen. However, when acrobat was mixed with the thiophanate methyl, the *Penicillium expansum* growth increased in the second passage. In passage 3rd, 4th and 5th, growth was observed to decrease (Table 2).

In vivo studies

The sensitive isolate (Pe-10) of *Penicillium expansum* was inoculated into pears after being dipped in a fungicide concentration. After 15 days, the percentage of infection zone diameter was measured.

The influence of continuous and alternating passage on establishing thiophanate methyl resistance in *Penicillium expansum* on pear. Thiophanate methyl was altered with Polyram, Dithane Z-78, Kocide, and acrobat for five successive passages. Results are indicated in (Table 3) that treatment of pear fruits for 5- successive passage increased the growth significantly. Similarly, the infection was also increased when thiophanate methyl treatment was altered with Polyram in the second passage; passage 3rd, 4th, and 5th decreased the growth of *Penicillium expansum*. In Dithane Z-78, Kocide and acrobat altered with thiophanate methyl decreased the infection of all five passages.

Polyram, Dithane Z-78, Kocide, and acrobat were combined with thiophanate methyl, and pears were treated for five consecutive passages. Results shown in (Table 4) that continuous use of thiophanate methyl, thiophanate methyl with Polyram, Dithane Z-78, Kocide, and acrobat decreased infection or inhibited the *P. expansum* in all passages except thiophanate methyl continuous infection of the pathogen increased infection in all five passages.

Table 3 Effect of continuous and alternate passage and passage on the development of thiophanate methyl resistance in *Penicillium expansum* on pear

S. No.	Fungicides (µg/ml)	Passage number				
		I	II	III	IV	V
1	Thiophanate methyl continuous (752.8µg/ml)	18.75	20.00	27.50	38.75	46.25
2	Thiophanate methyl altered Polyram	18.25	21.25	16.75	12.50	09.25
3	Thiophanate methyl altered Dithane	18.75	12.50	10.75	09.00	05.75
4	Thiophanate methyl altered Kocide	18.75	16.25	13.00	07.50	04.75
5	Thiophanate methyl altered Acrobat	18.75	15.00	13.75	10.50	07.50

a = PDI as % of control,
* = Significance at p= 0.05 and p=0.01 with Wilcoxon's (1939) sum rank
Test results were compared with the first passage to that of other passages

Table 4 Effect of continuous individual passage and passage on mixed fungicide on the development of thiophanate methyl resistance in *Penicillium expansum* on pear

S. No.	Fungicides (µg/ml)	Passage number				
		I	II	III	IV	V
1	Thiophanate methyl continuous (752.8µg/ml)	18.75	20.00	27.50	38.75	46.25
2	Thiophanate methyl + Polyram	15.00	10.50	09.25	06.25	04.25
3	Thiophanate methyl + Dithane	12.50	13.00	10.50	09.75	05.50
4	Thiophanate methyl + Kocide	13.75	12.50	10.75	08.50	06.00
5	Thiophanate methyl + Acrobat	12.50	08.25	05.75	04.50	02.50

a = PDI as % of control,
* = Significance at p= 0.05 and p=0.01 with Wilcoxon's (1939) sum rank
Test results were compared with the first passage to that of other passages

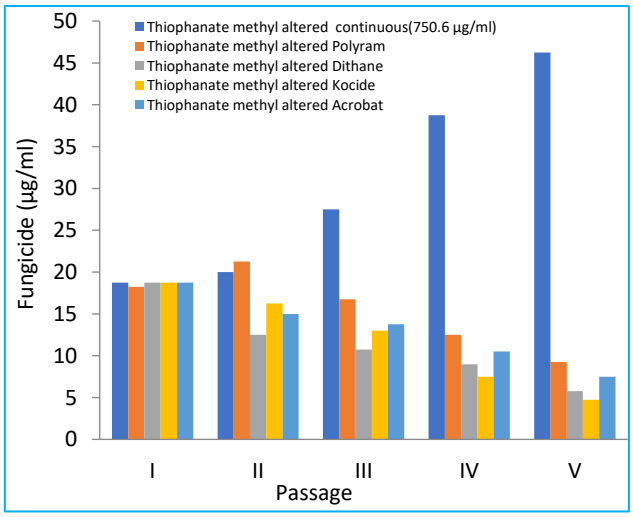

Fig 3 Effect of continuous and alternate passage and passage on the development of thiophanate methyl resistance in *P. expansum* on pear

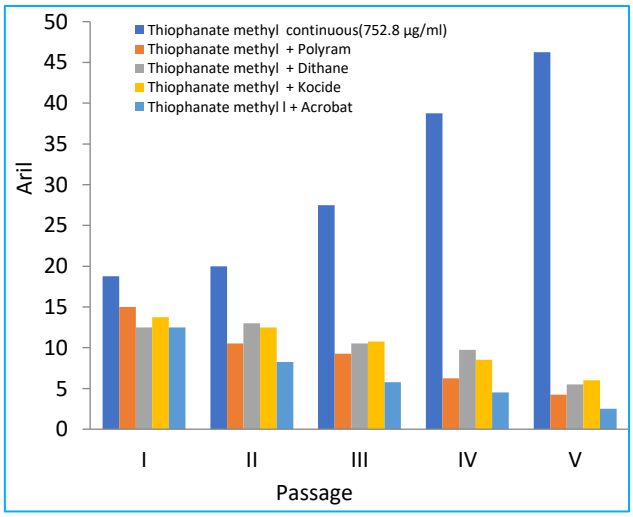

Fig 4 Effect of continuous individual passage and passage on mixed fungicide on the development of thiophanate methyl resistance in *P. expansum* on pear

All of them agree that resistant mutants emerge due to spontaneous mutation under the selection pressure of a fungicide. High resistance may develop in the *Penicillium expansum,* causing blue mold in the pear. In the present finding, thiophanate methyl, acrobat, Polyram, Kocide, and Dithane Z-78 are always used to manage blue mold and other fruit diseases [7-9]. Fungicide administration can potentially influence the establishment of resistance in plant pathogens. On agar plates and pear fruits, the effects of repeated exposure to *Penicillium expansum* (Pe-10 isolate) on thiophanate methyl were examined, either alone, in rotation, or in combination with other fungicides operating via distinct mechanisms.

In this investigation, the continuous passage of sensitive isolate on thiophanate methyl five successive passage increased the growth significantly at the 5th passage. This indicates that the development of thiophanate methyl resistance in the pathogen. But culturing of the isolate alternately or in a mixture with acrobat, Polyram, Kocide, and Dithane Z-78 completely inhibited the growth of sensitive isolate (Pe-10) on the potato dextrose agar plates and the fruits. Fungicides used alternately should have different modes of action [10]. The current study confirmed that using different specific site-inhibiting fungicides, such as acrobat, Polyram, Kocide, and Dithane Z-78, reduces the likelihood of resistance mutations in *Penicillium expansum*. Edifenphos may be used against *Septoria nodorum* and *Cercospora herpotrichoides*, two bacteria resistant to carbendazim [11]. In order to investigate potential new applications of fungicide proposed mathematical models [12]. The pathogen in this study likely developed resistance to thiophanate methyl, acrobat, Polyram, Kocide, and Dithane Z-78 in combination, as previously shown in *Macrophomina phaseolina* [13].

CONCLUSION

The continuous application of thiophanate methyl demonstrated an increase in resistance both *in vitro* and *in vivo*. The experiment revealed that alternating the use of thiophanate methyl with specific fungicides resulted in improved inhibition of the pathogen, both *in vitro* and *in vivo*. The most promising results were observed when thiophanate methyl was used in combination with select fungicides. This combination strategy demonstrated superior efficacy during both *in vivo* and *in vitro* applications, indicating a synergistic effect in managing the pathogen. Among the tested fungicides, the combination of acrobat with thiophanate methyl emerged as the most effective fungicide for the management of *Penicillium expansum*, the causative agent of blue mold disease in pears.

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