Field evaluation of Indofil M-45 against Fruit rot (*Colletotrichum* sp.) Leaf spot (*Alternaria* and *Cercospora*) disease of Pomegranate

Abstract:

The pomegranate (*Punica granatum* L.), is one of the ancient and highly praised favorite fruit belongs family Lythraceae of Myrtales order which is mainly grown in tropical and subtropical regions of the world. In recent years for the successful cultivation of pomegranate, faced by many constraints, among them, pest and diseases are the major ones. Common pathogens of pomegranate leaf and fruits include Anthracnose and Cercospora and Alternaria leaf spot and these pathogens cause severe loss to pomegranate crop. Therefore, a field experiment was carried out on the effect of Indofil M- 45 against leaf spots and fruit rot of pomegranate during 2018-19 and 2019-2020 at College of Horticulture, Hiriyur. Experimental results revealed that all the treatments significantly reduced the leaf spots and fruit rot disease severity over untreated control. Amongst all the treatments in both seasons. Indofil M-45 atat gm/liter was most effective in management leaf spots (15.50 and 16.17 PDI), anthracnose (10.55 and 8.33 PDI) with a yield of 4.18 tons/ha and 3.59 tons/ ha respectively followed by the same fungicides at 3.00gm/liter.

Key Words: Pomegranate, Anthracnose, Cercospora and Alternaria leaf spot and Indofil M-45

Introduction:

Pomegranate (*Punica granatum* L.) is one of the oldest known edible fruits and is capable of growing in different agro-climatic conditions ranging from tropical to sub-tropical (Levin, 2006; Jalikop, 2007). Though, it is native of Iran but cultivated extensively in Mediterranean and central Asian countries. It is highly suitable for growing under arid and semiarid regions due to its

versatile adaptability, hardy nature, low-cost maintenance and high returns. In the recent past its wide significance in health, nutrition and livelihood security has been recognized which resulted in heavy demand for fruit consumption not only in India but throughout the globe. In India, pomegranate is commercially cultivated in Maharashtra, Karnataka and Andhra Pradesh and the most important cultivar in this pomegranate belt is 'Bhagwa' which covers around 80% area under pomegranate in Maharashtra (Bassiri, 2018; Castagna et al., 2020; Wang et al., 2018). In last two decades, its cultivation has popularized in arid and semi-arid regions of India, not only because of its sweet acidic taste, precocious bearing, and better shelf-life but as a remunerative crop as well (Anon., 2004). Among these leaf/fruit spot caused by various organisms such as, *Colletotrichum gloeosporioides, Cercospora punicae, Alternaria alternata*, *Sphaceloma punicae*, *Drechslera* sp., and *Phomopsis* sp., take a heavy toll on the crop (Jamadar and Patil, 2007). This results in drastic reduction in the yield as well as ultimate marketability by way of severe spotting of the produce. Several conventional fungicides are being used by the farmers with no avail. Hence there is a need to explore chemical formulations with higher doses, which are highly efficient in managing these diseases effectively. Hence an attempt was made to identify the performance of the higher dose molecules against the leaf spots and fruit rot of pomegranate.

Material and methods:

Field experiment was conducted in two *Kharif* cropping seasons of 2018-19 and 2019-20 on the management of anthracnose (*Colletotrichum gloeosporioides*), Cercospora and Alternaria leaf spot (*Cercospora punicae* and (*Alternaria* spp.) on pomegranate crop at College of Horticulture, Hiriyur, UAHS, Shivamogga, Karnataka. The soil of the experimental field was red sandy loam which was acidic to neutral in reaction. The experiment was laid out with Randomized Block Design (RBD). The experiment consists of 5 treatments including control and were laid out in plots with size 8m X 6 m (48 m^2) with spacing 12 ft X 10 ft and the variety used was Super Bhagwa. The treatment fungicides were applied to the Pomegranate field at beginning of the disease's appearance. The spray schedule was repeated at 15 days intervals. The observation of incidence of Leaf spots, Fruit spots and Anthracnose diseases was assessed by using the 1-9 score chart and the percent disease index (PDI) was calculated for each spray as under.

The percent disease index (PDI)was calculated by the following formula which was given by Wheeler, 1969 and Fruit yield per plant wise were recorded

$$\text{Per cent disease index (PDI)} = \frac{\text{Sum of the individual disease ratings}}{\text{Number of leaves/fruits observed}} \times \frac{100}{\text{Maximum disease grade}}$$

Chart 1: Treatment details along with checks:		
	Treatments	**Formulation (ml or g/litre)**
1	Indofil M-45	2.0
2	Indofil M-45	3.0
3	Indofil M-45	4.0
4	Propineb 70% WP	3.0
5	Untreated check	NA

Chart 2 : Phytotoxicity

Sl. No	Treatments	Formulation (ml/g/Litre)
1	Indofil M-45	2.0g
2	Indofil M-45	3.0g
3	Indofil M-45	4.0 g
4	Untreated check	NA
5	Indofil M-45	8.0 g
6	Indofil M-45	16.0 g

Phytotoxicity on Pomegranate crop

Phytotoxicity observations were recorded at 0, 1, 3, 5, 7 & 10 days after each spray of different treatments as per phytotoxicity parameters

Chart 3: Scores for Phytotoxicity

Sr.	Phytotoxicity (%)	Score
1	No phytotoxicity	0
2	0-10	1
3	11-20	2
4	21-30	3
5	31-40	4
6	41-50	5
7	51-60	6
8	61-70	7
9	71-80	8
10	81-90	9
11	91-100	10

Statistical Analysis:

The experimental data collected were analyzed statistically for its significance of difference by the normal statistical procedure adopted for randomized block design. Data from the percent disease index and yield were analyzed by ANOVA. Percent data were transformed arcsine where necessary. Differences within the means were compared by using Fisher's LSD

(Least Significant Difference) test (Walter, 1997. The level of significance used in ‘F’ and ‘T’ test was P = 0.05 and P = 0.01. Critical differences were calculated wherever ‘F’ test was significant. The values percent disease index was subjected to angular transformation according to the table given by Sundarraj *et al*. (1974).

Results and Discussion:

Efficacy of Indofil M-45 against Fruit rot (*Colletotrichum* sp.) disease of Pomegranate during 2018-19 and 2019-2020

The efficacy of the different treatments during three sprays against fruit rot (*Colletotrichum* Sp.) in Pomegranate during 2018-19 is presented in Table-1.

Before the spray, the infestation of Fruit rot (*Colletotrichum* Sp.) was uniform in all the treatments and ranged PDI between 0.82 and 1.0. At 10 days after first application, Indofil M-45, the at 4.0 gm/liter was found superior in reducing the fruit rot incidence (3.58 PDI) of Pomegranate followed by Propineb 70% WP at 3.0 gm/lt (5.50 PDI). These were superior over rest of the treatments and these were followed by Indofil M-45 at 3.0 gm/liter recorded 6.28 PDI and IFC017 Indofil M-45 at 2.0 gm/liter recorded PDI 7.10 PDI. However, the untreated check recorded the highest fruit rot incidence by showing 15.13 PDI.

After second spray the treatment with Indofil M-45 at 4gm/liter was recorded 7.13 and Propineb at 3.0 gm/lt recorded 8.45 PDI respectively and were superior over the rest of the treatments. However, the untreated check recorded the highest fruit rot incidence by showing 25.63 PDI.

After the third spray schedule similar trend in the efficacy of treatments was recorded, wherein, Indofil M-45 at 4 gm/liter was recorded lowest fruit rot disease incidence caused by *Colletotrichum* sp. with 10.55 PDI and this plot recorded 76.22 percent reduction of disease over control plot which was followed by Propineb 70% WP which recorded PDI of 14.45 and Indofil M-45 at 3 gm/liter and 2 gm/liter recorded PDI of 15.75 and 16.23 respectively. However, the highest disease incidence was recorded in the untreated check (45.50 PDI).

The efficacy of the different treatments during three sprays against fruit rot (*Colletotrichum* Sp.) in Pomegranate during 2019-20 is presented in Table-2.

Before the spray, the infestation of Fruit rot (*Colletotrichum* Sp.) was uniform in all the treatments and ranged PDI between 0.57 and 0.82. At 10 days after first application of Indofil M-45, the at 4.0 gm/liter was found superior in reducing the fruit rot incidence of Pomegranate followed by Propineb 70% WP at 3.0 gm/lt (5.20 PDI). Then Indofil M-45 at 3.0 gm/liter recorded 6.83 PDI. These were superior over rest of the treatments and these were followed by Indofil M-45 at 2.0 gm/liter (7.10 PDI). However, the untreated check recorded highest fruit rot incidence by showing 15.13 PDI.

After second spray the treatment with Indofil M-45 at 4gm/liter was recorded PDI 5.78 and it was superior over rest of the treatments and Propineb at 3.0 gm/lt recorded 10.13 PDI. However, the untreated check recorded the highest fruit rot incidence by showing 27.50 PDI.

After the third spray schedule similar trend in the efficacy of treatments was recorded wherein, Indofil M-45 at 4 gm/liter was recorded lowest fruit rot disease incidence caused by *Colletotrichum* sp. with 8.33 PDI which was followed by Propineb 70% WP which recorded PDI of 15.85 and Indofil M-45 at 3 gm/liter and 2 gm/liter recorded PDI of 16.52 and 17.48 respectively. However, highest disease incidence was recorded in the untreated check (45.50 PDI).

Efficacy of Indofil M-45 against Leaf spot (*Alternaria and Cercospora*) disease of Pomegranate during 2018-19 and 2019-2020.

The efficacy of the different treatments during three sprays against Leaf spot (*Alternaria* and *Cercospora*) in Pomegranate during 2018-19 is presented in Table-3.

Before the spray, the infestation of Leaf spot (*Alternaria* and *Cercospora*) was uniform in all the treatments and ranged PDI between 0.82 and 1.00. At 10 days after first application, Indofil M-45 at 4.0 gm/liter and 3.0 gm/liter were found superior in reducing the leaf spot incidence (3.63 PDI and 3.88) of Pomegranate respectively which were superior over other treatments which were followed by Propineb 70% WP at 3.0 g/lt (5.25 PDI). Then Indofil M-45 at 2.0 gm/liter recorded 7.28 PDI. However, the untreated check recorded the highest fruit rot incidence by showing 14.75 PDI.

After second spray the treatment with Indofil M-45 at 4gm/liter and 3 gm/liter recorded PDI of 8.41 and 9.08 respectively which was followed by Propineb at 3.0 gm/lt recorded 10.35 PDI and were superior over rest of the treatments. However, the untreated check recorded highest fruit rot incidence by showing 25.25 PDI.

After third spray schedule similar trend in the efficacy of treatments was recorded wherein, Indofil M-45 at 4gm/liter and 3gm/liter was recorded lowest leaf spot disease incidence caused by *Alternaria* and *Cercospora* with 15.50 and 16.27 PDI respectively which was followed by Propineb 70% WP which recorded PDI of 17.40 and Indofil M-45 2 gm/liter recorded PDI of 19.15. However, the highest disease incidence was recorded in the untreated check (44.13 PDI) and in the plot treated with IFC017 (Indofil M-45) at 4gm/liter recorded 64.87 percent disease reduction over control.

The efficacy of the different treatment during three sprays against Leaf spot (*Alternaria* and *Cercospora*) in Pomegranate during 2019-20 is presented in Table-4

Before the spray, the infestation of Leaf spot (*Alternaria* and *Cercospora*) was uniform in all the treatments and ranged PDI between 0.57 and 0.82. At 10 days after first application of Indofil M-45, at 4.0 gm/liter and 3.0 gm/liter were found superior in reducing the leaf spot incidence (3.55 PDI and 3.73 PDI) of Pomegranate followed by Propineb 70% WP at 3.0 g/lt (5.30 PDI). Then Indofil M-45 at 2.0 gm/liter recorded 6.78 PDI. The untreated check recorded the highest fruit rot incidence by showing 14.88 PDI.

Similar trend in the efficacy of different treatment was recorded after second spray, wherein after second spray the treatment with Indofil M-45 at 4gm/liter and 3 gm/liter recorded

PDI of 9.45 and 9.89 respectively and were superior over rest of the treatments which was then followed by Propineb at 3.0 gm/lt which recorded PDI 11.63. However, the untreated check recorded the highest fruit rot incidence by showing 27.38 PDI.

After the third spray schedule similar trend in the efficacy of treatments was recorded wherein, Indofil M-45 at 4gm/liter and 3gm/liter recorded lowest leaf spot disease incidence caused by *Alternaria* and *Cercospora* with 16.13and 16.82 PDI respectively which was followed by Propineb 70% WP which recorded PDI of 20.23 and Indofil M-45 at2 gm/liter recorded PDI of 23.50. However, the highest disease incidence was recorded in the untreated check (45.25 PDI).

Fruit yield

The efficacy of the different treatments during three applications were found a difference in Pomegranate yield per plant during 2018-19 is presented in Table-1.

Application of Indofil M-45, the at 4.0 ml/liter recorded higher pomegranate fruit yield of 4.18 tons/ha and this treatment remained statistically superior over all the treatment. Propineb 70% WP at 3g/liter which has given yield of 3.58 tons/ha and Indofil M-45 at 3 ml/liter recorded fruit yield of 3.38 tons/ha where these two treatments were on par with each other. These were followed by the application of Indofil M-45 2ml/liter (3.19 tons/ha). However, lowest yield was recorded in the untreated check (2.32 tons/ha).

The efficacy of the different treatments during three applications were found a difference in Pomegranate yield per plant during 2019-20 is presented in Table-2.

Application of Indofil M-45, at 4.0 gm/liter recorded higher pomegranate fruit yield of 3.59 tons/ha and was on par with Propineb 70% WP at 3gm/liter which has given yield of 3.08 tons/ha. Both the treatments remained statistically superior over all the treatments. These were followed by the application of Indofil M-45 at 3 gm/liter and 2 gm/liter (2.97 tons/ha and 2.52 tons/ha). However, lowest yield was recorded in the untreated check (2.13 tons/ha).

Phytotoxic effect of Indofil M-45 for Phytotoxicity on Pomegranate crop during 2018-19 and 2019-2020.

Application of Indofil M-45 at 16.0gm/liter, 8.0gm/ liter, 4 gm/liter,3gm/liter and 2gm/liter dose rates and other tested chemicals for its phytotoxicity studies did not shown any phytotoxic symptoms like leaf injury, wilting, vein clearing, necrosis, epinasty and hyponasty at any days after treatments on pomegranate crop (Table 5).

Navale *et al*. (1998) found that Mancozeb, Copper oxy chloride, Ziram and Captan as the best fungicides for controlling leaf spot and fruit spots of pomegranate in mrigbahar caused by *Alternaria alternata, Cercospora sp., Colletotrichum gloeosporioides.* The present findings were also in agreement with the work of Gowder *et al.* (2017), Sachin and Sandeep (2016), Yadav *et al*. (2017) and Jayalakshmi *et al.* (2017). Mancozeb due to the reason of that being contact fungicide Mancozeb persist long on the stem (Suryanarayana and Rajarao 1988, Amrinder *et al.*, 2009 and Ghazanfar *et al.*, 2010) and plots that applied with longer intervals were protected. Mancozeb itself is not fungicidal and can effectively be considered a pro-fungicide which, when exposed to water, breaks down to release ethylene bis isothiocyanate sulfide (EBIS), which is then converted *viz.*, the action of UV light into ethylene bisisothiocyanate (EBI). Both EBIS and EBI are believed to be active toxicants and are thought to interfere with enzymes containing sulphydryl groups.

This fatal disruption of core enzymatic processes is postulated to inhibit or interfere with at least six different biochemical processes within the fungal cell cytoplasm and mitochondria (Kaars, 1984)

The direct effect of mancozeb upon core biochemical processes within the fungus results in inhibition of spore germination (Tate and Wood,1994; Wicks and Lee, 1984; Wong and Wilox, 2001). Mancozeb displays the characteristics of a typical multi-site protectant-only fungicide, in that following application onto the target plant, the compound remains on the leaf surface and does not penetrate through the cuticle to where systemic redistribution can occur (Kaars, 1982). Fortunately, mancozeb has an excellent record of crop safety over a wide range of crops and environmental conditions. Mancozeb does not show curative properties when sprayed onto plants where the disease has already been established. It is assumed this is because disease is already established inside the plant tissue where mancozeb cannot penetrate.

The rate of breakdown of mancozeb into EBIS and EBI can directly affect the residual activity of the compound on plant foliage. Each mancozeb particle consists of a zinc-rich shell surrounding a central nucleus of polymer-structured EBDC. This structure is extremely stable, and the low solubility of the zinc shell means EBDC can pass through this layer and be deposited on the leaf surface at a controlled rate (Kaars, 1982). Thus, the results of earlier workers are also in line with the results obtained in the present investigations.

Table.1: Bio-efficacy of Indofil M-45 against Fruit rot (*Colletotrichum* sp.) disease of Pomegranate during 2018-19

Tr. No.	Treatments	Dose Formulation (g or ml/liter)	Before spray	PDI 10 days after			% Reduction over control after 3rd spray	Yield tons/ha
				First spray	Second spray	Third spray		
T1	Indofil M-45	2.0	0.82 (4.93)	6.53* (14.79)	10.50 (18.89)	16.23 (23.76)	63.43	3.19 (10.12)
T2	Indofil M-45	3.0	0.82 (4.93)	6.28 (14.49)	10.25 (18.68)	15.75 (23.38)	64.50	3.38 (10.52)
T3	Indofil M-45	4.0	1.00 (5.97)	3.58 (10.89)	7.13 (15.47)	10.55 (18.96)	76.22	4.18 (11.74)
T4	Propineb 70% WP	3.0	0.98 (5.61)	5.50 (13.56)	8.45 (16.85)	14.45 (22.34)	67.43	3.58 (11.09)
T5	Untreated check	NA	0.94 (5.74)	15.00 (22.79)	25.63 (30.41)	44.38 (41.77)	0.00	2.32 (8.77)
	CDat5%		NS	1.20	1.50	1.13	-	0.99
	SEM		NS	0.38	0.48	0.36	-	0.32

* Mean of three replication   PDI- Per cent Disease Index   The values in the parenthesis are arc sine transformed

Table.2: Bio-efficacy of Indofil M-45 against Fruit rot (*Colletotrichum* sp.) disease of Pomegranate during 2019-20

Tr. No.	Treatments	Dose Formulation (gm or ml/liter)	Before spray	PDI 10 days after			% Reduction over control after 3rd spray	Yield tons/ha
				First spray	Second spray	Third spray		
T1	Indofil M-45	2.0	0.82* (4.93)	6.78 (15.06)	13.93 (21.89)	23.50 (29.00)	48.06	2.52 (9.12)
T2	Indofil M-45	3.0	0.57 (4.62)	3.73 (11.12)	9.89 (18.33)	16.82 (24.21)	62.84	2.97 (9.91)
T3	Indofil M-45	4.0	0.63 (4.22)	3.55 (10.85)	9.45 (17.91)	16.17 (23.71)	64.28	3.59 (10.92)
T4	Propineb 70% WP	3.0	0.63 (4.62)	5.30 (13.3)	11.63 (19.82)	20.23 (26.72)	55.30	3.08 (10.10)
T5	Untreated check	NA	0.75 (4.62)	14.88 (22.69)	27.38 (31.55)	45.25 (42.28)	0.000	2.13 (8.35)
	CDat5%		NS	1.29	2.08	1.11	-	0.81
	SEM		NS	0.42	0.67	0.36	-	0.26

* Mean of three replication PDI- Per cent Disease Index The values in the parenthesis are arc sine transformed

Table.3: Bio-efficacy of Indofil M-45 against Leaf spot (*Alternaria and Cercospora.*) disease of Pomegranate during 2018-19

* Mean of three replication PDI- Per cent Disease Index The values in the parenthesis are arc sine transformed

Tr. No.	Treatments	Dose Formulation (gm or ml/liter)	Before spray	PDI 10 days after			% Reduction over control after 3rd spray	Yield tons/ha
				First spray	Second spray	Third spray		
T1	Indofil M-45	2.0	0.82* (4.93)	7.10 (15.46)	11.43 (19.76)	17.48 (24.71)	61.59	2.52 (9.12)
T2	Indofil M-45	3.0	0.57 (4.62)	6.83 (15.14)	11.04 (19.38)	16.52 (23.98)	63.70	2.97 (9.91)
T3	Indofil M-45	4.0	0.63 (4.22)	3.58 (10.89)	5.78 (13.90)	8.33 (16.77)	81.70	3.59 (10.92)
T4	Propineb 70% WP	3.0	0.63 (4.62)	5.20 (13.17)	10.13 (18.54)	15.85 (23.47)	65.16	3.08 (10.10)
T5	Untreated check	NA	0.75 (4.62)	15.13 (22.89)	27.50 (31.63)	45.50 (42.42)	0.00	2.13 (8.35)
	CDat5%		NS	0.97	1.45	1.03	-	0.81
	SEM		NS	0.31	0.47	0.33	-	0.26

Table.4: Bio-efficacy of Indofil M-45 against Leaf spot (Alternaria and Cercospora) disease of Pomegranate during 2019-2020
* Mean of three replication   PDI- Per cent Disease Index   The values in the parenthesis are arc sine transformed

Tr. No.	Treatments	Dose Formulation (gm or ml/liter)	Before spray	PDI 10 days after			% Reduction over control after 3rd spray	Yield tons/ha
				First spray	Second spray	Third spray		
T1	Indofil M-45	2.0	0.82* (4.93)	7.28 (15.65)	14.13 (22.07)	19.15 (25.92)	56.60	3.19 (10.12)
T2	Indofil M-45	3.0	0.82 (4.93)	3.88 (11.34)	9.08 (17.53)	16.27 (23.79)	63.12	3.38 (10.52)
T3	Indofil M-45	4.0	1.00 (5.97)	3.63 (10.96)	8.41 (16.85)	15.50 (23.19)	64.87	4.18 (11.74)
T4	Propineb 70% WP	3.0	0.98 (5.61)	5.25 (13.23)	10.35 (18.77)	17.40 (24.66)	60.56	3.58 (11.09)
T5	Untreated check	NA	0.94 (5.74)	14.75 (22.59)	25.25 (30.17)	44.13 (41.63)	0.00	2.32 (8.77)
	CDat5%		NS	0.98	0.96	1.66	-	0.99
	SEM		NS	0.28	0.31	0.53	-	0.32

Table 5: Evaluation of IFC017 (Indofil M-45) for Phytotoxicity of Pomegranate during 2018-19 and 2019-2020

Sl.No.	Treatments	Dosage (gm /lt)	Phytotoxicity observations at 0, 1, 3, 5, 7 & 10 days after application (Scale: 0-9)																				
			Leaf tip injury			Wilting			Vein clearing			Necrosis			Epinasty			Yellowing			Hyponasty		
			R_1	R_2	R_3	R_1	R_2	R_3	R_1	R_2	R_3	R_1	R_2	R_3	R_1	R_2	R_3	R_1	R_2	R_3	R_1	R_2	R_3
T1	Indofil M-45	2.0g	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
T2	Indofil M-45	3.0g	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
T3	Indofil M-45	4.0 g	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
T5	Untreated check	NA	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
T6	Indofil M-45	8.0 g	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
T7	Indofil M-45	16.0 g	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00

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