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Antagonistic Effect of Three *Trichoderma* Species on the *Alternaria porri* Pathogen of Onion Blotch

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Abstract: Three *Trichoderma* species such as *T. harzianum*, *T. pseudokoningii* and *T. virens* were extensively studied on the inhibition of *Alternaria porri* pathogen of onion blotch disease. Three concentrations of Liquid Culture Filtrate (LCF) of these *Trichoderma* species were tested against Mycelial Growth (MG) and Conidial Germination (CG) of *A. porri*. The Percent Inhibition of Mycelial Growth (PIMG) and Percent Inhibition of Conidial Germination (PICG) was found to be the highest in the LCF of *T. virens*. The LCF of remaining two *Trichoderma* species were also showed good inhibitory effect on the *A. porri*. The PICG was higher than the PIMG. Two dual culture methods were applied to find out the PIMG. The highest PIMG was 75.80 (method-1) and 75.54 (method-2) occurred by *T. virens* on PDA. But on Sabouraud's, the highest PIMG was found 74.34 (*T. harzianum*) and 65.86 (*T. virens*) in method-1 and 2, respectively. The colony overgrowth was faster and slower for *T. harzianum* and *T. pseudokoningii*, respectively. *T. virens* was taken 9 days to colony overgrow in both 2 methods. Present study showed that different *Trichoderma* species have good antagonistic effect on the MG and CG of *A. porri*. In every case, *T. virens* was the best bio-control agent against *A. porri* pathogen of onion blotch disease.

Key words: *Alternaria porri* · antagonism · onion blotch · *Trichoderma* species · PIMG

INTRODUCTION

Onion (*Allium cepa* L.), belongs to the family Liliaceae, is one of the most important vegetable among the bulb crops grown in Bangladesh. Onions are used in cooking in various ways as an essential spice. Besides, it is rich in flavonoids like quercetin and sulfur compounds, such as allyl propyl disulphide that have been perceived benefits to human health [1]. Onion also has medicinal value as a possible cancer preventive [2, 3]. The world production of onions is now 39,400,000 Mt per year which is by far higher than the 14,000,000 Mt per year obtained in 1960. Average world yields increased from 12 Mt/hector in the early 1960s to 17 Mt/hector in 2001 [4]. About 30% of the onion grown in Bangladesh is produced from seed [5]. The total cultivation of onion in Bangladesh is of 95,000 acre`s and the production is 15,40,000 Mt [6]. Several diseases of onion have been recorded [7] and blotch contaminated by *Alternaria porri* (Ellis) Cif. is noted as the major disease in Bangladesh [8, 9]. The blotch disease is a major problem for seed production in tropical countries like Bangladesh [10]. It causes breaking of floral stalks and thus the seed production is seriously reduced [11]. Bulb and seed yields of onions were significantly reduce as a result of blotch [12]. About 20 to 25% of decrease seed yield has been recorded in India [13] and 41 to 44% in Bangladesh [14].

Therefore, this study was initiated to test the antagonistic effect between *A. porri* and *Trichoderma*. With a view to measure the role of *Trichoderma* as a bio-control agent against *A. porri* pathogen, this research was done and presented in this paper.

MATERIALS AND METHODS

Pathogen used: *Alternari porri* was isolated from the infected leaf of onion and cultured on Potato Dextrose Agar (PDA) medium. Ten days old culture of pathogen was used for each experiment.

***Trichoderma* species used:** Three species of *Trichoderma* namely *T. harzianum*, *T. pseudokoningii* and *T. virens* were isolated from the different soil and garbage samples, cultured on PDA medium. Five days old culture of *Trichoderma* was used for each experiment.

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Collection of Liquid Culture Filtrate (LCF) of *Trichoderma*: For harvesting LCF of three *Trichoderma* species, Potato Dextrose Broth (PDB) was used in conical flask and *Trichoderma* was cultured for 20 days at 25°C, on rotary shaker 140~150 rpm from which LCF were harvested. To collect the filtrate, the liquid cultures were firstly filtered through 2 layers of Whatman No. 1 filter paper to remove hyphal fragments and finally filtered using a 0.22 μm sized membrane filter. The filtrates were used for incorporation into PDA and PDB separately. Thus the samples were ready for further use.

Mycelial Growth (MG) and Conidial Germination (CG): The LCF of three *Trichoderma* species was mixed with PDA and PDB separately to have 25, 50 and 75% concentrations (v/v) of each LCF. After autoclaving, PDA mixtures were poured in sterilized Petri dishes. Conidia of *A. porri* were taken from 10 days old culture and placed in the center of each petri dish and MG readings were recorded after 10 days of incubation. The experiment was replicated 5 times and mycelial growth was measured [15]. In case of CG, conidia from the 10 days old culture of *A. porri* were taken and conidial suspensions (10^3/ml) were made at above concentration with LCF and PDB. The conidial suspension was taken in sterilized watch glass, a drop of conidial suspension was taken on separate grove slide and kept at 25°C in a moisture chamber for 24 hours. For control, MG and CG was counted in fresh PDA and PDB, respectively. After incubation period, a drop of lactophenol cotton blue was placed over conidial suspension on the slide and examined under (×400) power microscope for recording the percentage of CG. The two readings A_1 (control) and A_2 (treated) of MG/CG were transformed in to percent inhibition of conidial germination (PICG) and percent inhibition of mycelial growth (PIMG) using the formula [16], where PIMG = $(A_1-A_2)/A_1 \times 100$.

Screening by dual culture method: Two parameters were observed in this test, PIMG and the number of day taken for the antagonist totally overgrow the *A. porri* colony on PDA and Sabouraud's media separately. For each of the three *Trichoderma*, a 5 mm agar disc taken from 5 days old culture and placed at the periphery of the 90 mm culture plates. Then, same size another agar disc of *A. porri* was similarly placed at the periphery but on the opposing end of the same Petri dish. For control, *A. porri* was placed in a similar manner on fresh PDA and Sabouraud's plate. All pairings were carried out 5 replicates and incubated at 25°C. Antagonistic activity was assessed after 5 days of incubation by measuring the radius of the *A. porri* colony in the direction of the antagonist colony (R_2) and the radius of the *A. porri* colony in the control plate (R_1) shown in Fig. 1. The two readings were transformed in to PIMG using the formula [16]. The number of days taken for the antagonist to overgrow the whole colony of *A. porri* was recorded. Observation was continued on the dual culture plates after 5 days of incubation, when mycelial extensions of *A. porri* were measured and followed by calculation of PIMG. The colony overgrowth time was recorded for each and every *Trichoderma* even for the *Trichoderma* that took the highest time to overgrow the *A. porri* colony.

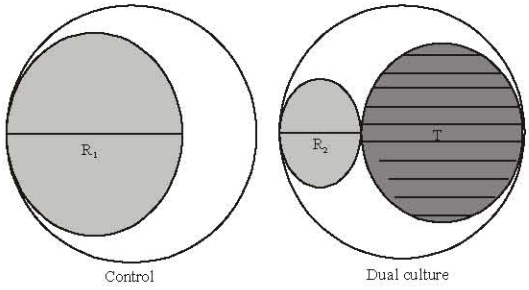

Fig. 1: Method of dual culture. R_1/R_2: Mycelial growth of *A. porri* and T: *Trichoderma*

Comparison of two screening methods: To test whether there was significant variation in screening methods with respect to the disc culture placement, two methods were carried out using three *Trichoderma*. The first method was as described earlier. In the second method, a 5 mm agar plug of the antagonist, *Trichoderma* was placed on 2 cm away from the periphery of the petri dish, an agar plug of same size test fungus *A. porri* was similarly placed 2 cm away from the edge of the Petri plate but opposite to *Trichoderma*. The plates were incubated in the same manner as for method 1. For control, a single agar plug of same size of *A. porri* was placed in a similar manner on fresh PDA and Sabouraud's plate but without *Trichoderma*. The radius of *A. porri* mycelium R_1, R_2 was recorded and PIMG determined after 5 days of incubation using the same formula [16].

RESULTS AND DISCUSSION

The three concentrations 25, 50 and 75% of LCF of three *Trichoderma* species were tested against MG and CG of *A. porri*. The highest PIMG and PICG was found 71.43 and 83.78 at 75% LCF of *T. virens*, respectively. The lowest PIMG and PICG was found to be 9.52 and

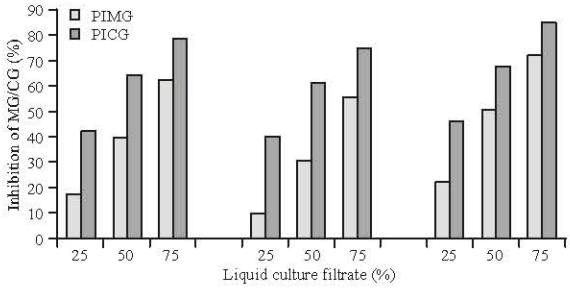

Fig. 2: Effect of liquid culture filtrate of *T. harzianum* (left), *T. pseudokoningii* (middle) and *T. virens* (right) on the mycelial growth (MG) and conidial germination (CG) of *A. porri*. MG and CG was calculated (n=5) after 10 days and 24 hours of incubation, respectively. PIMG: Percent inhibition of mycelial growth, PICG: Percent inhibition of conidial germination

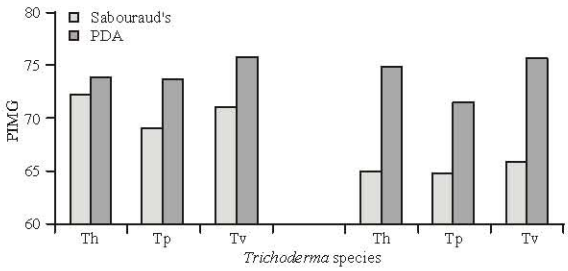

Fig. 3: Percent inhibition of mycelial growth (PIMG) of *A. porri* was calculated (n=5) after 5 days of incubation using method-1 (left) and method-2 (right). Th: *T. harzianum*, Tp: *T. pseudokoningii* and Tv: *T. virens*

Table 1: Effect of Liquid Culture Filtrate (LCF) of 3 *Trichoderma* species on the Mycelial Growth (MG) and Conidial Germination (CG) of *A. porri*

Used *Trichoderma*	LCF (%)	MG (mm)	PIMG	CG (%)	PICG
	25	52	17.46	43	41.89
T. harzianum	50	38	39.68	27	63.51
	75	24	61.90	16	78.38
	25	57	09.52	45	39.19
T. pseudokoningii	50	44	30.16	29	60.81
	75	28	55.56	19	74.32
	25	49	22.22	40	45.95
T. virens	50	31	50.79	24	67.57
	75	18	71.43	12	83.78
Control		63 (on PDA)		74 (in PDB)	

PIMG: Percent inhibition of mycelial growth, PICG: Percent inhibition of conidial germination. MG and CG was counted (n=5) after 10 days and 24 hours of incubation, respectively

Table 2: Percent inhibition of mycelial growth (PIMG) of *A. porri* in dual culture

Method	*Trichoderma* species used	PIMG Sabouraud's	PIMG PDA	Overgrowth of colony/day (PDA)
	T. harzianum	72.34	73.77	8
Method-1	*T. pseudokoningii*	68.88	73.77	10
	T. virens	70.83	75.80	9
	T. harzianum	64.86	75.00	9
Method-2	*T. pseudokoningii*	64.70	71.42	11
	T. virens	65.78	75.54	9

PIMG was measured (n=5) after 5 days of incubation at 25°C

39.19 at 25% LCF of *T. pseudokoningii*, respectively. *T. harzianum* was showed moderate inhibition both of MG and CG. The inhibition of MG and CG were increased with the increase of LCF concentration in every case. The PICG was more than PIMG. Among three *Trichoderma* species, LCF of *T. virens* was the best for both PIMG and PICG. To know whether the antagonistic activity of LCF of *Trichoderma* was diffusible as well as antifungal which inhibited the MG and CG of *A. porri* was very pronounced compared to the control plate (Table 1 and Fig. 2). The metabolites of *Trichoderma* could influence the outcome of the decay caused by basidiomycetes in freshly fell down pine [17]. Dennis and Webster [18] and Jinantara [19] showed that culture filtrate produced by *Trichoderma* contained inhibitory substances against microorganisms. Among the antibiotics produced by *T. harzianum* were 6-n-pentyl-2H-pyran-2-one, 6-n-pentenyl-2H-pyran-2-one, pyridone, anthraquinones, butenolides, isonitrin D and F, trichorzianines and furanone [20, 21]. *T. virens* was also shown to produce several other antibiotics such as gliotoxin, gliovirin, gliocladic acid, heptilidic acid (avocetin), viridin, viridiol and valinotricin [22].

Two dual culture methods were applied to the PIMG of *A. porri*. In method-1, PIMG was 72.34, 68.88 and 70.83 on Sabouraud's and 73.77, 73.77 and 75.80 on PDA medium. The highest PIMG on Sabouraud's and PDA was 72.34 and 75.80 for *T. harzianum* and *T. virens*, respectively. On PDA, similar PIMG (73.77) was occurred for *T. harzianum* and *T. pseudokoningii*. In method-2, the highest PIMG was 65.78 (Sabouraud's) and 75.54 (PDA) for *T. virens*. The number of colony overgrown day was recorded the lowest 8 in method-1 for *T. harzianum* and highest 11 in method-2 for *T. pseudokoningii*. *T. virens* was taken 9 days to overgrow the colony of *A. porri* in both methods (Table 2 and Fig. 3). Jun and Kim [23] reported that the antifungal activity of *T. virens* and

T. harzianum to *Pythium* spp. was stronger than that of *T. koningii*. Dharmaputra *et al.* [24] tested two isolates of *T. harzianum* and one isolates of *T. viride* against three isolates of *Ganoderma* from oil palms. All three *Trichoderma* isolates inhibited the mycelial growth of the pathogen but *T. harzianum* (isolate B10-1) showed the best performance among the three isolates. Due to the variable antagonistic potential of individual isolates, the first screening is to select the most active antagonist against that particular pathogen before a species or particular isolate of *Trichoderma* can be considered as a biocontrol agent [25]. Jinantara [19] reported that the three isolates of *T. harzianum* possessed different ability to attack *Sclerotium rolfsii* and this result was in agreement with [26] who found different isolates of *T. harzianum* could parasitize sclerotia of *S. rolfsii* at varying percentage inhibition.

CONCLUSION

It was confirmed by this experiment that the LCF produced by 3 *Trichoderma* species were diffusible and could prevent or inhibit the MG and CG of *A. porri*. Therefore, *Trichoderma* has a large potential effect as biocontrol agent against *A. porri* pathogen of onion blotch.

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