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Effect of chemicals and biological agents on branch canker disease in tea

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Branch canker caused by *Macrophoma* sp. is the most wide spread and serious stem disease of tea plants. In this study, we tried to determine the bioefficacy of different chemical fungicides and indigenous biological strains on branch canker disease in tea plant under glasshouse condition. The infected stem portions were collected and identified through 18S rRNA molecular methods. Bacterial strains were isolated from different tea growing areas and confirmed by16S rRNA technique. Fungal biocontrol strains were obtained from repository Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India. Among the fungicides tested, benomyl (70%) showed better results followed by copper oxychloride (65%). The biocontrol agents *Bacillus* sp. (50%) and *Trichoderma viride* (50%) yielded significant results. *Pseudomonas* sp. showed moderate activity (45%) followed by *Trichoderma harzianum* (30%). The integrated combination approach, propiconazole/*Bacillus amyloliquefaciens* and propiconazole/*B. subtilis* recorded effective control against branch canker disease under greenhouse condition followed by hexaconazole/*B. amyloliquefaciens.* Combination of fungicides companion (0.70), hexaconazole (0.63) and propiconazole (0.67) were found to be most effective. The organic fungicide tricure gave satisfactory control followed by *Gliocladium virens* against branch canker disease.

Keywords: Beverage, *Camellia sinensis*, Companion, Fungicides, *Gliocladium virens*, Hexaconazole, *Macrophoma* sp. Propiconazole, Tricure

Tea is the most popular, inexpensive beverage throughout the world because of its characteristic aroma and flavour produced from the shoots of the commercially cultivated tea plants (*Camellia sinensis* (L.) O. Kuntz). Tea is a monoculture crop, it provides a stable microclimate for several pests and diseases, which on an average leads to a crop loss of around 10-15%[1].

In India, tea is cultivated extensively in Assam and West Bengal, and also in certain parts of Himachal Pradesh and Uttar Pradesh. Tea gardens in south India are spread over the Western Ghats in Karnataka, Kerala and Tamil Nadu. Tea is grown in 0.58 million ha and it was estimated nearly 88,115 tea plantations are in India which includes Assam (55.4 %), West Bengal (24 %), Tamil Nadu (9.3 %), Kerala (8.3 %), Tripura (1.4 %) and Karnataka (0.49 %). In Tamil Nadu, tea grown areas are in the Nilgiris and the Anamallais[2].

Branch Canker is a stem disease caused by *Macrophoma theicola*. Commonly occurs in drought prone areas, it is fairly distributed in Sri Lanka. Its action on the crop is variable. In some conditions, it merely causes branch canker; in others, it kills back the younger shoots. In a few instances, it causes considerable intense damage in new clearings (about 3 or 4 years old). Low yield due to collar and branch canker caused by *Phomposis theae* and *M. theicola* have been reported from Central Africa[4]. The present work focuses on the effect of various chemical fungicides, indigenous bacterial strains and fungal biological control agents against the branch canker disease caused by *Macrophoma* sp. in greenhouse condition.

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Materials and Methods

Branch canker pathogen

The branch canker pathogen was isolated from different tea growing areas of south India. The branch specimen was washed with sterile distilled water and surface sterilized with 0.1% mercuric chloride for 1.0 min. The infected portion was cut into small pieces and then plated on water agar medium and after a day of incubation, the pathogen was transferred to PDA medium. The branch canker pathogen was characterized and morphologically identified by following the method described earlier by Petch[3]. The fungus was confirmed as *Macrophoma* sp. through 18S rRNA molecular technique (NCBI Accession No.

JQ234977 for NBCHE-6) using BLAST search method at NCBI[5].

Biocontrol strains

The bacterial biocontrol agents were isolated from different tea growing regions (Coonoor, Koppa and The Anamallais). Identification of morphological characteristics and biochemical tests were carried out as described in Stolp & Gadkari[6]. Bacterial biocontrol strains were conformed using 16S rRNA molecular method[7]. Molecular identification of indigenous bacteria strain codes were given as WR46-2 for *Bacillus* sp., WR5-4 for *Pseudomonas* sp., CS-2 for *B. subtilis* and WPIO4 for *B. amyloliquefaciens*. The bacterial sequences were submitted in NCBI Gen Bank Nucleotide Data Bases for *Bacillus* sp. (JN616373), *Pseudomonas* sp. (JQ319656), *B. subtilis* (KM527836) and *B. amyloliquefaciens* (KM853034). The fungal biocontrol agents, *Trichoderma harzianum, T. viride* and *Gliocladium virens*, were procured from Microbial Type Culture Collection and Genebank, (MTCC), CSIR-IMTECH, Chandigarh, India.

Evaluation of chemicals and biocontrol agents

The effect of different chemical fungicides, such as copper oxychloride 3.0 g/L (fytolon 50% WP), Benomyl 0.5 g/L (benofit 50% WP), tebuconazole 0.5 g/L (25.9 w/w folicur EW 250) and tridemorph 0.5 g/L (calixin 80% EC) were evaluated against branch canker disease under greenhouse condition and also biocontrol agent such as *Bacillus* sp. *Pseudomonas* sp. *T. harzianum, G. virens* and *T. viride* at 3 g/L were tested against branch canker disease under greenhouse condition.

Integrated management of branch canker disease

Systemic fungicides, such as hexaconazole 1.0 mL/L (contof 5% EC), propiconazole 0.7 mL/L (Tilt 25% EC), companion 2.0 g/L (carbendazim 12% + mancozeb 63% WP) and botanical fungicide of tricure 3.0 mL/L (0.03% azadiractin EC) were assessed and screened against branch canker disease under greenhouse condition. Biocontrol strains, such as *Bacillus subtilis*, *B. amyloliquefaciens* and *Gliocladium virens* were critically studied at equal concentrations of 3.0 g/L against branch canker disease.

Greenhouse experiment method

Twelve months old healthy nursery tea plants were taken to study the bioefficacy of chemical fungicides and biocontrol agents against branch canker disease, *Macrophoma* sp under greenhouse condition. The disease was induced in 25 plants by injuring the stem with a sterile scalpel and uniformly sprayed with *Macrophoma* sp. spore suspension (1×10^5 spore mL^{-1}) of 7 days old culture. The inoculated plants were kept in greenhouse conditions for disease development. After 43 days of incubation, the size of canker length was recorded as pre-assessment. Subsequently, the chemical fungicides and biocontrol were sprayed at 7 days interval. A control treatment was maintained to study the infection rate of *Macrophoma* sp. without chemical fungicides and biocontrol application. The application dosages were followed as per UPASI-TRF, TRI recommendations (Good Agriculture Practice). Three replications were maintained for each treatment and each replication consisting of 25 plants. After 4th spraying, branch canker disease protection level was calculated as percentage level (%) = Number of plants infected/Total number of plants per treatment; and the reduction of canker length was calculated using the formula: Wound Healing (WH) = (L1-L2), where, L1 – Pre- and L2- Post-assessment. All data obtained were subjected to statistical analysis of variance (ANOVA) and were used to evaluate differences between separate means. Differences with CD at 0.05 were considered statistically significant[8].

Results and Discussion

Evaluation of chemicals and biocontrol agents

The indigenous bacterial biocontrol strains were isolated from different tea area of southern India and fungal biocontrol agents were obtained from MTCC (Table 1). The selected biocontrol and chemicals were tested against branch canker disease causal organism by *Macrophoma* sp. under greenhouse condition. Benomyl (70%) was noticed as the highest disease protection level against branch canker disease followed by copper oxychloride (65%) (Table 2). The results are in agreement with Nepolean *et al*.[9]. Benomyl at lowest

Table 1 — Bacterial and fungal biocontrol agents

Bacterial strains	NCBI Accession No.	Different tea growing regior
Bacillus sp. (WR46-2)	JN616373	The Anamallai
Pseudomonas sp. (WR5-4)	JQ319656	Koppa
Bacillus amyloliquefaciens (WP1O4)	KM853034	The Anamallai
Bacillus subtilis (CS-2)	KM527836	Coonoor
Fungal strains	Collection center	Location
Trichoderma harzianum	MTCC	Chandigarh
Trichoderma viride	,,	,,
Gliocladium virens	,,	,,

concentration (0.01%) totally inhibited the wood rot pathogen *Hypoxylon serpens* followed by copper oxychloride. Moreover, the systemic fungicide of benomyl (0.2%) has been shown to have effective control on *Macrophomina* sp. blight of mung bean[10].

In the present study, tebuconazole (60%) and tridemorph (55%) showed good results against branch canker disease under greenhouse condition (Table 2). These findings are in accordance with Mareeswaran *et al.*[11] who have reported that tubeconazole inhibits the growth of *Macrophoma* sp. at various concentrations under *in vitro* condition. The biological agents, such as *Bacillus* sp. (50%) and *Trichoderma viride* (50%) showed significant results against branch canker disease (Table 2). Chowdappa *et al.*[12] have reported that *B. subtilis* and *T. harzianum* against the early and late blight in tomato plant and induced the systemic resistance. Many workers have observed that *Trichoderma* spp. control the soil borne fungal pathogens[13]. Das *et al.*[14] have reported that application of *T. viride* in *Rhizoctoina solani* injected soils reduced sheath blight pathogen of rice.

Table 2 — Potential of selected biocontrol agents and chemical fungicides on the control of *Macrophoma* sp. under greenhouse condition

Treatment details	Dosage in 10L	Branch canker. Disease protection level (%)
Benomyl (Benofit 50%WP)	5 g	70[a]
Copper oxychloride (Fytolon 50% WP)	30 g	65[b]
Tebuconazole 25.9%w/w (Folicur EW 250)	5 g	60[c]
Tridemorph (Calixin 80% EC)	5 g	55[d]
Bacillus sp. (WR46-2)	30 g	50[e]
Pseudomonas sp. (WR5-4)	30 g	45[f]
T. harzianum (MTTC)	30 g	30[g]
T. viride (MTTC)	30 g	50[e]
CD at P= 0.05	-	2.80

[Values are mean followed by the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 0.05 % level]

The present study revealed that *Pseudomonas* sp. (45%) yielded moderate disease protection level followed by *T. harzianum* (30%) against branch canker disease under greenhouse condition (Table 2). The results are in agreement with Mareeswaran *et al.*[15] the *Pseudomonas* sp. showed least antagonistic potential against branch canker pathogen under *in vitro* condition. Singh *et al.*[16] have reported that *T. harzianum* and *P. fluorescens* showed control of pea wilt caused by *Fusarium* sp.

Integrated management of branch canker disease

Integrated approach, i.e., chemical fungicides along with biological agents, to control the branch canker disease showed mixed results. Size of canker in particular reduced significantly after imposing the treatments under greenhouse condition. However, there were no significant difference in the treatments (Table 3). The present study has clearly indicated that integrated spraying of propiconazole/*Bacillus amyloliquefaciens* reduced the size of canker followed by propiconazole/*B. subtilis* against branch canker disease under greenhouse condition. The same effects were observed in the treatment hexaconazole/*B. amyloliquefaciens* and hexaconazole/*B. subtilis* (Table 3). The result are in agreement with Morang *et al.*[17] who have demonstrated that hexaconazole (100%) and propiconazole (98.51%) were more effective against brown root rot of *Fomes lamoensis* in tea plant at concentration of 100 mg/L. The integrated approach was reported earlier by Singh *et al.*[16] against pea wilt caused by *Fusarium* sp.

Table 3 — Alternate spraying schedule management of branch canker disease under greenhouse condition

Treatment details	Dosage in 10L	Assessment in cm		Disease Protection level
		Pre (L1)	Post (L2)	(L1-L2)
Propiconazole	7 mL	1.53	0.87[a]	0.67
Hexaconazole	10 mL	1.37	0.73[a]	0.63
Companion	20 g	1.80	1.10[a]	0.70
Propiconazole/*Bacillus amyloliquefaciens*	7 mL/30 g	2.43	1.03[a]	1.40
Propiconazole/*Bacillus subtilis*	7 mL/30 g	1.93	0.97[a]	0.97
Hexaconazole/*Bacillus amyloliquefaciens*	10 mL/30 g	2.00	1.10[a]	0.90
Hexaconazole/*Bacillus subtilis*	10 mL/30 g	2.20	1.30[a]	0.90
Gliocladium virens	30 g	1.73	1.17[a]	0.57
Tricure	30 mL	1.63	1.03[a]	0.60
Control		1.43	1.90[ab]	–0.47
CD at P = 0.05		0.70	0.60	-

[Values are mean followed by the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) a 0.05 % level]

Bacillus subtilis has been reported to control *Colletotrichum trifolii*, a causal agent of anthracnose of alfalfa (*Medicago sativa* L.) seedlings[18]. Furthermore, Yuan *et al*.[18] have also reported that *B. amyloliquefaciens* produced (VOCs) volatile compounds that act effectively against *F. oxysporum* f. sp. *cubense*.

In the present investigation too, combination fungicide of companion and propiconazole were found to be more effective against branch canker disease under greenhouse condition (Table 3). The results are in alignment with Mareeswaran & Neploean[19] the fungicides hexaconazole (35.99%), companion (100%) and propiconazole (75%) showed highest growth inhibitory effect against branch canker pathogen under *in vitro* level at 10 ppm.

The botanical fungicide of tricure (0.03% azadiractin EC) gave satisfactory control against branch canker disease followed by *Gliocladium virens* (Table 3). These findings are in agreement with Mareeswaran & Neploean[19] wherein the organic fungicide tricure (100%) showed complete growth inhibition against branch canker pathogen *Macrophoma* sp. under *in vitro* condition at 8% concentration. Also, *Gliocladium* sp. has been reported to control *Macrophomina phaseolina* in sesamum[20]. These results are supported by Mareeswaran and Premkumar[21,22] the indigenous biological agents, such as *Bacillus* sp, *B. amylo-liquefaciens*, *Pseudomonas* sp, *Trichoderma* sp. and chemical treatment of copper oxychloride, benomyl and companion were found to be good effective against branch canker disease under field condition.

Conclusion

In this study, we have evaluated control measures to determine the best option, whether separate application or integrated approach, for branch canker disease. Continuous application of chemicals treatment showed significantly difference in between the treatments, such as benomyl, copper oxychloride, tebuconazole and tridemorph. These results demonstrate that benomyl and integrated schedule of propiconazole/*Bacillus amyloliquefaciens* were found effective as a potential treatment against branch canker disease under greenhouse condition. The investigation mainly highlights the integrated approaches of branch canker disease and also integrated approach is not significantly difference in among the treatments.

Conflict of Interest

None to declare.

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