



Biodegradation of insecticides and herbicides by *Aspergillus* species: A review

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ABSTRACT

Use of synthetic pesticides during past decades has led to serious environmental pollution and residual toxicity to soil organisms. In view of this, efforts have been made all over to study degradation of pesticides and their residues by microorganisms. Bacterial degradation of pesticides has been extensively studied and several strains have been recommended for application and accelerated degradation of residues but fungal degradation has attracted less attention. Several filamentous fungal species belonging to ascomycetes have been found effective in degrading the insecticides and herbicides. Moreover the spore forming ability enable them more persistence in the nature even during harsh climate making them a better choice with less number of applications. It is a review article and mainly focuses on the bioremediation of pesticides using *Aspergillus* species.

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1. Introduction

Agricultural use of pesticides dates back to 2500 BC when these were applied for on-field and off-field protection of crops. The first known pesticide was elemental sulphur dusting of which used in ancient Sumer about 4,500 years ago in ancient Mesopotamia. During the 15th century, toxic chemicals such as arsenic, mercury and lead were applied to crops to kill pests. During the 17th century, nicotine sulphate was extracted from tobacco leaves to use as an insecticide (Ashman and Puri, 2002). Subsequently synthetic pesticides entered into agriculture during 1940s resulting in rapid progress and development of different pesticides of widely diverse chemical nature. Extensive use of the synthetic pesticides and their prolonged residual toxicity in the soil environment has affected the soil microflora and fauna leading to loss of soil productivity (Hewitt, 1998). This has become a major concern not only in our country but also throughout the world.

In India 15–20% of all produce is destroyed by insect pests (Bhalerao and Puranik, 2007). This emphasizes the paramount importance of insecticides in India in preventing

agricultural loss and enhancing production. The enormous use of pesticides, has added to environmental pollution. However, a number of bacteria, cyanobacteria and fungal species are known to metabolically degrade the insecticides and other organochemicals causing decrease in their environmental toxicity. Members of phycomycetes, ascomycetes and white-rot fungi (*Pleurotus ostreatus*, *Trametes versicolor*, *Trametes hirsutus*, *Bjerkandera adusta*, *Stereum hirsutum*, *Hypholoma fasciculare*, *Flammulina velutipes*, *Lentinus ododes*, *Penicillium steckii*, *Phlebia acanthocystis*, *Phlebia aurea*, *Phlebia lindtneri*, *Phlebia vrevispora*, *P. chrysosporium* and *P. sordid*) have been noted to be very useful for biodegradation of pesticides (Bending *et al.*, 2002; Jauregui *et al.*, 2003). Many a different species of *Aspergillus* can degrade the pesticides under ambient environmental set up, even when exposed to high concentrations (Anderegg and Madisen, 1983; Hasan, 1999; Bhalerao and Puranik, 2009; Jain and Garg, 2013; Jain *et al.*, 2014; Yadav *et al.*, 2015; Oliveira *et al.*, 2015). However, little attempt has been made to comprehensively evaluate the potential of *Aspergillus* species in degrading the pesticides in the field. This review presents the researches

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made on the evaluation of various species and strains of *Aspergillus* for biodegradation of pesticides and use of these fungi in bioremediation.

2. Degradation of insecticides

2.1 Organophosphates

Marine-derived fungus *Aspergillus sydowii* performs the degradation of organophosphorus insecticide methyl parathion (commercially named Folisuper 600 BR) even at high concentrations. Different concentrations (50-300 mg/l) of the insecticide could be effectively degraded by the fungus in solid medium but better degradation could be achieved in liquid medium. *A. sydowii* shows (98% degradation in 10 days and 100% degradation in 20 days) with a pesticide concentration of 50 mg/l (Alvarenga *et al.*, 2014). Two other isolates of *A. sydowii* (CBMAI 935 and CBMAI 1241) also degraded profenofos applied at 100 mg/l (da Silva *et al.*, 2013). These strains degraded up to 98% and 92% in 30 days in a concentration of 50 mg/l and 100 mg/l, respectively (da Silva *et al.*, 2013). The fungus could also utilize 1.65 g/l malathion and lincer as phosphorus sources (Hasan, 1999). *Aspergillus glaucus* degraded malathion to non-toxic levels when the insecticide was applied in stored grains (Anderegg and Madisen, 1983). Similarly soil derived fungus *Aspergillus oryzae*, isolated from the soil using a 100 mg/l of monocrotophos, grew well up to 500mg/l and showed tolerance upto 900 mg/l of monocrotophos in liquid medium (Bhalerao and Puranik, 2009)(Table 1).

Yadav *et al.* (2015) observed that chlorpyrifos is degraded very efficiently by *Aspergillus sp.* in continuous flow bioreactor as compared to degradation by bacteria. The operating range for continuous bioreactor is found to be in the range of 180 to 250 mg/l. d. This shows that fungus is a better agent for biodegradation of chlorpyrifos as compared to other microbes. Taking different OP insecticides (Pirimiphos-methyl, Pyrazophos, Dimethoate, Malathion, Lincer, Profenfos), Hasan (1999) reported that two isolated phosphatase-producing fungi *A. flavus* and *A. sydowii* caused a general degradation of the insecticides up to a concentration of 1000 mg/l. Soluble phosphorus increased distinctly under the action of these species by enhanced phosphatase activity. The mineralization of insecticide residues was higher in soil amended with wheat straw than in unamended soil.

A. fumigatus utilized pirimiphos-methyl and lincer, as sole phosphorus sources. More than 60 % of *A. flavus* and *A. sydowii* isolates utilized malathion and lincer (1.65 g/l) as phosphorus sources. In enrichment culture *A. sydowii* followed *A. niger* and *A. flavus* were best degrading species by producing more than 50% of biomass. *A. fumigatus* and

A. terreus also utilized the pesticides but produced less than 50 % biomass (Hasan, 1999). Liu *et al.* (2001) observed that *A. niger* degraded dimethoate by showing enhanced expression of OP acid anhydrase (Phosphotriesterase). The OP degrading activity of the fungus was significantly enhanced by Cu²⁺ as observed in bacteria (Mulbry and Karns, 1989; Liu *et al.*, 2001). The tolerant strain of *A. niger* degraded formothion and malathion with almost equal efficiency whereas it was unable to degrade parathion and dichlorovos indicating the chemical specificity of the enzyme (Liu *et al.*, 2001).

Fungi are also known to degrade OP insecticides by enhanced phosphatase activity (Hasan, 1999). *A. sydowii* phosphatase was highly active against pyrazophos followed by lincer and malathion. All added pesticides except profenfos could be degraded by the fungus during three weeks of exposure (Hasan, 1999). *A. flavus* and *A. sydowii* were the first fungi isolated from wheat straw capable of degrading organophosphate pesticides and utilizing these compounds as sole phosphorus and carbon sources by releasing phosphorus from these pesticides through the action of their phosphatases. These strains could be beneficial as fungal inoculums for efficient hydrolysis of pesticides (Hasan, 1999).

By the application of 150 mg/l concentration of monocrotophos at pH 8 and temperature 30°C Jain *et al.* (2014) reported that *A. falvus* caused significant (up to 1.16 fold) increase in degradation. The fungus degraded 91.59 ± 4.31% of pesticide in a concentration of 150 mg/l within 15 days. The released inorganic phosphate content remained almost same showing the metabolic utilization of the nutrient by the fungus (Jain *et al.*, 2014). *A. niger* and *A. flavus* degraded monocrotophos in phosphorus-free liquid medium with the degrading ability of *A. niger* being more than *A. flavus*. The implementation of degrading enzyme (extracellular hydrolase) from the fungus was more effective than application of whole cell (Jain and Garg, 2013).

Silambarasan and Abraham (2013a,b) observed that *A. terreus* showed 100% degradation of 300 mg/kg of chlorpyrifos and its major product within 24 hours and 48 hours respectively, with media supplemented with nutrient and media with no addition of nutrient but only supplemented with insecticide. Chlorpyrifos could also be efficiently degraded by *A. niger* both in nutrient enriched and nutrient deficient media. The degraded increased exponentially with prolongation of incubation indicating the enhanced synthesis of the degrading enzyme by the fungus and metabolic utilization of the insecticide (Mukherjee and Gopal, 1996).

Aspergillus niger utilised glyphosate mainly through the cleavage of carbon-phosphorus (C-P) bond, resulting in

the release of sarcosine and a phosphate group. The phosphate group was utilized as a source of phosphorus for fungi growth. The released sarcosine was possibly further degraded to other products. A small fraction of glyphosate was degraded through the cleavage of carbon-nitrogen(C-N) bond, with the release of aminomethyl phosphonic acid and also the growth of the fungus was enhanced in the presence of pesticide, which shows the degrading power of the fungus (Adelowo *et al.*, 2014).

Chlorfenvinphos, however on the other hand, was degraded to very low level by the use of the fungal species. Combination of both the species was much effective rather than using each fungus at a time indicating cooperativity between the species (Oliveira *et al.*, 2015). *A. niger* and *A. smithii* metabolically degraded dichlorvos efficiently in nutrient enriched medium. There was increased growth performance of the fungi in the culture with the insecticide on prolonged exposure indicating the utilization of the insecticide as a substrate by the fungus (Mohapatra, 2006). Effective degradation of diazinon has been achieved with *A. oryzae* and *A. niger* (Mostafa *et al.*, 1972).

2.2 *Organochlorines*

Among the various OC insecticides, extensive work has been done on the fungal degradation of endosulfan. Strains *A. sydoni* have been found to degrade endosulfan very efficiently and use it as a source of carbon in broth medium as well as in soil microcosm (Goswami *et al.*, 2009). It has been reported that the fungus degraded both α endosulfan and β endosulfan with almost equal efficiency (95% and 97%, respectively) through oxidative and hydrolytic pathways (Goswami *et al.*, 2009). *A. niger* degraded technical grade endosulfan to almost complete removal within 12 days at 400mg/ml concentration under laboratory condition (Bhalerao and Puranik, 2007). However, the fungal degradation of the insecticide caused formation of various less toxic products like endosulfan diol, endosulfan sulfate, and an unidentified metabolite. Nevertheless metabolic utilization of the insecticide and its degradation products was performed by the fungus as evidenced by the increased CO₂ evolution (Bhalerao and Puranik, 2007). Hussaini *et al.* (2013) observed that *A. niger* degraded endosulfan (59%) more efficiently than lindane (29%). DDT was, however, not degraded by the fungus. There is no report on the efficient fungal degradation of DDT showing its recalcitrant behaviour in the environment. However, microbial consortia can be tried for removal of the residual DDT from the environment. Indigenous *A. niger* (ARIFCC 1053) isolated by Bhalerao (2013) could tolerate and utilize higher concentration (1,000 mg/l) of endosulfan. In vitro degradation was marked with increase in the amount of

released chlorides, dehalogenase activity, and released proteins. The organism was able to degrade half of the initial endosulfan within 96 h of inoculation and complete degradation was achieved after 168 h of incubation. The study also identified sulfurous acid, glyoxal, and protonated formic acid, which, in the environment, are generally converted to CO₂, SO₂, and H₂O (Bhalerao, 2013).

Mukherjee and Mittal (2005) recorded degradation of endosulfan by *A. terreus*. The degradation was not very remarkable in the first three days but it increased quite drastically with prolongation. The strain *A. tamarii* JAS9 isolated from endosulfan spiked soil was able to tolerate higher doses of endosulfan up to 1300 mg/l and grew well up to 1000mg/l (Silambarasan and Abraham, 2013b). Another important feature was that these particular strains were capable of degrading endosulfan sulphate which is more persistent and hence need to be degraded (Silambarasan and Abraham, 2013a).

Microbial degradation of DDT has been recorded from 1960 but fungal degradation is limited. Many strains of *Trichoderma viride* metabolize DDT by producing DDE, DDA and DDNS (Singh and Dwivedi, 2004). Mehrotra *et al.* (2004) have reported that *A. flavus* and *A. parasiticus* converted DDT to DDE in nutrient enriched medium. The species were, however, not efficient degrader of the insecticide when compared to that performed by *T. viride*.

A. flavus and *Penicillium notatum* metabolically degraded aldrin and dieldrin on prolonged incubation and on increase of the inoculum density (Mehrotra *et al.*, 2004). Syntrophic activity of *Aspergillus* and bacteria had shown more efficient degradation of these chemicals than by individual strains. Similarly chlordane and heptachlor were found degraded to their epoxides by syntrophy of *A. niger* and *Pseudomonas urticae* (Singh and Dwivedi, 2004). *Aspergillus* species could also effectively degrade α -HCH in nutrient enriched media but metabolic use of the chemical has not been reported (Rani and Dhaniala, 2014; Javaid *et al.*, 2016).

2.3 *Pyrethroids*

No significant work has been done on the fungal degradation of pyrethroids though algal and cyanobacterial degradation has been reported (Samantarai, 2006; Chandrakala, 2016). Some literature are, however available, to show the efficiency of *Aspergillus* to degrade pyrethroids. *A. niger* could degrade 54.83 % of β -cypermethrin (50 mg/l) in 7 days and could completely degrade 100 mg/l of 3-phenoxybenzoic acid within 22 h, which is considerably higher than the reported degradation rates of some bacteria, such as *Ochrobactrum lupini* DG-S-01 (Chen *et al.*, 2011a)

and *Stenotrophomonas sp.* ZS-S-01 (Chen *et al.*, 2011b). Enzymatic activities of the fungal strain showed that it can effectively degrade α -cypermethrin and its metabolites except permethric acid, which makes it an important biodegradable organism (Deng *et al.*, 2015)

3. Degradation of herbicides

3.1 Chloroacetanilides

The microbial degradation of metolachlor was solely due to the mixed fungal culture of *Aspergillus flavus* and *Aspergillus terricola* in soil which was estimated by difference in degradation in uninoculated and inoculated sterile soils and the net effect was found to be 49.21% in a concentration of 20 μ g/g (Sanyal and Kulshrestha, 2003). Similarly, the overall degradation due to the combined effect of biotic and abiotic processes was up to 84.24% after 25 days of incubation in soil treated at the 50 mg/g level, out of which, as high as 50.80% degradation was due to the mixed fungal community and only 33.45% was due to other abiotic processes. The mixed culture of fungus was almost able to degrade 100% of pesticide but less efficiency was observed when applied separately (Sanyal and Kulshrestha, 2003).

Crude extract of *Aspergillus flavus* was very much effective at higher concentration of metolachlor causing about 48.2% degradation of the parent chemical within 6 h. The rate of degradation was found proportional to the volume of the extract added and the duration of incubation (Sanyal and Kulshrestha 2004). The evidence from the metabolites formed during the degradation of metolachlor showed that the parent chemical was hydrolyzed by dechlorination, hydroxylation, and dealkylation and that aniline was in minor fractions of the metabolites formed (Sanyal and Kulshrestha 2004).

3.2 Sulfonylureas

Aspergillus niger showed total biodegradation of two sulfonylureas- chlorsulfuron and metsulfuron methyl in nutrient rich medium and the degradation pathways were the sulfonylurea bridge cleavage and the hydroxylation of benzene ring (Boschin *et al.*, 2003).

Atrazine, diuron and isoproturon could not be degraded only by fungus like *A. fumigates* and *A. terreus* but a fungi-bacteria consortium was effective to remove these herbicides from the medium (Oliveira *et al.*, 2015). The above study indicated that the initial degradation was performed by bacteria and the subsequent metabolism by the fungi. *A. niger*, isolated from soil samples showed survival in liquid media even in high concentration of about 2mg/ml of

chlorimuron-ethyl. The degradation of the chemical by the fungus was by the extracellular enzymes, which converted it into simpler forms that enabled the microorganism to utilise it for growth and maintenance. Fungal consortium with *Aspergillus* as the major partner was more effective in removing chlorimuron-ethyl from soil and water (Sharma *et al.*, 2012).

3.3 OP herbicides

Higher tolerance levels of the local fungal strains (*A. niger* FGP1, *A. terreus* PDP1, *A. terreus* BGCZ3, *A. tamarii* PDCZ1 and *A. flavus* WDCZ2) against glyphosate was reported by Eman *et al.* (2013). The mycoremediation by these fungal strains from liquid media after 16 days showed that glyphosate was degraded rapidly in liquid media by *A. flavus* WDCZ2 (99.6%) followed by *A. tamarii* PDCZ1 (96.7%) and *A. flavus* WDCZ2 (90.6%) (Eman *et al.*, 2013). The maximum degradation of triclosan was achieved by *A. versicolor* which was up to 71.91% at 7.5 mg/l triclosan concentration (Tastan and Dönmez, 2015).

4. Conclusion

The literature showed that there are many different species of *Aspergillus*, which can degrade toxic agrochemicals very efficiently both in vitro and in vivo conditions. Such species should be identified and isolated to be utilized as biodegrading agents. The tolerant strains can be upgraded in the laboratory condition and can be exclusively used in agricultural field for degradation of these chemicals. Alternatively, a consortium of these organisms can also be utilized to achieve more effective degradation in less time and quantity.

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Table 1
Degradation of various pesticides by different strains of *Aspergillus*.

Pesticides	<i>Aspergillus</i> spp.	Important Intermediate Product	Reference
Methyl parathion	<i>A. sydowii</i> CBMAI 935	p-nitrophenol	Alvarenga <i>et al.</i> , 2014
Monocrotophos	<i>A. oryzae</i> , <i>A. niger</i> and <i>A. flavus</i>	Methylamine and Dimethyl phosphate	Bhalerao and Puranik, 2009; Jain and Garg, 2013; Jain <i>et al.</i> , 2014
Profenofos	<i>A. sydowii</i> CBMAI 935	Chlorophenol-glycose conjugate and MHPM	da Silva <i>et al.</i> , 2013
Chlorpyrifos	<i>Aspergillus</i> sp., <i>A. terreus</i> JAS1, <i>A. niger</i>	3,5,6-Trichloro-2-pyridinol and Diethyl thiophosphate	Mukherjee and Gopal, 1996; Silambarasan and Abraham, 2013a; Yadav <i>et al.</i> , 2015
pirimiphos-methyl	<i>A. fumigates</i> , <i>A. flavus</i> , <i>A. niger</i> and <i>A. sydowii</i>		Hasan, 1999
Pyrazophos	<i>A. flavus</i> , <i>A. niger</i> and <i>A. sydowii</i>	Phenyl hydrazine and diethyl thiophosphoric acid	Hasan, 1999
Chlorfenvinphos	<i>A. fumigates</i> and <i>A. terreus</i>	2,2,4-trichloroacetophenone	Oliveira <i>et al.</i> , 2015
Dimethoate	<i>A. flavus</i> , <i>A. niger</i> and <i>A. sydowii</i>	Dimethyl phosphate	Hasan, 1999
Malathion, lincer, profenfos	<i>A. glaucus</i> , <i>A. flavus</i> , <i>A. niger</i> and <i>A. sydowii</i>	Desmethyl malathion and dimethyl thiosulphate	Anderegg and Madisen, 1983; Hasan, 1999
Glyphosate	<i>A. flavus</i> , <i>A. tamarii</i> , <i>A. terreus</i> and <i>A. niger</i>	Aminomethylphosphonic Acid	Eman <i>et al.</i> , 2013; Adelowo <i>et al.</i> , 2014
β-cypermethrin	<i>A. niger</i>	3-phenoxybenzoic acid and chrysanthemic acid	Deng <i>et al.</i> , 2015
Metolachlor	<i>A. flavus</i> and <i>A. terricola</i>	2-hydroxy acetamide	Sanyal and Kulshrestha, 2003; Sanyal and Kulshrestha 2004
Chlorsulfuron, metsulfuron methyl	<i>A. niger</i>	triazine derivatives	Boschin <i>et al.</i> , 2003
α and β endosulfan, endosulfan diol, endosulfan sulphate and lindane	<i>A. sydowi</i> , <i>A. niger</i> , <i>A. niger</i> (ARIFCC 1053), <i>A. terreus</i> , <i>A. tamarii</i> JAS9	Endosulfan sulphate and endosulfan diol	Bhalerao and Puranik, Mukherjee and Mittal, 2005; 2007; Goswami <i>et al.</i> , 2009; Hussaini <i>et al.</i> , 2013; Bhalerao, 2013; Silambarasan and Abraham, 2013b
Atrazine, diuron, and isoproturon	<i>A. fumigates</i> and <i>A. terreus</i>	triazine derivatives	Oliveira <i>et al.</i> , 2015
Chlorimuron-ethyl	<i>A. niger</i>	ethyl-2-aminosulfonylbenzoate and 4-methoxy-6-chloro-2-amino-pyrimidine	Sharma <i>et al.</i> , 2012
Triclosan	<i>A. versicolor</i>	2,4-dichlorophenol	Tastan and Dönmez, 2015
DDT	<i>A. flavus</i> and <i>A. parasiticus</i>	DDE	Mehrotra <i>et al.</i> , 2004

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