Exploration of Potential Indigenous Non-phytopathogenic Fungi for Bio-organic Fertilizer Recycling from Organic Waste

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ARTICLE INFO

Received: 12 May 2022
Received in revised: 8 Jul 2021
Accepted: 25 Jul 2022
Published online: 18 Aug 2022
DOI: 10.32526/ennrj/20/202200110

Keywords:
Fungi/ Mixed culture/ Organic wastes/ *Penicillium*/ Seed germination/ *Trichoderma*

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ABSTRACT

Using potential microbes in biodegradable solid waste management is an emerging science. Microbes play a significant role in recycling of organic solid wastes. Therefore, the present project was carried out to isolate indigenous potential non-phytopathogenic fungi from local relevant decomposed substrates for the purpose of organic waste recycling as bio-organic fertilizer (BOF). A total of thirteen fungal strains were isolated. Seven of them were identified as *Trichoderma* spp., and the rest were *Penicillium* spp. Germination of mung bean (*Vigna radiata*), mustard (*Brassica campestris*), and wheat (*Triticum aestivum*) seeds were assessed by application of 13 fungal isolate suspensions. Significant increase of germination percent was achieved in mung bean (98.35%), mustard (96.65%), and wheat (93.35%) by fungal treatments RW-T02, PL-P01, and CD-T01/MSW-T05, respectively, compared to the controls. But radicle and plumule lengths were not promoted by fungal treatments in the majority of cases. Significantly, the longest radicle and plumule lengths of mung bean and mustard were found in control treatments. Conversely, in wheat the longest radicle and plumule length were achieved in treatments MSW-T05 and RW-T03, respectively. Based on superior performances of percent germination and radicle/plumule length, six fungal isolates were selected for compatibility performance in mixed cultures. In the compatibility tests, two fungal combinations (ABF and BCE) presented superior mutual intermingle appearances. Perhaps these combinations may play significant roles in biodegradation of organic wastes.

1. INTRODUCTION

Waste is not waste, but may be treated as a resource, if it is managed properly. Proper management of waste offers value added product and its recycling conserves congenial environment. One of the most acceptable and viable options of organic waste management is composting, which offers good quality product recognized as bio-organic fertilizer (Kauser and Khwairakpam, 2022). Bio-organic fertilizer (BOF) is not simply bio-fertilizer but it is rich in both beneficial microbes and organic substances. Well decomposed, fully biomatured and sanitized compost with potential microbes are known as bio-organic fertilizer, which is beneficial for soil health improvement and to ensure agricultural yield with high nutritional values (Heerden et al., 2002; Barua et al., 2018; Castiglione et al., 2021). Microbes enriched BOF is not only rich in decomposed organic substances but also beneficial microbes which enhance yield and nutritional qualities of the crop, and obviously it is superior to simply organic fertilizer (Molla et al., 2012).

Bangladesh is an agro-based and densely populated country. Therefore, a significant volume of waste is generated every day. In Dhaka City, approximately 4,000-4,500 tons of solid waste is generated per day (Bari et al., 2007), where the majority is biodegradable for composting. Proper environmentally friendly and economically viable disposal of such waste is a great concern to the relevant authority. Generation of potential technology for production of quality BOF may reduce the load of excessive usages of chemicals fertilizers for agronomic practices, as well as opening an avenue of sustainable environmentally friendly recycling of bio-decomposable wastes.

The potential microbes play vital roles in composting/bioconversion of organic wastes treated

Citation: Molla AH, Zahan H, Oliver MMH, Mosharaf MK. Exploration of potential indigenous non-phytopathogenic fungi for bio-organic fertilizer recycling from organic waste. Environ. Nat. Resour. J. 2022;20(6):598-610. (https://doi.org/10.32526/ennrj/20/202200110)

by a reliable process of waste management to reduce the aforesaid problems (Molla et al., 2006; Cerda et al., 2018). Additionally, the roles of microbes in biodegradation are considered as an important factor (Jung and Kim, 2016; Srivastava et al., 2020). Proper attention on using biological agents for composting is important, otherwise the obtained end product of composting (i.e., BOF) may cause severe damage to the applied crop. This implies that the use of microbes for composting (biodegradation) should be non-phytopathogenic. In biodegradation processes of organic wastes, the microbes used have greater degradation ability if they originated from a similar environment (Meda et al., 2021).

Moreover, the food toxicities/adulteration is a common phenomenon due to excessive usage of agrochemicals for agronomic practices. Continuous consumption of adulterated foods may invite several fatal diseases to human as well as other living beings. Possible alternatives to ensure the supply of bio-based plant growth enhancers (i.e., value added BOF) may help to produce safe food (crops) production by reducing usages of chemical fertilizers. Accordingly, the current interest of using bio-organic fertilizers and phyto-stimulators is inspiring the enhancement of crop yield and protection against crop damages as green technologies (Adesemoye et al., 2008; Castiglione et al., 2021). Moreover, degradation of organic substrates was enhanced more by application of a microbial consortia than a monoculture (Li et al., 2021). The most important determinant in the biodegradation process is a compatible consortium of microbes, which ensure quality compost for crop growth and development (Gutierrez-Correa and Tengerdy, 1997; Molla et al., 2004; Devi and Anu-Appaiah, 2021).

Therefore, the present study was undertaken to find potential, but non-phytopathogenic fungal isolates and compatible consortia which will be efficient for future use in degradation of organic wastes into bio-organic fertilizer as well as reduce the load of inorganic fertilizer usage, promote safe food production and conserve congenial friendly environment.

2. METHODOLOGY

2.1 Collection of relevant wastes for isolation of native filamentous fungal isolates

Six types of decomposed organic wastes materials were collected from local sources for filamentous fungal strains isolation. These were (1) Decomposed cow dung (CD), (2) Rotten rice straw (RW), (3) Decomposed poultry litter (PL), (4) Rotten wood (RW), (5) Decomposed municipal solid waste (MSW), and (6) Decomposed leaf litter (LL).

2.2 Isolation of fungal isolates as pure culture

The Rose Bengal Agar (RBA) media and technique was used for isolation of indigenous fungal strains (Martin, 1950). All reagents of RBA were dissolved in distilled water by subsequent stirring and gentle heating before being autoclaved (121ºC for 15 min). Exactly 2 mL of sterile (filtered) streptomycin (50 mg/mL) was added aseptically to molten RBA and mixed homogeneously. One milliliter of the 1,000-fold diluted sample (substrate used for isolation of fungi) was poured into a petri dish followed by approximately 15 mL of molten RBA and allowed to solidify. The plates were incubated at room temperature (28±2ºC) for 2 weeks and observed every day. The colonies (i.e., colony forming unit) were transferred onto 3.9% potato dextrose agar (PDA, Oxoid) plate for subculture to obtain the pure strains.

2.3 Identification of isolated fungal strains

Visual observation of petri dish cultures and macro and micro-morphological features of the reproductive organs of well grown fungal isolates/strains in slide culture (Molla et al., 2002a) were adopted for identification of fungal species by viewing in microscope and image processing unit. For visual observation the isolates were grown in 3.9% PDA (Oxoid) for 10 days and for studying reproductive organs in slide culture, the slides were kept 2-4 days at room temperature based on description of Molla (2002). The mode of mycelial growth, color, odor, and changes of medium color of each isolate were examined daily. The relevant code number was assigned to the tentatively identified fungal isolates based on source of origin and genus.

2.4 Preparation of fungal spore suspensions for screening against crop seed germination

A total of 13 fungal isolates/strains were selected based on fast and vigorous growth appearance in PDA plates. The fungal isolate was subcultured on potato dextrose agar (PDA, 3.9% Merck) plate for seven days at ambient temperature (28±2ºC). For preparing spore suspension/culture filtrate, each plate of fungal culture was washed with 10 mL of sterilized 0.01% Tween 20 solution, diluted twenty-fold with sterile water and filtered by 12.5 cm Whatman#1 filter

paper. The stocks of spore suspensions were preserved at 4ºC until used for subsequent activities.

2.5 Seed sterilization and germination of seeds against fungal spore suspensions

The germination of three crop seeds [Mung bean (*Vigna radiata*), Mustard (*Brassica campestris*), and Wheat (*Triticum aestivum*)] were examined by applying fungal spore suspensions. Uniform disease free crop seeds were surface sterilized by washing one minute in 95% ethanol followed by 2-3 min in 2% sodium hypochloride and finally washing six times with sterile distilled water (Molla et al., 2001a). Twenty-five seeds were soaked on filter paper with fungal spore suspensions in glass petri dishes. However, the filter of each petri dish was soaked with 5 mL of fungal suspension based on treatment before placing seeds on the petri dishes. Each fungal isolate was considered as a treatment and replicated five times. Sterile water soaked filter paper was used as a control treatment. Seeds were allowed to germinate in aseptic environment to attain a constant number of germinated seeds in a seed germinator in the Seed Technology Laboratory of BSMRAU (Bangabandhu Sheikh Mujibur Rahman Agricultural University). Concurrently, germination performance was recorded. Approximately more than 2 mm of radicle emerging from a seed coat was considered as a germinated seed. Germination percent, germination index, radicle length and plumule length were recorded and percent infected/spotted seedlings were considered for evaluation of induced phytopathogenic study of seedlings by fungi in seeds germination. Germination index was calculated based on the procedure of Islam et al. (2018).

2.6 Compatibility of fungal mixed culture

The six fungal isolates/strains were designated as A=CD-T01 (*Trichoderma* sp.), B=MSW-T04 (*Trichoderma* sp.), C=RW-T02 (*Trichoderma* sp.), D=RS-P01 (*Penicillium* sp.), E=MSW-T05 (*Trichoderma* sp.), and F=PL-P01 (*Penicillium* sp.) and selected from 13 fungal isolates/strains based on crop seeds germination.

A total of 12 combinations designated as ABC, ABF, ACE, ACF, ADE, ADF, BCE, BCF, BDE, BDF, CDE, and CDF using six isolates/strains (A, B, C, D, E, and F) were evaluated for compatibility performance. Each combination was replicated five times. Equal sizes of three different fungal blocks were placed in a freshly prepared PDA plate and allowed to grow in an incubator at 28ºC for 5-7 days. The study was exercised by adopting the technique described by Molla et al. (2001b).

The inoculated fungal plates were monitored daily after the second day of inoculation. Fungal isolates combinations with proper intermingled growth were selected for subsequent research activities.

2.7 Experimental design and statistical analysis

The experiments were done in glass petri dishes in an aseptic environment in factorial completely randomized design with five replications. Simple statistical tools along with R-software (version 4.1.3) were used for analysis of variance and mean comparison (LSD). Finally, the processed data were presented in tables and figures.

3. RESULTS AND DISCUSSION

3.1 Isolation and identification of filamentous fungal isolates/strains

Several numbers of filamentous fungal strains were isolated from six decomposed sources of organic substrates rich in cellulose and hemicellulose (Figure 1), but, finally, 13 pure cultures of filamentous fungal strains were recorded (Table 1). Others isolates were discarded based on their almost similar appearance at plate culture. Generally, cow dung, poultry litter, municipal solid wastes (MSW), etc. are being used for composting with the addition of bulking materials rich in cellulose and hemicellulose as carbon source such as rice straw, saw dust etc. Fungal isolates originated from cellulose and hemicellulose rich substrates would be effective for degradation of bulking materials as compost.

Among the isolated fungal strains, the species of *Trichoderma* and *Penicillium* were monitored, and the species *Trichoderma* was higher in number than the *Penicillium*. Only these two genera of fungal strains were recorded from six sources. Several authors reported different species and number of filamentous fungi isolated from different substrates. Findings of our previous study reported a maximum number of *Trichoderma* spp. in decomposed POME (Palm Oil Mill Effluent) wreckages and *Penicillium* spp. in decomposed sludge (Molla et al., 2002a). In another study, Ahirwar et al. (2017) isolated 5, 10, and 11 thermophilic and thermotolerant fungi from 6, 11, and 12 samples of litter, wheat straw and compost, respectively. Thirty-six fungal isolates under seven genera were isolated from four biogas plants and DNA sequences tools were used for their identification

(Young et al., 2018). The macro/micro-morphological structures of the reproductive organs of the studied fungi were used to identify the genera (Rifai, 1969; Bohacz and Kornillowicz-Kowalska, 2020).

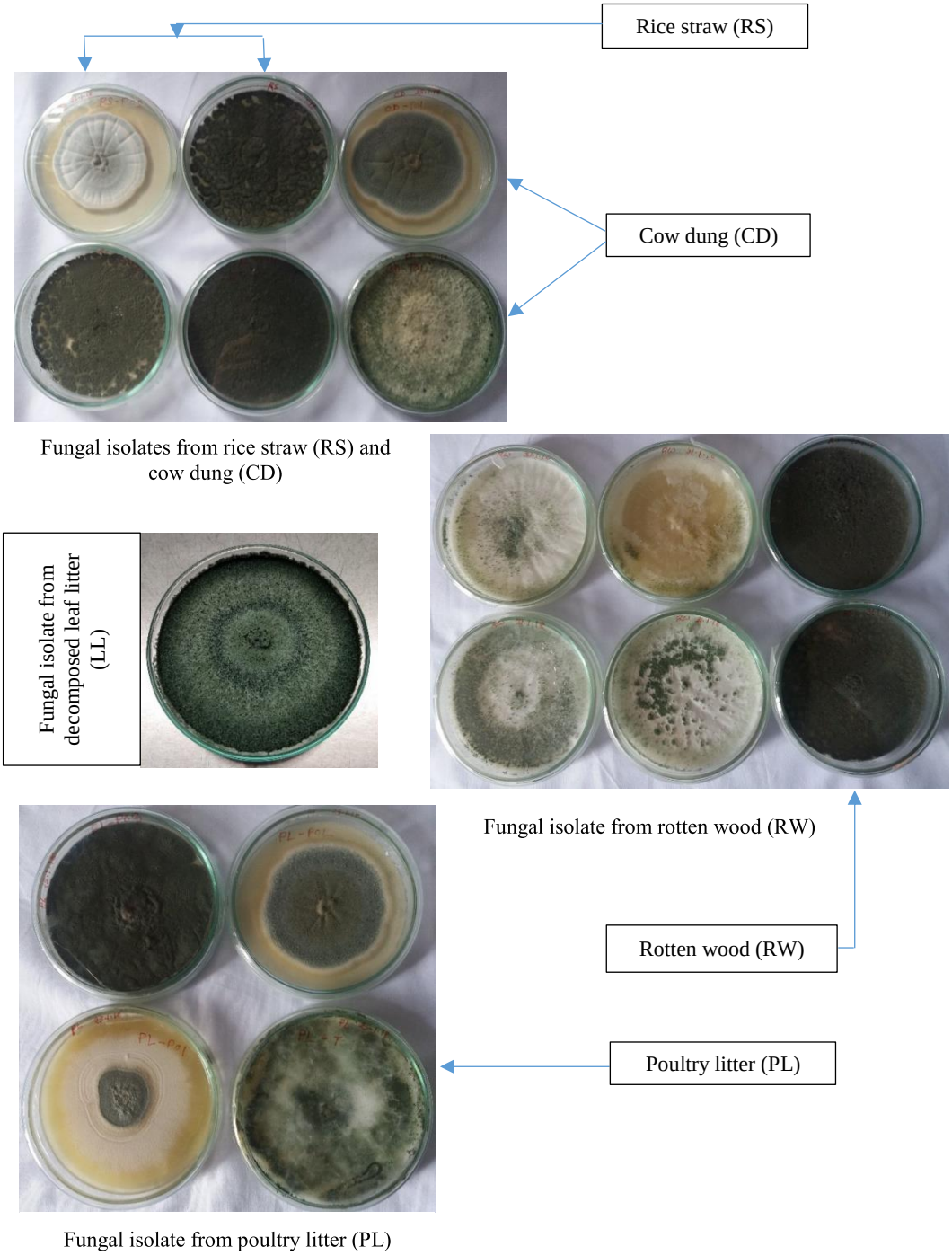
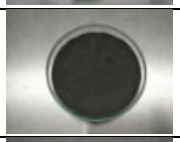
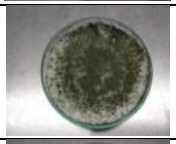
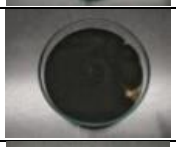
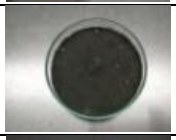


Figure 1. Isolated fungal species from different related native sources

3.2 Germination of crop seeds

Thirteen fungal isolates/species were used for three crop seeds [Mung bean (*Vigna radiata*), Mustard (*Brassica campestris*), and Wheat (*Triticum aestivum*)] germination to evaluate its performance (Figure 2). Performances of the applied 13 fungal isolates/species were examined by studying the parameters of percent germination, germination index, radicle length, and plumule length. The findings of the study have been presented and discussed below.

Table 1. Tentatively identified fungal isolates/strains

Isolate code	View in plate	Morphological description	Tentative identification
CD-P01		Smooth and uniform growth of fungal strain, initial color was dark ash, the central matured part became whitish. The young margin was relatively more off-white in color.	*Penicillium* sp.
CD-P03		It's growth was also smooth and uniform but color was more dark ask. Slow growing fungal strain.	*Penicillium* sp.
CD-T01		Relatively compact white i.e., milk colored, fast growing fungal mycelia mat was observed within 3-4 days after inoculation in PDA plate. Later sporadic green fungal biomass was developed.	*Trichoderma* sp.
RW-T02		Fluffy milk-colored fungal mycelia in nature. But the mycelia color was changed as olive green in color within 3-4 days and the green portion was expanded in mature stage.	*Trichoderma* sp.
RW-T03		Relatively green colored mycelia were appeared and later on the middle portion became whitish in color. The peripheral portion was quite green.	*Trichoderma* sp.
PL-P01		Smooth and uniform fungal mycelial growth was appeared. The matured central portion of mycelia became whitish in color. The peripheral young mycelia were off-white.	*Penicillium* sp.
PL-P03		Slow growing deep olive colored mycelial smooth mat was appeared and uniform growth and colored was maintained.	*Penicillium* sp.
PL-T01		Closely similar pattern of fungal mycelial growth was observed as CD-T01.	*Trichoderma* sp.
RS-P01		Slightly rough, harsh, deep greenish to olive colored slow growing mycelia was observed. At matured stage loose power of spores was noticed on mycelial mat.	*Penicillium* sp.
RS-P02		Almost similar pattern of fungal mycelial growth was observed as PL-P01. But the color of the whole was almost uniform.	*Penicillium* sp.
LL-T04		Initially deep green colored fluffy mycelial growth was observed. But at mature stage, the fungal biomass of central part of the plate became comparatively whitish in color and peripheral portion was quite green.	*Trichoderma* sp.
MSW-T04		Quite fluffy and fast growing white colored mycelial was appeared at early stage. But after 2-3 day later it was turned into olive color.	*Trichoderma* sp.
MSW-T05		More or less uneven but smooth fungal mycelial growth was observed. Moreover, it was slowing growing in nature.	*Trichoderma* sp.

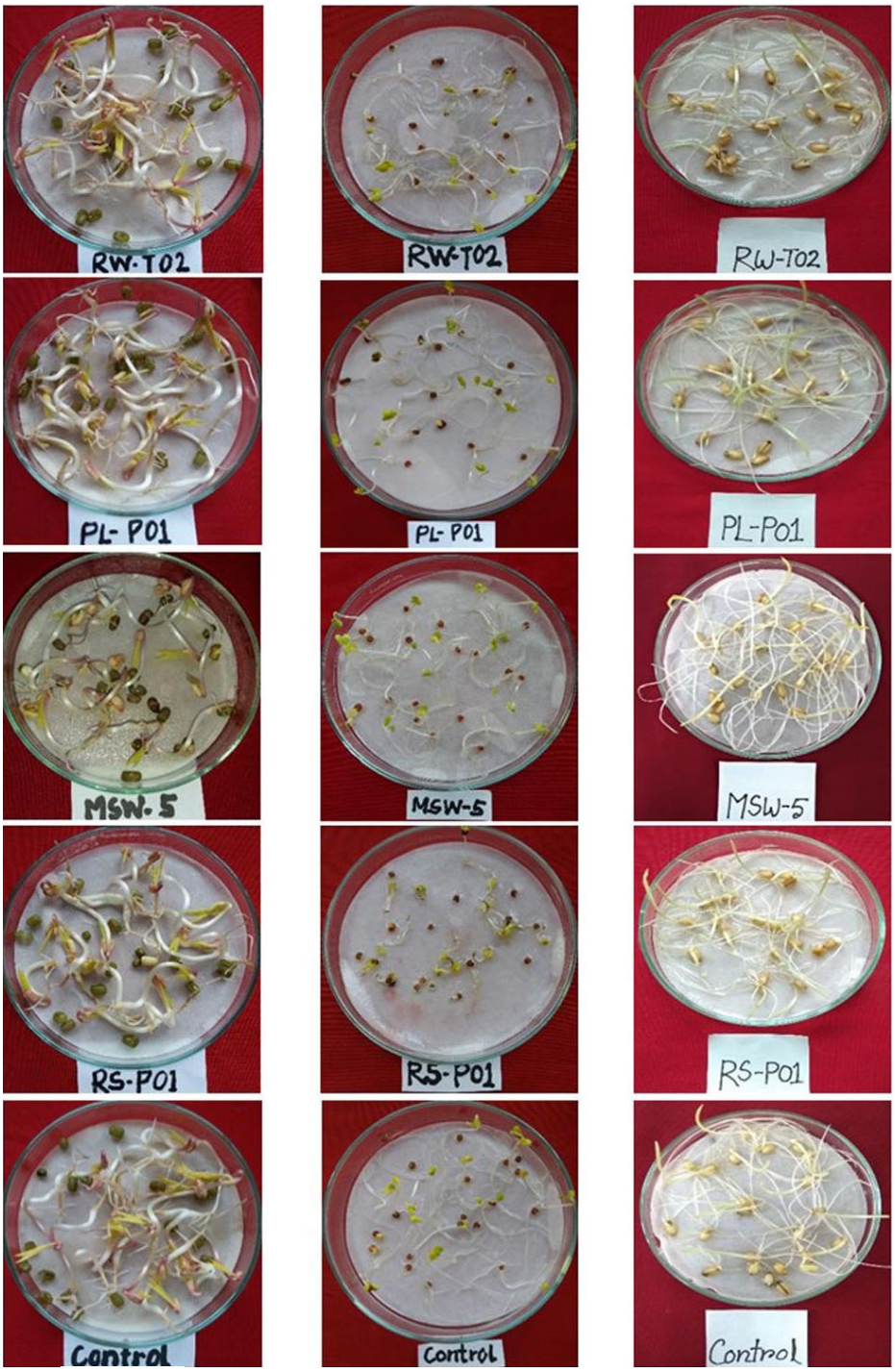


Figure 2. Germination performance of mung bean, mustard and wheat seeds at spores suspension of different fungal isolates/strains in petri dishes. (A-mung bean seed, B-mustard seed, and C-wheat seed)

3.2.1 Germination percentage

No germination of mung bean seeds (*Vigna radiata*) was noticed after 24 h, but at 48 h more than 30% seeds were germinated in all treatments (Table 2). Next, 60% germination of seeds was recorded at 72 h, and at 96 h the percent germination in all treatments was above 80%, except the treatment CD-P03 which was around 76%. The treatment RW-T02 provided the highest (98.35) percent of germination at 96 h (Table 2 and Figure 3) and the second highest (96.65%) was monitored at PL-P01 treatment. However, the control (water) treatment provided 91.61% of mung bean seed germination at 96 h.

Table 2. Germination percent of three crop seeds by application of several fungal suspensions

Treatment*	Germination (%) of three crop seeds											
	Mung bean				Mustard				Wheat			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
CD-P01	0.00	43.30	68.35	81.65^{e}	1.67	48.35	86.65	93.35^{abc}	26.65	80.00	86.65	86.65^{cd}
CD-P03	0.00	35.00	65.00	76.65^{f}	0.00	65.00	90.00	95.00^{ab}	46.65	85.00	85.00	85.00^{cd}
CD-T01	0.00	58.30	73.35	85.00^{de}	0.00	41.65	85.00	93.35^{abc}	18.35	88.35	93.35	93.35^{a}
RW-T02	0.00	71.65	95.00	98.35^{a}	0.00	33.35	73.35	91.65^{bcd}	26.65	80.00	83.35	83.35^{d}
RW-T03	0.00	35.00	78.35	83.35^{de}	0.00	25.00	78.35	93.35^{abc}	31.65	83.35	86.65	86.65^{cd}
PL-P01	0.00	75.00	91.65	96.65^{ab}	0.00	55.00	95.00	96.65^{a}	26.65	85.00	86.65	86.65^{cd}
PL-P03	0.00	41.65	70.00	83.35^{de}	1.67	58.35	85.00	90.00^{cd}	35.00	81.65	83.35	83.35^{d}
PL-T01	0.00	46.65	68.35	83.35^{de}	0.00	56.65	88.35	91.65^{bcd}	33.35	85.00	86.65	86.65^{cd}
RS-P01	0.00	58.30	80.00	81.65^{e}	0.00	35.00	51.65	75.00^{e}	33.35	90.00	91.65	91.65^{ab}
RS-P02	0.00	66.65	76.65	86.65^{d}	0.00	46.65	90.00	93.35^{abc}	30.00	85.00	88.35	88.35^{bc}
LL-T04	0.00	63.30	76.65	85.00^{de}	0.00	23.35	71.65	91.65^{bcd}	8.35	75.00	85.00	85.00^{cd}
MSW-T04	0.00	75.00	90.00	93.35^{bc}	0.00	31.65	71.65	91.65^{bcd}	31.65	85.00	86.65	86.65^{cd}
MSW-T05	0.00	56.65	70.00	81.65^{e}	0.00	46.65	88.35	90.00^{cd}	28.35	90.00	93.35	93.35^{a}
Control	0.00	56.65	91.65	91.65^{c}	0.00	38.35	80.00	88.35^{d}	36.65	76.65	78.35	78.35^{e}
				(p≤0.1)				(p≤0.001)				(p≤0.01)

*N.B. Fungal strains isolated from decomposed/rotten (CD-Cow dung, RW-Rotten wood, PL-Poultry litter, RS-Rice straw, LL-Decomposed leaf litter, and MSW-Municipal solid waste); P-*Penicillium* sp. and T-*Trichoderma* sp.

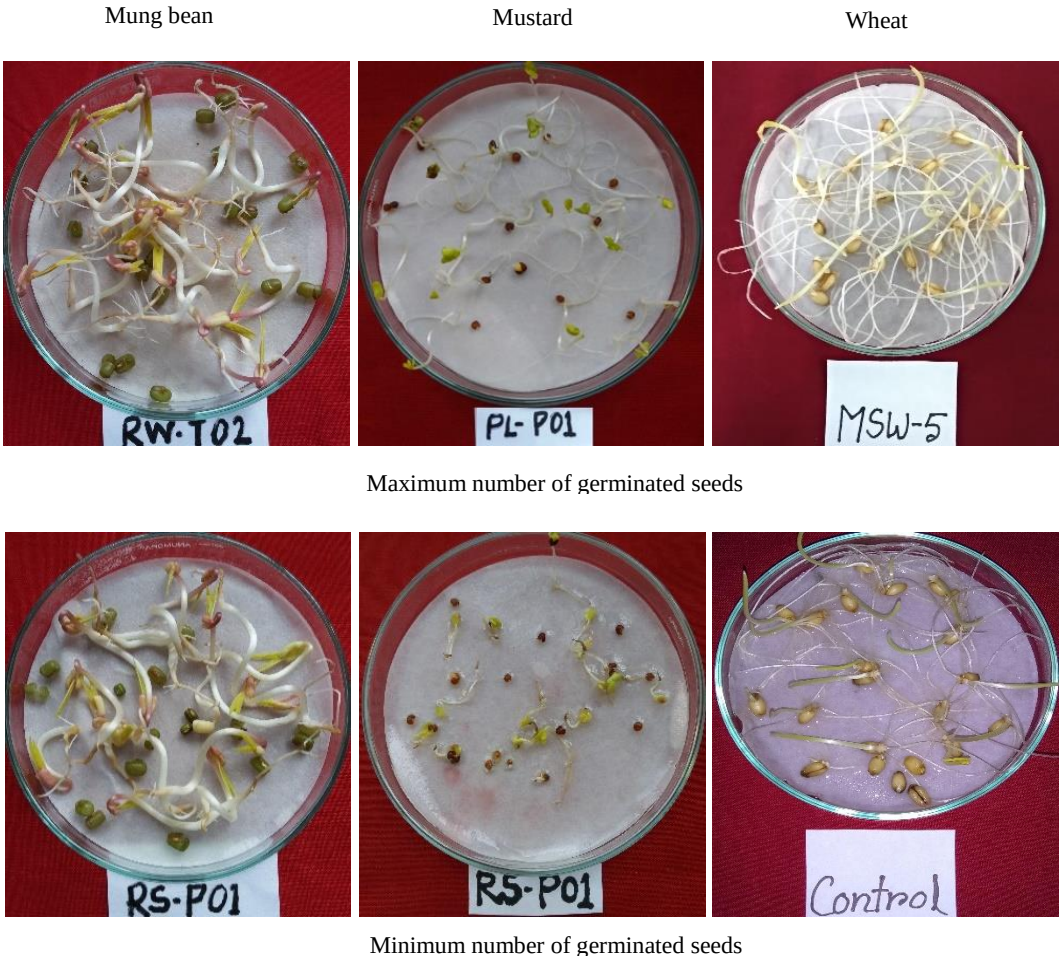


Figure 3. Maximum and minimum germination percent of three crop seeds while germination allowed by application of fungal suspension

In the case of mustard (*Brassica campestris*) at 24 h, 1.67% germination was monitored in treatments CD-P01 and PL-P03 (Table 2). After 48 h, 25% or more germinated seeds were recorded in all treatments. At 72 h, more than 50% germinated seeds were noticed. While at 96 h the percent germination in all treatments was above 88%, except the treatment RS-P01 which was around 75%. The treatment PL-P01 provided the highest (96.65) percent of germination at 96 h and second highest (95%) was treatment CD-P03. On the other hand, the treatment RW-T03 provided above 93% of seeds germination. But in control (water) treatment, only 88.35% of mustard seed germination was achieved at 96 h.

In wheat (*Triticum aestivum*) seed germination, the responses were not like mung bean and mustard. Germination of wheat seed was responded earlier at 24 h and completed by day 72 h and at 96 h the germination record was same as at 72 h (Table 2). At 72 h above 80% germination was recorded in all the treatments except the control (78.35%). The highest 93.35% germination was recorded in the treatments CD-T01 and MSW-T05. The second highest (91.65%) was achieved at treatment RS-P01. In all cases the germination percent of all crop seeds was significantly higher than the control treatment.

The applied fungal spore suspension treatment influenced germination of seed and germination index. Germination of some seeds by some isolates/strains was quite encouraging. The obtained findings conveyed that no particular isolate positively influenced in germination of all tested crop seeds. In the present study, the influence on germination of seeds by fungal suspension was crop specific. However, the germination of *Brassica campestris* seeds was promoted when a fungal suspension was applied (Molla and Khan, 2018). Microbes release different bio-molecules/growth promoting substances, i.e., phyto-hormones, which influence or promote germination of seeds and growth of seedlings (Kagithoju et al., 2013; Castiglione et al., 2021). In the present study, the fungal isolates PL-P01 and RW-T02 played remarkable roles in crop seeds germination in most of the cases. It implied that these isolates might release useful bio-enhancer plant metabolites which promote germination. Yue et al. (2019) studied seed germination and mortality of desert winter annual plants (*Brassica tournefortii*; *Plantago ovata*) with soil-borne fungi, and used fungi induced substantial seed germination and mortality in summer with ten strains demonstrating host-specificity. Increasing and decreasing of seeds germination were noticed by fungal application due to species host-specificity (Li et al., 2021). *Trichoderma* spp. promote seed germination and alleviated biotic and abiotic stresses in germinating seeds and seedlings by inducing physiological protection in plants against oxidative damage (Mastouri et al., 2010; Delgado-Sánchez et al., 2013). Like *Triochoderma* spp., *Penicillium* spp. promotes seed germination and seedling growth (Yamaji et al., 2005; Tamura et al., 2008; Delgado-Sánchez et al., 2013), and protects seedlings by producing antifungal compounds to keep antifungal activity under competitive condition (Yamaji et al., 2005). In another study, seed germination of *Strychnos potatorum* with *Penicillium* and *Aspergillus* species that the significant increase and early germination was noticed due to the culture exudates on metabolic activities that promote cell division of pre-germinated seeds, as well as production of growth regulating substances like gibberellins and auxins (Yoneyama et al., 1998; Kagithoju et al., 2013; Khokhar et al., 2013). In the present study, early germination appeared in wheat (*Triticum aestivum*), which may be most responsive to the released metabolites by the applied fungal isolates.

3.2.2 Germination index (GI)

No germination was recorded at 24 h in the case of mung bean. So, the germination index was zero for all the treatments at 24 h. The germination index at 72 h was 100 or above in each treatment. The treatment RW-T02 provided the highest (107.31) germination index at 96 h and a closer index (second highest) was recorded 105.46 for PL-P01 at the same period. Whereas the germination index of 100 was recorded in control treatment (Table 3). Conversely, the lowest GI (83.63) was monitored for the CD-P03 treatment.

In the case of mustard, at 24 h the germination index was zero for all the treatments, similar to mung bean (Table 3). However, the highest germination index was 118.75 and 109.39 recorded in treatment PL-P01 at 72 and 96 h, respectively. The second highest GI (107.53) was found in treatment CD-P03 at 96 h. On the other hand, the germination index in control treatment was 100.

In wheat seed (Table 3) germination, the responses were not like mung bean or mustard. Wheat seed germination was noticed at 24 h and completed by 72 h. At 72 h the highest germination index was recorded in the treatments CD-T01 and MSW-T05 (119.14), whereas in control it was 100 as usual. The

second highest GI (116.98) was recorded in treatment RS-P01 at 72 h.

The germination index (GI) is the percent expression of multiplied results of percent germination (Molla et al., 2002b). Therefore, it follows the similar trends as the germination percent. But GI is treated as a dependable measurement of phytotoxicity levels of seeds germination (DeVleeschauwer et al., 1981; Rao et al., 2014). Seed germination by fungal application either enhances (germination) due to release of growth regulating substances like gibberellins and auxins (Khokhar et al., 2013) or decreases due to liberation of mycotoxins (Khokhar et al., 2011; Rao et al., 2014), which is dependent on the strains of microbes. In the present study, the GI of mustard and wheat were influenced more than the control by all fungal treatment, but mung bean was affected by only some of the fungal isolates.

Table 3. Germination index of three crop seeds in germination with application of several fungal suspensions

Treatment*	Germination index (GI) of three crop seeds											
	Mung bean				Mustard				Wheat			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
CD-P01	0.00	76.43	74.58	89.09	0.00	126.08	108.31	105.66	72.72	104.37	110.59	110.59
CD-P03	0.00	61.78	70.92	83.63	0.00	169.49	112.50	107.53	127.29	110.89	108.49	108.49
CD-T01	0.00	102.91	80.03	92.75	0.00	108.60	106.25	105.66	50.07	115.26	119.14	119.14
RW-T02	0.00	126.48	103.66	107.31	0.00	86.96	91.69	103.74	72.72	104.37	106.38	106.38
RW-T03	0.00	61.78	85.49	90.94	0.00	65.19	97.93	105.66	86.36	108.74	110.59	110.59
PL-P01	0.00	132.39	100.00	105.46	0.00	143.42	118.75	109.39	72.72	110.89	110.59	110.59
PL-P03	0.00	73.52	76.38	90.94	0.00	152.15	106.25	101.87	95.49	106.52	106.38	106.38
PL-T01	0.00	82.35	74.58	90.94	0.00	147.72	110.44	103.74	90.99	110.89	110.59	110.59
RS-P01	0.00	102.91	87.29	89.09	0.00	91.26	64.56	84.89	90.99	117.42	116.98	116.98
RS-P02	0.00	117.65	83.63	94.55	0.00	121.64	112.50	105.66	81.86	110.89	112.76	112.76
LL-T04	0.00	111.74	83.63	92.75	0.00	60.89	89.56	103.74	22.78	97.85	108.49	108.49
MSW-T04	0.00	132.39	98.19	101.85	0.00	82.53	89.56	103.74	86.36	110.89	110.59	110.59
MSW-T05	0.00	100.00	76.38	89.09	0.00	121.64	110.44	101.87	77.35	117.42	119.14	119.14
Control	0.00	100.00	100.00	100.00	0.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

*N.B. Fungal strains isolated from decomposed/rotten (CD-Cow dung, RW-Rotten wood, PL-Poultry litter, RS-Rice straw, LL-Decomposed leaf litter, and MSW-Municipal solid waste); P-*Penicillium* sp. and T-*Trichoderma* sp.

3.2.3 Length of radicle and plumule

Radicle and plumule length of mung bean, mustard and wheat are shown in Table 4. In case of mung bean, the longest radicle length was observed in control treatment (9.08 cm) and which was followed by the treatment LL-T04 (9.06 cm). The shortest radicle length was recorded in the treatment PL-P03 (6.37 cm). In the case of mustard, the longest radicle length was observed in control treatment (5.89 cm) also which was followed by the treatment PL-T01 (4.47 cm). The shortest radicle length was recorded in the treatment RW-T03 (0.8 cm). Conversely, in wheat the longest radicle length was observed in treatment MSW-T05 (6.87 cm) which was followed by the control treatment (6.09 cm). The shortest radicle length was recorded in the treatment RW-T03 (3.18 cm).

In Table 4 the longest plumule length of mung bean was observed in control treatment (1.98 cm) which was followed by the treatment MSW-T05 (1.93 cm). The shortest plumule length was recorded in the treatment CD-T01 (1.43 cm). In mustard the longest plumule length was observed in control treatment (3.25 cm) which was followed by the treatment PL-P03 (2.62 cm). Conversely the shortest plumule length was recorded in the treatment RS-P01 (0.90 cm). On the other hand, in wheat the longest plumule length was observed in treatment RW-T03 (3.08 cm) which was followed by the control treatment (2.91 cm). The shortest plumule length was recorded in the treatment CD-P03 (2.15 cm). Both radicle and plumule length of mustard and mung bean attained significant height in the control than other treatments. It might happen that the fungal treatments released some sorts of metabolites that arrested the growth of radicle and plumule to some extent. Conversely in wheat the longest radicle and plumule lengths were recorded in MSW-T05 and RW-T03, respectively; which were statistically insignificant to the results attained in

control treatment. Moreover, both radicle and plumule length in wheat were enhanced by the treatments containing *Trichoderma* spp.

The germination of Millet (Dami Mallit) seeds by application of *Trichoderma* spp. and *Penicillium* sp. were examined and the *Trichoderma* strains enhanced the plumule length whereas, *Pencillium* strain decreased the plumule growth of all varieties in all concentrations compared to control (Hassan et al., 2013). In another study, the coleoptile length of *Elymus sibiricus* increased with application of endophytic fungi supernatant (Li et al., 2017). In the present study, the longest plumule length was achieved using fungal treatment (RW-T03) in wheat, but it was statistically similar to control and most of the fungal treatments presented the statistically similar results also. Mustard had the longest plumule length in the control, but it was statistically similar to most of the treatments. In mung bean, relatively poor plumule length was noticed in the most fungal treatments compared to control, perhaps due to release of some mycotoxins by the applied fungi (Khokhar et al., 2011). Among eight fungal treatments, *Fusarium oxysporum* and *Aspergillus flavus* presented reduced radicle and plumule length on fenugreek seed germination (Khokhar et al., 2011). It implied that responses of fungal suspension/supernatant are not similar to all crop seed germination, but seems to be crop specific. Besides, the radicle and plumule growth are more responsive to mycotoxins than germination of seeds.

Table 4. Radicle and plumule length of mung bean, mustard and wheat germinated seeds by different fungal isolates suspension at last day of observation

Treatment*	Radicle length (cm) at 96 h of germination			Plumule length (cm) at 96 h of germination		
	Mung bean	Mustard	Wheat	Mung bean	Mustard	Wheat
CD-P01	7.62^{ab}	3.81^{bc}	5.40^{bc}	1.73^{abc}	2.28^{ab}	2.39^{bcd}
CD-P03	7.64^{ab}	2.49^{de}	5.35^{bc}	1.77^{abc}	2.54^{ab}	2.15^{cd}
CD-T01	7.21^{ab}	1.60^{efg}	5.58^{abc}	1.43^{d}	1.84^{bc}	2.83^{ab}
RW-T02	6.68^{b}	4.39^{b}	5.21^{bc}	1.69^{bcd}	2.04^{abc}	2.16^{d}
RW-T03	8.01^{ab}	0.80^{g}	3.18^{d}	1.77^{abc}	1.43^{bc}	3.08^{a}
PL-P01	8.07^{ab}	4.41^{b}	5.40^{bc}	1.68^{bcd}	2.22^{ab}	2.31^{bcd}
PL-P03	6.37^{b}	1.23^{g}	5.52^{bc}	1.64^{cd}	2.62^{ab}	2.70^{abcd}
PL-T01	8.15^{ab}	4.47^{b}	4.97^{bc}	1.56^{cd}	2.07^{abc}	2.45^{bcd}
RS-P01	6.69^{b}	1.19^{g}	4.88^{bc}	1.71^{bc}	0.90^{c}	2.54^{abcd}
RS-P02	7.72^{ab}	3.53^{bcd}	5.58^{abc}	1.76^{abc}	2.30^{ab}	2.50^{abcd}
LL-T04	9.06^{a}	1.29^{fg}	4.61^{c}	1.82^{abc}	1.64^{bc}	2.57^{abcd}
MSW-T04	7.12^{ab}	2.77^{cd}	5.92^{ab}	1.73^{abc}	2.42^{ab}	2.80^{abc}
MSW-T05	7.41^{ab}	2.42^{def}	6.87^{a}	1.93^{ab}	2.27^{ab}	2.85^{ab}
Control	9.08^{a}	5.89^{a}	6.09^{ab}	1.98^{a}	3.25^{a}	2.91^{ab}
	($p \leq 0.1$)	($p \leq 0.001$)	($p \leq 0.01$)	($p \leq 0.05$)	($p \leq 0.1$))	($p \leq 0.1$)

*N.B. Fungal strains isolated from decomposed/rotten (CD-Cow dung, RW-Rotten wood, PL-Poultry litter, RS-Rice straw, LL-Decomposed leaf litter, and MSW-Municipal solid waste); P-*Penicillium* sp. and T-*Trichoderma* sp.

3.3 Compatibility performance of fungal mixed culture

A total of 12 combinations designated as ABC, ABF, ACE, ACF, ADE, ADF, BCE, BCF, BDE, BDF, CDE, and CDF using six fungal isolates (A, B, C, D, E, and F) were examined for their performance as mixed fungal growth. Compatible mixed cultures, which might ensure multiple enzymes in bioprocess system for enhancement of biodegradation, are more highly efficient than monocultures (Gutierrez-Correa and Tengerdy, 1997; Rahman et al., 2002). However, both mutual intermingle as well as inhibitory growth of fungal isolates were noticed. The combination ABF and BCE presented the maximum mutual intermingle growth (Figure 4). Mixed cultures of microbes can strengthen and accelerate the bioconversion process and the most important determinants in mixed culture are strain compatibility and nutritional status (Duenas et al., 1995; Gutierrez-Correa and Tengerdy, 1997; Molla et al., 2001b). Accordingly, these two combinations are considered for future research activities.

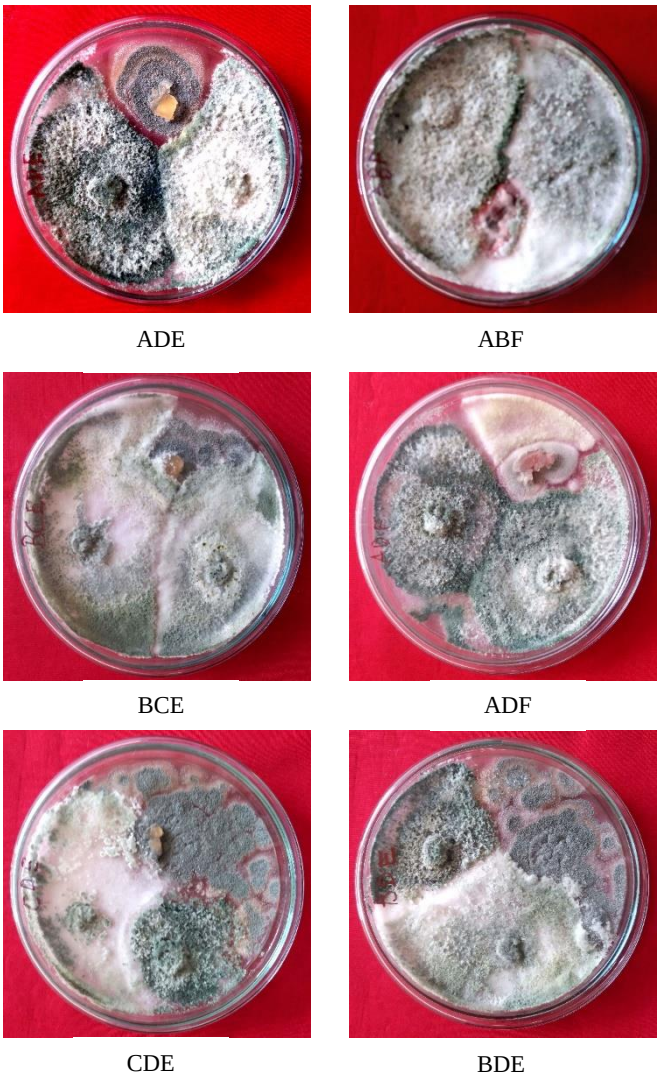


Figure 4. Features of mixed culture growth of different fungal isolate on PDA plates

4. CONCLUSION

Responses of fungal isolates/strains isolated from six native habitats showed dissimilar trends in germination of different seeds. Isolates/strains showed significant response to a crop seed germination, but not equally to all seed types. An isolate/strain significantly increased percent germination but did not affect radicle/plumule length. Discoloration and abnormal symptoms of germinated seeds were not noticed. ABF and BCE combinations of fungal isolates/strains may respond successfully in organic wastes degradation as a mixed culture due to its excellent intermingle performance. Perhaps these combinations (ABF and BCE) of fungal isolates may play significant roles in biodegradation of organic wastes into compost that will reduce load of inorganic fertilizers usages along with improved yield and quality of crop in future research.

ACKNOWLEDGEMENTS

The authors are grateful and extend sincere thanks to Research Management Wing (RMW) of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh for extending their cooperation and providing required fund to conduct research.

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