



## Short communications

**Effect of passage on the development of thiophanate methyl resistance in *Alternaria alternata* (Fries) Keissler causing brown spot of tobacco**Sonali M. Kumbhar<sup>1\*</sup>, S. K. Mengane<sup>1</sup> and S. T. Ingle<sup>2</sup><sup>1</sup>Department of Botany, M. H. Shinde Mahavidyalaya, Tisangi 416 206, Maharashtra, India<sup>2</sup>Department of Botany, DKASC College, Ichalkaranji 416 115, Maharashtra, India

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**Abstract**

This study was conducted to investigate the *in vivo* development of thiophanate-methyl resistance in *Alternaria alternata* (Fries) Keissler, the causal agent of brown spot disease in tobacco. The experiment evaluated the effects of repeated fungicide exposure through three different passage strategies: continuous, alternate and mixture. A sensitive isolate of *A. alternata* obtained from Jainyal village, Kolhapur, Maharashtra, was subjected to eight successive passages with thiophanate-methyl (200 ppm). Continuous exposure led to a significant increase in both fungicide resistance and disease infection. In contrast, alternate and mixture passage effectively suppressed the development of resistance and reduced infection rates. Alternating thiophanate-methyl with chlorothalonil and copper oxychloride (200 ppm each) resulted in inhibition of pathogen growth by the 2<sup>nd</sup> passage, reducing the disease infection and showed only 10% and 20% infection, respectively. Similarly, alternating thiophanate-methyl with carbendazim and mancozeb (200 ppm each) achieved a notable reduction in infection by the 1<sup>st</sup> passage. Mixtures of thiophanate-methyl with carbendazim and copper oxychloride separately, also suppressed pathogen growth in the 2<sup>nd</sup> passage, with corresponding infection levels of 10% and 20%. Notably, combining thiophanate-methyl with chlorothalonil and mancozeb (200 ppm each) led to complete inhibition of both pathogen growth and infection at the 1<sup>st</sup> passage. These findings highlight the effectiveness of fungicides used alternately and in mixed form, in decreasing resistance development and managing *A. alternata* infections in tobacco.

**Keywords:** *Alternaria alternata*, Mancozeb, Passage, Thiophanate-methyl, Tobacco

India is an agricultural country, recognized as the second-largest producer and manufacturer of tobacco and exports it on a large scale. According to the Tobacco Board of Andhra Pradesh (2023), India has exported the tobacco and its products approximately 2,91,181.74mt and has earned 9,739.75 crore during 2022-2023. Tobacco is used as traditional and folk medicine in various countries. The leaves are traditionally used as tranquilizers, spasmolytics, and help to kill parasites, as well as to treat poisonous bites and stings, strangulated hernia, orchitis, skin diseases, ringworms, wounds, and ulcers, and the plant itself has insecticidal properties. The use of mancozeb + carbendazim at the rate of 300ppm inhibited 96.90% mycelial growth of *A. alternata* *in vitro*, while under field conditions, the two sprays of mancozeb and carbendazim were used in mixture (0.2%), the blight of fennel disease is inhibited by 71.53% (Meena et al., 2020).

The management of *A. alternata* leaf spot on Ber under field conditions was studied by using three different fungicide mixtures: Tebuconazole 25% + Trifloxystrobin 50% WG, Azoxystrobin 18.2% + Difenoconazole 11.4% SC, and

Carbendazim 12% + Mancozeb 63% W Peach applied at 0.05%. These fungicide mixtures resulted in disease intensity reduction and effective disease control by 76.67%, 72.50%, and 65.42%, respectively. (Kumar and Singh, 2020). The disease leaf blight of blond Psyllium caused by *Alternaria alternata* was investigated under field conditions using a combination of Mancozeb and Carbendazim (0.2%) which resulted in a 31.07% reduction in disease intensity, while the application of Mancozeb alone at 0.25% reduced the disease intensity by 20.71% (Choudhary et al., 2017).

Under field conditions, the intensity of fruit rot disease of Chilli caused by *Alternaria alternata* was significantly reduced by 83.72% by applying two foliar sprays of the formulation of tebuconazole (50%) and trifloxystrobin (25%) at a concentration of 0.20% applied at an interval of 15 days (Ginoya and Gohel, 2015). Sequential applications of chlorothalonil (0.2%), thiophanate-methyl (0.10%), and copper oxychloride (0.3%) effectively mitigated the incidence of *Chrysanthemum* leaf blight disease caused by *A. alternata* (Fr.) Keissler (Kamanna et al., 2010). A mixture of Ziram and Mancozeb (0.3%) was highly effective in

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controlling *Alternaria* species which causes blight of Sunflower (Sindhamathar et al., 1976). The leaf spot disease of cauliflower caused by *Alternaria* was significantly reduced *in vivo* using Mancozeb and Azoxystrobin at concentrations of 0.25% and 0.1%, respectively (Valvi et al., 2019). The fungicide Score (Difenoconazole 25% EC), at a concentration of 0.05%, demonstrated the highest efficacy in controlling tomato blight (Kumar et al., 2017). In managing *Alternaria alternata* which causes Gerbera blight, various fungicides such as tricyclazole (0.1%), Bordeaux mixture (0.6%), and a combination of iprodione and carbendazim (0.1%) were effective in controlling the disease by 96.59%, 95.85% and 95.88% respectively under polyhouse conditions (Nagrle et al., 2012). In the management of chili leaf spot, sequential treatments with Indofil M-45 (0.2%), Indofil Z-78 (0.2%), Chlorothalonil (0.2%), and Bavistin (0.1%) resulted in disease control by 62.3%, 57.5%, 58.0%, 58.7% respectively (Kumar et al., 2013). In the study on damping-off and wilt diseases of watermelon caused by *Fusarium oxysporum* f. sp. *niveum*, reported that the fungicide thiophanate-methyl (Topsin-M) reduced pre-emergence damping-off and wilt incidence by 44.4% and 72.9%, respectively, under greenhouse conditions. Under field conditions, application of thiophanate-methyl resulted in a reduction of pre-emergence damping-off and wilt by 73.7% and 67.7%, respectively (Sallam, 2009).

A two-year study on white mold disease of snap bean, caused by *Sclerotinia sclerotiorum* was conducted, in the 2015 growing season, disease incidences of 26.9%, 25.2%, and 14.2% were recorded following applications of boscalid (126.2 g/ha), fluazinam (162.8 mL/ha), and thiophanate-methyl (35.9 mL/ha), respectively. In 2016, the same treatments resulted in substantially lower incidences of 1.9%, 0.6%, and 7.6%, respectively (Lehner et al., 2017). The management of black scurf disease in potato, caused by *Rhizoctonia solani* was demonstrated that the application of a fungicidal combination of thiophanate-methyl 45% + pyraclostrobin 5% FS at a rate of 20.0 mL/100 kg of seed was highly effective. This treatment led to a significant reduction in disease incidence, 83.7% in the 2012–13 season and 67.5% in 2013–14; as well as disease severity, which was reduced by 82.6% and 60.4% during the respective seasons (Lal et al., 2017). In the experiment of cumin wilt caused by *Fusarium oxysporum* f. sp. *cumini*, the efficacy of various fungicides applied as foliar sprays at a concentration of 2000 ppm. The treatments included carbendazim (50% WP), tebuconazole 50% + trifloxystrobin 25% (75 WG), pyraclostrobin 5% + metiram 55% WG, carbendazim 12% + mancozeb 63%, and thiophanate-methyl (70% WP). Disease control efficiencies were recorded as 80.51%, 67.84%, 64.63%, 76.80%, and 67.93%, respectively,

indicating significant suppression of the wilt pathogen (Jangir et al., 2022). The efficacy of fungicides against wilt diseases in pepper under both greenhouse and field conditions was demonstrated, under greenhouse trials, thiophanate-methyl (0.10%) achieved the highest disease control (83.10%) against *Verticillium* wilt caused by *Verticillium dahliae*, compared to untreated controls. In field experiments of *Fusarium* wilt caused by *Fusarium oxysporum*, the highest disease control was observed with captan (at 0.30% concentration) (95.60%), while thiophanate-methyl, applied at a 0.10% concentration, resulted in the lowest control efficacy (54.40%) (Mihajlović et al., 2021).

The application of fungicides remains a widely accepted practice for crop disease management. The continuous use of a single fungicide may lead to the development of pathogen resistance. Utilizing fungicides with different modes of action may help overcome this resistance and reduce the infection percentage. Consequently, this study aims to investigate the development of resistance to thiophanate-methyl in *A. alternata* and explore strategies to overcome this resistance by using fungicides with distinct mechanisms of action.

### **Passage effect in the establishment of resistance to thiophanate-methyl in *Alternaria alternata***

#### *Experimental site:*

The experiment was conducted from August 2023 to May 2024 at Lakhanapur village, Tehsil Nipani, District Belgaum, State of Karnataka, India (Pin code: 591 237). The village is situated at 16.4332° N latitude and 74.4013° E longitude. The location lies within the Northern Transition Agro-climatic Zone of Karnataka. The average annual rainfall is 1000mm to 1300mm. The soil type at the site is classified as deep black, silt-loamy soil.

#### *In vivo studies:*

The spore suspension (30ml,  $1 \times 10^6$  conidia/ml) of pathogen *A. alternata* was introduced on 30 days old tobacco plants sprayed with thiophanate-methyl (70% WP) (@200ppm) continuously, alternately, and in a mixture with other fungicides (@200ppm). The fungicides like chlorothalonil (75%WP), mancozeb (75%WP), carbendazim (50%WP) and copper oxychloride (50% WP) were used. The concentrations of spore suspension and all fungicides, age of test plant, were kept constant for every passage. Ten plants for each fungicidal treatment were used. The experiment was carried out in triplicate at above mentioned location. The percentage of infection over untreated control plants was calculated.

#### *Continuous, Alternate and Mixture passage*

To investigate the impact of continuous passage in the





Table 2. Exposure of *Alternaria alternata* to Thiophanate-methyl continuously and in a mixture with various fungicides(*In vivo*) Fungicides (200ppm)

	Passage Number and Percentage of infection (%)							
	1	2	3	4	5	6	7	8
Thiophanate-methyl (70% WP) continuous	20.00	30.00	40.00	50.00	50.00	60.00	60.00	70.00
Thiophanate-methyl(70% WP) + Chlorothalonil (75% WP)	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00
Thiophanate-methyl (70% WP) + Mancozeb (75% WP)	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00
Thiophanate-methyl (70% WP) + Carbendazim 50%	10.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00
Thiophanate-methyl (70% WP) + Copper oxychloride (50% WP)	20.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00

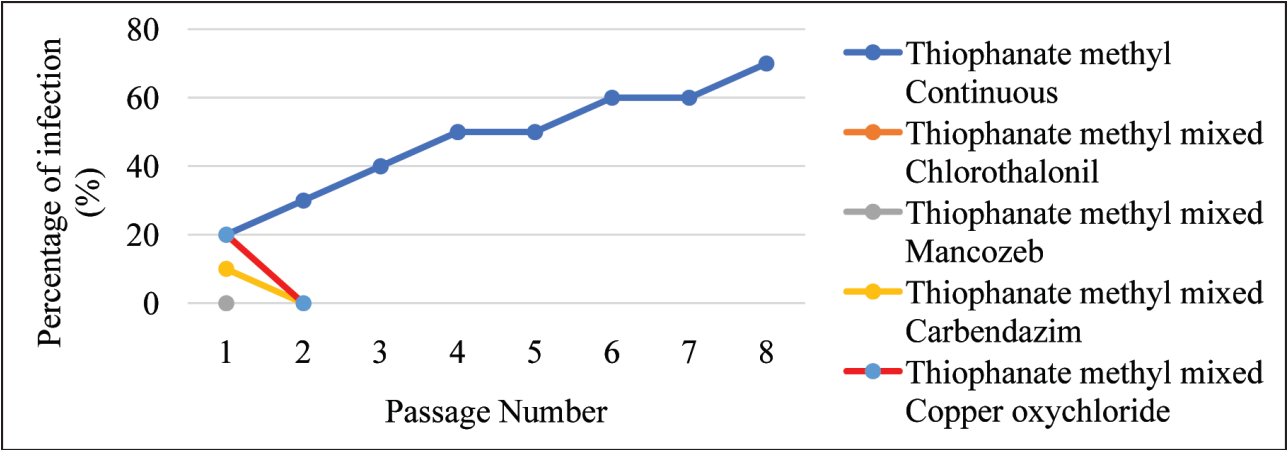


Figure 2. Dose response curve of *Alternaria alternata* to Thiophanate-methyl used continuously and in a mixture with different fungicides in developing the resistance till eight successive passages.

application of carbendazim with Ridomil, Dhanuka, and Kocide resulted in complete inhibition of the pathogen's growth by the second passage in an *in vivo* experiment. Vihol et al. (2009) evaluated the efficacy of various fungicides under field conditions and found that mancozeb (0.2%) was

the most effective in controlling blight of cumin caused by *Alternariaburnsii* followed by copper oxychloride (0.25%) and thiophanate-methyl (0.03%). Desai et al. (2015) reported that the management of pigeon pea wilt caused by *Fusarium udum* was highly effective when benomyl was alternated

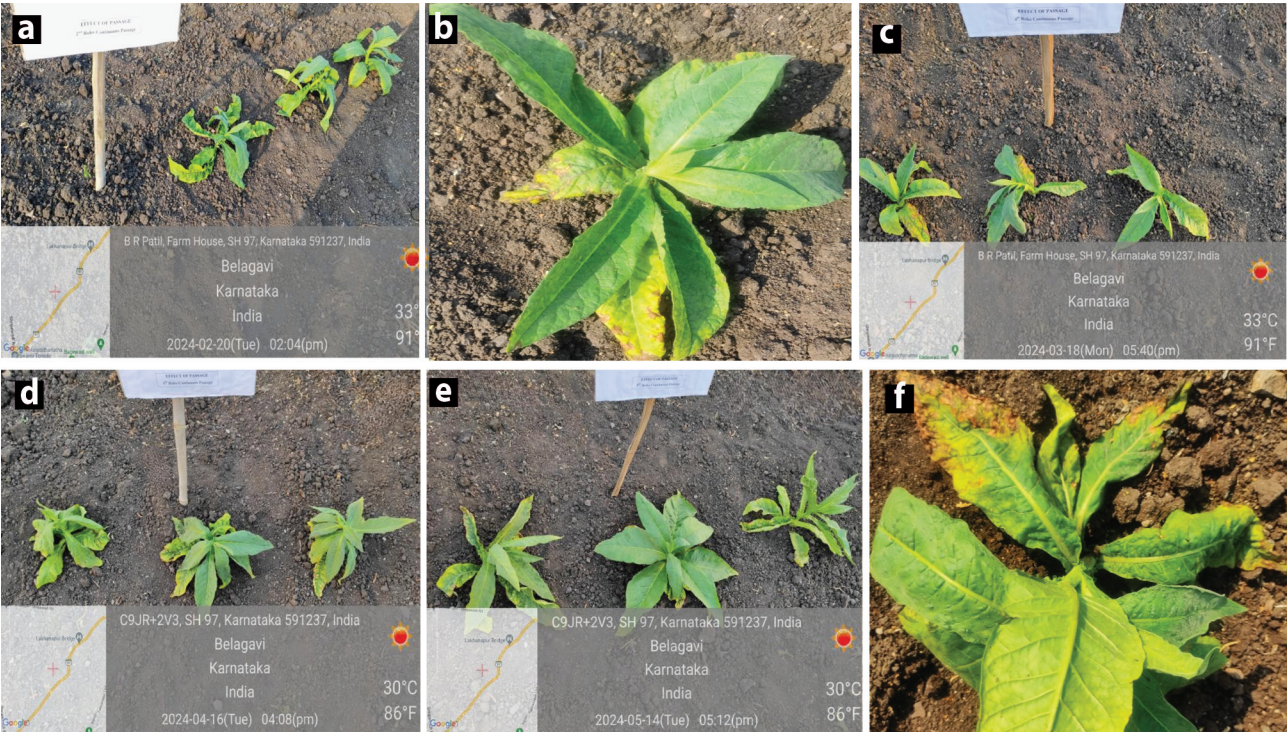


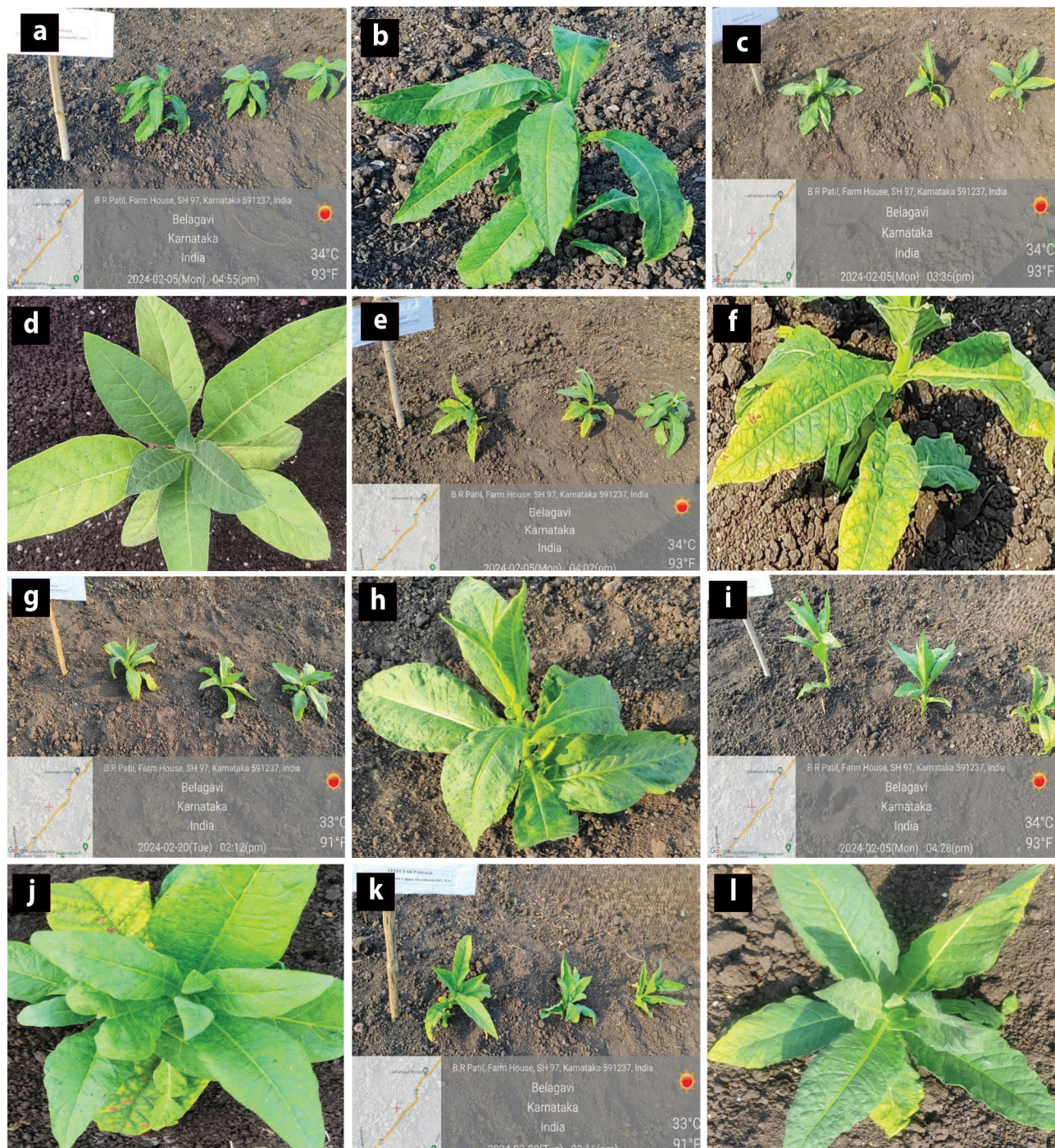
Figure 3. Effect of continuous passage on the development of thiophanate-methyl resistance in *Alternaria alternata*. a. 2<sup>nd</sup> Passage, Thiophanate-methyl continuous, b. 2<sup>nd</sup> Passage, Thiophanate-methyl continuous (plant showing symptoms), c. 4<sup>th</sup> Passage, Thiophanate-methyl continuous d. 6<sup>th</sup> Passage, Thiophanate-methyl continuous e. 8<sup>th</sup> Passage, Thiophanate-methyl continuous f. 8<sup>th</sup> Passage, Thiophanate-methyl continuous (plant showing symptoms)



with Roko (thiophanate-methyl), leading to complete elimination of infection (100%) by the third passage.

In the mixture passage, when thiophanate-methyl was combined with chlorothalonil and copper oxychloride (both

applied at 200 ppm) separately, and subsequently applied to the plants, a significant inhibition of pathogen growth was observed after the 2<sup>nd</sup> passage, resulting in reduction of infection percentage by 10% and 20%, respectively. In contrast, when thiophanate-methyl was mixed with

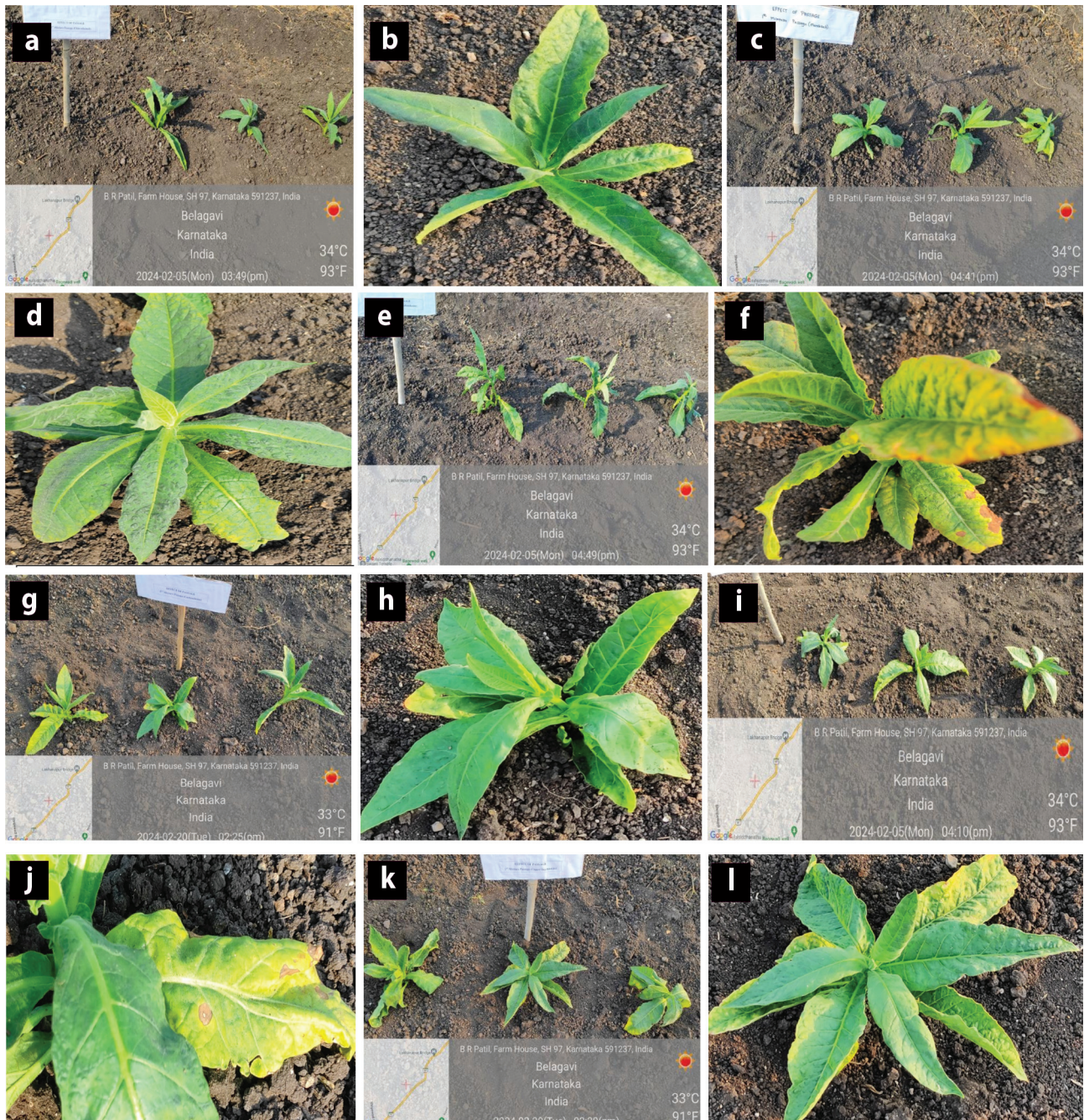


**Figure 4.** Effect of alternate passage on the development of Thiophanate-methyl resistance in *Alternaria alternata*. **a.** 1<sup>st</sup> Passage, Thiophanate-methyl alternate Carbendazim **b.** 1<sup>st</sup> Passage, Thiophanate- methyl alternate Carbendazim (plant without symptoms) **c.** 1<sup>st</sup> Passage, Thiophanate-methyl alternate Mancozeb **d.** 1<sup>st</sup> Passage, Thiophanate-methyl alternate Mancozeb (plant without symptoms) **e.** 1<sup>st</sup> Passage, Thiophanate-methyl alternate Chlorothalonil **f.** 1<sup>st</sup> Passage, Thiophanate-methyl alternate Chlorothalonil (plant showing symptoms) **g.** 2<sup>nd</sup> Passage, Thiophanate-methyl alternate Chlorothalonil **h.** 2<sup>nd</sup> Passage, Thiophanate-methyl alternate Chlorothalonil (plant without symptoms) **i.** 1<sup>st</sup> Passage, Thiophanate-methyl alternate Copper oxychloride **j.** 1<sup>st</sup> Passage, Thiophanate-methyl alternate Copper oxychloride (plant with symptoms) **k.** 2<sup>nd</sup> Passage, Thiophanate-methyl alternate Copper oxychloride (plant without symptoms) **l.** 2<sup>nd</sup> Passage, Thiophanate-methyl alternate Copper oxychloride



mancozeb and carbendazim (both applied at 200 ppm) separately and sprayed on plants, disease infection was completely inhibited at the 1<sup>st</sup> passage by delaying the thiophanate-methyl resistance in pathogen (Table 2, Fig. 2 & Fig.5).

*Alternaria alternata* (Fr.) Keissl. is the etiological agent of brown spot disease, which affects a wide range of crops, leading to both qualitative and quantitative yield losses. Due to severe disease conditions, farmers use various fungicides to manage its spread. The present study was conducted to evaluate the effect of different fungicides, applied in



**Figure 5.** Effect of Mixture passage on the development of Thiophanate-methyl resistance in *Alternaria alternata* **a.** 1<sup>st</sup> Mixture Passage, Thiophanate-methyl + Chlorothalonil **b.** 1<sup>st</sup> Mixture Passage, Thiophanate-methyl + Chlorothalonil (plant without symptoms) **c.** 1<sup>st</sup> Mixture Passage, Thiophanate-methyl + Mancozeb **d.** 1<sup>st</sup> Mixture Passage, Thiophanate-methyl + Mancozeb (plant without symptoms) **e.** 1<sup>st</sup> Mixture Passage, Thiophanate-methyl + Carbendazim **f.** 1<sup>st</sup> Mixture Passage, Thiophanate-methyl + Carbendazim (plant with symptoms) **g.** 2<sup>nd</sup> Mixture Passage, Thiophanate-methyl + Carbendazim **h.** 2<sup>nd</sup> Mixture Passage, Thiophanate-methyl + Carbendazim (plants without symptoms) **i.** 1<sup>st</sup> Mixture Passage, Thiophanate-methyl + Copper oxychloride **j.** 1<sup>st</sup> Mixture Passage, Thiophanate-methyl + Copper oxychloride (plant showing symptoms) **k.** 2<sup>nd</sup> Mixture Passage, Thiophanate-methyl + Copper oxychloride **l.** 2<sup>nd</sup> Mixture Passage, Thiophanate-methyl + Copper oxychloride (plant without symptoms)



continuous, alternate, and mixed forms, in inhibiting the development of *A. alternata* resistance to thiophanate-methyl and in reducing both disease infection and pathogen growth. Current investigation revealed the successful establishment of resistance in the pathogen by continuous application of thiophanate-methyl and the use of other fungicides like carbendazim, mancozeb, chlorothalonil, and copper oxychloride alternately and in a mixture with thiophanate methyl helped to slow down the thiophanate-methyl resistance in *A. alternata* and reduced pathogen growth. These findings are consistent with the observations of Dekker (1981), Mathivanan and Prabavathy (2007), Sable and Gangawane (2012), who stated that the use of fungicide combinations is an effective strategy for managing the development of fungicide-resistance in pathogens.

The disease resistance in *A. alternata* was decreased by using carbendazim (0.5%) in mixed and in alternate form with thiophanate-methyl, Dithane M-45, ridomil and curzate, and has been proven best to control root rot of fenugreek, investigated by Khandare (2014). Khilare and Gangawane (1998) reported the development of resistance by continuous application of thiophanate-methyl and then the use of thiophanate-methyl alternately and in mixed form with Captan and Dithane M-45 was found to reduce the resistance in *Penicillium digitatum* and proved effective in the management of green mold disease.

Field experiments were conducted by Romana et al. (2019) for three-years period to control *C. manginecans*, the pathogen responsible for mango quick decline. The application of Bavistin (carbendazim) in combination with thiophanate-methyl at a concentration of 750 ppm resulted in a significant reduction in disease incidence by 40% in 2014, 36% in 2015, and 39% in 2016. Baviskar and Rathod (2023) conducted an experimental study to control blue mold disease in pear. Thiophanate-methyl, when applied in combination with Polyram (Metiram 70% WG), Dithane Z-78, Kocide, and Acrobat, resulted in a significant reduction in the resistance of *Penicillium expansum* at 5<sup>th</sup> passage. The effect of mixture passage of carbendazim with fungicides such difenoconazole, myclobutanil and propiconazole on *Gloeosporium ampelophagum*, the causative agent of grape anthracnose, was studied by Khilare and Chavan (2011) and reported that the disease incidence was reduced at 5<sup>th</sup> passage treatment by decreasing the resistance in the pathogen. Mueller et al. (2004) reported that the application of thiophanate-methyl at rates of 0.56 kg a.i./ha and 1.12 kg a.i./ha during the reproductive growth stage of soybean significantly reduced the incidence of stem rot caused by *Sclerotinia sclerotiorum*, and also resulted in increased crop yield. Gowdar et al. (2021) evaluated the bio-efficacy of thiophanate-methyl ranging from 857 to 1143 g/ha, these

doses were found to be effective in controlling blast disease, sheath rot, and paddy grain discoloration in rice, resulting higher yield of rice. Malathi et al. (2004) demonstrated that pre-planting treatment of sugarcane setts in a 0.25% thiophanate-methyl suspension for a duration of 24 hours was significantly more effective in reducing debris-borne infections by *Colletotrichum falcatum* compared to 1 hour treatment at higher concentrations. Moreover, thiophanate-methyl treatment is better than the use of carbendazim in controlling red rot disease of sugarcane. Mathivanan and Prabavathy (2007) conducted an *in vivo* study demonstrating that a mixture of carbendazim and mancozeb at a concentration of 2.0 g/L was highly effective in reducing leaf blight of sunflower caused by *Alternaria helianthi*. Across three separate experiments, this treatment resulted in disease reductions of 55.7%, 32.7%, and 48.6%, respectively, compared to other fungicidal treatments and the unsprayed control.

This experiment demonstrated that the use of fungicides in alternating form or combinations of different fungicides at low concentrations was more effective than the application of individual fungicides in suppressing pathogen growth and reducing disease infection in the host plant. Moreover, the use of thiophanate methyl with carbendazim, mancozeb, chlorothalonil and copper oxychloride in different mode of actions yielded better results. Additionally, lower application rates in such mixtures can enhance crop safety and improve economic efficiency, making this approach a sustainable strategy within integrated disease management programs.

## Conclusion

The present study demonstrated that continuous application of thiophanate-methyl (70% WP) resulted in increased fungicide resistance in the pathogen which was indicated by increased percentage of infection upto the 8<sup>th</sup> passage. In contrast, alternating thiophanate-methyl with each fungicide such as, chlorothalonil, mancozeb, carbendazim and copper oxychloride and applying each individual fungicide separately in combination with thiophanate-methyl, effectively reduced the developed resistance, significantly inhibited pathogen infection and pathogen growth in infected tobacco plants.

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