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Original Article

Biodegradation of Lambda-Cyhalothrin Karate insecticide by *Aspergillus niger:* A novel fungus for Bioremediation

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Abstract

Pesticide uses are widely documented for controlling various pests that attenuate agricultural productivity. However, extensive use of pesticides is considered as one of the most important agents in polluting the environment. Karate is one of the synthetic insecticides that possesses Lambda cyhalothrin as an active component, which causes several environmental concerns. It has the characteristic of being persistent in the environment and its toxicity. Microbial degradation could be one of the efficient and eco-friendly methods for removing environmental contaminants. Aspergillus niger are the fungi which belong to the family Aspergillaceae is one of the well-studied fungus know to control other genera fungal infection in plants caused by various pathogenic necrotrophs. Here in this paper, we have documented how Aspergillus niger strain present is capable of degrading insecticide Karate. Growth of Aspergillus was observed at different timepoints, and an increase in biomass production was analysed by UV spectroscopy. Our findings have suggested through GC-MS that the intermediates are present, which occurs in the degradation pathway of Lambda cyhalothrin, confirming the capability of Aspergillus to degrade Karate. The current study highlights the potential usage of Aspergillus, which could practically be possible for suing in open field. This method of detoxification could be proven to be one of the efficient methods in the bioremediation process.

Keywords: *Degradation, Karate, Aspergillus niger, gas chromatography mass spectrometry*

Introduction

Pesticides are chemical substances used to prevent, control, destroy pest. They play a crucial role in modern agriculture and public health by controlling pests that threaten crops, livestock, and human well-being. In recent times, the use of various pesticides has become a fundamental aspect of agricultural practices, aiming to enhance crop production and fulfill the rapidly increasing food demand (Pujar et al. 2022). Pesticides are classified based on their target organisms into different categories, such as Insecticides, Herbicides, Fungicides, Rodenticides, Nematicides, Bactericides. Insecticides, a class of pesticides derived from both natural and synthetic sources, are utilized to eliminate insects or interfere with their developmental processes. The major classes of insecticides include organophosphates, carbamates, organochlorines, polychlorinated biphenyls (PCBs), neonicotinoids, and pyrethroids. Karate pesticide is a broad-spectrum insecticide containing Lambda-Cyhalothrin as active ingredient, a synthetic pyrethroid recognized for its strong neurotoxic activity against insects. Lambda-Cyhalothrin functions by disrupting the sodium channels in the insect nervous system, leading to paralysis and eventual death (Narahashi 2002). This pesticide is highly effective in controlling biting, chewing and sucking insects in cotton and other crops. Due to its fast action and residual effect, Karate is widely preferred by farmers. Karate pesticide has harmful effects on human health, causing symptoms such as itching, tingling, burning or numbness of exposed skin, a condition known as paresthesia. Excessive use of this pesticide significantly contributes to environmental pollution, while its continuous use leads to persistence and bioaccumulation in ecosystems, emphasizing the need for effective biodegradation strategies.

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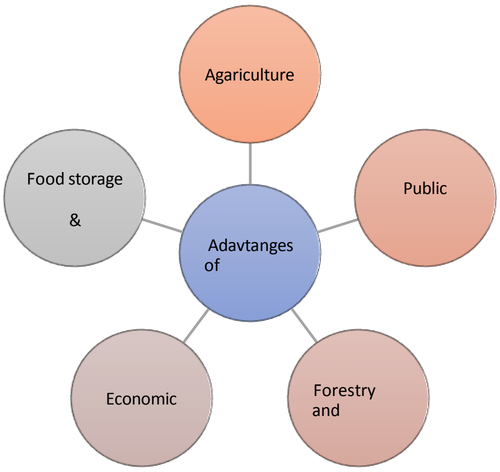

Figure. No. 1 Advantages of Pesticides

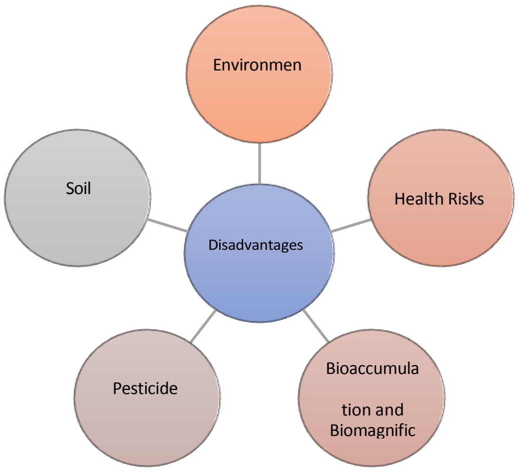

Figure. No. 2 Disadvantages of Pesticides

Figures 1 and 2 illustrate the advantages and disadvantages of pesticide usage. Pesticides play a crucial role in agriculture by protecting crops from pests, diseases, and weeds, thereby enhancing productivity and ensuring higher yields. Increased agricultural output contributes to improved financial returns for farmers. Additionally, pesticides are widely used in residential and commercial settings for controlling cockroaches, bedbugs, and rodents. However, excessive pesticide application poses significant challenges, including environmental pollution, acute and chronic health hazards, and the development of pesticide resistance. Moreover, persistent pesticide residues accumulate in food chains, leading to toxic effects on higher organisms, including humans (Sharma et al. 2019). Therefore, adopting responsible and sustainable pesticide usage, along with advancements in pesticide degradation methods, is crucial for long-term ecological and human health benefits. Pesticides are low degradable and therefore known to be toxic substances. Pesticide degradation can be achieved through various methods, including microbial degradation (bioremediation), chemical degradation, physical degradation and photodegradation (Rani et al. 2021). Among these approaches, bioremediation is considered as a key strategy, providing a sustainable and eco-friendly approach that utilizes microorganisms to degrade toxic compounds into less harmful substances (Peter et al. 2023, Sehrawat et al. 2021). Bhatt et al. 2021 studied that the microbial consortia are an essential for a degradation of the recalcitrant pesticides in the natural environments (Bhatt et al. 2021, Vaksmaa et al.2023). *Aspergillus niger*, a ubiquitous filamentous fungus, has gained attention for its remarkable enzymatic capabilities in breaking down various organic pollutants, including pesticides (Brenda et al. 2020). The researcher assessed the impact of *Aspergillus niger* as a remediation agent on lambda-cyhalothrin residues and associated heavy metals in *Lactuca sativa* (lettuce) leaves. The application of *A. niger* led to a significant reduction in lambda-cyhalothrin residue levels, highlighting its potential for bioremediation (Sanyaolu and Adetola 2018). Birolli et al. studied the ability of marine-derived fungi, including *Aspergillus niger*, to degrade lambda-cyhalothrin, resulting in the formation of less toxic metabolites (Birolli et al. 2018). Further research is required to elucidate the degradation mechanisms and efficiency of *Aspergillus niger* in breaking down lambda-cyhalothrin. So, the present study aims to investigate the role of *A. niger* in the degradation of soil treated Karate pesticide, evaluating its efficacy and potential application in sustainable bioremediation approaches.

Materials and method:

Pesticides, reagents, solvents, and culture media:

Technical grade 5EC Karate pesticide was used which is manufactured in Syngenta (India). The Nutrient agar, Nutrient broth, Sabouraud dextrose agar was purchased from Himedia company. The *Aspergillus niger* was inoculated in Sabouraud dextrose broth (Purchased from HiMedia Company) and incubated overnight at 28°C.Acetone, n-hexane were purchased from S D Fine Chem Limited.

Degradation of Karate

Nutrient broth was prepared, and a Karate pesticide (treated soil sample) was added to a conical flask. A forcep was sterilized using a burner and allowed to cool for a few seconds and then used to transfer a pure culture of *Aspergillus niger* into the broth. All procedures were carried out under aseptic conditions. The conical flasks were then incubated at 28°C for three days, five days and seven days on a rotary shaker at 120 rpm. A flask containing Karate (lambda- cyhalotharin) without fungus was treated as control (Ayyar and Kamble 2018).

Quantitative analysis

Quantitative analysis of pesticide degradation was carried out using different techniques, including UV-Visible spectroscopy and Gas Chromatography-Mass Spectrometry (GC-MS).

a UV visible spectroscopy:

A UV-Visible spectrophotometer was used to quantify the compounds and monitor changes in the Karate pesticide due to degradation. For this analysis, 400 µL of degraded Karate

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pesticide was mixed with 100 µL (10) of a suitable solvent, and absorbance was measured at 575 nm on the 3rd, 5th, and 7th days, respectively.

b Gas Chromatography-Mass Spectrometry (GC-MS):
GC-MS was carried out for identification of degradation metabolites. Extraction was carried out using a solution of n-hexane (distilled) and acetone (distilled) in a 2:1 ratio. The eluted material was collected in a 250 mL conical flask and later evaporated in a hot air oven. The dried residue was dissolved in 20 to 50 µL of n-hexane in small glass vials for GC-MS analysis. (Bhattacharyya et al.2023, Aktar et al.2009, Buvaneswari et al. 2018).

Results and Discussion:

Isolation of *Aspergillus niger* on Sabouraud's dextrose agar Plate:

Aspergillus niger spp was maintained on sabouraud dextrose agar plates. A loopful of pure strain of *Aspergillus niger* was taken and inoculated on Sabouraud dextrose agar plates, and incubated for 2-3 days at 28°C. *Aspergillus niger* are filamentous septate hyphae, were hypae are divided into segments by cross wall known as (septa) conidiophores (Fig.3). The vesicles sterigma are present in *Aspergillus niger* which appears in dark coloured moulds. The variation of colour can be observed from range black to brownish tint.

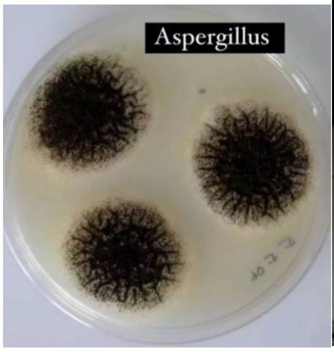


Figure.3. Strain of *Aspergillus niger* exhibiting brownish filamentous structure Figure.4. Karate Pesticide lambda- cyhalothrin

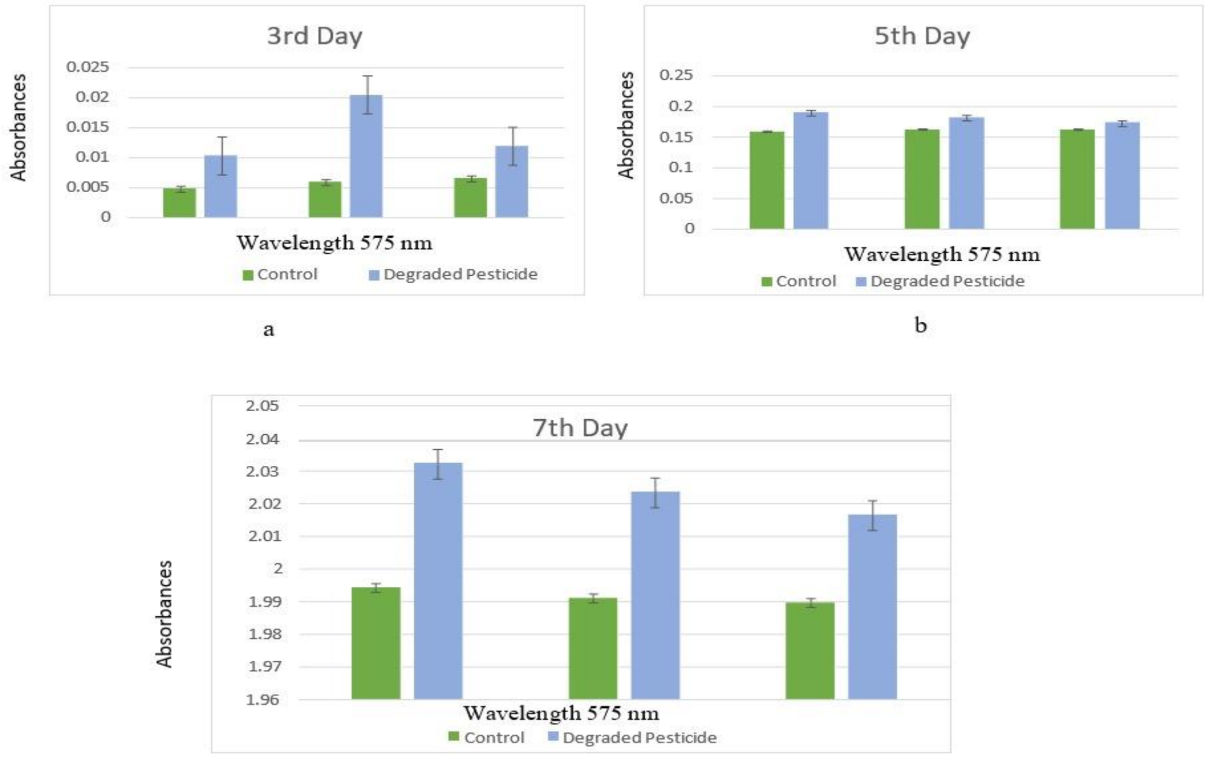
Karate insecticide containing the main ingredient lambda cyhalothrin was purchased from local market from Solapur city. Karate insecticides are mainly used in Okra, Brinjal and most vegetable crops (Rahman & Alam,2010). Soil samples were collected from the field where karate was sprayed. Nutrient broth was prepared. Nutrient broth with insecticide was treated as control. Pure culture of *Aspergillus Niger* spp fungi was growth and place into the medium of culture broth (Littera, Farkas & Urik, 2023). All methods were carried out in sterile conditions. A flask containing Karate (lambda- cyhalothrin) without fungus was treated as control. All flasks were maintained at 28° C at 120 rpm. The degradation was observed by the growth of the fungus in the nutrient media and the absorbance was recorded (Wang et al, 2025). Pesticide degradation was recorded at different time points such as 3rd, 5th and on 7th day. It was observed that on the 3rd day the lambda cyhalothrin was not degraded as there was no significant growth observed in the flask containing *Aspergillus niger* . However, on the day 5th and 7th there was a significant growth of fungus where the media turned to appear turbid fig.5. The turbidity clearly states that the degradation is initiated due to the growth of the hypae and biomass of the fungi (Miyazawa et al 2022). All experiments were carried out in three biological replicates. Degradation of the insecticide is represented in statistical graphs where increase in degradation clearly states on 5th and 7th day of the incubation time. The absorbance was recorded at the wavelength of 575 nm (graph.1).

a *3rd Day* b *5th Day* c *7th Day*

A.niger+Pesticide Control | A.niger+Pesticide Control | Control A.niger+Pesticide

Figure 5. a The figure displays the nutrient broth in the presence of *aspergillus niger* fungus and pesticide and the other flask shows the nutrient media and the pesticide which is treated as a control on 3rd day of incubation **Figure 5.b** shows the presence of aspergillus niger fungus and pesticide and the other flask shows the nutrient media and **Figure**

.5.c shows the media containing pesticide and the other flask showing pesticide along with the fungus.



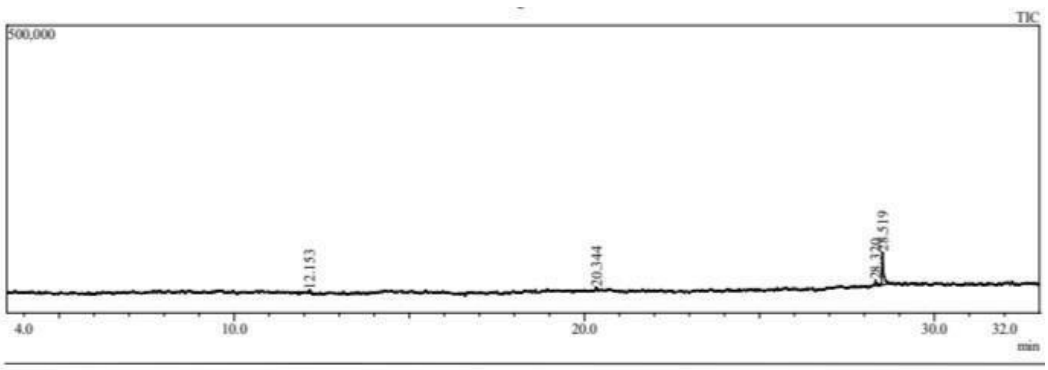
c

Graph. 1. a, b, c, Graphical representation of Karate degradation by *Aspergillus niger* on 3rd 5th and 7th day of incubation.

Gas chromatography mass spectrometry GCMS

Gas chromatography mass spectrometry GCMS is one of the analytical methods which could be used to analyse the samples of pesticides degradation Gonzalez et al, 2013. It is one of the efficient and effective methods where the intermediates of the breakdown of pesticides could be detected. The samples of degraded pesticides with the help of the fungus were collected on the day 7th. The samples were treated with the n- Hexane and samples were sent to Scientific instruments, laboratory at Solapur university. The graph 2, represents the chromatograms of the n-hexane treated sample of pesticide degradation by *A.niger*.

The results are shown graph 2 and table 1.



Graph 2. : Chromotogram of n-hexane treatment of degraded Karate pesticide (lambda -cyhalothrin)

The chromatograms of the fractions of n-hexane treated degraded Karate (lambda cyhalothrin) pesticide were obtained from the gas chromagraphy- mass spectrometry analysis with the peaks are shown in graph 4.

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Table 1: chemical compounds found in degraded Karate pesticide (lamda-cyhalothrin):

Peak Report TIC

Peak#	R.Time	Area	Area%	Name	Similarity
1	12.153	19321	7.18		34
2	20.344	18780	6.97	Benzaldehyde, 3-phenoxy-	87
3	28.320	39030	14.50	.lambda.-Cyhalothrin	89
4	28.519	192127	71.35	lic acid, 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethyl-, cyano(3-pheno	94
		269258	100.00		

Various compounds were obtained in the n-hexane treated samples of karate pesticide degradation process are represented in table no 1..The retentional time 12:153: has shown an unknown compound with a similarity of 34. At RT 20.344 Benzaldehyde 3- phenoxy was observed at a similarity of 87. This might be the intermediate in the breakdown process of karate that contains phenoxy benzene structure. An aldehyde group could be released due to the growth of aspergillus niger (Kumari & Reddy 2020) The presence of benzaldehyde, 3-phenoxy, suggests the breakdown of the pesticide into smaller aromatic compounds (Pankaj etal, 2022). Benzaldehyde, 3-phenoxy is the intermediate in the degradation process hwere it could enhance the breakdown into simpler compounds. Cyclopropane carboxylic acid 3-[2-chloro-3-[2-chloro-3,3,3- trifluro-1-propenyl]2,2-dimethyl-cyano [3 phenoxyphenyl] methyl ester is another compound found in the GS MS at RT 28 which displays 94 similarities. It can be concluded that cyclopropane is a ring structure compound which could be detected in the degradation involvement (Salleh et al, 2023), while the chloro group can be possibly the reason as a side product and explaining that the karate could be in a transformation process (Bhat et al, 2006). The formation of the cyclopropane carboxylic acid could be a result of fungal enzymatic action where it cleaves the ring structure evolving the chlorine as a byproduct (Chen et al,2015). Presence of lamba cyhalothrin indicates that the pesticide karate is not completely degraded and require more hypal growth of fungi or the incubation time for degradation (Mishra et al, 2016). Table 2 representing different compounds with there molecular formula and structure obtained in the GC MS results.

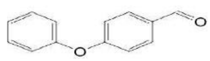
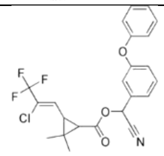
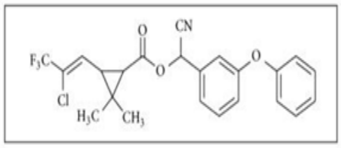
Sr. No	Name of the compound	Molecular formula of the compound	Structural formula of the compound
1	Benzaldehyde, 3phenoxy	Cl3H10O2	
2	Lambda-cyhalothrin	C23H19ClF3N O3	
3	Cyclopropane carboxylic acid,3-(2-chloro3,3,3- trufluoro-1propenyl)-2,2dimethyl-cyani(3phenoxyphenyl) methyl easter	C23H19ClFNO3	

Table 2. Represents the molecular compound name their molecular formula and the structural formula the Different compound found in n-hexane extract of degraded Karate pesticide (lambda- cyhalothrin.

Conclusion:

In this study the fungal growth in various samples at different timepoint suggest there could be a possibility of karate insecticide degradation. The statistical data obtained by UV Spectroscopy suggest an enhanced growth of biomass of fungal culture explaining the fact that *aspergillus niger* could be capable of degrading the insecticide karate containing Lamba cyhalothrin. The results obtained from the GCMS results could explain the degradation is initiated and a breakdown is observed by the presence of compound Benzaldehyde,3phenoxy. TheBenzaldehyde,3phenoxy could be serve the purpose of intermediate in the degradation pathway. Presence of Cyclopropane-carboxylic acid,3-(2-chloro-3,3,3- trifluoro-1- propenyl)2,2-dimethyl-cyano (3phenoxy phenyl) methyl ester explains the formation of chlorine group which could be as a side chain or byproduct of the degradation pathway. Identification of these products could lead us to confirm the ability of *aspergillus niger* to degrade the karate insecticide. This could lead as a significant implication in bioremediation. Microbial degradation could be a n answer for the ever-increasing pollution due to various pesticides. It could be proved as a n ecofriendly method in the bioremediation process. However deeper understanding of the complete degradation should be studied and how the implementation of *aspergillus niger* in open soil could be implied will be the future studies of interest.

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Conflicts of interest
The authors declare that there are no conflicts of interest regarding the publication of this paper.

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