



## Eco-friendly management of tomato late blight using botanicals, bio-control agents, compost tea and copper fungicides

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### ABSTRACT

Tomato is affected by the major disease late blight caused by *Phytophthora infestans* in Sikkim. *In vitro* experiment of biocontrol agents, botanicals and organically approved chemicals evaluated against *P. infestans* revealed that garlic, *Trichoderma harzianum*, copper oxychloride and copper hydroxide were effective. In field experiment conducted during 2014 and 2015, copper oxychloride @0.25% was found most effective followed by copper hydroxide @0.25% for the management of late blight. The pooled mean yield of tomato treated with copper oxychloride was recorded highest with 8.99 t/ha, 24.50 t/ha and 25.33 t/ha for open, rain shelter and tunnel, respectively followed by copper hydroxide. Hence, copper fungicides can be used for the management of late blight in tomato in organic farming.

**Key words:** Biocontrol agents, Copper fungicides, *Phytophthora infestans*, Late blight

Organic agriculture is gaining its importance and the market for organic products has been increasing significantly throughout the world (Suja 2013). During 2016, Sikkim has achieved a remarkable distinction of converting its entire cultivable land under organic certification (APEDA 2017). Tomato (*Solanum lycopersicum* L.) is one of the important vegetables in Sikkim it is grown in an area of 0.995 thousand ha producing 8.955 thousand tonnes (Anonymous 2014). Tomato production in Sikkim is hampered by wet and humid climate, high rainfall, poor soil fertility, non-availability of resistant varieties, lack of effective control measures, prevalence of pest and diseases. Late blight caused by *Phytophthora infestans* (Mont) de Bary is one of the most significant constraints for tomato production in Sikkim. Host resistance is an important control measure however, at present there are no tolerant and/or resistant tomato cultivars available against late blight pathogen, *P. infestans* worldwide (Islam *et al.* 2013). Farmers mainly rely upon systemic fungicides to control late blight but, the use of systemic fungicides are not permitted in organic agriculture. Therefore, there is a need to study the alternative approaches for disease control under organic conditions.

Some alternatives used in organic agriculture are plant derived products, bio-control agents, copper and sulphur based fungicides. But the literatures on alternative methods like biocontrol, botanicals are very much limited for the management of late blight in tomato. The fungicides like copper compounds have already been used in different parts of the country for management of late blight. However, it is quite essential to evaluate their efficacy against the disease under the climatic condition of Sikkim having high rainfall and high humidity which favours pathogen build up and make the management measures ineffective. In high rainfall areas open cultivation of tomato is also very problematic. Therefore, it is very important to test tomato under various conditions like tunnel, rain shelter and open conditions. Considering various facts related to climate, pathogen and plant protection aspects, the present investigation was carried out to test the different organic approaches for the management of late blight in tomato.

### MATERIALS AND METHODS

*In vitro* evaluation of botanical extracts against *P. infestans*: Thirteen locally available botanicals, viz. garlic (*Allium sativum*) 5%, ginger (*Zingiber officinale*) 5%, turmeric (*Curcuma longa*) 5%, chilaune (*Schima wallichii*) 5%, mugwort (*Artemisia vulgaris*) 5%, papaya (*Carica papaya*) 5%, periwinkle (*Catharanthus roseus*) 5%, tulsi (*Oscimum sanctum*) 5%, datura (*Datura stramonium*) 5%, salvia (*Salvia officianalis*) 5%, marigold (*Tagetes erecta*) 5%, pudina (*Mentha arvensis*) 5% and *Lantana camara* 5% were used for the study. The antifungal potential of aqueous botanical extracts against the pathogen *P. infestans* was

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studied using poisoned food technique in PDA amended with different plant extracts (Nene and Thapliyal 2000). Three replicated plates for each treatment was maintained and incubated at 18°C in a BOD incubator. Percent inhibition of the pathogen compared to control was calculated by using the formula:

$$I = (C-T)/C \times 100$$

where I, Per cent inhibition; C, Radial growth in control; T, Radial growth in treatment.

*In vitro evaluation of Trichoderma against P. infestans:* *Trichoderma* species isolated from the rhizosphere soil collected from different places of Sikkim were used. *In vitro*, a dual culture study was used to observe the effects of the *Trichoderma* isolates towards *P. infestans*. Percent inhibition of the pathogen compared to control was calculated by using the formula:

$$I = (C-T)/C \times 100$$

where I, Per cent inhibition; C, Growth of pathogen alone without antagonist (control); T, Growth of pathogen along with the antagonist.

*In vitro evaluation of fungicides against P. infestans:* *In vitro* investigation was carried out to evaluate antifungal action of different organically permitted fungicides, i.e. copper oxychloride 50% WP, copper hydroxide 77% WP, potassium permanganate (AR), sodium bicarbonate (AR), wettable sulphur 80% WP along with positive control metalaxyl 35% WS 0.1% against *Phytophthora infestans* using poisoned food technique (Nene and Thapliyal 2000). Per cent inhibition was calculated by using the following formula:

$$I = (C-T)/C \times 100$$

where I, Per cent inhibition; C, Radial growth in control; T, Radial growth in treatment.

*Mass multiplication and seed treatment:* *T. harzianum* broth (10 ml) containing 107 cfu/ml was added to the sterilized 1 kg talc contained in the polypropylene bags. Carboxy-methyl cellulose (CMC) @1% and Mannitol @3% was also added and mixed well. The inoculated substrate was periodically shaken during incubation at 28°C for 7 days. The bags were stored at room temperature after incubation. For seed treatment, seeds of tomato were treated with the bioformulation @10 g/kg of seed along rice glue for easy adherence.

*Effect of different treatments under field conditions:* Field experiment conducted during 2014 and 2015 at ICAR Research Farm, Sikkim Centre, Tadong, Gangtok (1350 amsl). Planting was done in the second week of March in both the years with tomato variety Romeo. The field trials were laid out in Randomized Block Design (RBD) with 3 replications in plots of 3 m × 2 m at the spacing of 80 cm × 40 cm between rows and plants. The tunnel and rainshelter was made at 5 m height and 3 m width. 0.5 m gap was made for tunnel from the soil to improve aeration and reduce humidity inside the tunnel. A total of 8 treatments

were used, viz. compost tea (1:5 w/v) as foliar spray, garlic 5%, *Trichoderma asperellum* (0.5% for spray and 10 g per kg seed for seed treatment), *Trichoderma harzianum* (0.5% for spray and 10 g per kg seed for seed treatment), copper oxychloride 50% WP @0.25%, copper hydroxide 77% WP @5%, metalaxyl 35% WS @0.1% and control with no spray. The plant extracts were prepared following the method given by Netam *et al.* (2011). Six sprays of above treatments were done immediately after the appearance of disease at 8 day interval. The data was recorded after the final spray. Disease severity was determined using a 1-6 severity scale (Gwary and Nahunnaro 1998) and the Per cent Disease Index (PDI) was calculated using standard formula. At physiological maturity, tomato fruits from each plot were harvested and weighed separately to determine fruit yield. All data were subjected to ANOVA.

$$PDI = \frac{\text{Sum of individual rating}}{\text{Number of plants examined} \times \text{Maximum disease grade in the scale}} \times 100$$

## RESULTS AND DISCUSSION

Rhizosphere soil from different tomato growing areas of Sikkim was collected and *Trichoderma* was isolated from the rhizosphere soil. Among them 15 *Trichoderma* isolates were selected for studying the *in vitro* efficacy of antagonists against *Phytophthora infestans*. The percent inhibition ranged from 42.66 to 70.33%. *Trichoderma harzianum* collected from ICAR, Tadong was the most effective in reducing the *Phytophthora infestans* colony growth (70.33%) followed by *Trichoderma asperellum* (62.33%) from Bermiok (Fig 1). *Trichoderma* spp. have been successfully used to control various plant pathogens in different crops. D'souza *et al.* (2001) reported that isolates of *T. harzianum* was most effective in inhibiting the growth of *Phytophthora parasitica* of betelvine under *in-vitro* conditions. Antagonistic microbes employ variety of mechanisms for inhibiting the growth of pathogenic organisms like competition, antibiosis, parasitism, direct penetration and production of volatile compounds (Agrios 1998). Strong lysis of the pathogen by *Trichoderma* spp. has been reported for *Sclerotium rolfsii* (Elad *et al.* 1980).

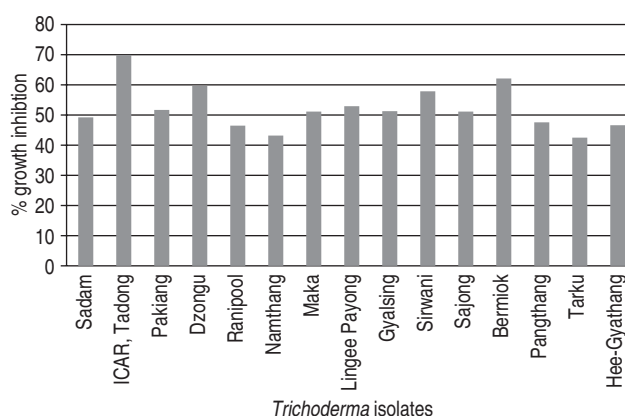


Fig. 1 Effect of *Trichoderma* against *Phytophthora infestans*

Plant essential oils have shown some bioactivity on *P. infestans* (Quintanilla *et al.* 2002). In the present study, the bulb extract of garlic (*Allium sativum*) @5% was found most effective with maximum per cent inhibition over control (47.76%) and closely followed by marigold (*Tagetes erecta*) (41.10%). Similar results of inhibition of colony growth by garlic bulb extract were observed in many plant pathogenic fungi. Ngadze (2014) reported that the water extracts of *Allium sativum* was active against *Phytophthora infestans in-vitro*. Allicin and its volatile compounds are responsible for antimicrobial properties of garlic bulb (Cao and Bruggen 2006). All the tested chemicals significantly suppressed the colony growth of *P. infestans*. Copper oxychloride @0.25% was most effective in reducing the colony growth of *P. infestans* with 87.52% and it was at par with copper hydroxide @0.25% (86.78%). Rani *et al.* (2016) reported that cupric sulphate 95% inhibited the mycelial growth of *Phytophthora infestans*.

The results (Table 1) revealed that copper oxychloride was the most effective with disease severity of 34.50%, 16.33%, and 19.23% in 2014 in open, rain shelter and high tunnel, respectively. This was followed by copper hydroxide @0.25% with disease severity of 35.76, 19.00, and 19.56 per cent in open, rain shelter and high tunnel respectively. In 2015 also, copper oxychloride @0.25% was found effective with disease severity of 34.66%, 18.16% and 21.33%. Copper oxychloride @0.25% was closely followed by the treatment of copper hydroxide with 37.00%, 20.30% and 24.33% disease severity. On the basis of two years mean data, the least late blight severity (34.48, 17.24 and 20.28%) was found in the plots treated with copper oxychloride @0.25% closely followed by copper hydroxide @0.25% (36.38, 19.65 and 21.94%). The yield of tomato treated with

copper oxychloride was highest with average yield of 8.99 t/ha, 24.50 t/ha and 25.33 t/ha for open, rain shelter and tunnel, respectively. This was followed by copper hydroxide with good average yield of 8.73 t/ha, 22.50 t/ha and 23.16 t/ha respectively (Table 2).

Neither biocontrol agents nor botanical treatments including compost tea extract were found effective against late blight. This may be due to their lower inhibitory activity against *P. infestans*, lack of persistence on tomato foliage surface because of continuous rainfall and also rapid spread of pathogen under congenial environment. Diniz *et al.* (2006) also reported that none of the plant extracts tested reduced the late blight in tomato. However, the severity of disease was minimum in the plots treated with standard fungicide metalaxyl @ 0.1% in this study. Metalaxyl a group of systemic fungicide have been widely employed to control late blight in potato and tomato and other diseases caused by oomycetes fungi (Shashidhara *et al.* 2009).

The efficacy of copper fungicides in management of various diseases is well documented (Teviotdale *et al.* 1989, Patel *et al.* 2014). The suppression of plant pathogens by the application of copper fungicides has been ascribed to the non-specific denaturation of proteins (Ware and Whitacre 2004). The toxic copper ion is absorbed by the germinating fungal spore. Alexandrov (2011) reported that copper fungicide Funguran (cupric hydroxide) suppressed the appearance of late blight of tomato. Platt *et al.* (1998) treated the plots of potatoes cv. Green Mountain with copper oxychloride and concluded that copper oxychloride reduced foliar and tuber blight and increased tuber yields relative to control plots. Our results are also congruent to the findings of Speiser *et al.* (2006) who reported that the copper fungicide treatment reduced foliar blight severity in potato cultivars

Table 1 Effect of organic treatments on the incidence of late blight in tomato

| Treatment   | Per cent disease severity (PDI) (%) |                  |                  |                  |                  |                  |                  |                  |                  |
|---|-------------------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|   | Open                                |                  |                  | Rain shelter     |                  |                  | Tunnel           |                  |                  |
|   | 2014                                | 2015             | Average          | 2014             | 2015             | Average          | 2014             | 2015             | Average          |
| Compost tea (1:5 w/v) as foliar spray                     | 92.63<br>(74.36)                    | 93.60<br>(75.46) | 93.11<br>(74.79) | 65.00<br>(53.76) | 68.00<br>(55.65) | 66.50<br>(54.63) | 70.33<br>(57.07) | 79.33<br>(79.33) | 74.83<br>(59.97) |
| Garlic 5%   | 95.76<br>(78.70)                    | 92.76<br>(74.46) | 94.26<br>(76.25) | 69.00<br>(56.20) | 70.66<br>(57.25) | 69.83<br>(56.68) | 71.00<br>(57.59) | 71.66<br>(71.66) | 71.33<br>(57.62) |
| <i>Trichoderma asperellum</i> 0.5%(Spray, seed treatment) | 91.93<br>(73.65)                    | 95.36<br>(77.60) | 93.64<br>(75.52) | 67.66<br>(55.56) | 71.00<br>(57.51) | 69.33<br>(56.37) | 65.00<br>(53.73) | 69.00<br>(69.00) | 67.00<br>(54.94) |
| <i>Trichoderma harzianum</i> 0.5%(Spray, seed treatment)  | 92.90<br>(74.54)                    | 94.30<br>(76.21) | 93.60<br>(75.36) | 64.33<br>(53.34) | 69.33<br>(56.42) | 66.83<br>(54.84) | 63.66<br>(53.25) | 67.33<br>(67.33) | 65.49<br>(54.03) |
| Copper oxychloride 0.25%                                  | 34.50<br>(35.96)                    | 34.66<br>(35.97) | 34.58<br>(36.01) | 16.33<br>(23.82) | 18.16<br>(25.22) | 17.24<br>(24.52) | 19.23<br>(26.00) | 21.33<br>(21.33) | 20.28<br>(26.75) |
| Copper hydroxide 0.25%                                    | 35.76<br>(36.73)                    | 37.00<br>(37.43) | 36.38<br>(37.09) | 19.00<br>(25.83) | 20.30<br>(26.77) | 19.65<br>(26.31) | 19.56<br>(26.22) | 24.33<br>(24.33) | 21.94<br>(27.90) |
| Metalaxyl 0.1%  | 29.41<br>(32.77)                    | 30.13<br>(33.21) | 29.77<br>(33.06) | 15.00<br>(22.73) | 14.00<br>(21.96) | 14.50<br>(22.38) | 16.43<br>(23.90) | 14.83<br>(14.83) | 15.63<br>(23.28) |
| Control   | 96.76<br>(80.00)                    | 98.10<br>(83.36) | 97.43<br>(80.85) | 73.33<br>(58.94) | 77.00<br>(61.69) | 75.16<br>(60.12) | 70.00<br>(56.97) | 78.33<br>(78.33) | 74.16<br>(59.52) |
| LCD (P= 0.05)   | 4.84                                | 6.05             | 3.75             | 5.86             | 6.08             | 1.95             | 8.27             | 11.38            | 4.04             |

Table 2 Effect of organic treatments on the fruit yield of tomato

| Treatment   | Open (kg) |       |         | Rain shelter (kg) |       |         | Tunnel (kg) |       |         |
|---|-----------|-------|---------|-------------------|-------|---------|-------------|-------|---------|
|   | 2014      | 2015  | Average | 2014              | 2015  | Average | 2014        | 2015  | Average |
| Compost tea (1:5 w/v) as foliar spray                     | 0.60      | 0.53  | 0.56    | 3.16              | 3.50  | 3.33    | 5.16        | 5.10  | 5.13    |
| Garlic 5%   | 0.46      | 0.56  | 0.51    | 4.83              | 5.00  | 4.91    | 4.83        | 3.60  | 4.21    |
| <i>Trichoderma asperellum</i> 0.5%(Spray, seed treatment) | 0.56      | 0.56  | 0.56    | 4.66              | 4.00  | 4.33    | 4.83        | 5.00  | 4.71    |
| <i>Trichoderma harzianum</i> 0.5%(Spray, seed treatment)  | 0.50      | 0.60  | 0.55    | 5.26              | 4.90  | 5.08    | 5.06        | 5.50  | 5.28    |
| Copper oxychloride 0.25%                                  | 9.83      | 8.16  | 8.99    | 25.66             | 24.00 | 24.50   | 26.00       | 24.66 | 25.33   |
| Copper hydroxide 0.25%                                    | 9.46      | 8.00  | 8.73    | 21.66             | 24.66 | 22.50   | 22.00       | 24.33 | 23.16   |
| Metalaxyl 0.1%  | 9.66      | 11.66 | 10.66   | 27.00             | 26.00 | 26.50   | 30.66       | 27.33 | 28.99   |
| Control   | 0.40      | 0.36  | 0.38    | 4.20              | 3.50  | 3.85    | 3.53        | 4.96  | 4.24    |
| LCD (P= 0.05)   | 0.989     | 1.57  | 1.88    | 3.10              | 5.44  | 2.20    | 2.81        | 4.77  | 2.79    |

by 27% and increased yield by 20% on average. Ghazanfar *et al.* (2010) reported that Kocide (copper hydroxide) was effective and reduced the late blight incidence in potato. Good coverage of the foliage with copper fungicides could control late blight in organic potato crop (Manuela and Hermeziu 2014). Narayan Bhat *et al.* (2007) showed that copper sulphate was highly effective reduced the lesion size of *Phytophthora infestans* in potato.

Our research is in conformity with findings of Olanya and Larkin (2006) who reported that aerated compost tea had no significant suppressive effects on *P. infestans*. No oil or biological treatment produced disease control comparable to the chemical control chlorothalonil. They also suggested the natural products and biological amendments tested are not sufficient for effective late blight control. The efficiency of various plant extracts and plant products was found less efficient under field conditions than copper based fungicides against late blight of potato (Cao *et al.* 2004). The incidence of late blight was highest in open conditions than under protected conditions. The main reason was that the plants were protected from rainfall under tunnel and rainshelter and thereby the removal of fungicides from the plant surface also was stopped under protected conditions. Powell *et al.* (2014) also reported that severity of late blight, caused by *Phytophthora infestans* was significantly lower in high-tunnel compared with open-field experimental plots. The severity of late blight was minimum in high tunnels due to less than optimal air temperature and relative humidity conditions for the growth of *P. infestans* (Kumar and Srivastava 1998, Inglis *et al.* 2011) and also the tunnel acts as a hindrance to sporangial spread inside the tunnel. The incidence of late blight was high in all the treatments except the fungicide treated one, this may due to high rainfall and humidity during the period under study. It has been reported that cool nights, warm days, and extended wet conditions from rain and fog can lead to late blight epidemics (Schumann and Arcy 2000, Solankey *et al.* 2017).

The findings of the present study revealed that copper fungicides can be used for the management of late blight

and increasing the yield in tomato grown under organic conditions when no other alternatives are available because of favourable weather conditions and virulent nature of pathogen. In high rainfall zones like Sikkim it is very much essential to grow them under protected conditions like rain shelter or tunnel for the effective management of late blight. Phytotoxic symptoms of any kind were not observed in copper fungicides treated plots in this study. As copper based fungicides are protectant in nature, spraying must be done before infection or immediately after the appearance of disease. Copper fungicides must be reapplied as plants grow to maintain coverage and prevent disease establishment. In wet weather copper fungicides should be applied as soon as the disease is observed or as soon as weather conditions are found to be favourable for disease development.

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