Association Analysis among Fungi Colonizing Wheat Crop Residues During Decomposition for Sustainable and Environment-Friendly Management of Renewable Natural Resources

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Abstract: The interspecific associations between selected fungal species were calculated based on assumption; the species occurring together in Petri dishes during isolation from litter might be associated as well. Each Petridis was considered as a quadrate for association analysis. The presence and absence of different species in Petri dishes at different sampling dates were recorded as done in grasslands by the quadrate method. In the present study, the interspecific association between 16 fungal species was evaluated, which was isolated during the decomposition of above-ground residues of wheat crop. A sum of 136 combinations was analyzed. The chi-square (χ^2) values were calculated to assess the significance of association (positive or negative), and the coefficient of association (V) was also enumerated to find out the extent of association between species in nature. Any organism was influenced by abiotic and biotic factors constituting its environment. The fungi are no exception; the colonization of a substrate by a fungus and its survival and multiplication depends upon the environment it gets. Therefore, before using any fungus as a bioinoculant, we must make sure that other microbes, especially the fungi already present on the substrate, do not negatively impact it. An analysis of the association between species colonizing the substrate would help determine the bioinoculant to be used for the degradation of organic waste in the interest of the environment to make it safe for everyone.

Keywords: association analysis; chi square (χ^2); coefficient of association (v); wheat crop; fungal decomposer.
Abbreviations: χ2-chi-square; V-Coefficient of association; CMC-Carboxymethyl cellulose.

1. Introduction

The fungi represent a large, diverse group of heterotrophic organisms, the majority of which are originated as saprobes in the soil and on the decomposing organic matter [1, 2]. These are eukaryotic, having internal membrane systems, membrane-bound organelles, and a well-defined cell wall made of large polysaccharides (glucan, mannan) and chitin [3, 4]. The

fungi are universal inhabitants of diverse ecosystems sharing morphologically alike characteristics [5-7]. The term 'fungicolous fungi' coined for fungi species that, over and over again, are coupled with other fungi [8, 9]. The association between biotic diversity and ecosystem function has received much attention among ecologists [10-13]. The various ecologists discussed earlier whether the positive relationship between diversity and function previously observed in fungi has been due to the importance of species diversity or whether individual species' specific properties are of overriding importance [14-16]. Westerdijk proposed the term association for the specific combinations of microfungi on decaying organic substrates [17]. Among many examples quoted were: (a) the occurrence of *Cladosporium herbarum, Alternaria tenuiis* and *Stemphylium* spp. on cereal stubble; (b) the occurrence of *C. herbarum, A. tenuiis,* and *Aureobasidium pullulans* on weathered cotton [17, 18].

The tribe found that the non-cellulolytic *Pythium oligandrum* grew on cellulose so long as it was introduced along with cellulolytic *Botryotrichum piluliferm* [19].Though numerous compounds of highly variable chemical structure enter into the constitution of any litter, a major part of the structural framework is comprised of carbohydrates [20, 21]. Therefore, carbohydrate biochemistry is likely to be the key factor, and any organism is influenced by the abiotic and biotic factors constituting its environment. Therefore, before using any fungus as a bioinoculant, other microbes, especially the fungi already present on the substrate, do not negatively impact it. It can be expected then that the decomposition of a substrate by a fungus or a fungal community depends upon its/their capacity to produce an enzyme(s), which can degrade the substrates' cell wall constituents [22]. Singh *et al*. had given "good enzyme-producing equipment" as one of the important characteristics contributing towards successful colonization of a dead organic substrate by the fungi because for the invasion of plant tissue by a fungus; the important characteristic is "the rate at which it can penetrate successive cell walls in a cellular tissue [23]. An analysis of the association between the species colonizing the substrate would be helpful in determining the bioinoculant to be used for the degradation of organic waste in the interest of the environment to make it safe for everyone. Moreover, studies reported that *Penicillium* and *Aspergillus* species can metabolize complex carbon compounds and the phenolic compounds present in the litter better than other species [24, 25].

Many mycologists have frequently observed fungal succession patterns during litter decomposition. Different fungal taxa dominate different stages of decay in individual ecosystems [26, 27]. However, it is unclear which biological features of fungi give rise to this pattern [28]. Several mycologists tested a longstanding hypothesis that fungal succession depends on species' evolutionary history, such that different fungal phyla prefer different decay stages [29, 30]. The study was designed to analyze the association between species colonizing the substrate would help determine the bioinoculant to be used for degradation of organic waste in the interest of the environment to make it safe for everyone. This may help select the right inoculants for manipulating the decomposition rate of post-harvested crop residues for sustainable natural resource utilization in eco-friendly agro farming practices. These were positively associated and thought to share common climatic and microhabitat requirements. Some mycoflora preferred the association of certain other fungal species, which played a positive key role in the growth and development of associated mycoflora. A study of the extent and nature of the association between microbes colonizing and decomposing a substrate might help manage crop residues through decomposition. Therefore, the study was taken into consideration.

2. Materials and Methods

The interspecific associations between selected fungal species were calculated based on the assumption that the species occurring together in Petri dishes during isolation from litter might be associated as well. For the purpose of association analysis, each Petri dish was considered as a quadrate. The presence and absence of different Petri dishes species at different sampling dates were recorded as is done in grasslands by the quadrate method [31]. The data for the presence and absence of the species in sampling units were arranged in a tabular form (two into two (2x2) contingency Table 1) for all species combinations.

Table 1. The contingency table of species X and species Y for all possible combinations.

Species Y \ Species X	Present	Absent
Present	a	b
Absent	c	d

Where: a= Number of Petri dishes in which both X and Y were present; b= Number of Petri dishes in which X was absent, but Y was present; c= Number of Petri dishes in which X was present, but Y was absent; d= Number of Petri dishes in which both X and Y were absent.

The chi-square (χ^2) values were calculated using the formula:

$$\chi^2 = \frac{n\,(ad - bc)^2}{(a + b)(c + d)(b + d)} \text{ where } n = a + b + c + d$$

If the observed value of χ^2 was greater than 3.84 0r 6.64, the association was considered to be significant or highly significant, respectively. If there was a positive association, most of the quadrates fall in the types 'a' and 'd'. However, during negative correlation was most of these falls in type 'b' and 'c'. If the value was less than 3.84, the association was considered to be insignificant. The strength of association between the two species was further estimated by calculating a coefficient of association, V [32].

$$V = \frac{ad - bc}{\sqrt{(a + b)(c + d)(a + c)(b + d)}}$$

3. Results and Discussion

The Chi-square (χ^2) values were calculated to assess the significance of association (positive or negative), and the coefficient of association (V) was also calculated to find out the extent of association (positive /negative) between the species in nature. In the present study, the interspecific association between 16 fungal species was evaluated; isolated during the decomposition of above-ground residues of wheat crop (Fig. 1; Table 2). About 136 possible combinations were analyzed, out of which 23 combinations were significant (i.e., 15 combinations positively significant, however 8 combinations negatively significant). The coefficient of association between various species combinations was summarized in Table 3. It showed 18 combinations with significant positive values (Fig. 2). On the contrary, 11 combinations with significant negative associations (Fig. 3).
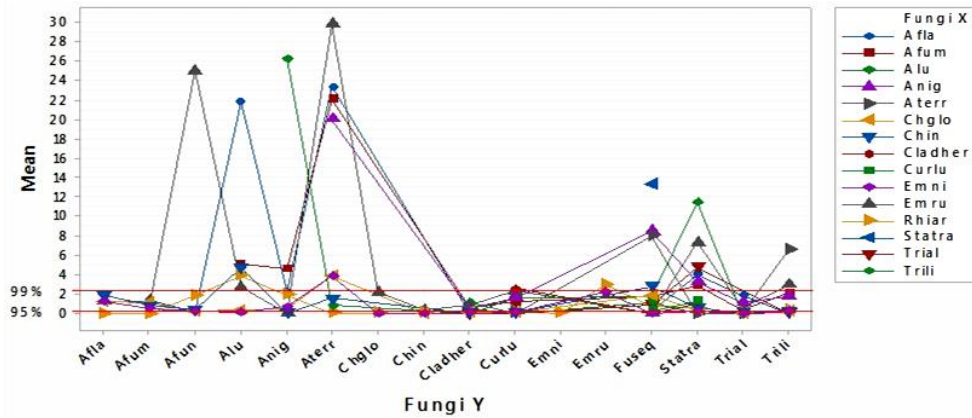
Figure 1. The interaction curve showing chi-square (χ2) values for association between various speciescombinations.Where: Rhiar= *Rhizopus arrhizus*; Emni= *Emericella nidulans*; Emru= *E. rugulosa*; Chglo= *Chaetomium globosum*; Chin=*C. indicum*; Afla= *Aspergillus falvus*; Afum= *A. fumigatus*; Afun= *A. funiculosus*; Alu= *A. luchuensis*; Anig= *A. niger*; Aterr= *A. terreus*; Trial= *Trichoderma album*; Trili= *T. lignorum*; Cladher= *Cladosporium herbarum*; Curlu=*Curvularia lunata*; Statra= *Stachybotrys atra*; Fuseq= *Fusarium equiseti*.
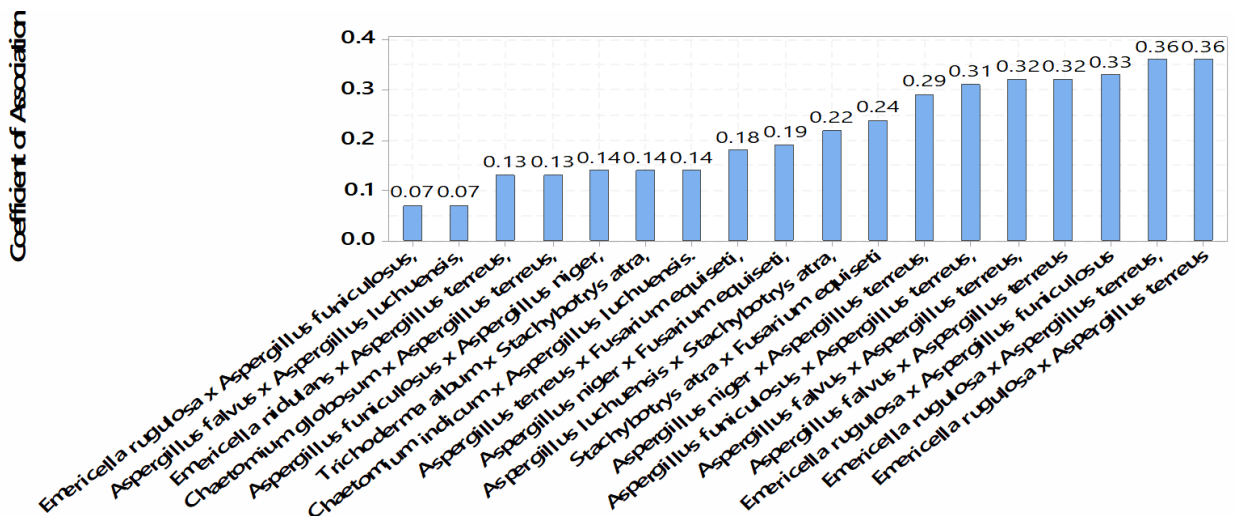

Figure 2. The bar diagram is showing a positive association of fungal species combination based on the value of the coefficient.

The fungal species combinations showed highly positive significant association after advanced numerical tools application based on coefficient values were *Emericella rugulosa* x *Aspergillus funiculosus*, *A. falvus* x *A. luchuensis*, *E. rugulosa* x *A. terreus*, *A. falvus* x *A. terreus*, *A. funiculosus* x *A.s terreus*, *A. niger* x *A. terreus*, *A. luchuensis* x *Stachybotrys atra*, *A. niger* x *Fusarium equiseti*, *A. terreus* x *F. equiseti*, *S. atra* x *F. equiseti*. However, significant positive associations were recorded against the combinations of *A. funiculosus* x *A. niger, E. nidulans* x *A. terreus, Chaetomium globosum* x *A. terreus, Trichoderma album* x *S. atra, C. indicum* x *A. luchuensis*. Onthe contrary, some fungal comnations reflected highly significant negative associations based on the values of coefficient such as *A. luchuensis* x *A. niger, E. rugulosa* x *S. atra, A. fumigatus* x *F. equiseti, A. fumigates* x *S. atra*. While *Rhizopus arrhizus* x *A. luchuensis, A. funiculosus* x *A. luchuensis, T. lignorum,* x *A. terreus, A. falvus* x *S. atra* showed the significant values for negative associations.The strongest positive association was recorded between *E. rugulosa* x *A. terreus* (V= +0.36) followed by *E. rugulosa* x *A. funiculosus* (V= +0.33)and *A. falvus* x *A. terreus* (V= +0.32). On the other hand strongest negative association was noticed between *A. luchuensis* x *A. niger* (V= -0.33) followed by *A. fumigates* x *F. equiseti* (V= -0.28) and *A. fumigates* x *S. atra* (V= -0.22).
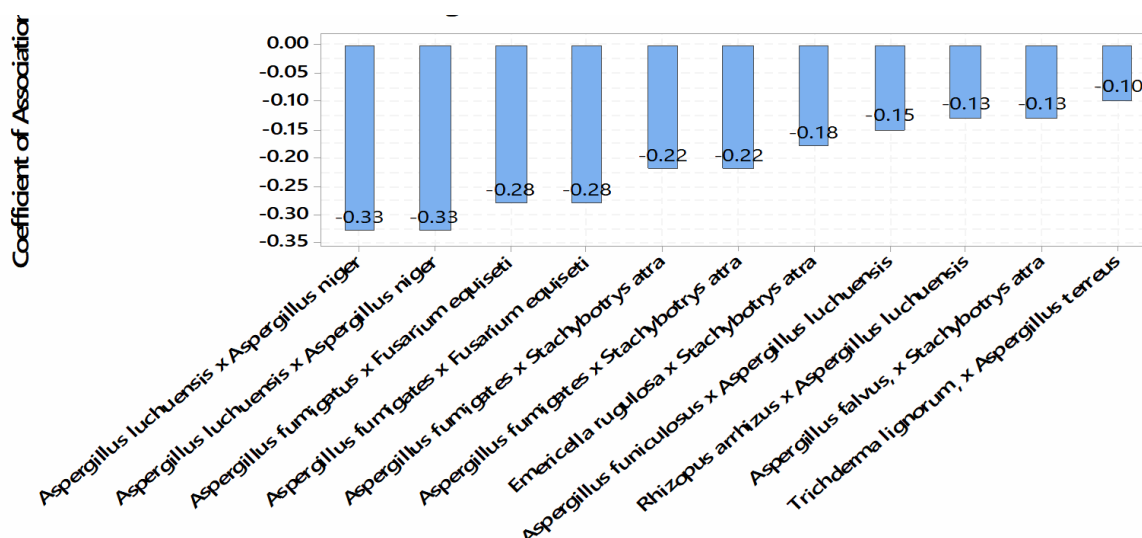
Figure 3. The bar diagram showing a negative association of fungal species combination based on the value of the coefficient.

The *Aspergillus niger* was found to have a highly significant positive association with *A. terreus*. The minimum temperature for the mycelial growth of *A. niger* has been found to be 11–13°C optimum 17–18°C and maximum 47–48°C [33-35]. The effect of biotic and abiotic factors on growth and aflatoxin productions have been widely studied using mathematical modeling [36, 37]. For *A. terreus*, The minimum, optimum and maximum temperature ranges have been determined as 11–13°C, 35–40°C, and 45–48°C, respectively [35, 38, 39]. Thus both the species occupy almost similar temperature niches. Both the species can grow at water potential between -210 and -260 bars [40-42]. Both the species tolerate the pH range 4–8 very well [43]. While *A. terreus* possesses C_i (insoluble cellulose) activity, *A. niger* does not possess it. On the other hand, *A. niger* possesses high C_x (carboxymethylcellulose) activity, *A. terreus* demonstrates only slight *in vitro* C_x activity. On a cellulosic substrate naturally, these two species together can complement each other. The tribe had found that the cellulolytic *Pythium oligandrum* grew on cellulose as long as it was introduced along with cellulolytic *Botryotrichum piluliferum* [19].

The *A. terreus* also had a very strong positive association with *A. flavus*. The optimum temperature range for the growth of *A. terreus,* i.e., 35–40°C falls well within the optimum temperature range of *A. flavus,* i.e., 25-42°C [35, 44]. The maximum temperature *A. terreus* is 45–48°C, while for *A. flavus* it is 47–48°C [39, 45-47]. Both of these show moderate growth from pH 4.0–8.0 [43]. The spores' formation was recorded at pH 5 to 7; however, peak spore formation was observed at a pH of 5.0. On the contrary lowest sporulation was recorded at pH 10.0. Thus the trends of spore formation were adversely affected by the pH of the alkaline range. Likewise, the lowest fresh weight of fungal mycelium was recorded at pH 10.0, which reflected that the higher the pH towards the alkaline range did not support the growth and development of *A. parasiticus*. Based on the finding, it has been proved that the alkaline medium might be helpful to inhibit the growth of mycelia growth and spore formation in *A. parasiticus* to protect crop damage due to such fungal species [48]. Both of these possess fairly good hemicellulolytic and Pectolytic activities and abilities to degrade insoluble cellulose and CMC (carboxymethyl cellulose) in a manner that might complement each other. Thus it is not a surprise that both the above-discussed combinations flourished together on a decomposing substrate.

In broad-spectrum, the *Aspergillus* species was notified a comparatively more tolerant myco-flora to pH towards the alkaline range; on the contrary, the *Penicillium* species showed its tolerance to a range of acidic pH [49, 50]. The findings based on pH make known to fungi could be able to pH ranged 3 to 8. The remarkable augmentation in mycelium's dry weight and trends of sporulation in liquid media were noticed at pH 5.5 and 6.5, respectively [49]. Thus the neutral to a weak acid range of pH of the medium was appropriate for mycelial growth and conidial production [50]. The optimum temperature for the growth of *Stachybotrys atra* is 23°C with a maximum between 37–40°C, while for *A. fumigatus*, these are 37–43°C and 63°C, respectively [51]. *S. atra* is known to produce antifungal metabolites that show antagonistic activities with several fungi. Thus, it is clear that the existence of a positive or negative association between two fungal species may not be purely a matter of chance [52]. A variety of environmental factors like temperature, pH, and physiological capabilities of species and antagonism or synergism between them determine whether any two species would grow together or attempt to exclude/avoid one another [53].

In view of the present study, we should give a second thought before recommending the use of *Trichoderma lignorum* if the residues are already colonized by sizeable populations of *A. terreus*; and that of *S. atra* if the residues are already colonized by *Emericella rugulosa*, *A. fumigatus*, or *A. flavus* [54]. *Trichoderma* species are one of the most common soil fungi, isolated from various habitats. Many *Trichoderma* strains are mesophilic and grow well in a wide range of temperatures from 15-35°C [55]. The contention draws support from earlier microbiologists' observations who showed that wheat straw failed to respond to microbial inoculation due to the presence of high populations of *Clostridium butyricum* [56]. On the other hand, co-inoculation of two positively associated species might also prove to be beneficial. Chatterjee and Nandi had noticed that mixtures of microorganisms could degrade lignin and holocellulose of wheat stubbles more efficiently than any individual species, and naturally, combinations of positively associated species might be a better choice in this context as compared to the negatively associated ones [57–62]. There appears to be a dearth of studies on the interactions of straw fungi on natural substrata.

Frankland emphasized comparative studies of fungal combinations to understand the successions of saprotrophic fungi [63]. These studies may have practical implications also. For example, Kanotra and Mathur could achieve improved enzyme titers by co-culturing a mutant strain of *T. reesei* with a strain of *Pleurotus sajor caju* with wheat straw as the substrate [60]. Gupte and Madamwar obtained improved hydrolytic and β- glucosidase activities by co-culturing two strains of *A. ellipticus* and *A. fumigates* [61].The association analyses by contingency tables and generalized linear modeling, chi-square values, and coefficient of associations were compared to infer relationships among combinations of fungal species. Both types of statistical approaches were highly congruent with the earlier report. They suggested fungal host preference or shared habitat preferences [64]. The fungi increased significantly in the presence of particular combinations among species, while most associations among fungal species were non-significant. On the contrary, other fungal species were negatively coupled with one and another, conceivably demonstrating diverse habitat preferences.

Table 2. The Chi-Square (χ2) values for an association between various species combinations.

Chi-Square (χ2) values for an association between various species combinations

Fungal sp.	Emni	Emru	Chglo	Chin	Afla	Afum	Afun	Alu	Anig	Aterr	Trial	Trili	Cladher	Curlu	Statra	Fuseq
Rhiar	0.04	2.95	0.04	0.06	0.03	0.0006	1.90	3.91*	1.94	0.07	0.06	0.41	0.11	0.23	0.58	0.74
Emni		2.22	0.01	0.02	1.24	0.51	0.22	0.11	0.64	3.87*	0.02	0.15	0.04	0.09	0.23	0.003
Emru			2.22	0.322	1.28	1.25	25.0**	2.67	0.03	29.80**	0.53	2.93	0.81	2.31	7.30**	0.002
Chglo				0.02	1.24	1.08	0.22	0.33	0.50	3.87*	0.02	0.16	0.04	0.09	0.41	1.86
Chin					1.88	0.78	0.34	4.73*	0.05	1.53	0.04	0.24	0.06	0.14	0.61	2.80
Afla						1.35	0.09	21.87**	2.22	23.33**	1.88	0.06	0.07	0.29	4.02*	0.22
Afum							0.14	0.35	0.02	0.06	0.87	0.37	1.20	2.68	11.74**	18.73**
Afun								5.07*	4.56*	22.07**	0.34	2.09	0.58	1.19	2.87	1.69
Alu									26.23**	0.80	0.04	0.11	0.001	1.60	11.46**	1.69
Anig										19.99**	1.15	1.70	0.48	1.61	3.25*	8.49**
Aterr											0.001	6.56*	0.45	0.08	0.02	7.97**
Trial												0.08	0.02	0.04	4.79*	0.92
Trili													1.13	0.08	0.007	1.18
Cladher														2.51	1.03	0.005
Curlu															1.28	0.26
Statra																13.30**

*Significant level 0.05; **Significant level 0.01;Rhiar= *Rhizopus arrhizus*; Emni= *Emericella nidulans*; Emru= *E. rugulosa*; Chglo= *Chaetomium globosum*; Chin=*C. indicum*; Afla= *Aspergillus falvus*; Afum= *A. fumigatus*; Afun= *A. funiculosus*; Alu= *A. luchuensis*; Anig= *A. niger*; Aterr= *A. terreus*; Trial= *Trichoderma album*; Trili= *T. lignorum*; Cladher= *Cladosporium herbarum*; Curlu=*Curvularia lunata*; Statra= *Stachybotrys atra*; Fuseq= *Fusarium equiseti*.

Table 3. The coefficient values of association between various fungal species combinations.

Coefficient of association between various species combinations

Fungal sp.	Emni	Emru	Chglo	Chin	Afla	Afum	Afun	Alu	Anig	Aterr	Trial	Trili	Cladher	Curlu	Statra	Fuseq
Rhiar	-0.04	+0.11	-0.01	-0.01	+0.01	-0.001	+0.09	-0.13	+0.09	+0.01	-0.01	-0.04	-0.11	-0.03	-0.05	-0.05
Emni		+0.09	-0.008	-0.01	+0.07	-0.04	-0.03	+0.02	-0.05	+0.13	-0.01	-0.02	-0.01	-0.02	-0.03	+0.04
Emru			+0.09	-0.03	+0.07	-0.07	+0.33	-0.10	-0.01	+0.36	-0.04	-0.11	-0.06	+0.10	-0.18	+0.003
Chglo				-0.01	+0.07	+0.06	-0.03	+0.03	-0.04	+0.13	-0.01	-0.02	-0.01	-0.02	-0.04	-0.09
Chin					+0.09	-0.05	-0.03	+0.14	-0.01	+0.08	-0.01	-0.03	-0.01	-0.02	-0.05	-0.11
Afla						-0.07	-0.006	+0.31	+0.09	+0.32	+0.09	-0.01	-0.005	+0.03	-0.13	-0.03
Afum							-0.02	+0.0002	+0.01	-0.01	-0.05	+0.04	+0.07	-0.10	-0.22	-0.28
Afun								-0.15	+0.14	+0.31	-0.03	-0.09	-0.05	-0.07	-0.11	+0.06
Alu									-0.33	-0.05	-0.01	-0.02	+0.002	-0.08	+0.22	+0.08
Anig										+0.29	+0.07	-0.08	-0.04	+0.08	-0.12	+0.19
Aterr											+0.002	-0.01	-0.04	-0.01	-0.05	+0.18
Trial												-0.01	-0.01	-0.01	+0.14	-0.06
Trili													+0.07	+0.01	+0.005	-0.07
Cladher														+0.10	-0.06	-0.11
Curlu															+0.04	-0.03
Statra																+0.24

Where: Rhiar= *Rhizopus arrhizus*; Emni= *Emericella nidulans*; Emru= *E. rugulosa*; Chglo= *Chaetomium globosum*; Chin= *C. indicum*; Afla= *Aspergillus falvus*; Afum= *A. fumigatus*; Afun= *A. funiculosus*; Alu= *A. luchuensis*; Anig= *A. niger*; Aterr= *A. terreus*; Trial= *Trichoderma album*; Trili= *T. lignorum*; Cladher= *Cladosporium herbarum*; Curlu= *Curvularia lunata*; Statra= *Stachybotrys atra*; Fuseq= *Fusarium equiseti*.

4. Conclusions

Understanding the type of association between fungal species, especially the decomposer, may help determine the inoculant species in relation to the dominant mycobiota already existing on the resources. This may help in the selection of the right inoculant for manipulating the rate of decomposition. These combinations were thought to be positively associated and shared widespread climatic and niche necessities. The investigation revealed that some fungal species were expected to associate with certain other species for their suitable growth and development. A study of the extent and nature of the association between microbes colonizing and decomposing a substrate might help manage crop residues through decomposition. It would be better to use those efficient fungal species as Inoculants to withstand the antagonism and competition from the fungal species existing on the residues. An analysis of the association between the species colonizing the substrate would help determine the bioinoculant to be used for the degradation of organic waste in the interest of the environment to make it safe for everyone.

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Conflicts of Interest

The authors declare no conflict of interest.

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