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Elucidation of Relationship between Phytophthora Leaf Blight and Fruit Rot in Tomato

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Abstract

Field experiments were carried out in order to determine the efficacy of traditional and new fungicides for preharvest and postharvest management to late blight disease of tomato. Preharvest management was followed by application of traditional fungicide and combination of fungicides. The combination of Mancozeb with Cymoxanil and Mancozeb with Phenamidone rendered fruit rot incidence between 8.0% and 9.3%, indicating most effective fungicides to manage this disease. Similarly, evaluation of type cultivar (determinate/indeterminate) response was done in the field. This results the intimate relation between infection in leaf and infection in fruit for the determinate cultivars ($R^2 = 0.96$). The experiments under high field epidemics (65.0% and 96.5% on leaves and fruits, respectively) have generated preliminary information on relationship between late blight severity on leaves and fruit rot incidence. Our study reveals late blight epidemics both on leaves and on fruits accelerated from the third week of February, which suggests special attention should be taken during this period. The laboratory experiments considered assessment of isolates of *Phytophthora*, and identification of most virulent isolate of the late blight fungus. Evaluation of infection and its progress on fruits, collected from fungicide treated plots and water sprayed plots, was made. Infection on fruits sampled from the plots treated with previously mentioned combination of fungicides had not progressed significantly ($P < 0.05$) compared with the fruits collected from plots treated with other fungicides. Occurrence of percent infection was estimated under laboratory in collections of fresh fruit (artificially inoculated) treated with fungicides. Similar result, as exhibited in the field experiments and experiment on infection progress, was obtained from the postharvest study. Postharvest management strategy considering fungicide application is discussed.

INTRODUCTION

Phytophthora, a lower fungus, is a destructive pathogen of solanaceous members infecting important crops like potato (*Solanum tuberosum*), tomato (*Lycopersicon esculentum*) and pepper (*Capsicum annuum*). *P. infestans* causes late blight, responsible for historical potato murrain in Ireland, in potato and tomato; while a different species *P. capsici* causes infection in pepper. Both of the species of this fungus may infect the roots, crown part, stem, leaves, and/or fruits. In tomato the first symptom usually appears on leaves as water-soaked pale brown/black, irregular lesions, and then occurrence of fruit rot and cracking in fruit under severe stage is often seen (fig. 1A,C and D). Young tissues are considered more succulent for this pathogen; therefore, disease progress is too much rapid in newly growing tomato leaves. Abundant moisture (dew) on the canopy leads sporulation of the pathogen, which is appeared as cottony growth on the underside of the affected leaves and/or on fruit. On other way, infection may occur in the roots or the collar region. At last water-soaked lesions are developed, which rapidly progress and cause girdling on base of the plant. This eventually leads to wilting and ultimately plant death. Lesion on mature fruits at postharvest causes severe infection on healthy fruits under humid/damp condition (fig. 1B). In current scenario late blight is a more challenging issue because of the appearance of new strains of *P. infestans* in India. New strains are identified in the Indo-Gangetic plain, which are found to be much more aggressive on tomato when comparison is made with the older strains. These strains prefer potato as a host under a

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condition when tomato is not grown in the area. To make this matter worse, these strains had developed resistance to several groups of systemic fungicides including Metalaxyl (Matuszak et al., 1994). This group of fungicide was earlier very effective against late blight and adopted by the growers for managing the disease. Our earlier studies mainly focused to find out the trend of infection and relationship with fruit rotting. Moreover, fungicidal application provided the information in reducing the fruit rot. Consequently, such information is essential for understanding epidemiology, and formulating management strategies to spread the infection on fruits. Therefore, this report may be helpful in order to save postharvest losses as well. One of the important focuses of this study was to establish the relationship between leaf and fruit infection by *P. infestans* in tomato. This work was designed in order to generate information on the strains of *P. infestans*, which are varied in the Indo-Gangetic plain. Secondly, information on the virulence of the said pathogen is limited. Even, the reaction of *P. infestans* in determinate and indeterminate tomato cultivars is not involved in a study to our knowledge. Therefore, the work was outlined to evaluate type cultivars of tomato that may be useful to breeders for development of resistance lines to *P. infestans*. Apart from these issues, a foremost work on saving the crop from preharvest and postharvest losses has also been performed. The current series of experiments were conducted to answer the questions whether late blight in tomato is (i) organ specific, (ii) type cultivar specific, and (iii) up to what extent this disease play role under postharvest condition.

MATERIALS AND METHODS

Trend of late blight severity and fruit rot incidence in the field

Field experiments were conducted during *Rabi* season of consecutive two years at the Vegetable Research Farm, Bihar Agricultural University, Sabour (Bihar). Field was ploughed 20-25 cm deep with soil turning plough; two to three cross harrowing and planking were done to make the field smooth, which ultimately results a well-leveled experimental area. The fertilizers were supplied in the experimental plots according to the normal fertilizer dose (120 kg N, 80 kg P and 60 kg K per hectare) follow in the region. Half amount of nitrogen was given at sowing and remaining at 45 days after transplanting. Phosphorus and potassium was supplied at sowing. Each treatment was replicated thrice with row to row and plant to plant distance 60 and 50 cm, respectively. Fruits having symptom of water soaked brownish lesions expressing rot appearance were considered for the assessment. Two field trials were conducted to satisfy our first objective, are described below:

Experiment 1. An experiment was conducted to understand the trend of late blight severity in the locality (fig. 2). The late blight severity was observed on the crop canopy and infected fruits were counted over time. For this experiment, a determinate cultivar *Kashi Vishesh* was grown in three plots with 20 plants (3 m row length). A separate field trial was laid upon, whereby spraying of different fungicides was performed on the determinate cultivar *Kashi Vishesh* (fig. 5). Various fungicides including mixed formulation e.g. Mancozeb + Metalaxyl, Mancozeb + Cymoxanil and Mancozeb + Phenamidone were sprayed on cultivar *Kashi Vishesh* and late blight severity was assessed.

Experiment 2. Reaction of late blight on type cultivar (determinate/indeterminate) was evaluated (fig. 3). The experiment was designed to recognize the relationship between type cultivar and late blight severity on leaves and fruit rot incidence. A trial was conducted consisting four cultivars including two determinate types (*H-86* & *BSS-488*) and two indeterminate types (*Pusa Rubi* & *Cherry Pink*). Each plot consisted of 4 rows (3 m length) of 5 plants planted. The crop was raised without application of fungicide, which offers natural infection under field condition in the area. Data were recorded on severity and fruit rotting incidence from last week of January to second week of March.

Germination test and pathogenicity assay

The surface contamination on tomato seed was removed by immersing the seeds in 70% ethyl alcohol for 10 s and then those were transferred into 0.5% sodium hypochlorite. Immediately after 2 min the dipped seeds were rinsed with sterile distilled water (SDW) for three times. The surface sterilized seeds were then aligned in the petriplates (moist chamber) and incubated at 25°C. The moist chambers were made with dry heat sterilized petriplates, where Whatman no. 1 filter papers placed inside the plates and moisten with SDW. Finally, percent germination was examined by counting germinating seeds over total seeds placed in the moist chamber. The seeds

were assumed germinated when the radicals attained a 10 cm length.

For pathogenicity assay two tests were individually conducted in order to identify most virulent isolate of *Phytophthora*. The tests, briefed below, were conducted under completely randomized design.

Detached-leaflet test. Incubation period and sporulation per lesion were measured to evaluate aggressiveness of isolate on the detached leaflets. Leaflets were taken from the fourth to sixth sub-terminal leaves of test plants so as to obtain leaflets of similar age and size. They were positioned in such a way that the adaxial side is touched with the moisten filter paper adjusted in the petriplates (15 × 100 mm). Each leaflet was then inoculated with a 25-µl drop of sporangial suspension (1×10^4 sporangia per ml). The inoculated petriplates (leaflets) were incubated at 16-18°C in the dark and regularly examined up to 6 days at 24 h interval, starting 24 h after inoculation. Appearance of first symptom was recorded and calculated using interpolation of consecutive data. Severity of infection was assessed on the basis of the proportion of leaflet with visible disease symptoms (Cohen, 1994). Rating was done on a 0-4 scale, where 0 was given for symptomless leaflet and 4 represented 96-100% blighted area of the leaflet. Collection of sporangia was achieved by inserting the leaflets in 10 ml of sporangial fixative (90:5:5 [vol/vol/vol], ethanol, glacial acetic acid, and formalin) and bottles were subjected to shaking at 15000 rpm for 5 min on an orbit shaker. Determination of the concentration of sporangia was done using a hemacytometer. Within a replication, five petriplates were used and the experiment was repeated three times keeping 3 replication at each experiment.

Root infection test. The virulence of *Phytophthora* can be recognized by evaluating isolate on roots (Ristaino, and Johnston, 1999). Pathogenicity tests were undertaken following Robertson (1968) with necessary modification. A healthy seedling (radical length 1 to 1.5 inch) grown in the moist chamber was placed in a petriplate containing Yeast Extract-Czapek's Agar(YECA, Vishunavat and Kolte, 2005). The strips of inoculum (5 mm wide) from 15-day old culture of *Phytophthora* grown on YECA were aseptically cut and placed across the rootlets at 5 mm behind the tip. These plates were incubated in dark at 16-18°C for 4 days and lesion length was measured. Six isolates with 3 replicates were assayed for this experiment; and the procedure was repeated thrice. Under each replication five plates were observed for the potential isolate. The selected isolate was taken to carry forward for artificial inoculation.

Determination of late blight severity on fruit

Tomato fruits brought from the field within a day of harvesting. The fruits were then subjected to hand washing with tap water and dried in shade. Additionally, all fruits were individually rinsed with 70% ethyl alcohol for 2 min and then 0.1% sodium hypochlorite for 2 min using a sterilized muslin cloth, and placed in the UV-rays treated egg tray, where 30 tomatoes of equal size were adjusted. Thereafter, the trays containing fresh tomato were treated with UV-rays for 45 min. The fruit containing trays were individually placed on a rotating chair in a closed chamber and moved clockwise at 10-12 rpm. This practice prohibited turbulent air and ensured uniform deposition of inoculum of the most virulent isolate of *Phytophthora* (1×10^4 sporangia per ml, recognized in pathogenicity assays) upon spraying over trays. Spraying of inoculum was performed with a hand sprayer: 8 pushes of the hand spray at 2 feet height from the tray (standardized previously in a preliminary study). Inoculated trays were incubated for 24 h in dark at 16-18°C ensuring successful infection of the pathogen. After 24 h, the inoculated trays were treated with fungicides namely Mancozeb, Mancozeb + Metalaxyl, Mancozeb + Cymoxanil and Mancozeb + Phenamidone.

The fruit trays sprayed with SDW was treated as control. Upon completion of fungicide spraying the trays were again incubated in dark at 16-18°C and observation was taken once in a day at 24 h interval from the accomplishment of treatment. The number of occurrence for each colony in different fruits was recorded started at 24 h after treatment, which was calculated and expressed in percent. Late blight severity and its progress were calculated using the formula (fig. 5):

Percent occurrence = N/C x 100
N = Total number of colony in the treated fruits
C = Total number of colony in control

The fungus was re-isolated from the artificially infected fruits and addressed the pathogenicity assay to satisfy Koch's postulate. The re-isolated pathogen rendered similar level of virulence as detected in pathogenicity assay. This experiment was conducted

two times following completely randomized design having 3 replication.

Assessment of fruit infection after harvesting

For assessment of fruit rot after harvesting, 90 fruits of uniform size were collected from each fungicide sprayed plots (ratio of infected and healthy fruits was 1:9). Similarly, the fruits were sampled from the plots that did not receive fungicide spray. The collected fruits were placed in plastic tray, previously rinsed with 0.1% formalin to disinfect saprophytes, and stored at room temperature (26±2°C). The collected fruits were monitored at 1,3,5 and 7 days after harvesting (DAH). Population of the infected fruit was counted, and incidence of fruit rot calculated using the formula:

$$\text{Percent fruit rot incidence} = (\text{Number of infected fruits}) / (\text{Total stored fruit}) \times 100$$

The experiment was repeated twice using completely randomized design with three replication.

RESULTS

Relationship between late blight epidemics on leaf and fruit

Under this study, only those fruits were considered for assessment showing symptoms of water-soaked lesion that enlarge rapidly into pale green to brownish-black and expand rapidly covering large area of the leaf (fig. 1). On the underside of larger lesions the pathogen is often visible during observation. Moreover, infection in fruit was observed as water-soaked spots that enlarge rapidly into brown to black lesions and, in severity, cracking of fruit was a common phenomenon. In this experiment, the severity of leaves and fruit rot incidence was recorded at weekly interval from fourth week of January to second week of March (fig. 2). At the initiation of disease, the severity was 4.5% and 2.1% recorded on leaves and fruits, respectively. The late blight severity was increased gradually but it progressed rapidly after third week of February. Overall, maximum percent infection was recorded in second week of March (65.0% severity on leaves and 96.5% incidence of fruits) when fruiting was extreme. The fruit rot incidence (72.2%) was recorded highest in *BSS-488* on second week of March.

Relation of leaf blight severity with fruit rot incidence between determinate cultivar and indeterminate cultivar was undertaken in this investigation (fig. 3). Close association of leaf blight severity and fruit rot incidence was detected for determinate cultivars (R^2 value for *H-86*: 0.964 and *BSS-488*: 0.965) compared to the indeterminate cultivars (R^2 value for *Pusa Ruby*: 0.597 and *Cherry Pink*: 0.632). The observation had begun immediately after appearance of symptom in each cultivar. In our investigation *BSS-488* (determinate cultivar) was found as the most susceptible cultivar rendering maximum infection both on leaves and fruits (63.4% and 72.2%, respectively). The indeterminate cultivars had expressed lowest maximum values of leaf blight severity and fruit rot incidence. Two indeterminate cultivars; *Pusa Ruby* shown maximum leaf blight severity (32.0%) but maximum fruit rot was detected in *Cherry Pink* (14.2%). Fruit rot incidence decreased at the end of March for both of the cultivars. However, the determinate type cultivars rendered maximum fruit rot incidence values at the last observation when fruiting was at its peak.

Late blight infection on leaf and fruit upon fungicide application

All the plots applied with fungicides against infection by *Phytophthora infestans* were reduced the numerical values of leaf blight severity and fruit rot incidence as compared to unsprayed plots (fig. 4). Higher leaf blight severity was recorded in plots sprayed with Mancozeb @ 0.2% and spraying of a mixed formulation of Mancozeb (Metalaxyl + Mancozeb @ 0.2%) that rendered 31% and 29.7% late blight severity, respectively. Similar impact of these fungicides was observed for fruit rot incidence. The other formulations of Mancozeb provided significantly lower leaf blight severity and fruit rot incidence ($P < 0.05$). Leaf blight severity was monitored 13.7% for Mancozeb + Cymoxanil and 15.0% for Mancozeb + Phenamidone. Analogous reaction of fruit rot incidence for the latest mentioned chemicals was found 8.0% and 9.3%, respectively. Unsprayed plots showed 57.7% leaf blight severity and 68.3% fruit rot incidence, respectively at last observation.

Postharvest fruit rot

The harvested fruits collected from different fungicide treated plots were assessed (Table 1). Laboratory findings indicated that fruits obtained from the plots treated with Mancozeb + Metalaxyl formulation exhibited 15.3% fruit rot incidence at 1-DAH, which was non-significant ($P < 0.05$) with plots treated with Mancozeb alone. The progress of fruit rot incidence was increased up to 7-DAH. However, the trend of fruit rot incidence was identical for the mixed formulation of Mancozeb with Cymoxanil and Phenamidone, where fruit rot incidence was increased with very poor rate up to 7-DAH. At all assessments, formulations of Mancozeb with Cymoxanil and Phenamidone were found to be non-significant ($P < 0.05$). The fruit rot incidence in control plots was maximum and increased at increasing rate up to 7 DAH, at this time more than 50% fruits were damaged due to infection by *Phytophthora*.

DISCUSSION

Tomato (*Lycopersicon esculentum*) is one of the most widely grown vegetable crops in the world, stands second after potato (Siddiqui et al., 2013; 2014). The crop is having the socio-economic importance to many countries including India. Therefore, pre and postharvest management of the crop to obtain healthy and bumper yield is one of the paramount objectives of the government, and other strategists (Siddiqui et al., 2015). It is well known that late blight is a dreaded disease that drastically affects the economic yield of tomato (fig. 1; Erwin and Ribeiro, 1996). This disease is favoured by humid and tropical environment favors. The facts considering that the subtropics, where the climate allows round-year cultivation, *Phytophthora* plays a key role being facultative parasite. Therefore, late blight disease of tomato is severe in the Indian subcontinent, where it is cultivated in moist, cool, and humid environments (Schumann and Arcy, 2005). The occurrence and severity of late blight are affected by moisture associated climatic factors (Adams and Stevenson, 1990). Our observation supports the role of micro-environment affecting disease severity. Leaf blight severity, presented in fig. 3, was found visually more on plants closer to ground (determinate type) comparing those plants attained taller height and defoliated the matured (relatively lower) leaves in the plant (indeterminate type).

Fig. 1. Symptom of late blight disease in tomato. A: healthy and primarily infected bunch of fruits; B: healthy and damaged fruits in the lot as postharvest collection kept for grading purpose; C: primary and late stages of symptom; D: cracking of fruit at severe infection.

Fungicide	Fruit rot incidence[a]			
	1-DAH[b]	3-DAH	5-DAH	7-DAH
Mancozeb	19.0 bc	23.0 c	28.7 c	36.7 c
Mancozeb + Metalaxyl	15.3 b	17.7 b	22.7 b	26.7 b
Mancozeb + Cymoxanil	5.7 a	7.7 a	10.0 a	14.7 a
Mancozeb + Phenamidone	6.3 a	8.0 a	11.7 a	17.0 a
Water spray (control)	29.0 d	36.0 d	43.7 d	51.7 d

[a] Within a data column, values followed the same letter are non-significant ($P = 0.05$).

Table 1. Late blight disease progress of postharvest tomato sampled from fungicide treated plots.

A result demonstrated by Platt (1985) where the author used Metalaxyl alone, which resulted in greatest increase in disease in the latter part of the growing season. The reason was later demonstrated by Matuszak et al., (1994) exploring resistance development of *Phytophthora* against this chemical. Under slight to moderate disease pressure Platt (1985) used Metalaxyl used in combination of Mancozeb provided significantly better disease control than Metalaxyl, if used alone. Disease was controlled significantly when plants were treated with Mancozeb or Metalaxyl along with Mancozeb, even moderate to high level of disease was developed in their study. In this relation our findings are also supported by the discussed study of Platt (1985). Our results (fig. 4) demonstrated that no significant difference ($P > 0.05$) was found when the plots were treated with Mancozeb alone or in combination with Metalaxyl. However, Mancozeb mixed with Cymoxanil and Mancozeb mixed with Phenamidone were equally effective to reduce the leaf blight severity and fruit rot incidence.

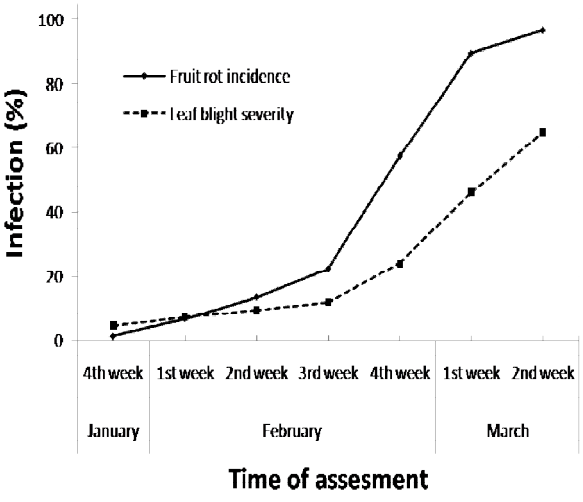

Fig. 2. Late blight epidemics on leaf and fruit under field condition

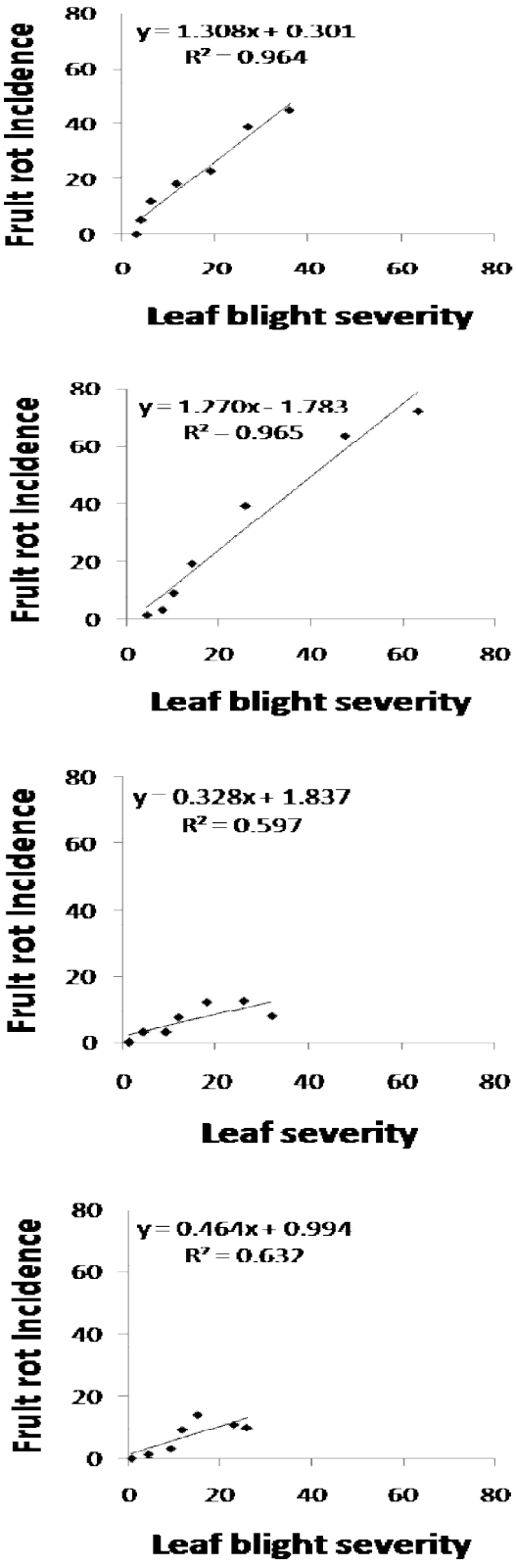

Fig. 3. Relationship between leaf blight severity and fruit rot incidence on determinate and indeterminate cultivar in tomato. A&B: determinate cultivar; C&D: indeterminate cultivar.

Postharvest rot of tomato fruit results in substantial economic losses across the globe. However, fungicidal treatment has been found significant to overcome the postharvest losses (Bolkan et al., 1990; Holliday, 1980). There is an escalating need of concern over the indiscriminate use of systemic fungicides because most of the fungicides have detrimental effects on environments and emergence of resistance in pathogen. Therefore, our findings entail that combination of non-systemic and systemic fungicides is a good alternative for controlling postharvest damage by the late blight fungus in tomato. Finally, minimizing postharvest losses due to late blight disease is of key concern particularly against developed new strains of *Phytophthora*. Our finding also advocates for strain-specific elaborative work for region oriented mandate. For postharvest management of fruit rot, it is impossible to eliminate postharvest losses, however, combination of systemic and non-systemic fungicides may save the stored fruit for a decent period (figs. 4 and 5, Table 1).

Kader (2002) advocated for reduction in postharvest loss by 50% to 60% against all postharvest pathogens, however, our results supported for a saving of 85% to 92% against late blight fruit rot (figs. 4 and 5). Late blight is a major postharvest disease in tomato because it has capability to rapid and extensive breakdown of stored fruit. However, a few strategies described in this paper may be incorporated in order to manipulate disease dynamics, and to better manage the crop from postharvest loss.

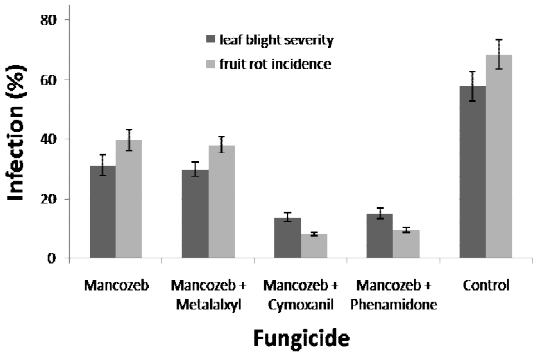

Fig. 4. Response of late blight disease on leaf and fruit against fungicide application. Error bars are standard errors of the mean.

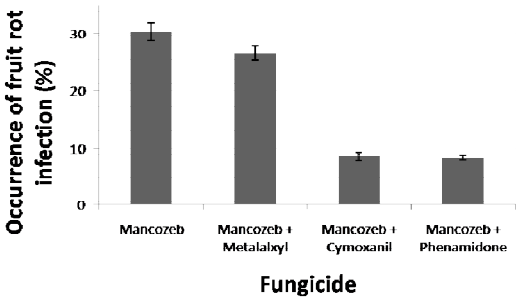

Fig. 5. Percent occurrence of infection on tomato fruit. Error bars are standard errors of the mean.

CONCLUSION

The marketing value of tomato is associated with severity of several factors like pathological, physiological and mechanical damages. Phytophthora blight in tomato is one of the major pathological constraints and responsible for significant reduction in yield. The infection in leaf, reducing photosynthate, contributes deduction in crop yield under field. Alongside, the postharvest loss is directly correlated with fruit infection that promotes fruit cracking. A better understanding of relationship between leaf and fruit disease dynamics could reduce the severity of loss. Our study explores importance of area-specific management upon identifying isolate(s) prevailing in the area. The disease could be managed with application of a mixed formulation of fungicide containing contact and systemic ingredients: this may discourage resistance development in Phytophthora for a locality. Under field experiments, relation between infection on leaf and on fruit for the determinate cultivars was found positively correlated indicating saving the canopy infection could save the crop from fruit yield damage. The disease may fluctuate with changing weather condition.

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