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Eco-friendly approach for killing of termites and cockroaches

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ABSTRACT

The presence of termites and cockroaches are common household pests, and they pose significant challenges in both residential and commercial settings due to their destructive behavior and potential health hazards. Conventional chemical pesticides often come with environmental and health concerns. Therefore, there is a growing need for effective and sustainable biocontrol agents to manage these pests. This study focuses on the development of a novel biocontrol agent derived from naturally occurring microorganisms and their metabolites. In this study, the enzymes chitinase, lipase and protease from bacteria are shown to have biotermiticide activity. The biocontrol agent demonstrates promising results in laboratory trials, exhibiting high efficacy in the elimination of both termites and cockroaches with the advantage of being easy, eco-friendly and economical. The biocontrol agent offers a sustainable solution to pest management, addressing concerns related to chemical pesticide usage and promoting ecological balance in pest control strategies.

Keywords: Biological, Eco-friendly, Environment, Enzymes, Pests

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INTRODUCTION

Termites are terrestrial social insects, they are classified in the super order *Neoptera*, they are polymorphic and hemimetabolous, with varying lengths from 3 to 25 mm (Constantino 2012; Gullan and Cranston 2017). They belong to the insect order *Isoptera*, in which "isos" means equal and "pteron" means wings and refers to the two pairs of identical wings in the adult termite (Throne and Carpenter 1992). They are morphologically soft-bodied, light-colored, polymorphic and cellulose-eating social insects living in large communities of several hundred to several million individuals. Termites are cosmopolitan but are most commonly present in oriental, neotropical and Ethiopian areas, being especially diverse in tropical forests (Constantino 2012). These insects typically feed on different forms and sources of lignocelluloses (alive, dead, or rotten), grasses, manure, roots, leaves, stems and soil (Eggleton 2010; Krishna 2013). Termites are insect pests of agricultural, ornamental crops and dry wood. Since they are social insects they have a strong inter-communication, due to which they are very active, with positive and negative effects on the environment. They are found in almost every type of soil in the world and have a broad range of species. They are major pests of different objects, such as timber, paper and various crops (Osipitan and Oseyemi 2012; Verma et al. 2009), and are efficient decomposers of wood and leaves in natural systems (Collins 1981; Noble et al. 2009).Termites are classified on the basis of their behavioral characters and habitats into woods inhabitant and ground inhabitant.

Control of termites by the use of chemicals involves their application to the wood or to the soil. The best method of eliminating dry wood termites is by using chemical fumigation in which the fumigant, usually sulfuryl fluoride, methyl bromide, or a combination of methyl bromide and carbon dioxide is pumped into the building. Different insecticides such as chlorpyrifos, bifenthrin, imidacloprid, endosulfan, and lindane are currently being used for the control of termites in stored wood as well as for crops (Su et al. 1999). The control of pests with chemical insecticides has generated several problems including resistance to insecticide, out-breaks of secondary pests normally held in check by natural enemies, risks for human safety and domestic animals, decrease in biodiversity, and other environmental concerns. These problems and sustainability of programs based predominantly on conventional insecticides have stimulated increased interest in integrated pest management. Sustainable agriculture in the 21st century will increasingly rely on alternative solutions for

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pest management that are eco-friendly and reduce the amount of human contact with chemical pesticides.

Cockroaches are very common household pests belonging to the class *Insecta* and order *Blattodea*. They are amongst the most primitive living winged insects, about 320 million years old. The word cockroach is derived from Spanish word *cucaracha*. The cockroach is characterized by a flattened oval body, long thread-like antennae, and a shining black or brown leathery integument. Male cockroaches usually have two pairs of wings, whereas females, in some species, are wingless or have vestigial wings. They migrate towards dark, warm, moist areas. Adult cockroaches can survive without water for about a month, and two or three months without food. Besides eating obvious foods, they also feed on leather, dried skin, and even starch from paper. Cockroaches not only spoil food, but also transfer pathogens and cause allergic reactions in humans (Brenner et al. 1995). There are over 4,700 species of cockroaches worldwide. Out of these only few species are commonly associated with humans.

Cockroaches are considered nuisance pests because they emit a repulsive odor, feed on anything that is edible to humans, hide in the cracks and crevices of various structures, and degrade the beauty of the household environment. They also have been associated in the transmission of various human diseases (Peterson et al. 1999). It is quite intriguing that cockroaches are able to endure and survive under conditions that are mostly harmful to human beings. Cockroach management is essential in terms of minimizing impacts on public health and the economy. There are a wide range of chemical pesticides that can be used to kill cockroaches, but many researchers have recognized the potential for the innovation of a safer and cheaper insecticides as an alternative for eradication of cockroaches (SzumLas 2002). Long term exposure to chemical pesticides causes various harmful effects in human beings such weakened immune system, difficulty in cellular respiration and may cause skin cancer (Anderson and Meade 2014). Continuous use of such chemical pesticides and fertilizers can also pollute the soil and decrease the soil fertility as well as it may reduce the growth of beneficial microbes in the soil (Shakir et al. 2016).

Biocontrol agents or biopesticides, are natural organisms or substances derived from living organisms that are used to control pests, including insects, weeds and pathogens. Unlike conventional chemical pesticides, biocontrol agents offer eco-friendly and sustainable alternatives for pest management. The use of biocontrol agents can reduce the reliability on synthetic chemical pesticides and minimize chemical residues in food and the environment. Biocontrol agents reduce the activity of the pathogens and damage the target organism; hence they are called natural enemies (Heydari and Pessarakli 2010). Many bacteria and fungi can be used as biocontrol agents. The major benefit of these biocontrol agents is that they only cause damage to the target organism and not the host (Savita and Anuradha 2019). Use of biocontrol agents is sustainable method having the potential to permanently reduce the pest organisms (Moazami 2019). Microbial biopesticides are used as biocontrol agents against a wide variety of agricultural insect pests (Ruiu 2018). These microorganisms are natural enemies of insects, they are target-specific, and promising in reducing the use of hazardous chemical pesticides (Majeed et al. 2017).

The microorganisms can be isolated from termite infested areas such as wood and soil, and they can be screened to check their termiticidal activity for subsequent use as biocontrol agents (Sindhu et al. 2011). The bacteria have the capability to produce different enzymes that can cause damage to the target insect. These enzymes include chitinases, proteases, lipases, etc.

MATERIALS AND METHODS

Collection of soil samples

Soil samples were collected from the surface of cow dung and termites infested wooden log from Pune, Maharashtra, India. Soil was collected in clean plastic bags, labeled and brought to the laboratory.

Isolation of the bacteria from soil samples

The soil samples were serially diluted up to 10^{-5} and 0.1 ml of 10^{-3}, 10^{-4} and 10^{-5} dilutions was spread on nutrient agar (NA) [NA (g/l): beef extract - 3.0, peptone - 5.0, NaCl - 8.0, pH - 7.2, 8.0, agar - 15.0, distilled water (DW) - 1000 ml] media plates. The plates were incubated at 37 °C for 24 hours. Single bacterial colony was streaked on NA media to get pure isolate. The bacterial isolates were maintained on NA slants.

Screening of bacterial isolates for enzymes responsible for killing of termites and cockroaches

The bacterial isolates were checked for the production of chitinase, lipase and protease enzymes. The colloidal chitin, lipid and casein agar media [Colloidal chitin (g/l) - KH_2PO_4 - 0.3, K_2HPO_4 - 0.7, $MgSO_4.7H_2O$ - 0.5, $FeSO_4.7H_2O$ - 0.01, $ZnSO_4.7H_2O$ - 0.001, $MnCl_2$ - 0.001, colloidal chitin - 10.0, agar - 30.0, DW - 1000 ml]; [Lipid agar (g/l) - peptone - 6.5, NaCl - 3.2, $CaCl.2H_2O$ - 0.065, oil - 1%, agar - 30.0, DW - 1000 ml] and [Casein agar (g/l) - soluble starch - 10.0, casein - 0.30, KNO_3 - 2.0, $MgSO_4.7H_2O$ - 0.05, K_2HPO_4 - 2.0, NaCl - 2.0, $CaCO_3$ - 0.02, $FeSO_4.7H_2O$ - 0.01, agar - 18.0, DW - 1000 ml] were prepared for chitinase, lipase and protease enzymes respectively. The bacterial isolates were spot inoculated on these media respectively and the plates were incubated at 37 °C for 24 to 48 hours. Gram's iodine reagent was added and the plates were observed for the zone of hydrolysis which indicated the production of chitinase, lipase and protease enzymes.

Characterization and identification of the potent bacteria isolates

The bacterial isolates producing chitinase, lipase and protease enzymes were characterized and identified by studying the morphological characters, Gram character, motility, oxidase and catalase enzymes production and biochemical tests such as sugar fermentation (Roy et al. 1983; Devis et al. 1983) and the Genus was identified by using Bergey's Manual of Determinative Bacteriology.

Extraction of chitinase, lipase and protease enzymes

The bacterial isolates producing chitinase, lipase and protease enzymes were enriched in colloidal chitin, lipase and casein broths respectively for two days at 37 °C. The control without inoculation was also kept. The broths were centrifuged at 10,000 rpm for 20 minutes and the supernatant were collected. The chitinase, lipase and protease enzymes were thus extracted which were in crude form.

Purification of the enzymes by ammonium sulphate precipitation

The extracted crude enzymes chitinase, lipase and protease were purified by ammonium sulphate precipitation method. For chitinase enzyme purification, 20 ml enriched chitin broth was centrifuged at 10,000 rpm for 20 minutes. The supernatant was collected and the pellet was discarded. Ammonium sulphate precipitation 80% saturation was used (Karunya et al. 2011). The supernatant 20 ml was measured and accordingly ammonium sulphate was weighed in gram with respect to the chart (80% saturation of ammonium sulphate, for 1000 ml = 387 g ammonium sulphate; for 20 ml = 7.74 g of ammonium sulphate). The supernatant was taken in a clean beaker. Icewas used to maintain cold temperature for ammonium sulphate precipitation. The beaker was placed on magnetic stirrer and ammonium sulphate was added pinch by pinch. The beaker was kept in the refrigerator overnight at 4 ºC. After this, the contents were centrifuged at 10,000 rpm for 20 minutes. The supernatant was collected measured and thus purified chitinase enzyme was obtained (Drewnowska et al. 2020; Lee et al. 2012). For lipase and protease enzymes purification, 20 ml enriched lipid and casein broths were centrifuged at 10,000 rpm for 20 minutes and the same process of ammonium sulphate precipitation was followed as above to get pure lipase and protease enzymes (Ali et al. 2023; Das and Prasad 2010; Merlyn et al. 2021; Patel et al. 2019; Rebecca et al. 2023).

Formulations for application in termite and cockroach infested area

The purified chitinase, lipase and protease enzymes were then used for killing of termites and cockroaches. After application of the purified enzyme formulation on termites and cockroaches, reduction in mobility and swelling in the stomach of the insects was observed. After a specific amount of time the mobility was completely stopped and the insect was observed to be died.

To evaluate the efficacy of the formulations on termites and cockroaches

To check the efficacy, the time required for the insect to die was noted. For this, 2 ml of purified chitinase, lipase and protease enzymes was used to see the effect on termites and cockroaches.

RESULTS

Collection of samples

The samples collected from termites infested wooden log and the surface of cow dung are shown in Fig. 1 and 2 respectively.

Isolation of bacteria from the samples

Total 20 bacterial isolates were obtained from wooden log and surface of cow dung samples.

Enzyme production by the bacterial isolates

The chitinase enzyme production was observed by isolate No. 1, lipase by isolates No. 2, 3 and 4 and protease by isolate No. 5. From the diameter of zone of hydrolysis, it was observed that lipase production was more by isolate No. 4 (Table 1). The chitinase, lipase and protease enzyme production by the bacterial isolates No. 1, 2, 3, 4 and 5 are shown in Fig. 3, 4 and 5.

Potent bacterial isolates

The five potent pure bacterial isolates for further study were labeled as No.1, 2, 3, 4 and 5 (Fig. 6).

Morphological and biochemical tests for characterization and identification of the bacterial isolates

The isolates No. 1, 4 and 5 were Gram positive filamentous rod, while isolates No. 2 and 3 were Gram negative short rod and Gram positive cocci respectively (Table 2). The oxidase production was seen by isolate No. 2, while all the isolates showed catalase production. From Bergey's Manual of Determinative Bacteriology, the isolates No. 1, 2, 3, 4 and 5 were *Nocardia*, *Pseudomonas*, *Nocardia*, *Micrococcus*, and *Nocardia* sp. respectively.

Purified enzymes

The enzymes viz., chitinase, lipase and protease purified by ammonium sulphate precipitation method are shown in Fig. 7, 8 and 9 respectively.

Effect of enzymes on termites and cockroaches

After using 2 ml of purified chitinase enzyme from isolate No. 1, the time required for killing of termites and cockroach was 3 and 5 minutes respectively (Fig. 10 and 11). This is because chitinase breaks down the chitin polymer, weakening the exoskeleton and making termites and cockroaches vulnerable to predation or desiccation (Gupta and Rajak 2016).

After using 2 ml of purified lipase enzyme from isolate No. 2, 3 and 4, the time required for killing of termites and cockroach was 3 and 5 minutes respectively (Fig. 12 and 13). Lipase breaks down lipids, disrupting the termites and cockroaches digestive processes and causing malnutrition or starvation (Khan and Akhtar 2013).

After using 2 ml of purified protease enzyme from isolate No. 5, the time required for killing of termites and cockroach was 3 and 5 minutes respectively (Fig. 14 and 15). Protease breaks down proteins, interfering with the termites and cockroaches metabolism and growth (Ahmad and Khan 2017).

DISCUSSION

In the present study we used bacteria which were isolated from termite infested areas such as cow dung sample, and damaged wooden log. There is a report on chitinase as a biotermiticide when it contacts the termite skin (Hussin and Majid 2020). This work is novel which describes the use of lipase, protease and chitinase to kill termites and cockroaches. The chitinase, lipase and protease enzymes target specific components of termites and cockroaches exoskeleton and digestive system, leading to their death or incapacitation.

CONCLUSION

The chitinase, lipase and protease enzymes from bacteria can help in the management of termites and cockroaches. This will be very easy, economical and eco-friendly approach in the control of termites and cockroaches having sustainable approach. This is effective method of the use of enzymes from bacteria to kill termites and cockroaches. Further research and development are warranted to optimize the formulation and application of this biocontrol agent for widespread implementation in pest management programs.

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COMPETING INTERESTS

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