Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(4): 1260-1266
Received: 18-05-2018
Accepted: 23-06-2018

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Field evaluation of different copper sources in a consortium of 'Copper-Chitosan-*Trichoderma*' for management of late blight disease of tomato

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Abstract
Present field experiment was conducted to compare the efficacy of different copper compounds viz.; CuOH (Technical grade), CuOH (Kocide) and Copper sulphate pentahydrate ($CuSO_4.5H_2O$) in combination with chitosan (Cs661) and Trichoderma *asperellum* (Tri) to find out the most effective copper source in the combination for late blight disease management in tomato. Among the three copper compounds, CuOH (TG) was found to be the most effective copper source in the combination. Minimum disease severity (2.33%) was recorded in plots treated with CuOH (TG)+Cs661(Ac)+Tri whereas maximum disease severity was recorded in control plot (40%) after 95 DAT. In terms of other attributes such as yield, reduction in disease progress (A and r value), the most effective treatment was CuOH (TG)+Cs661(Ac)+Tri, which was at par with standard fungicide mancozeb and significantly higher as compared to control. The preliminary results suggest that the combination variants having CuOH as copper source could be developed as effective combination products as Cu- based fungicides for safe and effective management of late blight disease of tomato.

Keywords: late blight, copper, chitosan, *Trichoderma, consortium*

Introduction
Since the dawn of recorded history, many plant disease outbreaks with far reaching consequences have occurred. Among these devastating outbreaks the 'late blight disease' holds its own significance. It has always been at the center stage of plant diseases. This disease signifies the official beginning of the science of plant pathology and was the first plant disease for which a micro-organism was proved to be the causal agent. Late blight caused by an oomycete *Phytophthora infestans* (Mont.) de Bary has historically been an important disease of potatoes and tomatoes. The disease poses very serious economic threat to the vast majority of potato production systems, as well as many tomato production systems worldwide (Madden, 1983) [1] and responsible for causing $6.7 billion yield losses annually (Nowicki *et al.*, 2012) [2].

Management of late blight pathogen under field conditions is a challenge since the pathogen has the ability to develop new races and is able to adapt to a wide range of environmental conditions. The control of the disease has traditionally relied on foliar applications of fungicides. These fungicides include copper salts, dithiocarbamates, bis dithiocarbamates, cyanoacetamide oxime and metalaxyl, etc. Although excessive use of these chemicals poses great threat at global level (Ragunanthan and Divakar, 1996) [3] and also risks several components of agro-ecosystem (Ghorbani *et al.*, 2004) [4]. The only effective fungicides currently permitted for blight control even in organic agriculture are copper-based products (Mizubuti *et al.*, 2007) [5].

However, the appearance of fungicide-resistant pathogen strains and negative environmental impacts associated with these practices has intensified the need for reducing chemical use and for alternative disease management methods. Bio-control agents (BCAs) could be one of the most promising and effective means for disease management strategy alone, or in amalgamation with reduced doses of chemicals and plant strengtheners in the management of plant pathogens resulting in least impact of the chemicals on the environment. Therefore, development of alternative or combination products that can naturally induce resistance in the plants and reduce the doses of copper is quite desirable.

One alternative approach could be strategic designing of a novel formulation involving lower doses of fungicide, plant strengtheners and biological plant protectant to manage this destructive disease. Such a combination could be an effective, low cost, environment friendly and consumer safe plant disease management strategy for *P. infestans*. Based on this analogy in a parallel study a formulation of copper- chitosan- *Trichoderma* (Cu-Chi-Tri) was developed and evaluated over multiple years for management of late blight of potato under field conditions (Erayya *et al*, 2018). It was observed that, a combination involving low dose of a fungicide (CuOH), a biocontrol agent (*Trichoderma*) and a plant defense activator compound (chitosan) was found equally effective with the standard recommended fungicides in restricting the growth of the pathogen and therefore, resulted in reduced disease severity and increased tuber yield (Erraya, 2014) [6] (Sajeesh, 2015) [7]. Hence, the present investigation was undertaken to evaluate efficacy of the combination for management of tomato late blight and was also focused to compare efficacy of different copper compounds in the combination.

Materials and Methods

Experimental site

The field trial was conducted in Bihar Agricultural University, Sabour, (Bihar) during cropping season 2016-17.

Ingredients of the consortium

Chitosan

Chitosan extracted from crustacean's chitin, was supplied in both solid (CS651) and liquid formulations (CS661; acetic acid based and Hydrochloric acid based) by Mahtani Chitosan Private limited, Veraval, Gujarat. The chitosan used in the study had 80 percent degree of deacetylation (DDA) and was soluble at 1 percent concentration in diluted acid (10 percent acetic acid) and at a pH below 6. Chitosan solid formulation (CS651) and liquid formulation (CS661 based on dilution in acetic acid and HCl acid) were used at 500ppm concentration.

Copper compounds

***Copper hydroxide* [CuOH (TG)]:** Copper hydroxide technical grade [CuOH (TG)] having 67 per cent a.i. was supplied by Spiess Urania, Germany with 99.7% copper as copper hydroxide (CuOH) and 0.3 per cent impurities.

Kocide [CuOH (Ko)]: Commercially available copper fungicide Kocide 77% WP (sold by DuPont Pvt. Ltd.) was also used. In the formulation copper hydroxide was 61.4 percent and rest other was inert materials.

Copper sulphate pentahydrate ($CuSO_4.5H_2O$): $CuSO_4$ of analytical grade was also used as one of the copper source in the treatments. Compound was having 99 percent copper as $CuSO_4$.

Trichoderma: Copper tolerant (upto 1000 ppm) and chitosanolytic fungus, *Trichoderma asperellum* (Tri) was obtained from culture repository of Biocontrol lab, Department of Plant Pathology, Govind Ballabh Pant University of Agriculture &Technology, Pantnagar. For field experiment the talc based formulation of *T. asperellum* (Tri) was used.

Field evaluation of efficacy of different copper sources in the consortium of 'Copper-Chitosan-*Trichoderma*' for management of late blight disease of tomato

Experimental details

The experiment consisted of 10 treatments (Table No. 1) including check and mode of applications was seed treatment + foliar spray (S+F). The trial was carried out in Randomized Block Design (RBD) and replicated three times. The dimension of each plot was 3×1.8 m^2. Late blight susceptible variety Kashi vishesh was used for the experiment in disease sick plots. Field was ploughed 20-25 cm deep with soil turning plough followed by two to three cross harrowings and plankings to make field smooth and leveled. Pre-planting irrigation was given to ensure good field preparation and moisture. N.P.K. fertilizers were applied @ 120 Kg N/ha, 200 kgP_2O_5/ha and 120 kgK_2O/ha, respectively. Nitrogen, as urea, was applied in two split doses: half as basal dose, applied at the time of last ploughing and remaining half at the time of earthing-up after emergence. A spacing of 60 cm x 20 cm spacing was maintained between rows and plants. Seedling dip treatments were given prior to planting. Sowing was done on 24th November 2016. After four weeks of germination irrigation was given as required. Two hand weedings were done during the crop season.

Table 1: Treatments for field trial on the management of tomato late blight

Sr. No.	Treatments*	Dose
T1	CuOH (TG) + Cs661 (Ac) + Tri	500 ppm + 500 ppm + 1%
T2	CuOH (TG) + Cs661 (HCl) + Tri	500 ppm + 00 ppm + 1%
T3	CuOH + Cs651 + Tri	500 ppm + 500 ppm + 1%
T4	CuOH (Ko) + Cs661(Ac) + Tri [tuber treatment with Cs661(Ac) + Tri followed by foliar spray with CuOH (Ko) + Cs661(Ac) + Tri]	500 ppm + 500 ppm + 1%
T5	CuOH (Ko) + Cs661(Acetic acid based) + Tri [tuber treatment with CuOH (Ko) + Cs661(Ac) + Tri followed by foliar spray with CuOH (Ko) + Cs661(Ac) + Tri]	500 ppm + 500 ppm + 1%
T6	$CuSO_4$ + Cs661 (Ac) + Tri	500 ppm + 500 ppm + 1%
T7	CuOH (Ko) 77% WP	500 ppm
T8	CuOH (Ko) 77% WP	2000 ppm
T9	Mancozeb 75% WP	2000 ppm
T10	Untreated Control (Water spray)	-

*CuOH (TG): Copper hydroxide technical grade; Cs661 (Ac): Acetic acid based liquid formulation of chitosan; Cs661 (HCl): Hydrochloric acid based liquid formulation of chitosan; Cs651: Powdered formulation of chitosan; $CuSO_4$: Copper sulphate; Tri: *Trichoderma asperellum*.

The mode of application of treatments was seedling dip treatment followed by foliar spray. For execution of these sprays, power-operated (ASPEE VBD09) knap-sack sprayer with hollow cone nozzle was used. It was maintained at a constant pressure of 40 psi and gave a uniform medium size droplet (226-325 micron). These sprays were conducted from December end 2016 to February 2017.

Disease observation

Under field conditions natural occurrence of late blight of tomato had been inconsistent since environmental conditions especially temperature, humidity and rainfall play a vital role in occurrence, prevalence and severity of the disease. The development of the disease was studied on the basis of symptoms on the leaves and fruits. During the growing season disease is recorded at five days interval using foliar blight assessment key given by Henfling 1987 [8] The disease progress curve of different treatments was made graphically to know the time of appearance of the late blight disease, amount of inoculum and effectiveness of management measures. To evaluate the epidemic the Area under Disease Progress Curve (AUDPC) (A-value) and the apparent rate of infection (r-value) was calculated. For estimating tomato yield five pickings were conducted at an interval of 10 days. The yield was expressed in kg/plot.

Statistical analysis

Experiment was carried out in Randomized Complete Block Design (RCBD) with one treatment factor and three replicates. Data recorded were first transformed to make them homogenous before analysis and the treatment were compared by means of critical differences at 5 per cent level of significance. Analysis of variance (ANOVA) test was performed for all experiment using GBPUA&T's statistical software STPR.

Results and Discussions

First disease symptom was observed in control plots on 5-01-2017 i.e., at 40 DAT (days after transplanting). However, during the period December 2016 to January 2017, environmental conditions did not favour late blight development at BAU, Sabour and hence disease pressure was low i.e., late blight severity reached upto 40% only in control plot at 95 DAT.

Disease severity

First observation on disease severity was taken on 65 DAT with control plot showing a disease severity of 0.33% which reached to 40% at 95 DAT. Among different treatments, minimum disease severity (2.33% at 95 DAT) was recorded in plots treated with standard fungicide mancozeb (2000 ppm) and in the combination, CuOH (TG) + Cs661 (Ac) + Tri. In case of the combination variants having CuOH (Ko) as copper source, disease severity was significantly less as compared to control. Disease severity of 15% at 95 DAT was recorded in case of CuOH (Ko) + Cs661 (Ac) + Tri [seedling treatment with Cs661 (Ac) + Tri followed by foliar spray with CuOH (Ko) + Cs661 (Ac) + Tri]. However, 13.33% disease severity in case of CuOH (Ko) + Cs661 (Ac) + Tri (1%) [Seedling treatment with CuOH (Ko) + Cs661 (Ac) + Tri followed by foliar spray with CuOH (Ko) + Cs661 (Ac) + Tri] was obtained. The combination variant having $CuSO_4$ as copper source was least effective variant among all the other variants, in which 31.66% severity was recorded at 95 DAT (Table 2, Fig: 1).

Area under disease progress curve (A-value) and apparent rate of infection (r-value)

A-value was found to be maximum (772.27) in control plots. It was minimum (25.42) in case of CuOH (TG) + Cs661 (Ac) + Tri followed by 25.69 in case of mancozeb (2000 ppm). Variants having CuOH (Ko) as copper source, i.e. CuOH (Ko) + Cs661 (Ac) + Tri [seedling treatment with Cs661 (Ac) + Tri followed by foliar spray with CuOH (Ko) + Cs661 (Ac) + Tri] showed A-value of 204.96. While, treatment CuOH (Ko) + Cs661 (Ac) + Tri [seedling treatment with CuOH (Ko) + Cs661 (Ac) + Tri followed by foliar spray with CuOH (Ko) + Cs661 (Ac) + Tri] showed 'A-value' of 147.42. Plots receiving triple combination spray having $CuSO_4$ as copper source showed 'A-value' of 517.75 (Table 2; Fig 2).

The r-value was also found to follow similar trend with maximum (0.160) in case of control plot while it was minimum (0.100) in case of mancozeb (2000 ppm) followed by 0.110 in case of CuOH (TG) + Cs661 (Ac) + Tri and CuOH (TG) + Cs661 (HCl) + Tri (Table 2; Fig 2). This data implies that disease progress was comparatively rapid in case of control plots and comparatively slower in case of standard fungicide mancozeb (2000 ppm).

Table 2: Efficacy of different copper compounds (in terms of disease severity, 'A- value' and 'r-value') in the consortium for tomato late blight management.

Sr. No.	*Treatments	Mean disease severity (%)							A-value	r-value
		65 DAS	70 DAS	75 DAS	80 DAS	85 DAS	90 DAS	95DAS		
1.	CuOH (TG) @ 500 ppm + Cs661 (Ac) @ 500 ppm + Tri @ (1%)	0.1 (1.81)	0.1 (1.81)	0.13 (2.06)	0.40 (3.12)	2.03 (6.82)	2.33 (8.13)	2.33 (8.13)	25.42	0.110
2.	CuOH (TG) @ 500 ppm + Cs661 (HCl) @ 500 ppm + Tri@ (1%)	0.13 (2.06)	0.16 (2.31)	0.20 (2.56)	0.70 (4.43)	2.33 (8.13)	2.33 (8.13)	2.66 (8.93)	27.37	0.110
3.	CuOH (TG) @ 500 ppm + Cs651 @ 500 ppm+ Tri @ (1%)	0.10 (1.81)	0.10 (1.81)	0.13 (2.06)	1.00 (5.73)	2.33 (8.13)	2.66 (8.93)	3.00 (9.72)	38.10	0.112
4.	CuOH (Ko) @ 500 ppm + Cs661 (Ac) @ 500 ppm + Tri @ (1%) [seedling treatment with Cs661 (Ac) + Tri followed by foliar spray with CuOH (Ko) + Cs661 (Ac) + Tri]	0.20 (1.95)	0.66 (4.61)	2.00 (7.01)	3.66 (10.52)	3.66 (10.52)	11.66 (18.61)	15.00 (22.28)	204.96	0.139
5.	CuOH (Ko) @ 500 ppm + Cs661 (Ac) @ 500 ppm + Tri @ (1%) [seedling treatment with CuOH (Ko) + Cs661 (Ac) + Tri followed by foliar spray with CuOH (Ko) + Cs661 (Ac) + Tri]	0.66 (1.20)	0.36 (2.51)	1.00 (5.73)	2.33 (8.13)	2.33 (8.13)	8.33 (16.59)	13.33 (20.45)	147.42	0.135
6.	$CuSO_4$ @ 500 ppm + Cs661 (Ac) @ 500 ppm + Tri @ (1%)	0.20 (1.95)	0.70 (4.43)	2.33 (8.13)	10.00 (17.46)	18.33 (24.99)	26.66 (31.07)	31.66 (34.23)	517.75	0.147
7.	CuOH (Ko) @ 500 ppm	0.06 (1.20)	0.36 (2.51)	0.70 (4.43)	2.33 (8.13)	3.66 (10.52)	10.33 (16.22)	26.66 (31.07)	215.39	0.145

8.	CuOH (Ko) @ 2000 ppm	0.03 (0.60)	0.66 (1.20)	0.70 (4.43)	1.00 (5.73)	2.33 (8.13)	3.66 (10.52)	4.00 (11.32)	63.49	0.130
9.	Mancozeb @2000 ppm	0.06 (1.20)	0.06 (1.20)	0.40 (3.12)	0.66 (4.61)	1.00 (5.73)	2.33 (8.13)	2.33 (8.13)	25.69	0.100
10.	Untreated Control (Water spray)	0.33 (2.70)	3.50 (9.96)	8.33 (16.59)	18.33 (25.30)	26.66 (31.07)	33.33 (35.21)	40.00 (39.14)	772.27	0.160
		SEm±				CD at 5%			CV	
	Treatments	0.77				2.16			26.72	
	Days	0.64				1.80				
	Treatment × Days	2.04				5.72				

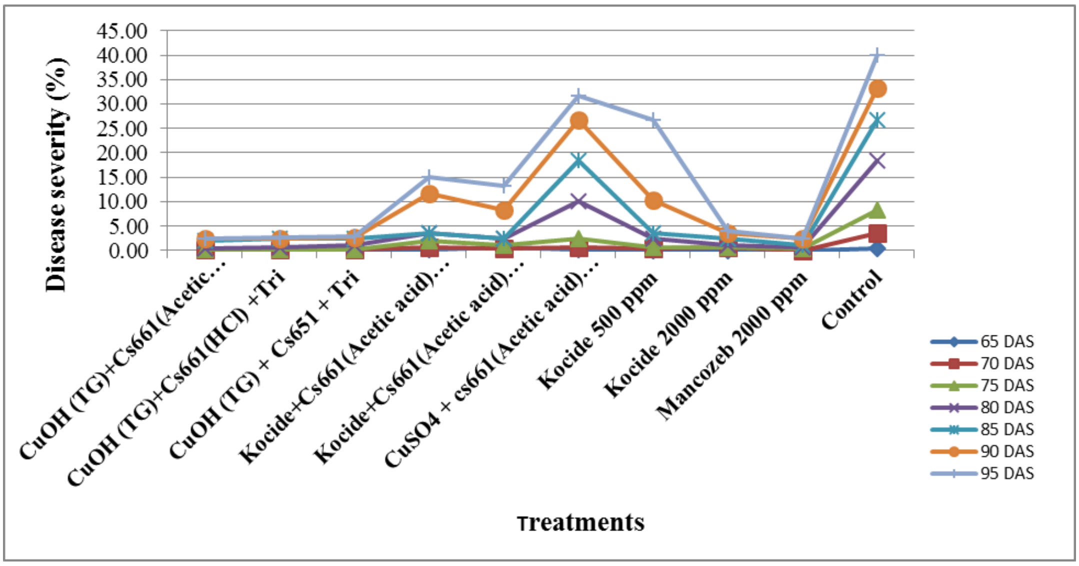

Fig 1: Tomato late blight disease progress in different treatments in the field trial

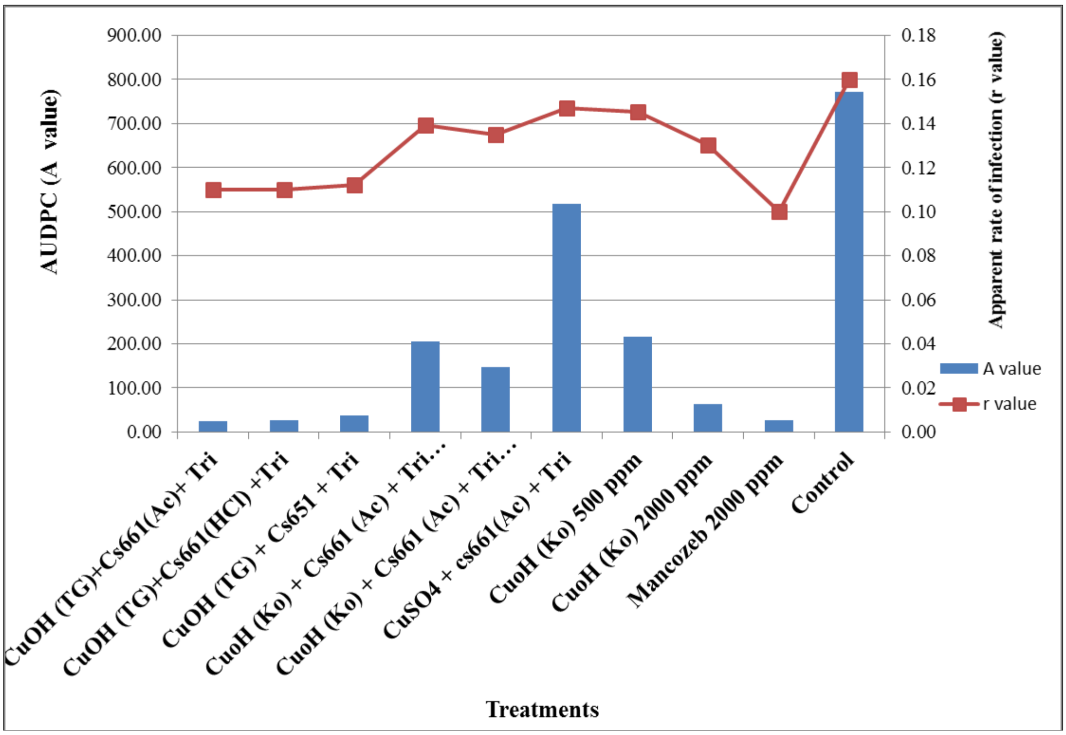

Fig 2: 'A' and 'r- values' of tomato late blight disease in different treatments in the field trial

Tomato yield

Maximum tomato yield of 10.66 kg/plot was obtained in standard fungicide mancozeb 2000 ppm treated plots, followed by 9.82 kg/plot in CuOH (TG) + Cs661 (Ac) + Tri treated plots. Yield obtained in plots treated with CuOH (Ko) + Cs661 (Ac) + Tri [seed treatment with Cs661 (Ac) + Tri followed by foliar spray with CuOH (Ko) + Cs661 (Ac) + Tri] was 6.43 kg/plot. While in case of CuOH (Ko) + Cs661(Ac) + Tri [seed treatment with CuOH (Ko) + Cs661 (Ac) + Tri followed by foliar spray with CuOH (Ko) + Cs661 (Ac) + Tri] yield obtained was 7.06 kg/plot. In plots treated with the consortium variant having CuSO4 as copper source, the yield obtained was 4.85 kg/plot. Minimum yield of 4.47 kg/plot was recorded in case of control plots (Table 3).

Table 3: Tomato yield in different treatments in the field trial

Sr. No.	*Treatments	Mean Yield (kg/plot)	% Increase over control
1.	CuOH (TG) @ 500 ppm + Cs661 (Ac) @ 500 ppm + Tri @ (1%)	9.82	119.68
2.	CuOH (TG) @ 500 ppm + Cs661 (HCl) @ 500 ppm + Tri@ (1%)	9.46	111.63
3.	CuOH (TG) @ 500 ppm + Cs651 @ 500 ppm+ Tri @ (1%)	8.91	99.32
4.	CuOH (Ko) @ 500 ppm + Cs661 (Ac) @ 500 ppm + Tri @ (1%) [seedling treatment with Cs661 (Ac) + Tri followed by foliar spray with CuOH (Ko) + Cs661 (Ac) + Tri]	6.43	43.84
5.	CuOH (Ko) @ 500 ppm + Cs661 (Ac) @ 500 ppm + Tri @ (1%) [seedling treatment with CuOH (Ko) + Cs661 (Ac) + Tri followed by foliar spray with CuOH (Ko) + Cs661 (Ac) + Tri]	7.06	57.94
6.	$CuSO_4$ @ 500 ppm + Cs661 (Ac) @ 500 ppm + Tri @ (1%)	4.85	8.50
7.	CuOH (Ko) @ 500 ppm	4.86	8.72
8.	CuOH (Ko) @ 2000 ppm	8.56	91.49
9.	Mancozeb @2000 ppm	10.66	138.47
10.	Untreated Control (Water spray)	4.47	0.00
	SEm±	1.22	
	CD at 5%	3.64	
	CV	28.39	

Pearson correlation analysis of AUDPC with apparent rate of infection (r) and yield

A positive correlation was found between A-value and r-value (0.77114). A-value had a negative correlation (-0.82296) with yield. Also it was found that 'r-value' also had a negative correlation (-0.96831) with tomato yield (Table 4). Hence this correlation proves the statistical validity of the data obtained from the experiment.

Table 4: Correlation analysis of AUDPC with apparent rate of infection (r) and yield in different treatments in the field trial for management of tomato late blight.

		AUDPC	Apparent rate of infection (r)
Apparent rate of infection (r)	Pearson Correlation	0.77114*	-
	Sig. (2-tailed)	0.0090	-
	N	10	-
Tomato yield (kg/treatment)	Pearson Correlation	-0.82296*	-0.96831*
	Sig. (2-tailed)	0.0035	0.0001
	N	10	10

*Correlation is significant at the 0.05 level (2-tailed)

Research on late blight disease is not only important for scientific interest but also important from economic as well as environmental perspective. A consortium of copper, chitosan and biocontrol agent focusing towards integrated management of late blight disease was developed and found effective in controlling late blight of potato (Sajeesh *et al.*, 2016) [9] (Erraya 2014) [6] (Bhardwaj 2016) [10]. This kind of integrated approach could lead to more effective and environment friendly management of late blight disease as it employs low dose of chemical fungicide (copper hydroxide in this case); plant strengthening and anti-microbial compound chitosan; and anti-pathogenic fungus *T. asperellum*. All the three components have the ability to combat the pathogen, *Phytophthora infestans*. Copper is a well-established fungicide for late blight management. Cu^{2+} ion reacts with sulfhydryl group of certain amino acids thus causing denaturation of cell proteins. Chitosan exhibits a variety of beneficial properties such as plant growth promotion and elicitation of plant defense reactions (Hadwiger *et al.*, 1988) [11]. Fungicidal activity of chitosan has been documented against various species of fungi and oomycetes (Muzzarelli *et al.*, 1990) [12] (Vasyukova *et al.*, 2005) [13]. The use of *Trichoderma asperellum* as a bio-control agent against plant pathogens has long been known. It acts directly through the mechanism of myco-parasitism or indirectly by competing for nutrients and space, modifying environmental conditions or promoting plant growth and plant defensive mechanisms and antibiosis (Vinale *et al*., 2008) [14].

In this field trial different copper sources were evaluated to check their efficacy in the consortium. It was found that CuOH (TG) was performing well as compared to CuOH (Ko) and $CuSO_4$ in the combination for management of late blight of tomato. The performance of the consortium variants having CuOH (Ko) as copper source was comparatively lower than variants having CuOH (TG) as copper source but it performed significantly better as compared to variants having $CuSO_4$ as copper source. Also performance of the consortium variants having CuOH (Ko) as copper source was significantly higher in terms of disease reduction and yield obtained as compared to control plots but the performance was lower as compared to standard fungicides. Hence, using CuOH (Ko) as copper source in the consortium is not beneficial. Thus, the consortium variant having CuOH (TG) as copper source is the best possible alternative to the standard fungicides. It should also be noted that CuOH (TG) is easily available in the Indian market which would facilitate the industrial production of triple combination at competitive prices.

The difference in the efficacy of different variants (difference in terms of copper source used) of the consortium might be due to the kind of interaction taking place between different components in the combination. The two components, copper and chitosan interact through adsorption or chelation mechanism of copper on chitosan particles. Use of chitosan as a heavy metal chelator compound has been described by many workers. Chitosan has been used in the fresh and salt water purification processes as metal and mineral chelator (Hadrami *et al.*, 2010) [15]. These abilities could be explored when chitosan is applied to plants to prevent diseases because it can chelate nutrients and minerals (*i.e.*, Fe, Cu), preventing pathogens from accessing them and could also chelate copper ions on their surface making them available on host surface for longer duration.

Also Hadwiger and McBride (2006) [16] reported that low level of copper plus chitosan resulted in significant decrease in late blight severity than each one alone and use of low level of copper resulted in reduced symptom. The present study is in conformity with the results obtained by Hadwiger and McBride (2006) but a new aspect has been added i.e using a third component, *Trichoderma* along with chitosan and low doses of copper for late blight management. Also the study highlights that all the copper compounds did not yield good results in the combination because interaction between the components lead to synergism that controls the disease. Chang and kim (2012) [17] tested two preparations of chitosan (SH-1 and H-1) against late blight of potato and found that both these preparation were highly effective in reducing late blight and thus can be adopted in organic potato production along with other disease control agents in an integrated manner. Also biological control of potato late blight has been done in the past using microbial antagonists e.g., *Trichoderma harzianum* and *T. viride* but to a limited extent as *Trichoderma* formulations alone were not effective for control of this disease (Singh 1968) [18], (Mukherji and Garg, 1988) [19] thus supporting the results of present study that *Trichoderma asperellum* along with chitosan and copper are effective in managing late blight. Hence the results obtained in this study are in close conformity with the above said research being done on the application of the combination of copper-chitosan-*Trichoderma* for late blight disease management.

Conclusion
The variant of the combination of copper, chitosan and Trichoderma having CuOH (TG) as copper source, was significantly better in terms of disease severity reduction, slow progress of disease and increased tomato yield. Consequently, the combination involving lower doses of CuOH (TG), plant defence inducer (chitosan) and bio-control agent (*Trichoderma*) showed promise in the effective disease management strategy as obtained with higher doses of standard fungicide by reducing the concentration of chemicals in the combination. Thus, CuOH based consortium could be used as an alternative to chemical fungicide for efficient management of late blight disease under organic cultivation. This, however, requires further research to supplement the present findings and conclusions.

Acknowledgements
Authors thank Department of Biotechnology, GOI for funding the "2+2 Indo-German project'' under which the present research was undertaken. Thanks are also due to the project partners at University of Munster, Germany, Spiess Urania Chemicals Gmbh, Hamburg, Sri-Biotech and Mahatani-Chitosan Pvt. Ltd., Hyderabad for providing inputs for the experiments. Also, authors thank the Director Research, Bihar Agricultural University, Sabour (Bihar) for providing necessary help and support in the conduct of field trials.

References
1. Madden LV. Measuring and modeling crop losses at the field level. Phytopathology. 1983; 73(11):1591-1596.
2. Nowicki M, Follad MR, Nowakowska M, Kozik EU. Potato and tomato late blight caused by *Phytophthora infestans*: an overview of pathology and resistance breeding. Plant Disease. 2012; 96:4-17.
3. Ragunathan V, Divakar BJ. Integrated pest management strategies. In: Molecular biology of the biological control of pests and disease of plants, ed. by M. Gunasekaran and D. J. Weber. CRC Press, Florida. 1996, 191-194.
4. Bhardwaj NR. Elucidating the role of *Trichoderma* in the triple combination 'Copper-Chitosan-*Trichoderma*' for the management of late blight disease of potato (*Solanum tuberosum* L.). Ph.D. Thesis, G.B Pant University of Agriculture and Technology, Pantnagar, Uttarakhand (India), 2016.
5. Ghorbani R, Wilcockson SJ, Glotis C, Leifert C. Potato late blight management in organic agriculture. Outlooks on Pest Management. 2004; 15:176-280.
6. Mizubuti ESG, Junior VL, Forbes GA. Management of late blight of potato with alternative products. Pest Technology. 2007; 1(2):106-116.
7. Erayya. Designing a triple combination of Copper, *Trichoderma* and Chitosan and its evaluation against late blight of potato caused by *Phytophthora infestans* (Mont.) de Bary. Ph.D. Thesis, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand (India). 2014, 157.
8. Sajeesh PK. Cu-Chi-Tri: A triple combination for the management of late blight disease of potato (*Solanum tuberosum* L.). Ph.D. Thesis, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand (India), 2015
9. Henfling JW. Late blight of potato: *Phytophthora infestans.* Technical Information Bulletin 4. International Potato Center Peru. 1987, 25.
10. Sajeesh PK, Bhardwaj NR, Balodi R, Kumar J. Field evaluation of triple combination of copper, chitosan and *Trichoderma* for management of late blight disease of potato under hill condition. Advances in Life Sciences. 2016; 5(7):2771-2778.
11. Bhardwaj NR. Elucidating the role of *Trichoderma* in the triple combination 'Copper-Chitosan-*Trichoderma*' for the management of late blight disease of potato (*Solanum tuberosum* L.). Ph.D. Thesis, G.B Pant University of Agriculture and Technology, Pantnagar, Uttarakhand (India), 2016.
12. Hadwiger LA, Chiang C, Victory S, Horovitz D. The molecular biology of chitosan in plant-pathogen interactions and its application to agriculture. In: Skjâk, G., Anthonsen, B. and Sandford, P. (eds) Chitin and chitosan: sources, chemistry, biochemistry, physical properties and applications. Elsevier Applied Sciences. Amsterdam. 1988, 119-l38
13. Muzzarelli R, Tarsi R, Filippini O, Giovanetti E, Biagini G, Varaldo PE. Antimicrobial agents and microther. 1990; 34(10).
14. Vasyukova NI, Chalenko GI, Gerasimova NG, Perekhod EA, Ozeretskovskaya OL, Irina AV *et al*. Chitin and chitosan derivatives as elicitors of potato resistance to late blight. Applied Biochemistry Microbiology. 2005; 36:372-376.
15. Vinale F, Sivasithamparam K, Ghisalberti EL, Marra R, Barbetti MJ, Li H. A novel role for *Trichoderma* secondary metabolites in the interactions with plants. Physiological and Molecular Plant Pathology. 2008; 72:80-86.
16. Hadrami AEl, Adam LR, Hadrami IEl, Daayf F. Chitosan in plant protection. Marine Drugs. 2010; 8:968-987; doi:10.3390/md8040968.
17. Hadwiger LA, McBride PO. Low level copper plus chitosan applications provide protection against late

blight of potato. Plant Health Progress. 2006; doi: 10.1094/PHP2006040601RS.
18. Chang T, Kim BS. Application of chitosan preparation for eco-friendly control of potato late blight. Research in Plant Disease. 2012; 18:338-348.
19. Singh JS. Biological control of *Phytophthora infestans*. In: Biocontrol of Plant Disease (eds. K.G. Mukerji and K.L. Garg) CRC Press Inc., Boca Raton. Florida, 1986, 174.
20. Mukerji KG, Garg KL. Biocontrol of Plant Disease, CRC Press, Inc., Boca Raton. Florida. 1988, 3.