

Original Research Article

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Efficacy of Cow by Products and Fungicides against *A. solani* causing Early Leaf Spot of Tomato *in vitro* and *in vivo* Conditions

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ABSTRACT

Tomato (*Solanum lycopersicum* L.) is the third most important crop in India that suffers from various biotic and abiotic stresses. Among the biotic stresses early blight caused by *Alternaria solani* [(Ell. and Mart.) Jones and Groot] is one of the most destructive diseases of tomato which leads losses up to 78 per cent. In the present study efficacy of cow by product and fungicides were evaluated against the pathogen *in vitro* and *in vivo* conditions. Among cow by products, cow urine showed the maximum growth inhibition of 78.44 per cent (20% concentration) *in vitro*. Among fungicides, Propiconazole 250 EC showed maximum growth inhibition of 96.67 per cent (250ppm) followed by Mancozeb 50%WP which showed growth inhibition of 84.44 per cent *in vitro*. To validate the efficacy of cow by products and fungicides, a trial was also conducted in the field conditions also. It was found that foliar application of Propiconazole @ 0.2% was found highly effective as it gave minimum disease intensity (6.5 percent) and maximum fruit yield (17.77 t/ha) and 10% concentration of combined effect of seed treatment and foliar application of cow urine was most effective as it gave minimum disease intensity (28.70 percent) and maximum fruit yield (14.72 t/ha). The effective components found in this study can be utilized as integrated disease management for *Alternaria solani* or early blight of tomato.

Keywords

Alternaria solani,
Propiconazole,
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urine

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Introduction

Tomato (*Solanum lycopersicum* L.) known as poor man's orange having high nutritive value and its fruit may be consumed either in fresh or in processed forms. It is rich in vitamin A, B and C and minerals (Khosro, 1994). It is the third most important crop in India after potato

and onion in terms of area and production having 8.14 lakh ha and 20.51 mt respectively with the total productivity of 25.20 t ha⁻¹ (Anonymous 2018-19) out of this Rajasthan contributes significant proportion having production of 9.02 mt with the productivity of 4.05 t ha⁻¹ over an area of 20366 hectare (2016-17) (Anonymous). Despite of this

abundance in quantity, its quality deteriorates and lags behind to meet up its domestic consumption due to various abiotic and biotic factors as it is affected by many fungal, bacterial, viral and nematodes diseases that affect tomato production and decrease its economical value. Among the various diseases, early blight that is caused by *Alternaria solani* (Ellis and Martin) Jones and Grout is one of the most destructive diseases of tomato that mainly occurs in the tropical as well as in subtropical regions. It causes loss both at pre and post-harvest stages that leads to reduction of 35 to 78 percent in yield (Jones *et al.*, 1993). Early blight of tomato caused by *A. solani* was first recorded in 1882 in New Jersey, USA (Bose and Som, 1986). In India, this disease was first noticed by Butler in Faizabad, U.P in 1905. Datar and Mayee, (1981) reported the losses due to this disease from different part of India to the extent of 48-80 percent.

Abhinandan *et al.*, (2004) conducted a survey in Punjab during 2001 and reported that at Tapa District, maximum disease intensity of 49.5 percent was observed and minimum disease intensity of 8.2 percent was observed at Babakala District. The disease is air borne and its pathogen is soil inhabiting. Andrus *et al.*, (1945) proved the pathogenicity of *Alternaria* on tomato by using mycelial fragments as a source of inoculum of *A. solani*. *Alternaria* is saprophytic, endophytic and pathogenic in nature. The fungus survives in the crop debris in or on the soil; disease is favoured by high humidity, dew and rainfall. The spores are disseminated by water, wind, insects and other means include man and machinery. Under favourable conditions bull eyed pattern in which concentric rings with brown to black spot appear on lower side of infected leaves. Jambhulkar *et al.*, (2016) stated that the conidia survive on the soil surface as well as on old dry lower leaves of the plant and spread when suitable climatic

condition prevails. The mycelium of fungus is haploid and septate. Morphologically it is branched, at initial growth stage it is light brown in colour while fully grown mycelium shown dark black in colour. The conidiophores are short, 50-90µm in length and are of dark black in colour. Conidia are characterized by their size (120-296 x 12-20 µm), beak, muriform, dark colour and are borne singly (Bose and Som, 1986). The conidia contain 5-10 transverse septa and 1-5 longitudinal septa (Singh, 1987).

In this study it is thought essential to test the efficacy of the most promising chemicals against early blight of tomato. However, in the recent years, the increase use of potentially hazardous fungicides in agriculture has been the subject of growing concern for both environmentalist and public health authorities. Therefore, novel approaches are required that uses low amount of chemicals which results in reduction of pollution hazards as well as the cost of production. So it is prerequisite to control plant disease by the integration of several methods. An integrated management approach that includes use of cow by products as well as chemicals for the management of early blight of tomato is evaluated in this report. Keeping in view of occurrence and losses incurred due to this disease and also lack of information in efficacy of some fungicides and botanicals against it was evaluated in this study.

Materials and Methods

This present study was conducted in the Laboratory of bio agent in college of agriculture, SKRAU Bikaner in order to develop integrated approach for management of early leaf spot of tomato via plant extracts, bioagents and by application of novel fungicides and cow by products. The pathogen was isolated by following Koch

postulates from infected leaves of tomato and then these leaves were cut into small pieces of about 1.5 cm- 2 cm. surface sterilization was done with 0.1% mercuric chloride, then washed three times with distilled water and placed on Petri plates having potato dextrose agar (PDA). These Petri plates were incubated at $26 \pm 1^\circ\text{C}$ for one week for sporulation. Pure culture was obtained by single spore technique by incubating at 28°C for a week. On the basis of symptomology and conidial characteristics, fungus was identified as *A. solani*, causal organism of early leaf spot of tomato..

Evaluation of novel fungicides and cow by products *in vitro*

Eight different fungicides *viz.*, Propiconazole 250 EC, Copper oxychloride 50WP, Chlorothalonil, Mancozeb 50WP, Mancozeb 64% + Thiophenate methyl 12%, Thiophenate methyl 15% + Copper oxychloride 40WP, Carbendazin 25% + Iprodione 25%, Carbendazin 12% + Mancozeb 63 % and cow by product (Cow urine, Cow dung and Butter milk) were evaluated for their efficacy against *A. solani* at four different concentrations *viz.*, 100, 150, 200, 250 ppm and 5, 10, 15 and 20% respectively under *in-vitro* condition through food poisoning technique.

Preparation of fungicides preparation

Molten sterilized potato dextrose agar was used as nutrient medium and required quantity of each fungicide and cow by product was added separately so as to get a desired concentration. The desired solution was added to PDA. About 15ml poisoned medium was poured to each of the 90mm Petri plates and allowed for solidification. A 5mm bit of *A. solani* was carefully cut by using cork borer and transferred aseptically to the centre of each Petri dish containing poisoned solid medium. Control was

maintained by growing the culture on PDA without fungicide and cow by product. The plates were incubated at $28 \pm 1^\circ\text{C}$ for seven days and the diameter of colony was recorded after seven days growth.

Evaluation of novel fungicides and cow by products *in vivo*

A field trial was carried out in field of college of agriculture SKRAU Bikaner, for management of early leaf spot of tomato in variety (S-22) using novel fungicides and cow by products in Kharif 2018. All the fungicides were applied @ 0.2% as foliar spray whereas one treatment of Saaf Carbendazim 12% + Mancozeb 63% was applied as seedling dipping (1hour) before transplanting @ 0.2%. Similarly cow by products (Cow dung, Cow urine and Butter milk) were applied as foliar spray as well as seedling dipping @10% concentration. Total thirteen treatments including control were tested following Randomized Block Design having plot size (3 m x 3 m). Each treatment was replicated thrice. The experiment was conducted under artificial inoculation conditions. Observations of per cent disease intensity and per cent disease control were recorded periodically in each plot as well as the fruit yield (t ha^{-1}) was recorded after harvesting of the crop.

Statistical analysis

Percent growth inhibition was calculated to evaluate the efficacy of plant extracts, bioagents, fungicides and cow by products against *A. solani in vitro* by formula given by Bliss (1934).

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

C = Mycelial growth of *A. solani* in control (mm)

T= Mycelial growth of *A. solani* in treatment (mm)

Per cent disease index (PDI) and disease control in various treatments were calculated by formula given by Wheeler (1969)

$$\text{Percent Disease index (\%)} = \frac{\text{Sum of individual ratings}}{\text{No. of leaves X Maximum disease Scale}} \times 100$$

The data of per cent disease incidence in all the experiments were transformed to their Arcsin values. The statistical analysis of the data of all the laboratory experiments was done by following Completely Randomized Design whereas the data of field experiments were analyzed by following Randomized Block Design (Cochran and Cox, 1957).

Results and Discussion

Efficacy of novel fungicides on mycelial growth of *A. solani* in vitro

It was evident from table 2 that all the fungicides significantly inhibited the growth of *A. solani* on PDA and the percent inhibition increases gradually with the increase of concentration. At 100 ppm concentration maximum per cent of inhibition was shown by Propiconazole 25%EC (88.89%), followed by Mancozeb 50% WP (64.44%) and Carbendazin 12% + Mancozeb 63% (60%) and minimum per cent of inhibition was shown by Chlorothalonil 75% WP (38.88%).

At 150 ppm, the similar trends of growth inhibition were reported. But Thiophenate methyl 15% + Copper oxychloride 40%WP and Carbendazin 25% + Iprodione 25% were equally effective (55.55%) against *A. solani*. Maximum growth inhibition was recorded in Propiconazole 25%EC (96.67%) followed by Mancozeb 50%WP (84.44%), Carbendazin 12% + Mancozeb 63 % (72.22%) whereas least per cent of inhibition was shown by Mancozeb 64% +Thiophenate methyl 12% (62.22%) at 250 ppm.

Efficacy of cow by products on mycelial growth of *A. solani* in vitro

It is evident from the table 2 that all the cow byproducts significantly inhibited the growth of *A. solani* on PDA. Percent inhibition increases with increase in concentration. 20% concentration was most effective in growth inhibition and maximum growth inhibition was observed by cow urine (78.49%) followed by cow dung (71.58%) and butter milk (42.56%).

Efficacy of novel fungicides and cow by products on *A. solani* in vivo

All the fungicides and cow by products were found to be significantly superior over control in management of disease. The minimum disease intensity of 6.50% was observed with Propiconazole 25%EC followed by 9.75% with Mancozeb 64% + Thiophenate methyl 12%, Thiophenate methyl 15% + Copper oxychloride 40%WP (13.56%)

The disease intensity of combi-product Mancozeb 64% + Thiophenate methyl 12% (9.75%) was less than Mancozeb 50%WP (26.20%) due to the complementary interaction between Mancozeb and Thiophenate methyl as compare to Mancozeb alone.

Similar trend was observed in efficacy of combination of Thiophenate methyl 15% + Copper oxychloride 40%WP .Among the cow by products, data presented in table 4 revealed that combined effect of seed treatment as well as foliar application @ 10 per cent.

The minimum disease intensity of 28.70% was observed in the treatment of cow urine followed by cow dung (35.43%) whereas maximum disease intensity was of butter milk (37.28%).

Table.1 Per cent growth inhibition of novel fungicides against *A. solani* under *in vitro*

Treatment	100ppm Conc.	150 ppm Conc.	200 ppm Conc.	250 ppm Conc.
	Growth Inhibition (%)	Growth Inhibition (%)	Growth Inhibition (%)	Growth Inhibition (%)
Propiconazole 250 EC	88.89 (70.01)*	92.22 (73.97)	95.55 (77.72)	96.67 (79.08)
Copper oxychloride 50WP	44.44 (41.60)	52.22 (46.17)	67.77 (55.10)	70.00 (56.77)
Chlorothalonil 75% WP	38.88 (38.58)	44.44 (41.61)	61.11 (51.38)	64.44 (53.20)
Mancozeb 50 WP	64.44 (53.20)	72.23 (58.10)	81.11 (64.18)	84.44 (66.62)
Mancozeb 64% +Thiophenate methyl 12%	45.50 (42.21)	54.44 (47.36)	60.00 (51.93)	62.22 (52.38)
Thiophenate methyl 15% + Copper oxychloride 40WP	42.22 (40.42)	55.55 (47.96)	64.44 (53.21)	68.89 (55.74)
Carbendazin25%+ Iprodione 25%	46.67 (42.81)	55.55 (47.96)	64.44 (53.22)	66.66 (56.93)
Carbendazin12%+ Mancozeb 63 %	60.00 (50.75)	64.44 (53.22)	70.00 (56.78)	72.22 (58.16)
Control	-	-	-	-
S.Em ±	1.234	1.502	1.413	1.546
CD (P=0.05)	3.732	4.543	4.272	4.676
CV%	4.505	5.00	4.223	4.475

*Figures in parentheses are angular transformed values

Table.2 Per cent growth inhibition of cow by products against *Alternaria solani* *in vitro*

Treatments	5% Conc. Growth Inhibition (%)	10% Conc. Growth Inhibition (%)	15% Conc. Growth Inhibition (%)	20% Conc. Growth Inhibition (%)
Cow dung	34.44 *(35.91)	42.22 (40.50)	58.72 (50.13)	71.58 (57.58)
Cow urine	47 (42.78)	55.55 (47.94)	67 (54.54)	78.49 (61.73)
Butter milk	15 (22.24)	27.29 (31.07)	36.48 (37.26)	42.56 (44.36)
Control	0	0	0	0
S.Em±	1.014	0.864	0.607	0.867
CD (P=0.05)	3.126	2.66	1.873	2.672
CV%	8.04	5.78	3.43	4.24

*Figures in parentheses are angular transformed values

Table.3 Effect of novel fungicides and cow by products on early blight of tomato caused by *A. solani* *in vivo*

Treatment	Disease Intensity (%)	Disease Control (%)	Fruit yield (t ha ⁻¹)	Increase in yield (%)
Propiconazole 25EC	6.50 *(14.77)	89.28	17.77 *(24.91)	68.34
Copper oxychloride 50WP	28.12 (31.89)	53.64	14.24 (22.09)	34.94
Chlorothalonil 75WP	22.47 (28.29)	62.95	15.04 (22.76)	42.57
Mancozeb 50 WP	26.20 (30.67)	56.80	14.41 (22.31)	36.63
Mancozeb 64% + Thiophenate methyl 12%	9.75 (18.19)	83.92	17.35 (24.58)	64.51
Thiophenate methyl 15% + Copper oxychloride 40WP	13.56 (21.49)	77.65	16.62 (24.03)	57.58
Carbendazim 25%+ Iprodione 25%	20.14 (26.66)	66.80	15.79 (23.41)	49.68
Carbendazim 12%+ Mancozeb 63 %	17.62 (24.82)	70.95	16.28 (23.78)	54.30
Carbendazim 12%+ Mancozeb 63 %	31.74 (34.29)	47.67	13.89 (21.77)	31.71
Cow urine	28.7 (29.66)	52.68	14.72 (22.48)	39.60
Cow dung	35.43 (36.53)	41.58	12.91 (21.06)	22.40
Butter milk	37.28 (37.63)	38.53	12.25 (20.45)	16.17
Control	60.65 (51.15)	0	10.5 (18.95)	
S.Em ±	1.901		1.086	
CD (P=0.05)	5.549		3.169	
CV%	11.02		8.36	

*Figures in parentheses are angular transformed values

Yield data presented in table 3 illustrated that maximum fruit yield of 17.77 t ha⁻¹ was recorded from Propiconazole 25%EC, followed by Mancozeb 64% + Thiophenate methyl 12% (17.35 t ha⁻¹), Thiophenate methyl 15% + Copper oxychloride 40%WP (16.62 t ha⁻¹) as compared to control (10.5 t ha⁻¹). Among the cow by products, 14.72 t ha⁻¹ fruit yield of tomato was observed in the treatment of cow urine as seed treatment and foliar application. Minimum yield of 12.25 t ha⁻¹ was observed in butter milk.

Early leaf spot of tomato is a foliar disease. The fungus survives in the crop debris in or on the soil; disease is favoured by high humidity, dew and rainfall. The spores are disseminated by water, wind, insects and other means includes man and machinery. The disease is air borne and soil inhabiting which is responsible for leaf blight, seedling collar rot and fruit rot of tomato (Datar and Mayee, 1981). The symptoms of the early blight disease appear as brown to dark leathery necrotic spots that appear first on the

leaflets (Locke, 1949). Walker (1952) reported oval or angular shaped spots of 0.3 to 0.4cm diameter with usually narrow chlorotic zone around the spot. As the spots mature, concentric rings of raised and depressed brown tissue are evident.

Management of *A. solani* should be done at initial stage and it should be done by organic and by natural ways as tomato is consumed directly or in processed form. So present study conferred the efficacy of novel fungicides as well as of cow by products *in vitro* and *in vivo*. Biological control also has its own limitations such as its effects are slow

and its durability is uncertain. Even the use of biocontrol agents exhibit inconsistent performance resulting in their limited commercial use for suppression of disease. Considering these limitation it is necessary to adopt chemical control as we cannot totally depend on biological control. This study evaluated the efficacy of novel fungicides as well as of cow by products *in vitro* and *in vivo* both. Out of the eight fungicides tested, Propiconazole followed by Mancozeb, Saaf and Thiophenate methyl 15% + Copper oxychloride 40WP were highly effective at 250 ppm in inhibition of mycelial growth of *A. solani*.

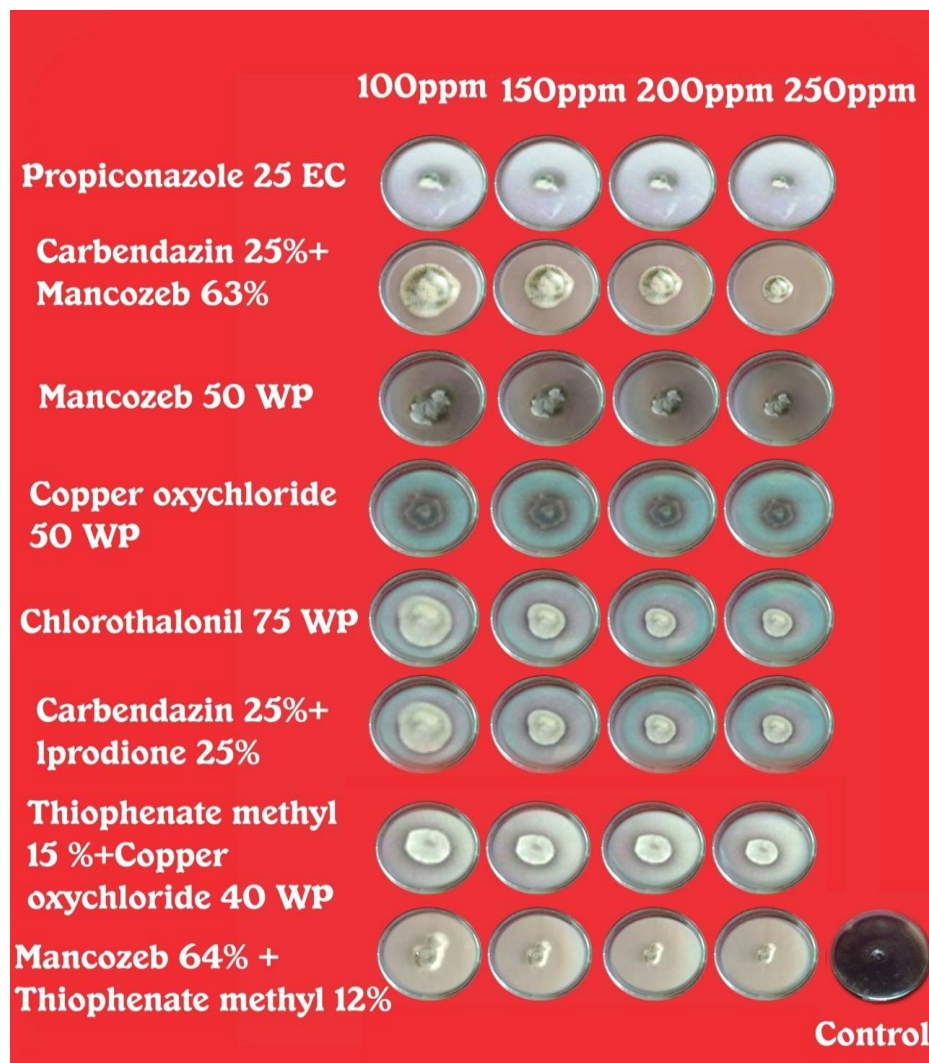


Plate.1 Efficacy of novel fungicides against *Alternaria solani* under *in vitro*

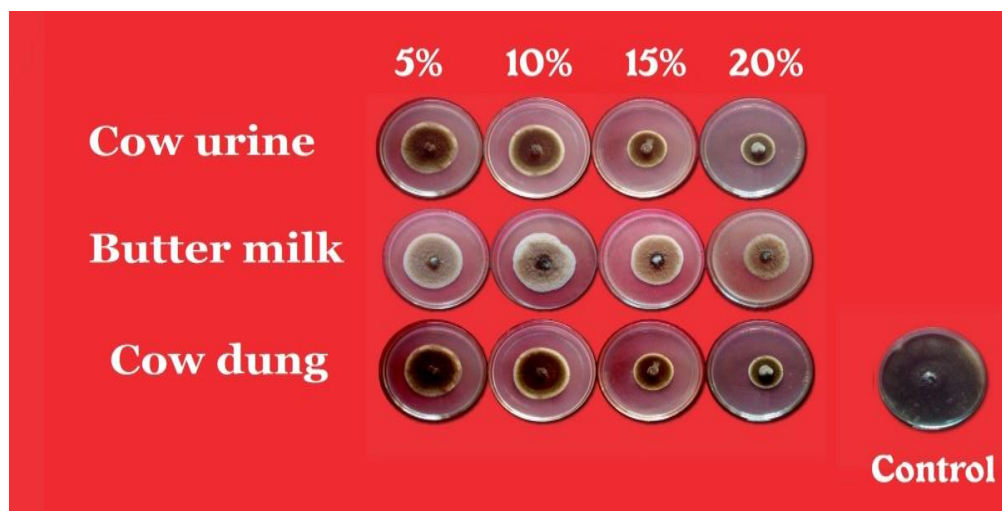


Plate.2 Efficacy of cow by products against *Alternaria solani* under *in vitro*

Sharma and Gaur (2009) evaluated the efficacy of nine fungicides against *A. alternata* under *in vitro* condition. Among the tested fungicides; Prochloraz (95.3%) was the most effective in inhibiting mycelial growth followed by Propineb (65.8%), Saaf (60.5%) and Mancozeb (57.8%). Kumar *et al.*, (2017) reported that the most effective fungicide was Difenconazole (Score) which inhibit the mycelial growth up to 78.61 per cent which was followed by Carbendazim (76.67 per cent).

Antifungal activity of cow by products was evaluated and their efficacy varied from 78.49 % (Cow urine) to 42.56% (Butter milk) at 20% concentration *in vitro*. Deshmukh *et al.*, (2012) evaluated the antifungal activity of cow urine at different concentrations: 5%, 10%, 15% and 20% against many pathogens like *Fusarium oxysporum*, *Penicillium notatum*, *Aspergillus*, *Alternaria solani* that confirms our finding. Among that, hyphal growth of *Alternaria* was inhibited and was merely 1.9 cm, 1.8 cm and no growth at 10%, 15%, and 20% concentration respectively whereas in control, hyphal growth of *Alternaria* was 2 cm. Sharma *et al.*, (2010) evaluated the efficacy of cow by products viz., cow urine and cow dung at different concentrations (0.5%, 2%, 3.5% and 5%) and

observed the spore germination behaviour of four fungal species. The conidial growth of *Alternaria alternata* (29.75 mm), *Fusarium oxysporium* (63.50 mm), *Colletotrichum capsici* (25.25 mm) and *Curvularia lunata* (32.50 mm) were used for their germination attributes.

It is evident from our finding that cow by product particularly cow urine has the antifungal activity and it can be used for management of early leaf blight of tomato as an alternative of fungicides which are hazardous and toxic for environment as well as for human health because tomato fruit is directly consumed by them as a salad and also in processed forms.

In vitro, effect of fungicides @ 0.2% foliar spray and combined effect of seedling dipping and foliar spray of cow by products at 10% on disease intensity and on yield was calculated. The minimum disease intensity was observed as 6.50% with Propiconazole 250 EC followed by 9.75% with Mancozeb 64% + Thiophenate methyl 12%. Among the cow by products, the minimum disease intensity was observed with cow urine (28.70%) followed by cow dung (35.43). The maximum yield was estimated from Propiconazole (17.77 t/ha), followed by Mancozeb 64%

+Thiophenate methyl 12% (17.35 t/ha). Among the cow by products, the estimated yield of cow urine was 14.72 t /ha followed by cow dung 12.91 t /ha.

Patil *et al.*, (2001) evaluated efficacy of fungicides, namely carbendazim (0.05%) and mancozeb (0.25%), the lowest percent disease incidence (PDI) was observed in carbendazim (13.9) and mancozeb (15.4) treatments. Kumar *et al.*, (2018) stated that Fenamidone 10% + Mancozeb 50% (0.2%) was most effective followed by Propiconazole 25% EC, Dimethomorph 9% + Mancozeb 60%, Cymoxanil 8% + Mancozeb 64% (0.2%) and Mancozeb (0.25%) and also economical in reducing severity of the early blight and increasing yield over control.

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