RESEARCH ARTICLE

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EVALUATION OF *Trichoderma* spp. AGAINST WIRESTEM DISEASE OF CAULIFLOWER CAUSED BY *Rhizoctonia solani*

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ABSTRACT

The biological effectiveness of twenty-eight native isolates of Trichoderma along with two known species (Trichoderma viride and Trichoderma harzianum) were tested against Rhizoctonia solani using dual culture technique. Of them, seven isolates and the two species of Trichoderma were tested in pot culture under net-house conditions against wirestem disease of cauliflower caused by R. solani. Inhibitory effects on dual culture were statistically very significant for all tested Trichoderma. Thirteen Trichoderma isolates exhibited more than 90% bio-control index and twenty-three isolates had more or at par with T. viride and T. harzianum. In an in-vitro trial, isolate from Dhankuta (Pakhribas) was most effective against R. solani as evident by the highest bio-control index at 6 days after incubation. Similar to this, in an in-vivo experiment, tested isolates of Trichoderma contributed more or less in the plant canopy area, root length and shoot circumference attributes. Trichoderma isolates significantly contributed to root dry matter and biomass while there was a highly significant difference in reduction of disease severity. T. harzianum and the isolate from Dhankuta (Pakhribas) were most effective for the control of wirestem disease.

1. INTRODUCTION

Trichoderma is among the most potential antagonists against a wide range of pathogens that causes diseases in various crops (Srivastava *et al*., 2015, Pandey *et al*., 2019). In 1932, Weinding first established the potential of *Trichoderma* as a bio-control agent and suggested that this genus of fungi has parasitic properties toward soil-borne plant pathogens like *R. solani* (Howell, 2003). *Trichoderma* is able to produce various volatile compounds like antibiotics that inhibit the growth of other fungi along with hyphal interactions (Dennis & Webster, 1971). *Trichoderma* spp. exert mycoparasitism, antibiosis, and competition against plant pathogens and induce resistance, and promote growth in plants (Naik & Rani, 2008, Mohiddin *et al*., 2010). Biocontrol of different *Trichoderma* spp. are known to be species specific owing to the ability to parasitize different phytopathogenic fungi. Some efficient species are; *T. harzianum, T. koningii, T. longibrachiatum, T. pseudokoningii, T. virens, T. viride, T. polysporum, T. atroviride, T. brevicompactum, T. reesei* etc. (Abdel-lateif, 2017, Puyam, 2016, Srivastava *et al*., 2014). This article aims at evaluating native *Trichoderma* isolates isolated from the soils against soil borne pathogen, *R. solani*.

2. MATERIALS AND METHODS

In order to assess the native *Trichoderma* isolates/species, *in-vitro* and *in-vivo* studies were conducted. First, the native isolates/species were screened in the laboratory against the *R. solani* by dual culture method. Under net-house conditions, the effective isolates/species were tested for wirestem disease on cauliflower (inoculated with *R. solani*) in pot cultures.

2.1. Dual culture of isolates with R. solani

Twenty-eight native isolates of *Trichoderma* spp. isolated from the soils of various locations of Nepal and two known species (*T. viride* and *T. harzianum*) obtained from the laboratory of National Plant Pathology Research Centre (NPPRC), Nepal Agricultural Research Council (NARC), Khumaltar, Lalitpur were evaluated for their biocontrol efficacy against *R. solani in-vitro* by

dual culture method (Table 1). The test was performed in the Central Agricultural Laboratory, Hariharbhawan, Lalitpur from December, 2018 to January, 2019. The experiment was conducted in a completely randomized design (CRD) with three replications (one plate as one replication) where different isolates/species were used as the treatments. The *R. solani* was provided by NPPRC, Khumaltar, Lalitpur.

Table 1. *Trichoderma* species and isolates used in the dual culture experiment

Isolates/Species	Location	Source/Fresh isolation from
Trichoderma-TP 1	Palpa 1	Maize field
Trichoderma-TRD 1	Rajhena, Dang 1	Maize field
Trichoderma-TSB	Sangha, Bhaktapur	Salla forest
Trichoderma-TP 2	Palpa 2	Maize field
Trichoderma-TRD 2	Rajhena, Dang 2	Maize field
Trichoderma-TNK	Nagarjun, Kathmandu	Fallow land
Trichoderma-TKB	Khajura, Banke	Rice field
Trichoderma-TPR	Padariya, Rupandehi	Vegetable field
Trichoderma-TRK	Raniban, Kathmandu	Sal forest area
Trichoderma-TI	Illam	Vegetable field
Trichoderma-TRS	Rajbiraj, Saptari	Legume field
Trichoderma-TGL	Godawari, Lalitpur	Fallow grassland
Trichoderma-TRJ	Rajikot, Jumla	Apple orchard
Trichoderma-TKK	Koteshwor, Kathmandu	Fallow land
Trichoderma-TKB	Khala, Baglung	Forest
Trichoderma-TKS	Kapurkot, Salyan	Ginger field
Trichoderma-TRC 1	Rampur, Chitwan 1	Fallow grassland
Trichoderma-TNK	Naikap, Kathmandu	Vegetable field
Trichoderma-TTS	Tarahara, Sunsari	Rice field
Trichoderma-TRC 2	Rampur, Chitwan (Sectoria)	Fallow grassland
Trichoderma-TK	Kabhrepalanchok	Potato field
Trichoderma-TLK	Lumle, Kaski	Potato field
Trichoderma-TD	Dhulikhel, Kabhrepalanchok	Vegetable field
Trichoderma-TMP	Malepatan, Kaski	Vegetable field
Trichoderma-TMC	Mangalpur, Chitwan	Rice field
Trichoderma-TGM	Galeshwor, Myagdi	Maize field
Trichoderma-TSP	Sarshadhara, Parbat	Forest
Trichoderma-TPD	Pakhribas, Dhankuta	Vegetable field
T. viride	-	NPPRC
T. harzianum	-	NPPRC

Both the pathogen and the antagonists were cultured and maintained in Borosil Petri plate (9 cm diameter) each containing approximately 20 ml of potato dextrose agar (PDA) medium. A 5-mm sized mycelial disc of five-day-old *R. solani* was placed 3-cm apart from the periphery in Petri plates. After 24 hours, 5-mm sized mycelial discs of three-day-old *Trichoderma* were inoculated in the same way, 3-cm apart from the *R. solani* and 3-cm from the periphery.

The inoculated Petri plates were incubated at 24±2°C. Radial growth of the *Trichoderma* isolates and *R. solani* were measured using a measuring scale and the area of each colony was measured by sketching on the plastic sheet and further processing was done to the final area recording using ImageJ 1.52a to study antagonist behavior at 6th day after inoculation (Figure 1).

Trichoderma as bio-control agents were evaluated by comparing bio-control index (BCI) which was computed using the colony area of *Trichoderma* isolates and test *R. solani* by the formula given by Szekeres *et al*. (2006).

$$BCI\ (\%) = \frac{\text{Area occupied by bio-control agent only}}{\text{Area occupied by colonies of both bio-control agent and test pathogen}} \times 100$$

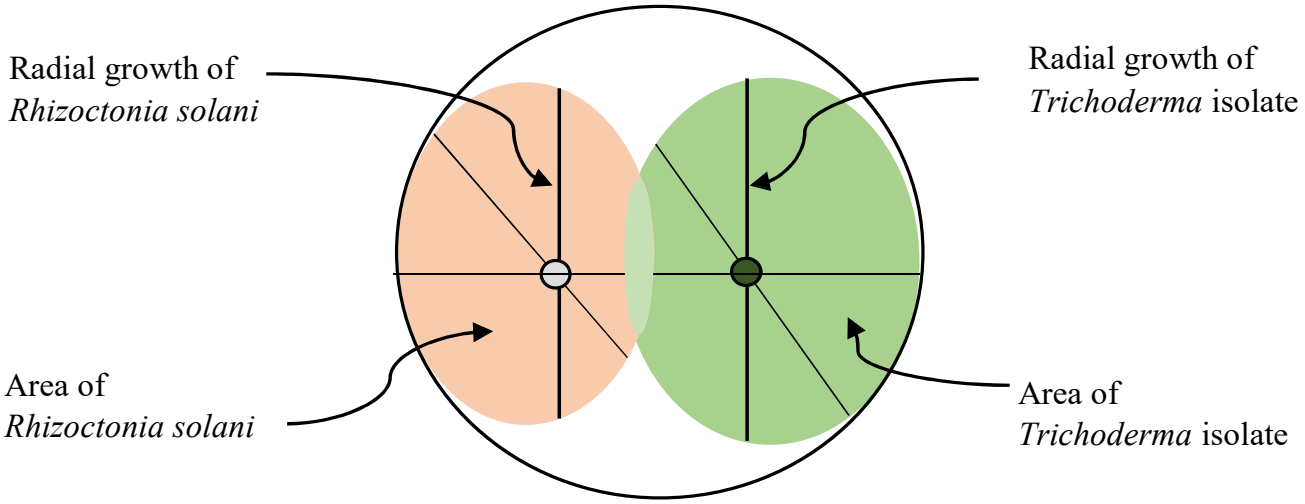

Figure 1. Measurement of growth of *R. solani* and *Trichoderma* isolate in dual culture Petri plate

2.2. Pot Culture under Net-House Conditions

Two known species (*T. viride* and *T. harzianum*) and seven native isolates, which were found effective in dual cultures, were evaluated for their effectiveness against wirestem disease on cauliflower (inoculated with *R. solani*) in earthen pot culture under net-house conditions (Table 2). The experiment was conducted at NPPRC, Khumaltar, Lalitpur from May, 2019 to September, 2019. The experiment laid out in CRD was replicated thrice and each experimental unit contained five plants.

Table 2. *Trichoderma* species and isolates (as treatment) used in the pot culture experiment

Isolates/Species	Location	Source/Fresh isolation from
Trichoderma-TPD	Pakhribas, Dhankuta	Vegetable field
Trichoderma-TTS	Tarahara, Sunsari	Rice field
Trichoderma-TRK	Raniban, Kathmandu	Sal forest area
Trichoderma-TRC 1	Rampur, Chitwan	Fallow grassland
Trichoderma-TLK	Lumle, Kaski	Potato field
Trichoderma-TRD 2	Rajhena, Dang	Maize field
Trichoderma-TRJ	Rajikot, Jumla	Apple orchard
T. viride	-	NPPRC
T. harzianum	-	NPPRC
Control (Without antagonist)	-	-

Approximately, 6.0-6.5 kg of sterilized pot mixture was filled in each earthen pot and inoculated with five mycelial discs (5-mm diameter grown on PDA) of *R. solani* in the upper layer of the soil 35 days before transplantation of cauliflower seedling. Twenty grams of *Trichoderma* mass culture prepared in finger-millet was inoculated in the upper layer of the soil 30 days before transplantation for colonization in soil. Moisture was maintained by proper irrigation. Twenty days after transplantation, 100 ml suspension of *Trichoderma* isolates (10^6) grown on PDA Petri plates was drenched.

Canopy of the plant, shoot circumference, root length, root dry matter, biomass and disease severity were recorded at 80 days after transplanting. The disease assessment was carried out by scoring the lesions on collar region (Figure 2) of plants based on the standard scoring scale of 0-4 given by Keinath & Farnham (2001) and disease severity percent as given:

0 = healthy, no symptoms of wire-stem

1 = superficial stem necrosis, longitudinal cracks ≥ 1 cm long and marginal discoloration along the crack

2 = stem partially girdled, ≤ 75% of the stem circumference girdled

3 = stem girdled, > 75% to 100% of the stem circumference girdled

4 = stem completely girdled and plant stunted

$$\text{Disease Severity (\%)} = \frac{\text{Sum of all numeric rating i.e. scoring}}{\text{Total no. of plant observed x Maximum rating}} \times 100$$

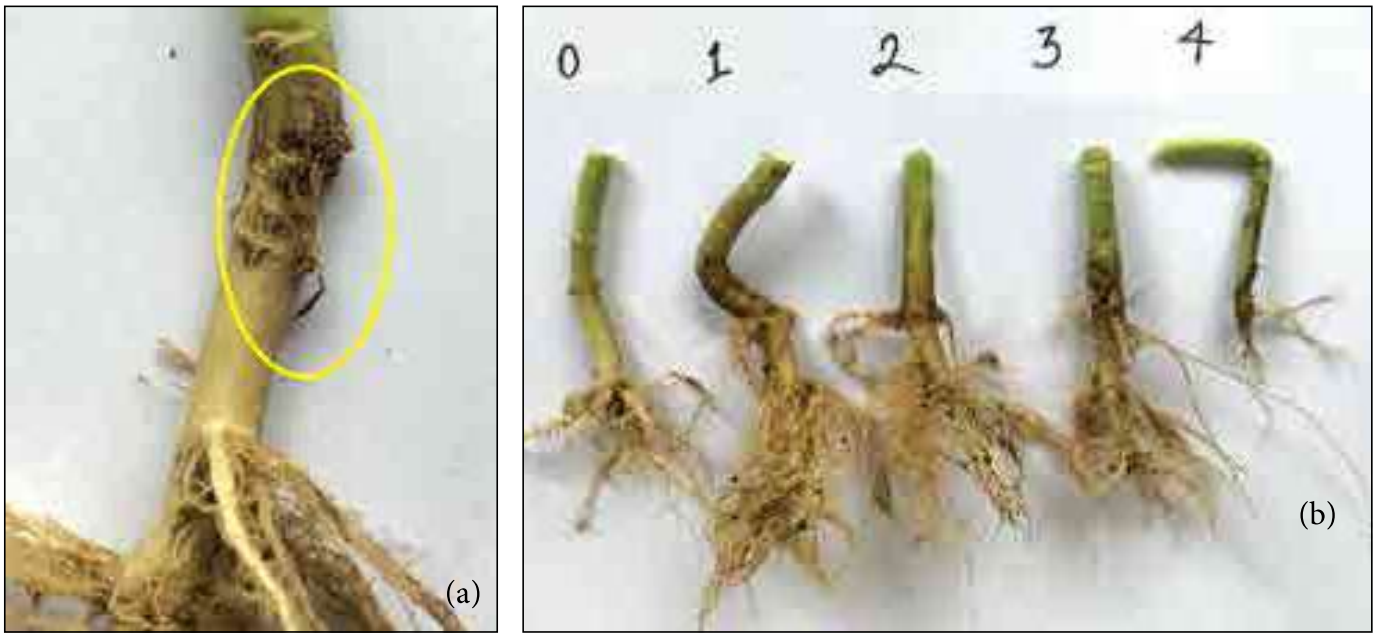

Figure 2. Wirestem disease assessment, (a) longitudinal cracks, and (b) scoring scale

2.3. Statistical Analysis

The data recorded were tabulated in Microsoft Excel 2013 data worksheets. R-Studio version 1.2.1335 was used for the statistical analysis of the data with agricolae 1.3-1 and lsmeans 2.30-0 for analyais of variance (ANOVA). The F-test was performed to compare the mean of treatment effects. For mean seperation, Duncan's multiple range test (DMRT) was performed at 5% level of significance. The Microsoft Excel 2013 and SPSS were used for tabular and graphical representation.

3. RESULTS AND DISCUSSION

3.1 Antagonism in-vitro

In dual culture test for *in-vitro* antagonism, inhibition zones [Figure 3(a)] were formed between some of the *Trichoderma* isolates and the pathogen, *R. solani* while most of the *Trichoderma* isolates appeared to mask the pathogen i.e. overgrowth [Figure 3(b)] on the colonies of the *R. solani* .

Figure 3. Interaction of *R. solani* and *Trichoderma* isolate, (a) inhibition zone in between, and (b) *Trichoderma* isolate overgrowth on *R. solani*

All tested *Trichoderma* isolates significantly inhibited or overcame the growth of the *R. solani.* BCI was highly significant among the tested *Trichoderma* isolates (Table 3). *Trichoderma* isolate of Pakhribas, Dhankuta had the highest BCI (100%) followed by isolate Padariya, Rupandehi (98.32%) while isolate Palpa-1 (49.43%) had the least BCI. Thirteen tested isolates exhibited BCI more than 90% and twenty-three isolates had more or at par with *T. viride* (96.25%) and *T. harzianum* (88.37%). Those twenty-three isolates were found to overgrow on the colony of *R. solani* completely in 10 days after inoculation.

In dual culture, some *Trichoderma* isolates formed brownish inhibition zones when came in contact with *R. solani* while most *Trichoderma* isolates appeared to overgrow the colony of the pathogen. The browning zones formed as a result of mycelia being parasitized through the mechanism of coiling, encircling and lysis of *R. solani* hyphae by *Trichoderma* (Agrios, 2005, Askew & Laing, 1994). Timila *et al*. (2015) have identified and reported this mechanism by native *Trichoderma* isolates from Nepal. In the present study, the tested *Trichoderma* isolates were found varied in their abilities to suppress *R. solani*. It demonstrates that not all *Trichoderma* are equally effective as biocontrol agents. Similar results have been explained by several authors (Amin *et al*., 2010, Seema & Devaki, 2012, Gveroska, 2013, Bastakoti *et al*., 2017, Manandhar *et al*., 2019). The faster growth of *Trichoderma* during competition for resources and space, as well as mycotoxin production, and contribute to the pathogen's suppression (Howell, 2003, Timila *et al*., 2015, Waghunde *et al*., 2016). Further, *Trichoderma* causes hyphal lysis and inactivation of enzymes limiting the development and activity of the pathogen, *R. solani* (Naik & Rani, 2008). In antagonism confirmation, BCI considered the advantages of antagonism based on different mechanisms like competition for space and nutrition, production of antifungal metabolites and mycoparasitism, into consideration by enabling development during incubation time (Szekeres *et al*., 2006).

Table 3. BCI in dual culture between *Trichoderma* isolates and *R. solani* at 6 days after inoculation

Isolates/Species	BCI (%)
Trichoderma-TP 1	49.43^{n} (44.65)
Trichoderma-TRD 1	87.74^{efghijk} (69.68)
Trichoderma-TSB	78.98^{jklm} (62.75)
Trichoderma-TP 2	94.75^{bcde} (78.31)
Trichoderma-TRD 2	94.75^{bcdef} (77.35)
Trichoderma-TNK	69.02^{m} (56.29)
Trichoderma-TKB	75.70^{klm} (60.50)
Trichoderma-TPR	98.32^{ab} (82.71)
Trichoderma-TRK	97.73^{ab} (82.39)
Trichoderma-TI	92.11^{bcdefgh} (73.92)
Trichoderma-TRS	92.79^{bc} (79.78)
Trichoderma-TGL	82.09^{hijklm} (65.10)
Trichoderma-TRJ	89.60^{cdefghi} (72.90)
Trichoderma-TKK	86.03^{fghijkl} (68.17)
Trichoderma-TKB	68.89^{m} (56.15)
Trichoderma-TKS	90.58^{bcdefgh} (74.18)
Trichoderma-TRC 1	91.47^{bcdefg} (75.42)
Trichoderma-TNK	88.66^{cdefghij} (70.64)
Trichoderma-TTS	93.60^{bcdefg} (75.48)
Trichoderma-TRC 2	90.06^{cdefghij} (72.02)
Trichoderma-TK	81.01^{ijklm} (64.24)
Trichoderma-TLK	97.73^{ab} (83.16)
Trichoderma-TD	86.66^{fghijkl} (68.78)
Trichoderma-TMP	85.27^{ghijkl} (67.65)
Trichoderma-TMC	76.18^{klm} (60.80)
Trichoderma-TGM	84.47^{ghijkl} (66.85)
Trichoderma-TSP	74.13l^{m} (59.45)
Trichoderma-TPD	100.00^{a} (89.96)
T. viride	96.25^{bcd} (79.45)
T. harzianum	88.37^{defghij} (70.23)
Control (Without antagonist)	0.05^{o} (1.28)
F-test	***
S.Em±	3.32
LSD (≤0.05)	9.43
CV (%)	8.49

Value of BCI in the parentheses were arc sine transformed; Means in a column having same small letter(s) do not differ significantly at 5 percent probability by DMRT; *** = highly significant; S.Em = Standard error of means; LSD (≤0.05) = Least significant difference, showing 5% level of significance; CV (%) = Coefficient of variation percentage

3.2 Effect Of Trichoderma Isolates Against Wirestem Disease Of Cauliflower

Bio-control efficacy of *Trichoderma* isolates against *R. solani* was studied *in-vivo* on wirestem disease of cauliflower plants. The effect was significant on root dry matter, biomass and disease severity over the control while on plant canopy, root length, and shoot circumference, the effect was non-significant among the treatments (Table 4). All the *Trichoderma* isolates were found at par with known *T. viride* (17.54%) and *T. harzianum* (18.11%) for increase in root dry matter. *Trichoderma* isolate of Rajhena gave the highest (62.35%) increase in plant biomass followed by Tarahara (60.31%) and Pakhribas (55.38%). The reduction of disease severity was highest in *T. harzianum* (47.22%) treated plants followed by *Trichoderma* isolate of Pakhribas (38.88%), Rajikot (36.12%), Tarahara (33.33%) and *T. viride* (33.33%), which were not significantly different from each other. However, all *Trichoderma* isolates were found to have the potential to raise yield attributing character as revealed by increased canopy, root and shoot systems and biomass compared to control. Increased root growth and biomass were observed in pot culture study. This might be due to the enhanced nutrient utilization by plants, and thus more tolerance to pathogens reflected by their linear relationship as positively correlated (Figure 4). Considering the fact that *Trichoderma* was also found to solubilize macro- and micro-elements of plant nutrients along with a significant increase in biomass (Li *et al*., 2015). Also, probably due to displacement and control of deleterious micro-flora (Harman, 2000) or production of secondary metabolites including, growth hormones, endochitinase, proteolytic enzymes gave symbiotic relationship with plants–microbes interactions (Ranveer *et al*., 2018) which might have resulted for significant growth.

Similarly, *Trichoderma* contributed to reducing the *R. solani* disease significantly on cauliflower plants. Similar to this, Uddin *et al*. (2011) and Rehman *et al*. (2012) noted decreased damping-off incidence of *R. solani* incidence and significantly improved cauliflower growth vigor as compared to control. Dabbas *et al*. (2009) also reported lowest disease intensity and highest percent disease management over control. This could have been the effect of induced systemic resistance by the action of *Trichoderma* on plant (Harman *et al*., 2004).

Table 4. Different attributes and disease severity of cauliflower treated with different *Trichoderma* isolates/species in soils inoculated with *R. solani* in pot culture

Isolates/Species	Plant canopy area (cm^2)	Root length (cm)	Root dry matter (%)	Shoot circumference (mm)	Plant biomass (g)	Disease severity (%)
Trichoderma-TPD	364.54	14.58	24.88^{a}	30.6	192.41^{a}	36.67^{d}
Trichoderma-TTS	430.15	16.09	25.88^{a}	30.67	198.51^{a}	40.00^{bcd}
Trichoderma-TRK	381.39	13.8	23.49^{a}	26.15	144.75^{bc}	48.33^{bc}
Trichoderma-TRC 1	398.95	14.13	23.52^{a}	29.25	166.83^{ab}	50.00^{ab}
Trichoderma-TLK	452.98	14.3	23.09^{ab}	27.81	181.65^{ab}	50.00^{ab}
Trichoderma-TRD 2	370.20	14.61	25.79^{a}	30.08	201.03^{a}	50.00^{ab}
Trichoderma-TRJ	386.79	14.69	25.88^{a}	27.77	183.52^{ab}	38.33^{cd}
T. viride	410.33	15.12	23.57^{a}	26.89	191.83^{a}	40.00^{bcd}
T. harzianum	344.23	15.63	23.68^{a}	28.06	193.01^{a}	31.67^{d}
Control (without antagonist)	320.34	13.55	20.05^{b}	24.62	123.83^{c}	60.00^{a}
F-test	ns	ns	*	ns	**	***
S.Em±	39.94	0.82	1.08	1.97	13.25	2.09
LSD (≤0.05)	-	-	3.18	-	39.09	6.16
CV%	17.92	9.73	7.78	12.12	12.91	8.66

Means in a column having same small letter(s) do not differ significantly at 5% percent probability by DMRT, ns = not significant; * = significant, ** = significant, *** = highly significant; S.Em = Standard error of means; LSD (≤0.05) = Least significant difference, showing 5% level of significance; CV (%) = Coefficient of variation percentage.

3.3. Correlation between disease severity percent and different attributes in pot culture

Plant canopy area, root length, root dry matter, shoot circumference and plant biomass were negatively correlated with disease severity (Table 5). Disease severity was found contributing 18.71%, 17.33% and 13.92% reduction in plant biomass, root dry matter and root length, respectively (Figure 4). The results clearly demonstrate the effect of the disease on the crop plant, which can directly affect crop yield. Plant canopy area and shoot circumference were not affected.

Table 5. Correlations between different attributes of cauliflower and disease severity by *R. solani* in pot culture

	Disease severity	Canopy area	Root length	Root dry matter	Shoot circumference	Plant biomass
Disease severity	-					
Canopy area	-0.028	-				
Root length	-0.373*	0.100	-			
Root dry matter	-0.416*	0.184	0.379*	-		
Shoot circumference	-0.106	0.435*	0.541**	0.534**	-	
Plant biomass	-0.433*	0.136	0.612**	0.688**	0.626**	-

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

**. Correlation is significant at the 0.01 level (2-tailed).

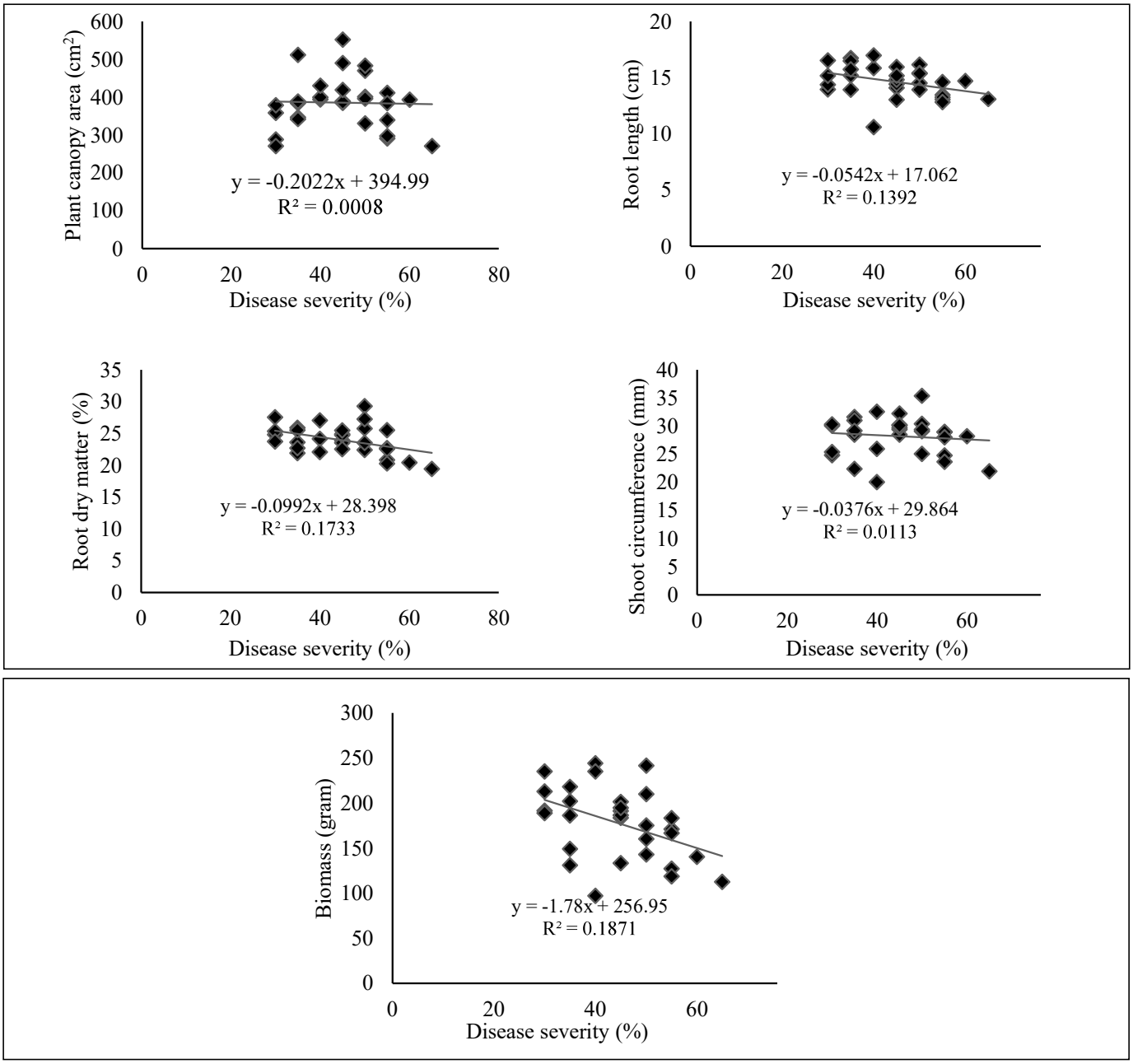

Figure 4. Simple linear relationship between disease severity by *R. solani* and different attributes of cauliflower in pot culture

4. CONCLUSION

From the present experimental studies, it can be concluded that native isolates of *Trichoderma* spp. are potential for the management of diseases caused by *R. solani* on cauliflower. Of the isolates tested, we found *T. harzanium* and Dhankuta (Pakhribas) isolate the most effective in managing wirestem disease. These *Trichoderma* with proper identity need to be promoted for commercial production and use in farmers' field.

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REFERENCES

Abdel-lateif, K. S. (2017). *Trichoderma* as biological control weapon against soil borne plant pathogens. *African Journal of Biotechnology, 16*(50), 2299-2306.

Agrios, G. N. (2005). *Plant Pathology*. 5th Edition. Elsevier Academic Press, New York.

Amin, F., Razdan, V. K., Mohiddin, F. A., Bhat, K. A., & Banday, S. (2010). Potential of *Trichoderma* species as biocontrol agents of soil borne fungal propagules. *Journal of Phytology, 2*(10), 38-41.

Askew, D. J., & Laing, M. D. (1994). The *in-vitro* screening of 118 *Trichoderma* isolates for antagonism to *Rhizoctonia solani* and an evaluation of different environmental sites of *Trichoderma* as sources of aggressive strains. *Plant and Soil, 159*, 277-281.

Bastakoti, S., Belbase, S., Manandhar, S., & Arjyal, C. (2017). *Trichoderma* species as biocontrol agent against soil borne fungal pathogens. *Nepal Journal of Biotechnology, 5*(1), 39-45.

Dabbas, M. R., Singh, D. P., & Yadav, J. R. (2009). Management of *Rhizoctonia* root rot of cauliflower through IDM practices. *International Journal of Plant Protection, 2*(1), 128-130.

Dennis, C., & Webster, J. (1971). Antagonistic properties of species-groups of *Trichoderma*. *Transactions of the British Mycological Society, 57*(1), 41-48. doi: 10.1016/s0007-1536(71)80078-5

Gveroska, B. (2013). *In-vitro* biocontrol activity of *Trichoderma harzianum* against some pathogenic fungi on tobacco. *Journal of Food and Environment Safety, 12*(1), 95-104.

Harman, G. E. (2000). Myths and dogmas of biocontrol: changes in perception derived from research on *Trichoderma harzianum* T-22. *Plant Disease, 84*(4), 377-393.

Harman, G. E., Howell, C. R., Viterbo, A., Chet, I., & Lorito, M. (2004). *Trichoderma* species-opportunistic, avirulent plant symbionts. *Nature Reviews Microbiology, 2*, 43-56.

Howell, C. R. (2003). Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evolution of current concepts. *Plant Disease, 87*(1), 4-10. doi: 10.1094/pdis.2003.87.1.4

Keinath, A. P., & Farnham, M. W. (2001). Effect of wirestem severity on survival on and head production of transplanted broccoli and cabbage. *Plant Disease, 85*, 639-563.

Li, R-X., Cai, F., Pang, G., Shen, Q-R., Li., R., & Chen, W. (2015). Solubilisation of phosphate and micronutrients by *Trichoderma harzianum* and its relationship with the promotion of tomato plant growth. *Public Library of Science, 10(*6), 1-16. doi: 10.1371/journal.pone.0130081

Manandhar, S., Pant, B., Manandhar, C., & Baidya, S. (2019). *In-vitro* evaluation of bio-control agents against soil borne plant pathogens. *Journal of Nepal Agricultural Research Council, 5*, 68-72.

Mohiddin, F. A., Khan, M. R., Khan, S. M., & Bhat, B. H. (2010). Why *Trichoderma* is considered super hero (super fungus) against the evil parasites? *Plant Pathology Journal, 9*(3), 92-102.

Naik, M. K., & Rani, G. D. (2008). *Advances in Soil Borne Plant Diseases.* New India Publishing Agency, Pltam Pura, New Delhi, India.

Pandey, N., Adhikari, M., & Bhantana, B. (2019). *Trichoderma* and its prospects in agriculture of Nepal: an overview. *International Journal of Applied Sciences and Biotechnology, 7*(3), 309-316.

Puyam, A. (2016). Advent of *Trichoderma* as a bio-control agent - A review. *Journal of Applied and Natural Science, 8*(2), 1100-1109.

Ranveer, K. K., Victor, A., Yogendra, S. G., & Vivek, K. (2018). *Trichoderma*: a most common biofertilizer with multiple roles in agriculture. *Biomedical Journal of Scientific & Technical Research, 4*(5). doi: 10.26717/BJSTR.2018.04.001107

Rehman, S. U., Lawrence, R., Kumar, E. J., & Badri, Z. A. (2012). Comparative efficacy of *Trichoderma viride*, *T. harzianum* and carbendazim against damping-off disease of cauliflower caused by *Rhizoctonia solani* Kuhn. *Journal of Biopesticides, 5*(1), 23-27.

Seema, M., & Devaki, N. S. (2012). In vitro evaluation of biological control agents against *Rhizoctonia solani*. *Journal of Agricultural Technology, 8*(1), 233-240.

Srivastava, M., Pandey, S., Shahid, M., Kumar, V., Singh, A., Trivedi, S., & Srivastava, Y. K. (2015). *Trichoderma*: a magical weapon against soil borne pathogens. *African Journal of Agricultural Research, 10*(10), 4591-4598. doi: 10.5897/ajar2015.10192

Srivastava, M., Shahid, M., Pandey, S., Singh, A., Kumar, V., Gupta, S., & Maurya, M. (2014). *Trichoderma* genome to genomics: A review. *Journal of Data Mining in Genomics & Proteomics, 5*(3), 162. doi:10.4172/2153-0602.1000162

Szekeres, A., Leitgeb, B., Kredics, L., Manczinger, L., & Vagvolgyi, C. (2006). A nobel, image analysis-based method for the evaluation of *in-vitro* anatagonism. *Journal of Microbiological Methods, 65,* 619-622. doi: 10.1016/j.mimet.2005.09.014

Timila, R. D., Manandhar, S., Manandhar, C., & Mahto, B. N. (2015). The *Trichoderma* spp.: a biological control agents from Nepalese soil. *Proceeding of Workshop,* 294-300. Nepal Agricultural Research Council, Nepal.

Uddin, M. M., Akhtar, N., Islam, M. T., & Faruq, A. N. (2011). Effect of *Trichoderma harzianum* and some selected soil amendments on damping-off disease of cabbage and cauliflower. *Journal of Experimental Biosciences, 2*(1), 37-42.

Waghunde, R. R., Shelake, R. M., & Sabalpara, A. N. (2016). *Trichoderma*: a significant fungus for agriculture and environment. *African Journal of Agricultural Research, 11*(22), 1952-1965.