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GREEN SYNTHESIS OF PESTICIDE FROM PLANT EXTRACT AND ITS EFFECTIVENESS AGAINST MEALYBUG

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Abstract

Most of the pesticides are harmful to both environment and mankind. In this green synthesis of pesticide, the pesticide is synthesized from plant extract and is applied on a plant affected by mealybugs. The pesticide extract was tested against Staphylococcus aureus, Pseudomonas aeruginosa and Aspergillus niger using disc diffusion method. The result showed that the tested bacteria and fungi could moderately inhibit by prepared green pesticide.

Keywords: green synthesis, pesticide, mealybugs.

Introduction:

A pesticide is any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pests[3]. The first known pesticide was elemental sulfur dusting used about 4,500 years ago in ancient Mesopotamia. The Rigveda, which is about 4,000 years old, mentions the use of poisonous plants for pest control[1]. By the 15th century, toxic chemicals such as arsenic, mercury, and lead were being applied to crops to kill pests. In the 17th century, nicotine sulphate was extracted from tobacco leaves for use as an insecticide. Ideally a pesticide must be lethal to the targeted pests, but not to non-target species, including mankind[2]. Pesticide usage is no longer safe, as toxic chemicals are used to prevent pests. Pesticide can penetrate into the soil and ground water which can end up in drinking water and pesticide spray can drift and pollute the air[4]. In 1962, Rachel Carson published the book "Silent Spring", in which she mentioned problems that could arise from the indiscriminate use of pesticides. This book inspired widespread concern about the impact of pesticides on the human healthand the environment[5]. According to a report by the United Nations Environment Program(UNEP) and the World Health Organization (WHO), pesticides are responsible for poisoning around three million people and causing ~200,000 deaths each year, worldwide[6]. A less toxic and an ecologically friendly approach on the pesticide use is organic pest control method[7]. This method utilizes organic pesticides which are made from natural sources. As organic pesticides are mainly plant based, they are

biodegradable hence does not affect the environment. Natural pesticides can be prepared using neem, tobacco, garlic, onion and so on.

. Mealybugs are plant pests that feed by piercing-sucking mouthparts. They are classified in the Pseudococcidae. The common name "mealybug" is derived from the fine powdery "mealy" wax that covers the body. Adult female mealybugs are wingless and adult male mealybugs are winged. Because mealybugs feed on plant juices they produce honeydew. Honeydew (a sugary, sticky substance) promotes the growth of black sooty mold. These sootymolds may inhibit plant photosynthesis[13].

Materials and Method

1. Materials

Garlic cloves, Chrysanthemum flower head, Rice water, Neem and Tobacco leaves

2. Synthesis of Pesticide

The pesticide was prepared using garlic cloves, chrysanthemum flower heads, neem and tobacco leaves. The chosen substances were then dried in theshades for a period of three weeks. The dried substances were grinded using mortar and pestle.The grinded mixture is then fermented with rice water in an air tight bottle or container for few(5 to 10) days. For 1g of the pesticide mixture 10ml of rice water is added. The liquid pesticide is then sprayed into the infected plant.

Fig.1 Synthesized Pesticide

3. Antimicrobial Study

Antimicrobial activity can be defined as a collective term for all active principles (agents) that inhibit the growth of bacteria, prevent the formation of microbial colonies and may destroy microorganisms[8]. Antimicrobial susceptibility tests are used to determine which specific antibiotics a particular bacteria or fungus is sensitive to[9]. Antimicrobial susceptibility testing can be performed using a variety of formats, the most common being disk diffusion, agar dilution, broth macro dilution, broth micro dilution and a concentration gradient test[10].

3.1 Agar Disc Diffusion Test

The antimicrobial screening of the pesticide was carried out by determiningthe zone of inhibition using agar disc diffusion method[11]. In agar diffusion method (ADM), theplant extract to be tested diffuses from its reservoir through the agar medium seeded with the test microorganism. Generally, the reservoir is a filter paper disc, which is placed on top of anagar surface[12]. The pesticide extract was tested against Staphylococcus aureus, Pseudomonas

aeruginosa and Aspergillus niger.

3.2 Bacterial Inoculums Preparation

Inoculum of Staphylococcus aureus, Pseudomonas aeruginosa and Aspergillus niger were prepared individually in a respective broth and kept for incubation at suitable temperature.

3.3 Antibacterial Test

Antibacterial activity of a molecule is completely associated with the compounds that provincially kill bacteria or slow down their rate of growth, without being extensively toxic to nearby tissues[8].The antimicrobial agents could be classified as the agentsthat can either be bactericidal, which kill bacteria, or bacteriostatic, which slow down the growth of bacteria. Antibacterial agents are the most important in fighting infectious diseases[8]. For the test conduction, the medium was prepared by dissolving 38 g of Muller Hinton Agar Medium in 1000 ml of distilled water. The dissolved medium was autoclaved at 15 Lbs pressure at 1210C for 15 minutes (pH 7.3). The autoclaved medium wascooled, mixed well and poured into petriplates (25 ml/plate), the plates were swabbed with Pathogenic Bacteria Culture viz. analysis (Pseudomonas aeruginosa, Staphylococcus aureus). Finally, about 25 µL, 50 µL, 75 µL, 100 µL of sample (Aqueous extractof pesticide) was loaded onto the disc and were then placed on the surface of Muller- Hinton medium and the plates were kept for incubation at 37°C for 24 hours. At the end of incubation, inhibition zones were examined around the disc and measured with transparent ruler in milli metres. The size of the zone of inhibition (including disc) was measured in milli meters. The absence of zone inhibition was interpreted as the absence of activity[13]. The activities are expressed as resistant, if the zone of inhibition was less than 7 mm, intermediate (8-10 mm) and sensitive if more than 11 mm.

3.4 Antifungal activity

3.4.1 Test Organism

The test fungi used for antifungal analysis, Aspergillus niger were isolatedfrom the environment.

3.4.2 Antifungal Assay by Well Diffusion Method

Antibiotic susceptibility tests were determined by agar disc diffusion method[42]. Fungi strains Aspergillus niger were swabbed using sterile cotton swabs in SDA agarplate. 25 µL ,50 µL,75 µL,100 µL of sample (Aqueous extract of pesticide) was loaded onto the disc of SDA medium and the compound was allowed to diffuse for 5 minutes and the plateswere kept for incubation at 22°C for 48 hours. At the end of incubation, inhibition zones were examined around the disc and measured with transparent ruler in milli meter.

RESULT AND DISCUSION

1. Application of the Prepared Pesticide on Mealy bugs

The experiment is conducted on the plant Solanum lycopersicum (tomato). After the plant reaches a certain height of 20 to 30 cm, it is introduced with Phenacoccus solenopsis (mealybugs). The pests are allowed to grow in the plant for about 7 days.

Fig.1 Introduction of mealybugs

Fig.2 After a week of the bugs introduction

Fig.**2**, shows that the mealy bugs have multiplied in number and plant leaves are greatly affected by them. By this time the pesticide was sprayed on the plant on a regular basis of twice a week.

Fig.3 After a Week of Pesticide Application

Fig.4 After One Month

It is evident from Fig.3 that the number of pests were decreased. The pesticide is sprayed for few more weeks. After continuous application the mealybugs are completely gone from the plant and new growth can be seen. From Fig.4 the flourishing growth of the plant can be seen and the tomato plant has produced flower buds. Though it took several weeks the pesticide has shown great resultsagainst mealy bugs.

2. Antibacterial Activity

Table 1. The Zone of Inhibition of Pesticide Against Tested Bacteria

Bacteria Strains Name and Zone inhibition (mm)	
Pseudomonas aeruginosa	Staphylococcus aureus
NA	NA
NA	NA
9	7
7	Nil
Control 12mm	8mm
Control 12mm	8mm

Table 1 shows the activity of pesticide against isolated bacteria strains Pseudomonas sps and Staphylococcus sps. The zone of inhibition is found to be 9 mm and 7 mm for both Pseudomonas sps and Staphylococcus sps. This indicated that, the synthesized green pesticide showed a moderate activity against these species of bacteria.

Fig.5 Antibacterial Study of the Pesticide against Pseudomonas aeruginosa and Staphylococcus aureus

Fig.5 shows the activity of synthesized green pesticide against isolated bacterial strains Pseudomonas aeruginosa and Staphylococcus aureus. The results showed that the tested bacteria could moderately inhibit by synthesized green pesticide.

3. Antifungal Activity

Table 2. The Zone of Inhibition of Pesticide Against Tested Fungi

Sample Zone of Incubation (mm)
Aspergillus Niger
NA
7
8
NA
Control 12mm
Control 8mm

Table 2 shows the activity of pesticide against the fungi Aspergillus niger. The zone of inhibition is found to be 7 for 50 µl and 8 for 75 µl. This indicated that the synthesized green pesticide showed a moderate activity against Aspergillus Niger.

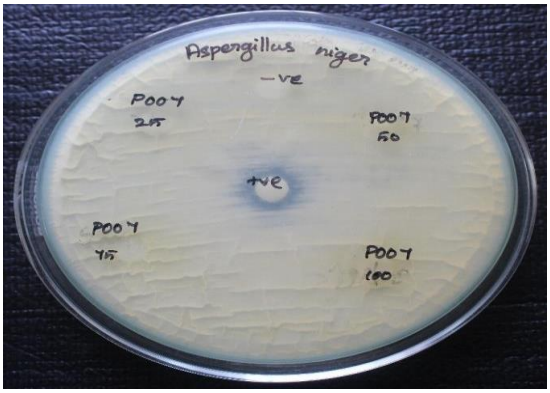

Fig.6 Antifungal Activity of the Pesticide against Aspergillus niger

Fig.6 shows the activity of synthesized green pesticide against isolated fungi Aspergillus niger. The results showed that the tested fungi could moderately inhibit by synthesized green pesticide.

Summary and Conclusion

It is a prominent fact that most of the pesticides available in the market is harmful to human beings and the surrounding. There is a need for an alternative solution, this project follows a less toxic approach by using only natural ingredients to synthesize a pesticideand using it in the pest affected plant. After the application of the pesticide against mealybugs(in the tomato plant), the plant become free of pests thus showing its effectiveness against mealybugs. The pesticide sample was then investigated to evaluate its antimicrobial activity against two bacteria (Pseudomonas aeruginosa and Staphylococcus aureus) and a fungus (Aspergillus niger) using disc diffusion method. In this study, the application of green pesticide against Pseudomonas aeruginosa, Staphylococcus aureus and Aspergillus niger**.** The result showed that the tested bacteria and fungi could moderately inhibit by prepared green pesticide. The synthesized pesticide can be said as green synthesis since it is eco-friendly, cost effective, has only natural ingredients, biodegradable, metal free and pollution free to the environment.

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