Degradation of polymers by fungi isolated from dumpsites

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Abstract

The present study aims at identifying the ability of nine fungal species isolated from the garbage of different sites to degrade polyethylene, polystyrene, and polyurethane when treated separately with the drench and mulching methods, each one sterilized and unsterilized for four months. The species considerably decomposed the polymers by these methods. Polystyrene demonstrated the highest degradation compared to the other polymers, particularly by sterilized and unsterilized drench methods. Seven fungal species showed greater than 50% weight loss of polystyrene when treated with these methods. *Aspergillus flavus* instigated the highest weight loss (74.78 ± 2.85%) by the unsterilized drench method. Of nine, three species caused more than 50% weight loss of polyurethane by the unsterilized drench method. *A. niger* reduced more than half the weight of the polymer by the sterilized drench method. In this study, polyethylene was found least degraded compared to polystyrene and polyurethane by the selected fungal species. Of nine, only two species, viz. *Aspergillus flavus* and *A. niger* caused a higher than 50% weight loss of polyethylene only by sterilized drench method. Scanning Electron Microscopy (SEM) images of six highly degraded polymer samples revealed the formation of spores, hyphae, cracks, and crevices on their surfaces.

Highlights

1. The study investigates the plastic degradation by fungal species
2. The fungi were collected from plastic wastes along with soil from dumping sites.
3. The experiment divulges encouraging results when the plastics were treated with fungi
4. The fungi cracks the plastic and formed their spores on the surface of plastic

1. Introduction

Nonbiodegradable plastic waste is a global environmental concern due to its adverse consequences on the entire biosphere (Thompson 2017) since the bacterial and fungal species do not generally decompose them. On the contrary, worldwide plastic waste has been continuously increasing since the last few decades due to the wide-ranging consumption of plastic products. The first synthetic plastic, namely *Bakelit*, was produced in 1907. The commercial production of plastic was sluggish before World War II. Plastic production skyrocketed globally after the 1950s for the packaging of food, agricultural and industrial products due to its great demand. The global annual production of plastic swelled over the subsequent 65 years to almost 200-folds, that is, 381 million tons in 2015 (Ritchie and Roser 2018). Sixty to ninety-nine million tons of mismanaged wastes of plastic were estimated to dispose of into the living planet during 2015 (Lebreton and Andrady 2019).

The continuous accumulation of plastic waste has badly affected both the terrestrial and aquatic ecosystems (Yogalakshmi and Singh 2020; Ali and Shams 2015; Perkins 2014; Barnes 2002). Plastic waste from metropolitan garbage, sewage sludge, and agricultural plastic mulch greatly influence the soil ecosystem, particularly soil micro-organisms (Wanner 2021; Chae and An 2018; He et al. 2018). The growing accumulation of plastic residues in the land is presently a global concern due to the application of plastic mulch in agricultural lands for higher yield, which damages soil health and threatens sustainable food production (Zhang et al. 2020). Furthermore, plastic pollution causes the deaths of many coastal and marine turtles, birds, mammals, and fish mainly through ingestion and entanglement (Roman et al. 2021; Roman et al. 2020; Wilcox et al. 2018).

Consequently, many researchers started working to determine bacterial and fungal species that demonstrate the ability to decompose plastic waste (Ali et al., 2021). Plastic degradation by fungal species has been more encouraging than by bacterial species that persuaded many researchers to identify the fungal species that can cause the degradation of various types of plastics by using different methods (Muhonja et al. 2018; Barratt et al. 2003).

Aspergillus niger decomposes thermally oxidized high-density polyethylene (Mathur et al., 2011) that reveals high potential in decomposing low-density polyethylene under natural conditions (Esmaeili et al., 2013). *A. clavatus* degrades low-density polyethylene when incubated in an aqueous medium for 90 days (Gajendiran et al., 2016). *A. oryzae* causes a weight reduction of low-density polyethylene when placed in a shaker incubator (Muhonja et al., 2018). *A. flavus* and *A. tubingensis* consume high-density polyethylene as a carbon source without pro-oxidant and pretreatment (Devi et al., 2015). *Rhizopus oryzae* decreases the weight of thermally treated low-density polyethylene and substantially reduces its tensile strength sustainably (Awasthi et al., 2017). *Zalerion maritimum*, a marine fungus, can utilize polyethylene as a carbon source in a minimal growth medium (Paço et al., 2017). The endophytic fungi degrade the gamma-irradiated low-density polyethylene strips (Sheik et al., 2015).

Khan et al. (2017) stated that *Aspergillus tubingensis* decomposes the polyester polyurethane film into smaller pieces in a liquid medium after two months. Taghavi et al. (2021) reported higher surface degradation of plastics under a mixed microbial system. They found the highest weight loss of polyethylene when incubated with *A. flavus* in unstimulated mix conditions. Barratt et al. (2003) reported that *Geomyces pannorum, Nectria gliocladioides*, and *Penicillium ochrochloron* degrade polyester polyurethane and reduce its tensile strength up to 60% under a varied range of soil water holding capacity. Álvarez-Barragán et al. (2016) stated that Cladosporium *cladosporioides* significantly reduces the weight of polyurethane after 14 days of incubation. Urzo et al. (2017) found that *Lasiodiplodia theobromae, Penicillium janthinellum, Fusarium verticillioides*, and *Paecilomyces puntonii* trim down the weight of polyurethane and consume it as the sole nitrogen and carbon.

Studies on the degradation of polystyrene by fungal species are insufficient compared to polyethylene and polyurethane. However, few studies have demonstrated the degradation of polystyrene by fungal species. For instance, *Cephalosporium* sp. and *Mucor* sp. reduce 2.17 ± 0.16% and 1.81 ± 0.13% weight of polystyrene respectively when incubated with them separately for eight weeks (Chaudhary and Vijayakumar 2019). Tsujiyama and Takada (2004) reported a slow degradation of polystyrene by *Phanerochaete chrysosporium*, a white-rot fungus.

In the present study, the fungal species and strains were taken from the surface of polyethylene, polystyrene, and polyurethane, living in several localities of the University of Karachi. These polymers are the most widely exploited disposable plastics as household packaging materials in the city. Polyethylene and polystyrene are thermoplastic, and polyurethane is a thermosetting plastic. The present study will help identify the potential of the fungi for the decomposition of different types of plastics and the determination of new approaches for their degradation.

2. Material And Method

The samples of polyethylene, polystyrene, and polyurethane were collected along with the soil samples from six garbage dumping sites of Karachi University. Three replicates of each type of plastic and the soil samples extracted from each site for the study. Two sets of utilized polystyrene food boxes were from the debris of several places to reveal any difference in its degradation by varied fungal species.

The samples were taken to the laboratory and were kept separately for 24 hours at room temperature. Each plastic sample was washed with distilled water and then cut into small pieces with sterilized scissors. The washed plastic pieces were weighed and placed separately on Potato Dextrose Agar (PDA) plates, supplemented with streptomycin and penicillin. The PDA plates were incubated at 28°C for five to six days to identify the fungal species using the references (Barnett and Hunter 1998; Raper and Thom 1949; Visagie et al. 2014).

The fungi were isolated from the soil samples by soil dilution technique (Nash and Snyder 1962; Urooj et al. 2018). The soil samples were collected from 10 cm depth at each site and put in separate test tubes with 10mL sterilized water, which was diluted up to a 1: 10 ratio. Each diluted sample of 0.1mL was placed on different streptomycin and penicillin augmented PDA Petri-dishes and was stored at 28°C for five days to let the fungi grow. The fungal species were recognized using the references cited earlier.

Test fungal species were grown in the conical flasks of 250 ml that consisted of 100 ml of Czapek's Dox broth and immunized with a 5 mm disc of test fungi cut from briskly growing culture. The flasks were kept safe at room temperature (25–30°C). Un-used polyethylene, polystyrene, and polyurethane were procured to run the pot experiment.

The pot experiment was performed by taking 1 kg soil in each pot to study the plastic degrading properties of fungi. In the sterilized pot experiment, the soil samples and plastic were sterile, while in the unsterilized pot experiment, the soil samples and the plastic were without sterilization. The plastic samples were sterilized with 1% bleach for 3 minutes, then with 70% alcohol for 3 minutes, and finally washed with distilled water.

In Drench Method, the fungal broth drained over the plastic, and in Mulching Method, the fungal broth drained over the soil surface. The degradation of buried plastics was observed with a one-month interval. The buried plastics were taken out after four months from the soil since the cracks and roughness on the surface of plastics were not distinct earlier. The plastics were then washed with water to observe them through dissecting microscope for further verification of the degrading plastics, which showed noticeable deterioration and unevenness on the surface of plastics. Every piece of the plastic was washed separately with distilled water and dried at room temperature, and then measured the weight loss due to different fungal species. The weight loss is a measure of biodegradation of polymer (Taghavi et al. 2021; Chaudhary and Vijayakumar 2019; Muhonja et al. 2018; Álvarez-Barragán et al. 2016)

The plastic samples, which demonstrated the highest degradation, were selected for a detailed investigation like the interaction of fungal hyphae with plastic through Scanning Electron Microscopy (SEM).

Analysis of Variance (ANOVA) and Duncan Multiple Range Test were applied using SPSS 16 for analyzing the data.

3. Results

Figure 1 demonstrates the weight loss of polyethylene when treated with nine different fungal species by employing four designated methods. The analysis of variance (ANOVA) reveals a highly significant variation in reducing the weight of the plastic by fungal species ($p < 0.001$) when treated with varied methods ($p < 0.004$). *Aspergillus flavus* showed the highest degradation (56.13 %) of the polymer when treated with the sterilized drench method, followed by *A. niger* when treated the polymer (50.71 %) in the same way.

Of nine, five species, viz., *Aspergillus flavus, A. niger, Penicillium expansum, Macrophomina phaseolina,* and *Fusarium solani* divulged the highest degradation of the polymer when treated with sterilized drench method among four designated methods. *A. oryzae, Penicillium decumbens,* and *P. frequentans* with the un-sterilized mulching method and *Penicillium rubrum* with the sterilized mulching method divulged the highest polymer degradation among the designated methods.

Figure 2 exhibits the weight loss of polystyrene when treated with nine fungal species by applying four methods. The statistical analysis discloses a highly significant variation in reducing the weight of the plastic by fungal species ($p < 0.001$) when treated with different methods ($p < 0.001$). *Aspergillus flavus* demonstrated the highest polystyrene degradation (74.78%) when treated with unsterilized drench method, followed by *A. niger* when treated the polymer (71.88%) with sterilized drench method.

Of nine, seven species, viz., *Aspergillus flavus, A. oryzae, Penicillium decumbens, P. frequentans, P. rubrum, P. expansum,* and *Macrophomina phaseolina* showed the highest degradation of the polymer *when treated with the un-sterilized drench method. Two species, viz., Aspergillus niger* and *Fusarium solani* with sterilized drench method divulged the maximum degradation of the polymer among the designated methods. Overall, the unsterilized drench method was the most effective for polystyrene degradation by fungal species.

Figure 3 discloses polyurethane degradation when treated with nine fungal species separately using four methods. The statistical analysis demonstrated a significant variation in the weight loss of the plastic by fungal species ($p < 0.02$) when treated with four methods ($p < 0.001$). *Macrophomina expansum* showed the highest polyurethane degradation (70%) when treated with the unsterilized drench method, followed by Aspergillus flavus (65.75%) when treated the same way.

Of nine, six species, viz., *Aspergillus flavus, Penicillium decumbens, P. frequentans, P. rubrum, P. expansum,* and *Macrophomina phaseolina* when treated with un-sterilized drench method and three species, viz., Aspergillus oryzae, *A. niger*, and *Fusarium solani* when treated with sterilized drench method demonstrated the highest degradation of the polymer amongst the designated methods. Overall, the unsterilized drench method was the most effective for polyurethane degradation by different fungal species.

Table 1 divulges the weight losses in terms of the percentage of three types of plastics when treated with nine different fungal species using four methods. Duncan Multiple Range Test was used to determine the statistical difference of the mean value, presented in this table. The mean values with different alphabets differ significantly ($P < 0.05$).

Image 1 exhibits the degraded surface of *Aspergillus flavus* treated polystyrene through Scanning Electron Microscope. Image 2 shows the degraded polyurethane when treated with the same species. The depiction reveals the penetration of the fungal species and the spread of the spores on the entire surface of polymers during four months.

4. Discussion

4.1 Polystyrene

The present study demonstrates that many fungal species substantially degraded polystyrene when treated with sterilized and unsterilized drench methods. Seven species, viz. *Aspergillus flavus, A. niger, Penicillium decumbens, P. rubrum, P. expansum, Macrophomina phaseolina, and Fusarium solani* were responsible for more than 50% weight loss of the polymer when treated with the sterilized drench and unsterilized drench methods for four months. *A. flavus* demonstrated the highest weight loss (74.78 ± 2.85%) of the polymer when treated with the unsterilized drench method. Furthermore, *A. flavus, A. niger*, and *Macrophomina phaseolina* caused a weight loss of more than 50% by the unsterilized mulching method. Moreover, *A. niger* resulted in 50.14 ± 15.17% weight loss of polystyrene when treated with the sterilized mulching method. All the nine species demonstrated higher than 30% weight loss of polystyrene when treated with all the four designated methods separately.

4.2 Polyurethane

Of nine fungal species, only three species, viz. *Aspergillus flavus, Penicillium rubrum*, and *P. expansum* caused more than 50% weight loss of the polymer when treated with an unsterilized drench method. *A. niger* caused more than 50% weight reduction of the polymer when treated with the sterilized drench method. Mathur and Prasad (2012) reported a 60.6% weight loss of the polymer when incubated with a shaking culture of *A. flavus* with 4.8×10^6 spores ml for 30 days. Oviedo-Anchundia et al. (2021) reported a 28.34% weight loss of this polymer by *Penicillium spp*.

Not a single fungal species caused higher than 50% weight loss of polyurethane by the sterilized mulching and unsterilized mulching methods. Three fungal species, viz., *Penicillium decumbens, P. frequentans*, and Aspergillus oryzae were responsible for less than 20% weight loss of the polymer when separately treated with the sterilized and unsterilized mulching methods. Furthermore, *Macrophomina phaseolina, Penicillium rubrum, and Fusarium solani* reduced less than 20% weight of polyurethane when treated with the sterile mulching method.

4.3 Polyethylene

The present study reveals that polyethylene is least affected by the selected fungal species when treated with the designated drench and mulching methods than the other plastic samples. Of nine species, only two species, viz. *Aspergillus flavus* and *A. niger* caused a higher than 50% weight loss of the polymer when treated with the sterilized drench method. These two species have not reduced the weight of polyethylene to fifty percent by the other designated methods.

Furthermore, the other seven fungal species have not reduced the weight of polyethylene to fifty percent by any of the four designated methods. *Penicillium decumbens and P. frequentans* reduced less than 20% weight of polyethylene by unsterilized drench and sterilized mulching methods. *Fusarium solani* dropped less than 20% weight of the polymer by unsterilized and sterilized mulching methods. *Penicillium rubrum* plummeted less than 20% weight of the polymer by the unsterilized mulching method.

Muhonja et al. (2018) reported that *A. oryzae* brought about 36.4 ± 5.53% weight loss of polyethylene in four months, which is comparable to the present study. *A. oryzae* resulted in a weight loss of 40.71 ± 2.73% (unsterilized mulching), 29.99 ± 2.33% (sterilized drench), 28.57 ± 22.99% (unsterilized drench), 22.14 ± 2.73% (sterilized mulching).

5. Conclusion

As a whole, almost all the fungal species demonstrated the highest degradation of polystyrene compared to the other polymers by all the designated methods. Drench methods are the most effective method for reducing the weight of the polymers compared to the mulching methods. Of nine, seven species reduced the weight of polystyrene by more than 50% when treated with the sterilized and unsterilized methods. On the contrary, only two species with the sterile drench method reduced polyethylene weight by more than 50%. Three species with the unsterilized mulching and one species with the unsterilized mulching methods slashed more than half of the polyurethane weight. *Aspergillus flavus* with the unsterilized drench method was the most effective in bringing down the polystyrene weight. This species with the sterilized drench method was also the most effective in reducing the polyethylene weight. *Penicillium expansum* was the most worthwhile in reducing the weight of polyurethane when treated with the unsterilized drench method. The present study can help decrease the continuously increasing various kinds of polymers in the environment.

Declarations

Authors' Contribution: Iqra Bashir designed and performed the experiment, and wrote the manuscript; Zafar Iqbal Shams supervised the overall study and helped in writing the manuscript; Syed Ehteshamul-Haque, being mycologist, provided technical assistance and laboratory facilities; Faizah Urooj helped in designing and performing the experiment; Hafiza Farhat helped in identifying the fungal strains. All authors read and approved the final manuscript

Conflict of interest: On behalf of all authors, the corresponding author states no conflict of interest.

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Tables

	Sterilized Drench			Sterilized Mulching			Un- Sterilized Drench		
Fungal species	Polyethylene	Polystyrene	Polyurethane	Polyethylene	Polystyrene	Polyurethane	Polyethylene	Polystyrene	Polyurethan
Control	^{a}1.42 ±1.64	^{a}1.28 ±1.55	^{a}3.75 ±4.78	^{a}2.85 ±2.33	^{a}1.81 ±1.77	^{a}3.75 ±4.78	^{a}1.42 ±1.64	^{a}1.10 ±1.42	^{a}3.75 ±4.78
Aspergillus flavus	^{h}56.13 ±3.21	^{e}f57.62 ±9.61	^{cd}36.95 ±11.76	^{bc}23.99 ±2.29	^{g}49.86 ±10.52	^{d}34.00 ±3.23	^{e}22.70 ±5.49	^{e}74.78 ±2.85	^{d}65.75 ±3.3
A. oryzae	^{ef}39.99 ±2.33	^{b}39.53 ±15.14	^{cd}41.25 ±4.78	^{bc}22.14 ±2.73	^{e}48.42 ±12.84	^{b}18.75 ±11.08	^{de}28.57 ±22.99	^{de}61.78 ±18.05	^{bc}35.00 ±4.(
A niger	^{gh}50.71 ±2.73	^{h}71.88 ±9.65	^{e}57.5 ±2.88	^{bc}22.85 ±4.03	^{c}50.14 ±15.17	^{bc}23.75 ±4.78	^{de}29.28 ±4.87	^{e}50.67 ±8.50	^{c}40.00 ±5.7
Penicillium decumbens	^{bc}25.71 ±2.33	^{d}53.71 ±18.97	^{b}27.5 ±2.88	^{b}11.42 ±5.21	^{cd}30.03 ±19.95	^{bc}16.25 ±4.78	^{b}17.14 ±2.33	^{b}54.53 ±28.44	^{bc}40.00 ±4.(
P. frequentans	^{cde}33.56 ±2.73	^{c}49.78 ±9.45	^{b}28.75 ±4.78	^{b}18.56 ±3.68	^{d}33.53 ±18.91	^{c}12.5 ±2.88	^{cd}18.56 ±3.68	^{bc}55.10 ±24.77	^{b}43.75 ±4.7
P. rubrum	^{b}22.85 ±4.03	^{c}51.49 ±1.53	^{bc}31.25 ±4.78	^{c}37.14 ±2.33	^{d}36.17 ±3.10	^{d}32.5 ±2.88	^{g}32.13 ±2.73	^{c}54.96 ±19.15	^{d}61.25 ±6.2
P. expansum	^{def}39.28 ±14.82	^{g}66.39 ±8.56	^{d}42.5 ±2.88	^{bc}22.85 ±2.33	^{f}44.89 ±1.60	^{e}35.00 ±9.12	^{de}25.71 ±2.33	^{d}69.64 ±7.78	^{d}70.00 ±4.0
Macrophomina phaseolina	^{fg}46.42 ±1.43	^{de}54.96 ±20.31	^{cd}38.75 ±4.78	^{bc}31.42 ±2.33	^{d}34.60 ±11.96	^{bc}17.50 ±6.45	^{f}26.42 ±1.43	^{c}56.71 ±14.43	^{bc}38.75 ±4.7
Fusarium solani	^{cd}31.42 ±2.33	^{f}57.71 ±2.01	^{d}43.75 ±11.08	^{bc}17.14 ±2.33	^{b}36.14 ±14.20	^{b}20.00 ±4.08	^{c}22.85 ±2.33	^{c}45.60 ±8.24	^{bc}33.75 ±4.7

Table 1: Weight losses (%) of three different types of plastics when treated with nine different fungal species using four different methods. According to Duncan Multiple Range Test, mean values with the same alphabet do not differ significantly.

Figures

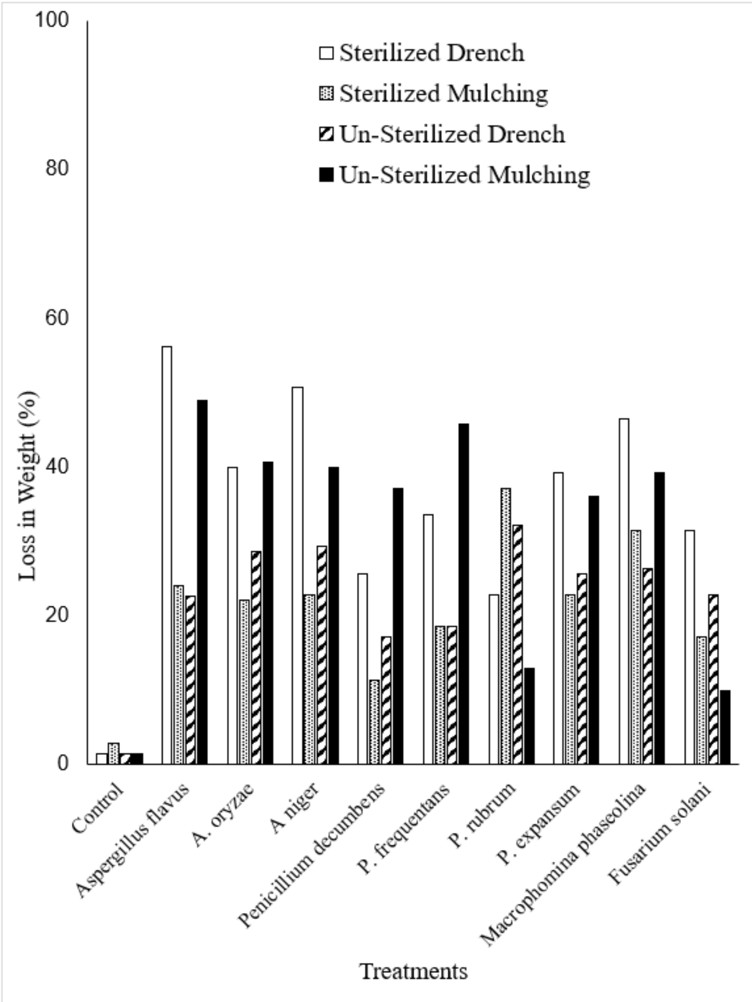

Figure 1

Treatment of Polyethylene by different fungal species using varied methods. The control represents untreated plastic.

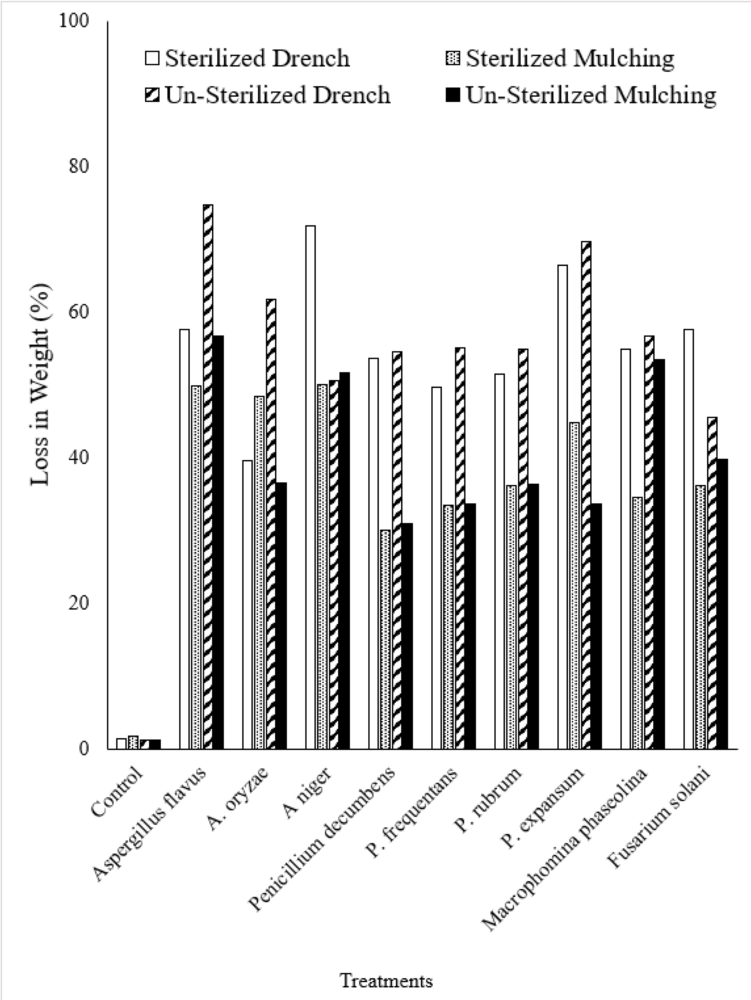

Figure 2

Treatment of Polystyrene by different fungal species using varied methods. The control represents untreated plastic.

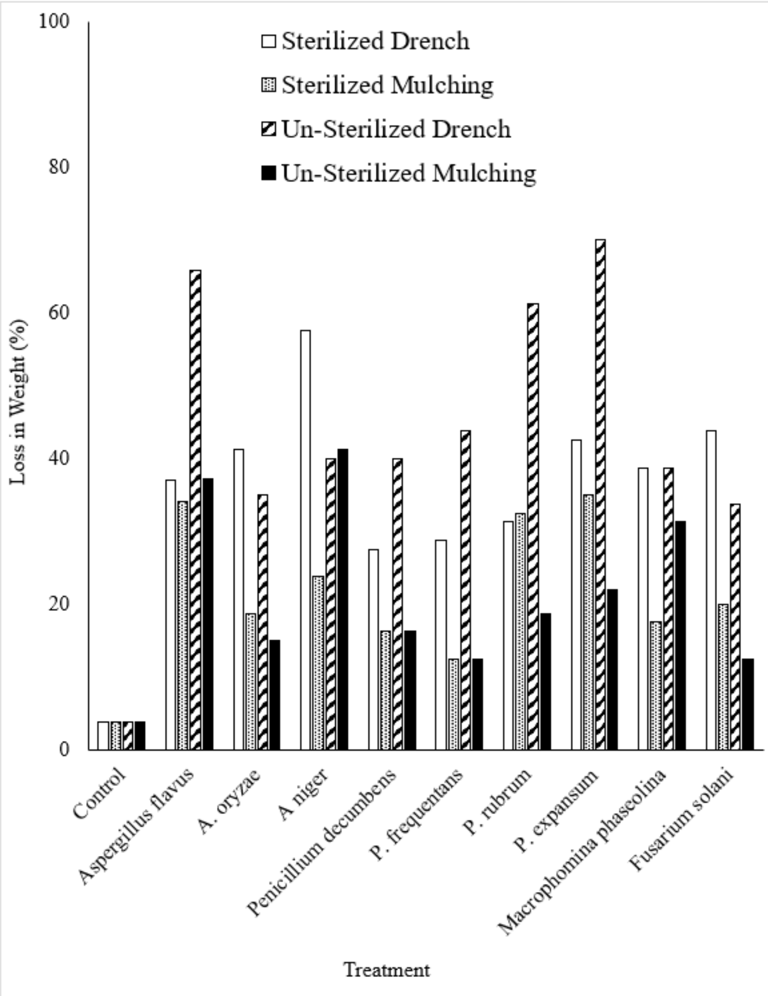

Figure 3

Treatment of Polyurethane by different fungal species using varied methods. The control represents untreated plastic.

Supplementary Files

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- Images.docx