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Research Article

Invitro Efficacy of Trichoderma Isolates and Commercial Fungicides against *Exobasidium vexans*, Causal Agent of Blister Blight in Tea

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Abstract

Blister blight is one of the most significant foliar fungal diseases of tea plant caused by *Exobasidium vexans.* An invitro experimentation on efficacy of biofungicides and chemical fungicides was carried out at Agriculture Research Station, Pakhribas from November 2020 to January 2021 to evaluate and analyze effectivity of two *Trichoderma* spp and five commonly used chemical fungicides against Blister Blight pathogen. Isolates of *Trichoderma viride* and *Trichoderma harzianum* were tested against *E. vexans* using dual culture technique. Five Chemical fungicides namely Copper oxychloride 50WP, Hexaconazole 5% EC, Metalaxyl 8% WP+ Mancozeb 64% WP, Carbendazim 50% WP, Carboxin 37.5% WS + Thiram 37.5% WS were tested at four different concentrations viz. 50 ppm, 100 ppm, 200 ppm and 500 ppm using poisoned food technique. The experiment was carried out in completely randomized design with three replications for each treatment. Both *Trichoderma viride* and *Trichoderma harzianum* demonstrated significant effect on mycelial growth reduction of *E. vexans*. *Trichoderma viride* and *Trichoderma harzianum* exhibited 70.87% and 66.98% inhibition in growth of *E. vexans* respectively. Similarly, Hexaconazole, Carbendazim, Carboxin + Thiram provided complete (100%) inhibition on pathogen growth regardless of concentration. Least inhibition (36%) on pathogen growth was recorded at 50 ppm of Copper Oxychloride. All chemical fungicides provided significant difference on reducing growth of pathogen in comparison to control at all concentration. It is recommended that *Trichoderma* spp be taken as a priority regarding its environmental benefit and furthermore if application of chemical fungicides is necessitated, rationalized use be done at lower dose and with appropriate timing.

Keywords: Blister blight; *Exobasidium vexans*; Fungicides; *Trichoderma viride*; *Trichoderma harzianum*

Introduction

Tea, a beverage widely regarded as one of the most popular drink worldwide is prepared by brewing processed leaves and shoots of tea plant, *Camellia sinensis* (Kumar and Shruthi, 2014). *Camellia sinensis* is a shrub type plant that can grow as a tree, but to simplify plucking and for profuse growth of new shoots, plants are pruned at regular intervals. The classification system by Ming (2000), describes currently cultivated tea plant into two varieties *Camellia sinensis* var. *sinensis* (China type tea) and *Camellia sinensis* var. *assamica* (Masters) Chang (Assam type tea). *C. assamica* subsp. *Lasiocalyx* (cambod type tea) considered to be a third variety is a hybrid between China and Assam type teas (Wambulwa *et al*., 2016 ; Meegahakumbura *et al*., 2018). In Nepal, all three type *C. sinensis* var. *sinensis*, *C. sinensis* var. *assamica* and *C. assamica* subsp. *lasiocalyx* are cultivated. But commercial cultivation is of the only first two in Nepal (Shrestha, 2016).

Agriculture, in its entirety, is not a separate and isolated entity, rather it is made up of several small and integrated entities bundled together. Crops like tea are such entities which comprised 2.85 % share in Nepal's total export in FY 2076/77 which was 3.30 percent in the previous fiscal year (MoFDC, 2019). Tea subsector contribution to the national gross domestic products (GDPs) is about 0.0105% and 0.0347% to the agricultural gross domestic product (AGDP) (CBS, 2014). Tea alone contributes a fair share of percentage in revenue generation of country. Since leaves are the marketable product, and subject of interest, any disruption on growth, stress and damage on leaves due to any pathogen or pest becomes of utmost importance and impacts leaf quality, plant's health and ultimately affects yield adversely. As is with any other crop *Camellia sinensis* is vulnerable to several diseases and pests. Some of the most common diseases that affect tea quality and quantity include grey blight (*Pestalotiopsis*), brown blight (*Colletotrichum*), Blister blight (*Exobasidium vexans*), leaf spots (*Cylindrocladium, Calonectria, Chochliobolus*) and red rust (*Cephaleuros virescens*) (Lehmann-Danzinger, 2000).

Among these, blister blight caused by a fungus *Exobasidium vexans* Massee is one of the most widespread diseases of tea (Ajay and Baby 2010). It mainly attacks young leaves and has caused huge losses in several parts of the world with 25-35% annual loss (Karunarathna *et al*., 2018). Reported for the first time from Upper Assam, India in the year 1868 (Watt and Mann 1903), blister blight has incurred losses upto 43% annually (Ordish, 1952, as cited in Ram and Mouli, 1983). It was first reported in Nepal in 1948 (Chaliha and Kalita 2020). Its incidence and severity is very high in shaded area (Shanmuganathan and Sabanayagam, 1961) and in monsoon season when moisture is high in the environment and is more prevalent in plantations at higher altitude (Agnihothrudu and Moulli, 1991 as cited in Barman *et al.*, 2020). Initial symptoms develop first on young and tender leaves and shoots and as a first stage translucent pale-yellow spots develop and later the spots enlarge (Sen *et al*., 2020). The spots on the upper side of leaf start shrinking into a shallow depression and on the underside typical blister lesions form which are convex in nature. On the lower side of the leaf, dense white and velvety growth appears and spores are produced there. The velvety growth is due to the presence of dense clusters of vertical sterile hyphae (Gadd and Loos 1948). The young stem swells, bends, distorts, breaks and ultimately dies back (Barman *et al*., 2020). When symptoms turn more severe the young leaves curl and roll very irregularly. Processing diseased leaf reduces quality, due to several biochemical changes in them (Baby *et al*., 1998) and hence adds more to the already affected business.

In tea, losses due to diseases is very high compared to losses by pest (Lehmann-Danzinger, 2000). The severity of attack has resulted in farmers using chemical fungicides which in many cases is unrationalized. Kalauni & Joshi (2019) analysed that among pesticides used in Nepal, fungicides shared a major chunk with 49%, followed by insecticide (33%), herbicide(14%) and then others (4%). Sharma *et al*. (2013) reported a higher (2,100 g, a.i./ha) pesticide application rate in tea farms, some 15 times, more than the national average rate of 142 g, a.i./ha. Irrational use of excessive pesticides in tea, or dependence incurs more problems with regards to poisoning, environmental hazard, residue and resistance development by pathogen. Considering the importance of tea and in its own context to our economy and trade, it is important that these problems be looked into. Disease on tea does not just attack on leaf but on different facets, on harvest, yield and economic aspects which is why when disease incidences rise, it necessitates looking for management strategies with low effect on environment. Due to the easiness, quickness and efficiency, chemical fungicides are a very easy option but its judicious use is very necessary. Over use, misuse, residual remains of pesticides has been reported from all over Nepal. For a very long-time pesticide application has provided a short-term relief, but with onset of organic certification and fungicidal toxicity on leaves due to over application, use of alternatives is encouraged, and rational use of chemical fungicides is preferred. And around this, bio-fungicides, which have been an integral part of environment are the perfect alternative to be used as an option in disease control.

Trichoderma spp are one such option. These fungi antagonize plant pathogen by using mechanisms like mycoparasitism, antibiosis and competition for nutrients and space, modification of the environmental conditions, and/or stimulating plant growth and plant defense mechanisms. (Benítez *et al*., 2004). *Trichoderma spp*. are soil borne and filamentous fungi, cosmopolitan in nature and frequently grown in all types of soil, manure and decaying woods (Samuels, 1996). They are plant symbionts and function as parasites and antagonists of many phytopathogenic fungi, thus protecting plants from disease. These fungi now are most commonly grown on selective media and visually identified based on morphological characters. Depending upon the strain, the use of *Trichoderma* in agriculture can provide numerous advantages.

This study will evaluate comparative efficiency of both biocontrol measures and chemical fungicides which would be helpful when later tested in field. Invitro study can ascertain and shortlist results for further research at a larger scale. Study of efficacy of these alternative approaches, and its benefit can prove as an option to conventional control methods and further promote rational application of Fungicides. This research was conducted with a hope that, it would help us provide efficacy of fungicides at different doses, provide us a comparison between bio-fungicides and

chemical fungicides and how it fared against fungal pathogens.

Materials and Methods

Experimental Site

This experiment was conducted in Plant Protection laboratory of Agriculture Research Station, Dhankuta. Dhankuta, along with other hilly districts, is a major tea growing district in eastern Nepal. This experiment was carried out in order to identify foliar diseases in the Station's plantation and then screen the pathogen against different fungicidal concentration and biocontrol agents. Blister blight was profusely prevalent in Tagdah variety and samples were brought in and used in the laboratory.

Isolation and determination of pathogen

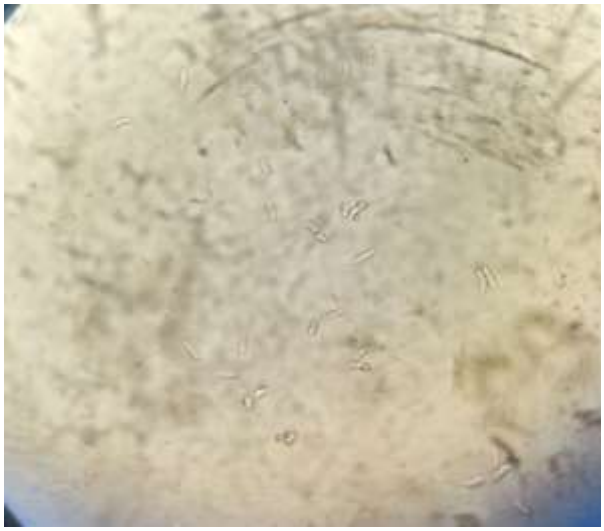

Fig. 1: Spores of *Exobasidium vexans* under microscope

Diseased samples with characteristic blister blight symptoms were cut into small pieces of about 2mm size and sterilized using 1% NaOC1 for 30 seconds and then subsequently washed with sterile distilled water 3 to 4 times. The sterilized diseased sections were placed in moisture chamber and incubated for 3 days at 26±1°C for profused fruiting. Then the sections were teased and examined under microscope (Fig. 1). In the Section with characteristic symptom of blister blight the pathogen were identified as *Exobasidium vexans* according to their morphological and microscopic character as identified by Gadd & loos (1948), ellipsoid, hyaline basidiospores with distinct septation only when they are mature, (Sen *et al*., 2020) and several two-celled thin-walled conidia. After determination the sections were aseptically transferred to petriplates with PDA media and incubated at 27±1 °C in an incubator.

Obtaining and Maintaining Pure Culture

After determination of pathogen the diseased section was plated in PDA plates. PDA plates were prepared using 39 gm of commercial PDA powder in a litre of distilled water, autoclaved at 121°C for 20 minutes at 15 lbs pressure then poured into sterilized petriplates under laminar air flow bench. Pure culture of *Exobasidium vexans*, was obtained after 4 subsequent subculturing.

Research Methodology and Research Design

The experiment was conducted using a completely randomized design (CRD), each treatment was allocated randomly and replicated 3 times and a control was placed along with every treatment. Growth observations were taken and Percent inhibition of fungal growth was calculated using the following formula (Vincent, 1947):

$$\text{Percent growth inhibition (\%)} = \frac{(C-T)}{C} x100$$

Where, C= colony growth in control plate

T = colony growth in treated plate

In-Vitro Evaluation of Commercial Fungicides

For the In-Vitro efficacy test, 5 commercially available fungicide; single and binary mixture of fungicides were selected. PDA media was autoclaved and after calculation, required calculated amount of fungicide was mixed and 50 ppm, 100 ppm, 200 ppm, and 500 ppm of poisoned media was prepared and poured in petridish (Table 1). The actively growing 5 mm fungal disc of *E. vexans* was cut and placed using sterilized cork borer.

Table 1: Selected commercial fungicides

1.	Copper Oxychloride 50% WP
2.	Carbendazim 50% WP
3.	Metalaxyl 8% WP + Mancozeb 64 % WP
4.	Carboxin 37.5% WS + Thiram 37.5% WS
5.	Hexaconazole 5 % EC

Dual culture test

Trichoderma viride and *Trichoderma harzianum* were tested for their biocontrol potentiality against *Exobasidium vexans* using dual culture technique. Commercial package of *T. harzianum* was taken and placed using sterilized spatula, over PDA media in petriplate and incubated at 27±1°C in an incubator, and after subculturing, pure culture of *T. harzianum* was obtained. Similarly, Pure culture of *T. viride* maintained in plant pathology laboratory was subcultured on a fresh PDA plate. For dual culture test, a sterilized cork borer was used to cut an actively growing 6mm mycelial section of 6 days old *E. vexans*, and placed about a cm away from the edge of a petriplate, and at opposite equidistant position, 6mm section of actively growing mycelial section of a week-old *T. harzianum* was placed. Similarly, 6mm section of actively growing mycelia of 7 days old *E. vexans* and *T. viride* culture was cut and placed at equidistant position. This was replicated 3 times

and a control was placed where only the 6 mm section of 6 days old *E. vexans* was allowed to grow.

Data recording and Analysis

The recorded data were fitted and analysed using MS excel and GenStat software 15.1.0.8035 version. The data and data were presented in different graphs and in tabular form.

Result and Discussion

Five fungicides at 4 different concentrations were tested against *Exobasidium vexans*, causal agent of blister blight in tea using poisoned food technique. The results recorded were analysed and then presented in tabular forms and in figures.

In-Vitro Evaluation of Fungicides Against Exobasidium vexans

Table 2 indicates growth in treated plates showed significant effect $p<0.001$ when compared over growth in control plates. Figures with the different letters indicate that they are significantly different at P = 0.05. Fungal growth inhibition ranged from 36% to 100%. Maximum inhibition (100%) was observed in Hexaconazole, a combination of Carboxin + Thiram and Carbendazim irrespective of their concentration. The results are in favor with findings in Southern India (Chaliha and Kalita 2020) who reported antisporulant activities, spore size reduction, viability of *Exobasidium vexans* by fungicides like Hexaconazole. Inhibition in spore germination results in diminished viability of spore which then reduces the chances of pathogenicity.

Table 2: Efficacy of different fungicidal concentration on mean colony growth of Exobasidium vexans after 10 days.

Fungicides	Mean colony growth (cm) at different concentration ppm			
	50 ppm	100 ppm	200 ppm	500 ppm
Copper oxychloride	1.5733b	1.2250c	0.6500ef	0.0000h
Carbendazim	0.0000h	0.0000h	0.0000h	0.0000h
Metalaxyl + Mancozeb	0.7733de	0.8000d	0.6000f	0.3500g
Carboxin + Thiram	0.0000h	0.0000h	0.0000h	0.0000h
Hexaconazole	0.0000h	0.0000h	0.0000h	0.0000h
Control (without fungicide)	2.4583a			
Grand mean	0.4014			
CV	18.8 %			
LSD (p = 0.05)	0.12435			
Standard error mean	0.04357			

*Values are the mean of three replications. Different letter following means indicate significant difference at P = 0.05

Table 3: Growth inhibition percentage of different fungicidal concentrations against *Exobasidium vexans.*

Fungicides	Growth inhibition (%) at different concentration (ppm)				Mean growth inhibition (%)
	50 ppm	100 ppm	200 ppm	500 ppm	
Copper oxychloride	36	50.16	73.55	100	64.92
Carbendazim	100	100	100	100	100
Metalaxyl+ Mancozeb	68.54	67.45	75.59	85.76	74.335
Carboxin + Thiram	100	100	100	100	100
Hexaconazole	100	100	100	100	100
Control (without fungicide)	0				0

Minimum inhibition in fungal growth was found in treatment of 50ppm of copper oxychloride (36%). In case of Copper oxychloride and Metalaxyl + Mancozeb, inhibition of fungal growth increased with increase in concentration. This also furthers and strengthens the argument of using Copper oxychloride below threshold level. At 500 ppm, Copper oxychloride provided complete inhibition in the growth of *Exobasidium vexans* while Metalaxyl 8% WP + Mancozeb 64% WP, provided about 86% inhibition. Copper fungicides work by acting on the nonspecific denaturation of cellular proteins. It disrupts the function of proteins and enzymes after absorption and results in cell damage and membrane leakage (Husak, 2015). As the concentration decreased, more mycelial growth of pathogen was observed. Systemic fungicides can be locally systemic and systemic, but regardless, these are mostly target based. Systemic fungicides like Hexaconazole, Carboxin, Metalaxyl, Carbendazim, penetrate host tissue and act on cellular sites of pathogen and then inhibit metabolic processes. These fungicides are generally narrow spectrum and act on target pathogen, also one of the reasons why they provide higher protection. Fungicides also alter structure, function of fugal cell membrane. Particularly, why Hexaconazole at lower doses was found highly effective is due to its ergosterol biosynthesis inhibiting (EBI) nature (Li *et al*., 2013)**.** EBIs have antisporulant activity and provide significant reduction in spore size, viability, and inhibits spore germination. The findings are in sync with what is known about EBI's antisporulant action, the suppression of viability (Baby *et al*., 2004)

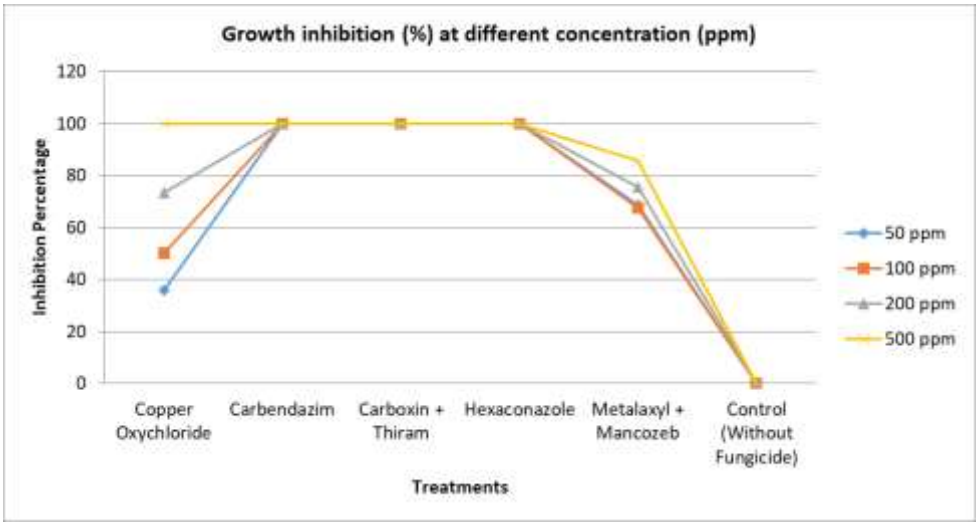

Fig. 2: Inhibition Percentage of fungicides on mean colony growth of *Exobasidium vexans.*

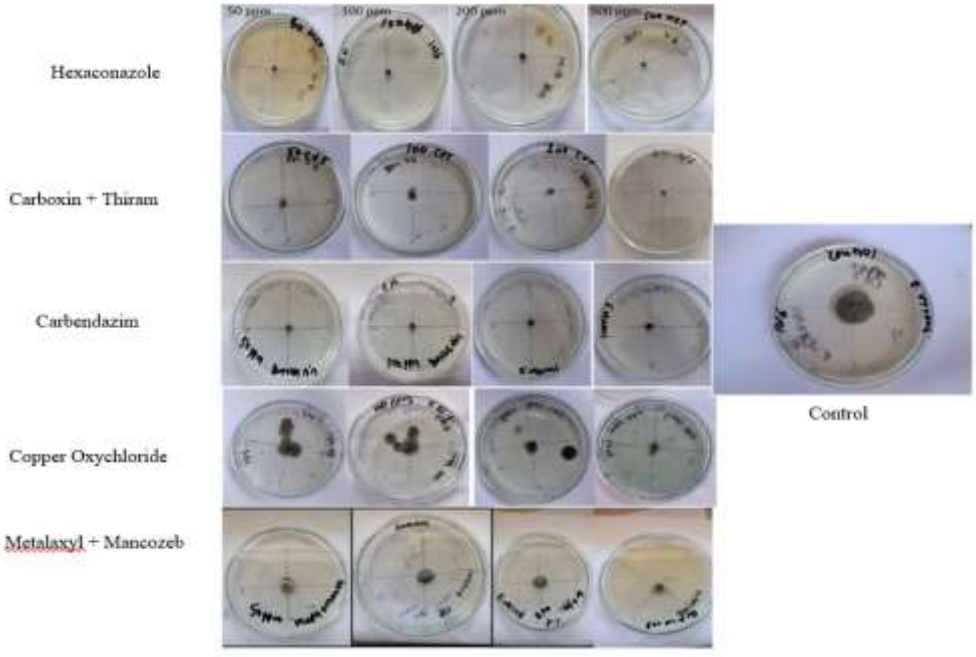

Fig. 3: Effect of different fungicides on mycelial growth of *E. vexans.*

Bioefficacy test of Trichoderma spp against Exobasidium vexans

Trichoderma viride and *Trichoderma harzianum* were selected to test their efficacy against *E. vexans* as a biocontrol agent. On 6th day of the dual culture, growth of *E. vexans* stopped completely and upon measurement, *Trichoderma harzianum* exhibited 66.98% inhibition in pathogen growth which indicated a good potentiality. Growth of *Exobasidium vexans* was significantly different in the treated plates than the growth in control plates. Similarly, in case of *Trichoderma viride*, on day 10, growth of pathogen completely stopped with 70.87 % inhibition. Biocontrol agents like *Trichoderma* utilize several mechanisms in controlling, modifying pathogen growth and functioning. *Trichoderma spp*. are known to suppress the pathogen by hyperparasitism and cell wall lysis (Baby and Chandramouli, 1996; Highley and Ricard, 1988). These results are encouraging and a very positive indication that biological agents do provide preventive protection against pathogen. In tea particularly, this result showcases that biological agents in an integrated approach can be used as a tool in Blister Blight management.

Table 4. Mean radial growth of Exobasidium vexans against Trichoderma viride and Trichoderma harzianum.

Treatment	Mean radial growth of *E. vexans* (cm)	inhibition percentage of growth (%)
T viride	0.5000b	70.87
T harzianum	0.5667b	66.98
Control	1.7167a	
CV%	8	
Grand mean	0.928	
SEM	0.0430	
LSD	0.1489	

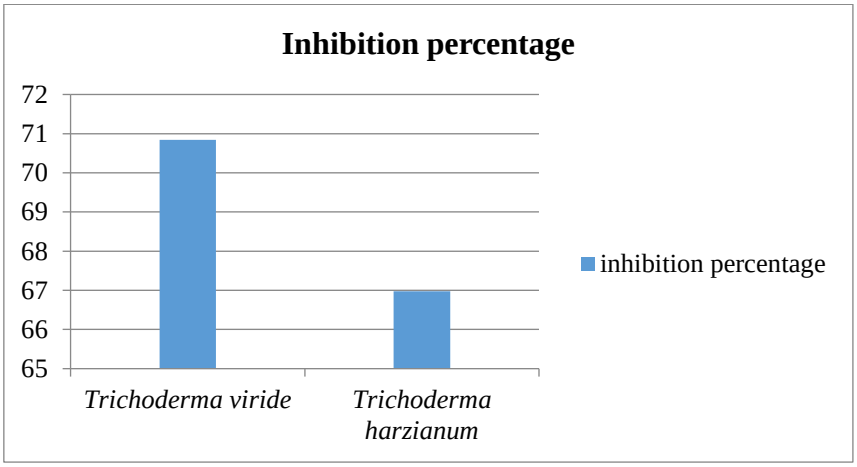

Fig. 4: Inhibition percentage of *Trichoderma* spp against *Exobasidium vexans.*

Conclusion

The study exhibited significant growth suppression of pathogen by both *T. harzianum* and *T. viride.* Complete inhibition was observed only in case of chemical fungicides. Chemical fungicides, regardless of their concentration provided significant growth suppression, emphasizing on judicious and rational use of appropriate chemical fungicide. Hexaconazole, Carbendazim and Carboxin 37.5% + Thiram 37.5% completely inhibited pathogen growth at all concentration. Copper oxychloride at 50 ppm provided least inhibition but as the concentration increased the pathogen growth reduced. Same pattern of growth inhibition was seen in Metalaxyl + Mancozeb treated plates. Based on the findings, Copper oxychloride and Metalaxyl + Mancozeb exhibited growth reduction as concentration increased but every treatment was significantly different to control. This attempt at identifying specific management approach should be further replicated and integrated with other approaches in managing this pathogen in field. These results need to be further verified, tested under in-vivo conditions to find out the actual degree of inhibition in field. Biotic stresses like these need several efforts of tolerant cultivars development to diseases, abiotic stresses. This

study has shown that both biocontrol agents *T. harzianum* and *T. viride* exhibit higher inhibition in growth of *Exobasidium vexans* and should be prioritized especially in a crop like Tea and be integrated with tested fungicides at lower concentration. Several fungicides provided uniform effective result at both higher and lower concentration indicating that no further indiscriminate use of fungicide be encouraged.

Author's Contribution

Karun Adhikari designed the research plan, performed data acquisition and drafted the manuscript; Karun Adhikari & Ashis Rai analysed and interpreted the data. Final form of manuscript was approved by both of the authors.

Conflict of Interest

The authors declare that there is no conflict of interest with the present publication.

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References

Ajay D, Baby UI (2010) Induction of systemic resistance to Exobasidium vexans in tea through SAR elicitors. *Phytoparasitica* **38**: 53–60. DOI: 10.1007/s12600-009-0068-x

Baby UI, Balasubramanian S, Ajay D, Premkumar R (2004) Effect of ergosterol biosynthesis inhibitors on blister blight disease, the tea plant and quality of made tea. *Crop Prot* **23**: 795–800. DOI: 10.1016/j.cropro.2004.01.001

Baby UI, Chandramouli B (1996) Biological antagonism of *Trichoderma* and *Gliocladium* spp. against certain primary root pathogens of tea. *J Plant Crops* **24**: 249–255.

Baby UI, Ravichandran R, Ganesan V, Pathiban R, Sukumar S (1998) Effect of blister blight disease on the biochemical and quality constituents of green leaf and CTC tea. *Trop Agric* **75**

Barman A, Nath A, Thakur D (2020) Identification and characterization of fungi associated with blister blight lesions of tea (Camellia sinensis L. Kuntze) isolated from Meghalaya, *India. Microbiol Res* **240**. DOI: 10.1016/j.micres.2020.126561

Benítez T, Rincón AM, Limón MC, Codón AC (2004) Biocontrol mechanisms of Trichoderma strains. *Int Microbiol* **7**: 249–260. DOI: 10.2436/im.v7i4.9480

CBS (2014) Statistical Information in Nepalese Agriculture

Chaliha C, Kalita E (2020) Blister Blight Disease of Tea: An Enigma. In: Diagnostics of Plant Diseases. *IntechOpen* DOI: 10.5772/intechopen.95362

Gadd CH, Loos CA (1948) The basidiospores of Exobasidium vexans. *Trans Br Mycol Soc* **31**: 229–233. DOI: 10.1016/s0007-1536(48)80004-5

Highley TL, Ricard J (1988) Antagonism of Trichoderma spp. and Gliocladium virens against wood decay fungi. *Mater und Org* **23**: 157–169.

Husak V (2015) Copper and Copper-Containing Pesticides: Metabolism, Toxicity and Oxidative Stress. *J Vasyl Stefanyk Precarpathian Natl Univ* **2**: 38–50. DOI: 10.15330/jpnu.2.1.38-50

Kalauni D, Joshi A (2019) Pesticides Import, Use, Consumption and Residue Status Among Food Crops in Nepal: a Review. *Big Data Agric* **1**: 21–25. DOI: 10.26480/bda.01.2019.21.25

Karunarathna KHT, Mewan KM, Weerasena OVDSJ, Perera SACN, Edirisinghe ENU, Jayasoma AA (2018) Understanding the genetic relationships and breeding patterns of Sri Lankan tea cultivars with genomic and EST-SSR markers. *Sci Hortic (Amsterdam)* **240**: 72–80. DOI: 10.1016/j.scienta.2018.05.051

Kumar PV, Shruthi BS (2014) Tea : An Oral Elixir. *Sch Acad J Pharm* **3**: 9–18.

Lehmann-Danzinger H (2000) Diseases and Pests of Tea: Overview and Possibilities of Integrated Pest and Disease Management. *J Agric Trop Subtrop* **101**: 13–38.

Li Y, Dong F, Liu X, Xu J, Chen X, Han Y, Liang X, Zheng Y (2013) Studies of Enantiomeric Degradation of the Triazole Fungicide Hexaconazole in Tomato, Cucumber, and Field Soil by Chiral Liquid Chromatography–Tandem Mass Spectrometry. *Chirality* **43**: 34–43. DOI: 10.1002/chir.22121

Meegahakumbura MK, Wambulwa MC, Li MM, Thapa KK, Sun YS, Möller M, Xu JC, Yang JB, Liu J, Liu BY, Li DZ, Gao LM (2018) Domestication origin and breeding history of the tea plant (Camellia sinensis) in China and India based on nuclear microsatellites and cpDNA sequence data. *Front Plant Sci* **8**: 1–12. DOI: 10.3389/fpls.2017.02270

MoFDC (2019) Nepal Foreign Trade Statistics Fiscal Year 2019/20 (2076/77). https://customs.gov.np/storage/files/1/FTS/Annual_FTS_pdf.pdf

Samuels GJ (1996) Trichoderma: A review of biology and systematics of the genus. *Mycol Res* **100**: 923–935. DOI: 10.1016/S0953-7562(96)80043-8

Sen S, Rai M, Das D, Chandra S, Acharya K (2020) Blister blight a threatened problem in tea industry: A review. *J King Saud Univ - Sci* **32**: 3265–3272. DOI: 10.1016/j.jksus.2020.09.008

Shanmuganathan N, Sabanayagam V (1961) The influence of sunshine and rain on tea blister blight, Exobasidium vexans Massee. *Ann appl Biol* **49**:306–315. DOI: 10.1111/j.1744-7348.1961.tb03616.x

Sharma D, Thapa R, Manandhar H, Shrestha S, Pradhan S (2013) Use of Pesticides in Nepal and Impact on Human Health and Environment. *J Agric Environ* **14**: 171. DOI: 10.3126/aej.v14i0.19797

Shrestha S (2016) Tea cultivation manual. https://www.academia.edu/41555522/Tea_Cultivation_Manual_Nepali

Venkata Ram CS, Chandra Mouli B (1983) Interaction of dosage, spray interval and fungicide action in blister blight disease control in tea. *Crop Prot* **2**: 27–36. DOI: 10.1016/0261-2194(83)90023-6

Wambulwa MC, Meegahakumbura MK, Chalo R, Kamunya S, Muchugi A, Xu JC, Liu J, Li DZ, Gao LM (2016) Nuclear microsatellites reveal the genetic architecture and breeding history of tea germplasm of East Africa. *Tree Genet Genomes* **12**: 1–10. DOI: 10.1007/s11295-015-0963-x

Watt G, Mann H (1903) The pests and blights of the tea plant / by Sir George Watt and Harold H. Mann. | Wellcome Collection. https://wellcomecollection.org/works/ef5mfjaq. Accessed 11 Aug 2021.