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ISOLATION AND SCREENING OF POTENTIAL FUNGI FROM CONTAF SPRAYED VINEYARDS

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Abstract: The present study, was carried out to isolate and screen the potential fungi from the contaf sprayed vineyards. Isolation of the soil fungi was done one day before the contaf spray and thereafter on Day 1, Day 5, Day 10, and Day 15 of spray. *Aspergillus niger*, *Aspergillus flavus*, *Penicillium sps.* are observed as the prominent species. There was a steep decline in the number of species after spray. Preliminary screening of the fungi was done *in vitro* at 5 ppm fungicide concentrations. The selected fungi was re-screened at higher fungicide concentrations such as 7 ppm, 10 ppm and 15 ppm. Radial growth of the fungi at 15 ppm of fungicide concentration was measured. The radial growth of the mycelium, thus shows the ability of the fungus to utilize the fungicide as nutrient source.

KeyWords- *Systemic fungicide, Aspergillus niger, Aspergillus flavus, Biodegradation, Nutrient source.*

INTRODUCTION

The increase in population every year is posing a challenge to the demand for food, fuel, water, energy and better living conditions. Crop production is generally improved by the use of chemical fertilizers and pesticides. A large amount of pesticides such as herbicides, insecticides, fungicides, nematicides etc. are used in agricultural activities. The use of pesticides, improved the quality and yield of crops but resulted in environmental pollution. A survey on pesticide application has shown that only 1% of the pesticide will reach the target pest [1]. The remaining will reach the soil and persist in the environment creating ground and surface water pollution, contaminating the soil and other environmental deteriorations. The process of bioremediation currently, is the most promising approach to reduce environmental pollution as soil microorganisms are self-sustaining and inexpensive. Bioremediation exploit the catabolic capacity of microorganisms in degradation. The microbes offer an ecofriendly and economical options for mineralization of contaminants or their transformation into less harmful non-hazardous compounds. The microorganisms degrade pesticides in all types of environments; still environmental pollution exists as persistent problem [2]. The microbes are capable of utilizing pesticides as energy sources [3].

Fungicide application, inhibit the growth of fungi or fungal spores. Fungicides are applied as granules, gas, and dust and in liquid form. The effectiveness of formulated product is due to active ingredient along with inert ingredient. Systemic fungicides applied, control the fungal pathogen remote from the point of application. Systemic fungicides are effective against fungal diseases such as mildews and leaf spots. Contaf, a triazole systemic fungicide is generally applied as foliar sprays on chillies, tomato and vineyards. They reach the soil by drifting during application, rain washing of the fungicide from the foliage and by fallen plant material to the ground. The present study was done, to isolate the potential fungi from the contaf sprayed vineyards, capable of degrading the fungicide *in vitro*.

MATERIALS AND METHODS

Soil sample collection

Soil samples were collected in sterile polypropylene covers from contaf sprayed vineyards, of shamshabad area of Telangana state. Soil samples are collected before and after contaf spray, on day 1 ,7, 10 and 15th day. The fungi are isolated employing dilution plate method using Potato Dextrose Agar medium.

Dilution plate technique

One gram of soil sample was weighed and taken into 10 ml sterile water. It was shaken thoroughly and kept undisturbed for 5 minutes. One ml of the supernatant was taken and added to 9 ml of sterile water and successive dilutions were made up to 10^4 accordingly. One ml each of the final dilution supernatant was aseptically transferred on to sterile petri plate before adding the cool and melted medium. The plates were gently swirled to distribute the soil solution and then the medium was added. The plates were incubated for five to seven days. Fungal colonies enumerated after the incubation period.

Isolation of fungi from contaf sprayed vine yards

Isolation of fungi was done by Dilution plate technique. Slides were prepared for identification, before transferring the fungi to PDA slants for preservation and future use. A diversity of mycoflora was found to associate with the contaf sprayed vine yards. Most of the fungi declined after the fungicide spray and only few like *Aspergillus niger*, *Aspergillus flavus, Penicillium sps.* sustained to grow even after 15 days. Cultures were maintained on potato dextrose agar slants and stored in refrigerator for future use. Sub culturing was done regularly at an interval of every 3 months.

Screening of potential contaf degrading fungi

Isolated fungi were inoculated on to Czapek-Dox agar media amended with 5 ppm of contaf. Preliminary screening was done at a concentration of 5 ppm. Fungi were inoculated aseptically on to the solidified agar medium amended with fungicide. Petri plates thus, inoculated were incubated at room temperature (26 ± 2C). Fungal colony diameter was measured in millimeters. The selected fungi were re-screened at higher fungicide concentrations for identifying the potential fungi that can degrade the fungicide.

RESULTS AND DISCUSSION

The fungicides sprayed generally not only affect the target organisms , but also exert negative pressure on the microorganisms present in the soil, on their reach to the soil. This would alter the equilibrium of soil microbes in the soil. Repeated application of fungicide may create selective pressure on the fungi to adapt to changed environment and the fungicide applied.

Fig.1. Contaf sprayed vineyard of Shamshabad area

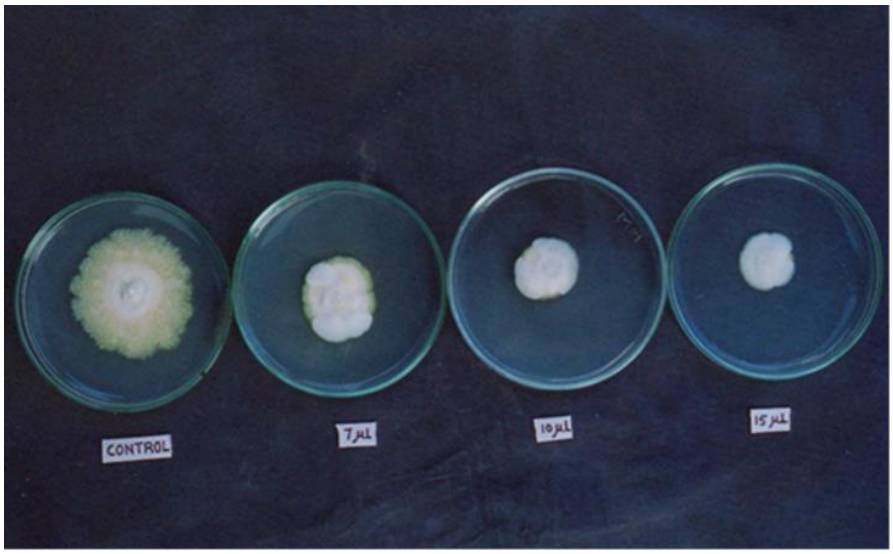

Fig.2.Growth of *Penicillium sps.* on Czapek-Dox agar medium amended with contaf at different concentrations.

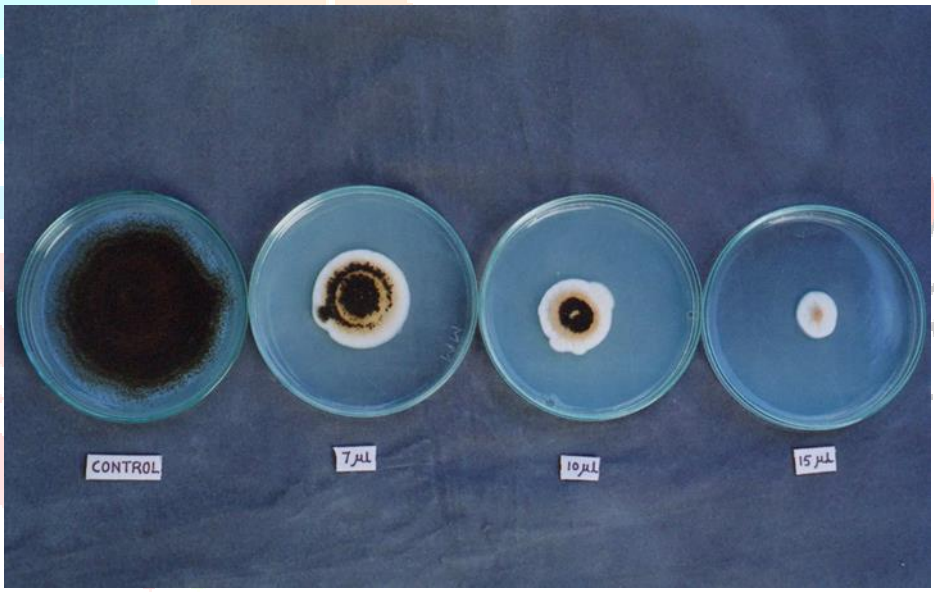

Fig.3. *Aspergillus niger* grown on Czapek-Dox agar medium amended with contaf at different concentrations.

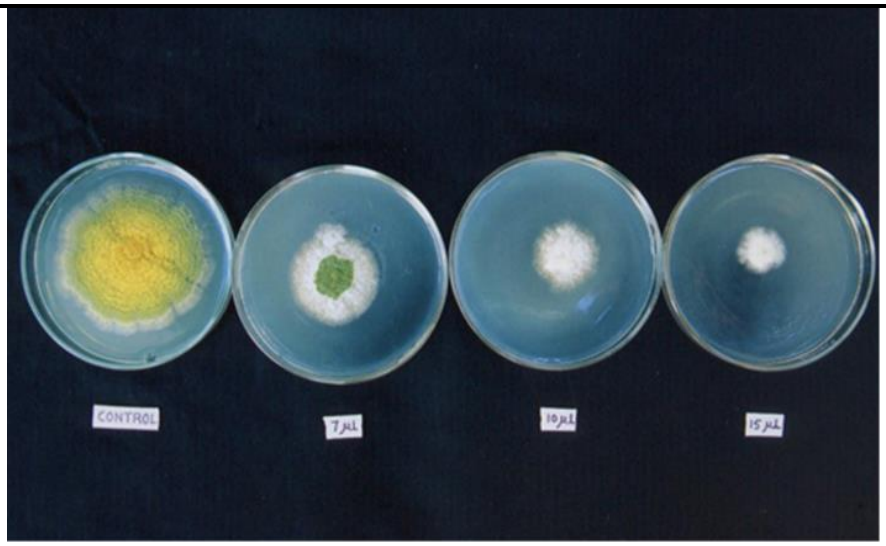

Fig.4. Growth of *Aspergillus flavus* on Czapek-Dox agar medium amended with contaf at different concentrations.

Mycoflora of the Contaf sprayed vineyards were estimated by serial dilution method. *Aspergillus* species were predominant in the vineyard soils. Fungicide spray affected the soil fungi considerably as it is evident from the decrease in the number of colonies from the day one of fungicide spray. Species of *Aspergillus* and *Curvularia clavata* remained in the soil up to 5 days. However, *Aspergillus niger* and *Aspergillus flavus* was consistently present even after 2 weeks of fungicide spray. Fig. 2, Fig.3, and Fig. 4. shows the growth of *Penicillium*, *Aspergillus niger* and *Aspergillus flavus* at fungicide concentration of 7, 10, 15 ppm . The fungi might be utilizing the fungicide as a nutrient source. The fungicide may serve as carbon, nitrogen or phosphorus source.

Aspergillus niger YAT degraded β-cypermethrin (β-CY) and its intermediates completely by co-metabolism and mineralization process [4]. Similarly, detoxification of Chlorpyriphos by fungal Species *Aspergillus niger* was observed in cotton soils [5]. *Aspergillus niger*, *Trichoderma viride* and *Helminthosporium sp.* degraded carbofuran and formed an intermediary compound hydroxy carbofuran [6]. Certain fungi *viz.*, *Trichoderma harzianum*, *Aspergillus niger*, *Phanerochaete chrysosporium* and *Mucor thermohyalospora* actively degraded endosulfan [7-9].

The metabolism of carbofuran in pure liquid cultures was showed by *Aspergillus niger*, *Fusarium graminearum* [10]. The biotransformation of linalool was observed, with submerged shaking cultures of *Aspergillus niger* ATCC9142 to furanoid and pyranoid linalool oxides [11]. The metolachor metabolism was studied using a mixed fungal culture of *Aspergillus flavus* and *A. terricola* isolated from a metolachlor-acclimated field soil [12]. *Aspergillus niger* showed potential ability to degrade diazinon [13]. There are reports showing bioremediation of malathion residues in water by *Aspergillus flavus* [14]. *Trichoderma virgatum*, *Aspergillus sydowi* and *Penicillium sp*. showed the ability to degrade organochlorine compounds mainly by methylation of pentachlorophenol into pentachloroanisol [15-16]. Fungal isolates, *Aspergillus niger*, *Ganoderma austral*, *Trichosporon* , *Verticillium dahaliae* showed significant ability to degrade endosulfan, lindane, chlorpyrifos malathion [17]. There are reports on the differential breakdown of mixtures of pesticides such as chlorpyrifos, linuron, metribuzin and simazine, trifluralin and dieldrin, and polyaromatic hydrocarbons by different microbial inoculants, especially basidiomycetes including *Trametes*, *Peniophora*, *Irpex*, *Picnosporus*, and *Phanerochaete* species [18]. *Aspergillus tamarii* and *Botryosphaeria laricina* isolated from the agricultural field were able to degrade the toxicant endosulfan and and its harmful metabolites like endosulfan sulfate, alpha endosulfan, and beta endosulfan by using them as a source of carbon and energy[19].

Glyphosate, a xenobiotic compound was effectively degraded by fungus-like *Penicillium spiculisporus*, *Aspergillus flavus*, and *Penicillium verruculosum* isolated from herbicide contaminated farms [20].

CONCLUSION

An extensive use of pesticides, cause deterioration of soil quality, acidification, denitrifiction, leaching and reduced biodiversity, disrupting the ecosystem. Microbes prove to be an economical and ecofriendly option to reduce the contamination. Contaf sprayed vineyards harboured a variety of fungi. However, the number of fungi decreased on fungicide treatment. Among the isolated fungi, *Aspergillus niger* and *A. flavus* showed sustained growth at 15 ppm concentrations of the fungicide. The isolated fungal species from the vineyard soils were subjected to degradation of the fungicide *in vitro*. Further research, will throw the light on the utilization of fungicide as nutrient source by fungi and the intermediates formed by biodegradation and their toxicity in the soil.

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