Influence crude secondary metabolite of entomopathogenic B. bassiana and T. harizanium fungi on mortality larvae stages of Culex pipiens

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Abstract

Culex pipiens mosquitos are thought to be vectors for many arboviruses, including West Nile virus and encephalitis virus, which have a global impact on human health. The natural management of this pest's aquatic stages is critical for sustaining an insecticide-free environment. The current study focused on the biological and biochemical effects of the entomopathogenic fungi Beauveria bassiana and Trichoderma harizanium on Culex pipiens laboratory colony larvae of instar. The results showed the effect of overlapping the concentrations of secondary metabolite for fungi Beauveria bassaina and Trichoderma harizanium on mortality of the four larval instars of C.pipiens mosquito which seems the exceed of B. bassaina more than T. harizanium in mortality of four larva instars and for all concentrations . The concentration 150 ppm demonstrated more concentrations in mortality for all fungi after incubation period (21) days.

Keywords: *Culex pipiens, Beauveruia bassiana, Trichoderma harizanium, entomopathogenic fungi.*

1- INTRODUCTION

Mosquitoes are dipteran insects that act as biological and mechanical vectors for parasites and pathogens that cause communicable diseases. They can spread enzootic or even epizootic diseases such as malaria, dengue fever, and filariasis. To implement long-term vector control strategies, the World Health Organization (WHO) developed the Global Vector Control Response (GVCR, 2017-2030) [1]. Chemical control of vector insects, despite its effectiveness, poses a health, environmental, and climatic risk. Insecticide resistance and unsustainable interventions pose obstacles to achieving sustainable development goals. The Culicidae are almost all bloodsuckers that spread a variety of serious diseases [2]. The Cx. pipiens is the most interesting because of its widespread distribution in tropical and subtropical countries, which has an economic impact. Vector control strategies typically target the aquatic stages of mosquitoes in their breeding habitat to counteract adult resurgence during

adult control. Chemical larvicides that target mosquito breeding sites cause resistance in the targeted species as well as long-term secondary effects that harm aquatic fauna [3]. Exploring eco-friendly and biological control methods is essential for developing an alternative larval control strategy. Entomopathogenic microorganisms play an important role in alternative insect pest control methods. The use of entomopathogenic fungi to control insect pests has yielded promising results, with Beauveria bassiana[4, 5] and Metarhizium anisopliae [6] being self-sustaining and efficient alternatives for controlling Cx. pipiens. Both species have a wide variety of isolates that vary in host specificity and origin. These fungi will help to maintain the ecological balance in the surrounding aquatic habitats by controlling the aquatic stages of mosquitoes. [7]. The current research looks at the efficacy of two Entomopathogenic microorganisms against Cx. pipiens larvae.

2- Materials and Methods

2-1- Collection, breeding and diagnosis of mosquitoes

Two pools were chosen to collect eggs and larvae of Culex pipiens located in Al Muthanna Province one in Samawah city and the other in Rumaitha city, where mosquitoes abound in these pools because contain organic materials. The samples have been taken in several times and different sites in the pools during October 2021 and September 2022 by using nets long arm making from tulle cloths, they were put in glass cans 1.5 m with cover holes and transported to the laboratory, the samples were emptied in Plastic ponds with diameter (70x40x15) cm and provided with tap water which leaved for the period 48 hours to remove the chlorine , add food of larvae with percentage 3:1 (protein powder and yeast) with 2 grams for all ponds and covered with tulle cloths [8].

2-2-Fungus Culture

Beauveria bassiana (Balsamo), and Trichoderma harizaniumsolates were obtained from the Mycology Center, Ministry of Science, and Technology, Iraq. The isolates were cultured in flasks on Sabouraud dextrose yeast agar (SDYA) medium [8], which contained 40 g glucose, 20 g peptone, 20 g agar, and 2 g yeast extract dissolved in 1000 ml of distilled water. The flasks were autoclaved for 15-20 minutes at 1210C The media was poured into Petri dishes and ready for inoculation [9].

2-3- Effect crude results secondary metabolite of fungi in different incubation on death rate of larval stages of Cx. pipiens mosquitoes

An estimation bio-active for crude results secondary metabolite of fungi in different incubation (7,14 and 21) days and used the same concentrations that preparative previously in item (3-3) for each fungus on death rate larvae stages of Cx. pipiens mosquitoes. The death rates have been calculated for the periods 24, 48, and 72) hours [8].

2-4-Statistical analysis

One way ANOVA at $p \leq 0.05$ was used to analyse the triplicate data means followed by a Multiple Comparison Test (MCT) to indicate the signfcant differences between means using a statistical analysis system (SAS, 2003).

3- Results

The tables (1-3) showed the effect of overlapping the concentrations of secondary metabolite for fungi Beauveria bassaina and Trichoderma harizanium on mortality of the

four larval instars of C.pipiens mosquito which seems the exceed of B. bassaina more than T. harizanium in mortality of four larva instars and for all concentrations . The concentration 150 ppm demonstrated more concentrations in mortality for all fungi after incubation periods (21) days , and on the other side the effect of secondary metabolite for fungi after incubation period 21 days is the highest ratio in larva death for all used concentrations, so mortality rate which cause by B. bassaina when used the 150 ppm for the first, second, third and fourth instar of larva were increased mortality to 81.11%, 67.77%, 59.99% and 64.44% respectively with period incubation (21) days, while the rate of mortality which cause by T. harizanium when used 150 ppm for the first, second, third and fourth instar of larva were and increased mortality to 65.55%, 64.44%, 55.55% 57.77% respectively with period incubation (21) days, and no mortality when control treatment. With regards interference between the factor study concentration and exposure period, concentration 150 ppm highest rate of mortality after exposure period 72 hours and with incubation period 21 days when the first larva of instar, mortality reached 81.11% for B. bassaina fungus and 65.55% for T. harizanium fungus in the table(1)

Table (1) effect of different concentrations from secondary metabolite for B. bassiana and T. harizanium on mortality larvae instars of C. pipiens after period of incubation (21 days)

Larval instar	Concentration (ppm)	The mortality for the larva instar according exposure period							
First		***B. bassiana***				***T. harizanium***			
		24h	48h	72h	Rate	24h	48h	72h	Rate
	25	26.66	40	50	38.88	23.33	30	40	31.11
	50	33.33	46.66	60	46.66	30	40	46.66	38.88
	75	43.33	56.66	66.66	55.55	33.33	43.33	53.33	43.33
	100	50	63.33	73.33	62.22	36.66	50	60	48.88
	125	53.33	63.33	80	65.55	46.66	53.33	70	56.66
	150	70	83.33	90	81.11	53.33	63.33	80	65.55
	Rate	46.1	58.88	69.99		37.21	46.66	58.33	
Second	25	30	40	46.66	38.88	16.66	26.66	33.33	25.55
	50	33.33	43.33	50	42.22	20	30	40	30
	75	40	46.66	56.66	47.77	30	33.33	46.66	36.66
	100	46.66	56.66	70	57.77	33.33	40	53.33	42.22
	125	46.66	56.66	73.33	58.88	43.33	53.33	63.33	53.33
	150	53.33	70	80	67.77	53.33	66.66	73.33	64.44
	Rate	41.66	52.21	62.77		32.77	41.66	51.66	
Third	25	20	30	33.33	27.77	20	23.33	30	24.44
	50	26.66	30	40	32.22	30	33.33	36.66	33.33
	75	26.66	33.33	43.33	34.44	26.66	40	46.66	37.77
	100	33.33	43.33	53.33	43.33	33.33	43.33	50	42.22
	125	46.66	53.33	63.33	54.44	30	46.66	56.66	44.44
	150	46.66	60	73.33	59.99	46.66	53.33	66.66	55.55
	Rate	33.32	41.66	51.1		31.1	32.99	47.77	
Fourth	25	16.66	26.66	30	24.44	16.66	20	26.66	21.1
	50	20	26.66	36.66	27.77	20	23.33	33.33	25.55
	75	23.33	33.33	43.33	33.33	20	30	40	30
	100	33.33	46.66	56.66	45.55	26.66	40	50	38.88
	125	50	60	70	60	36.66	46.66	56.66	46.66
	150	53.33	66.66	73.33	64.44	50	56.66	66.66	57.77

	Rate	32.77	43.32	51.66		28.33	36.1	41.1	

L.S.D. 0.05(con.)=14.12, L.S.D. 0.05 (ex.p.)=8.12, L.S.D.0.05 (interference.)=11.08

4- Disscusion

The raised rate of mortality even arrived to the incubation period 21 days. It indicated the bioactive toxic materials have been reached the climax in the 21 days period incubation where it is confirmed current study. It was highest concentrations for yield secondary metabolite of fungi (enzymes, toxins, Fatty acids) after incubation period 21 days, the relationship between all the concentrations result crude secondary metabolite for fungi, periods exposure, old larvae and rate of mortality this is one side and another side the relationship between sensitivity stages and type mosquitoes it has been took foreword same with it got in the use suspension method against the instars of larvae for the C.pipens mosquito, and it confirmed with the statistical analysis. This result has been supported through significant differences between the treatments. The results of current study agree with [10] when he used amount 20 gram from extract crude beauvericin toxin that result B.bassiana against larvae Ae. aegypti that perform to mortality with percentage 86%, in [11] conducted on mortality reach 100% when used crude extract for compound Tolypin that result from fungi Tolypocladium niveum with concentration 100 mg per litter against larvae Cx. pipiens and Anopheles maculipinnis mosquitoes, also the result agreed with the results that got researchers [12] rate of mortality reach 80% for Anopheles stephensi when larvae exposure to beauvericin compound by concentration 10 mg/ml. The researcher [13] has been used three fungi secondary metabolite to control Cx. quinquefasciatus where it was more effect on first larvae instar from the rest of the instars. The investigator [14] has been showed the use ribotoxin extract from B. bassiana to inhibit insect immune response. A toxic protein bassiacridin secreted from B. bassiana was isolated and toxic for locusts. [15] found the use patulin compound that produce from Penicillium citrinum by concentration 10 mg/ml against third instars of larvae of Cx. quinquefasciatus perform to the rate of mortality by 90%. [16] demonstrated percentage by 100% when exposure first instar larvae for An. stephensi mosquitoes to crude yields secondary metabolite of M. anisopliae fungi.

With regard to Trichoderma is a genus contains a lot of filamentous fungi that used as a biological control agent in agriculture domain on different pests and it has ability to parasite them (mycoparasitism) between other mechanism of action [17]. In last years, the possibility of using Trichoderma genus as a biological control in insect aspect has been deem, both directly or indirectly. Several studies have been done that T. genus is able to of controlling insect pests straightway during parasitism and can produce different of insecticidal secondary metabolite, antifeedant and repellent metabolite. Another side indirectly during the active of plant defense responses by attractiveness of natural enemies of insect symbiotic groups [18]. The information about genus T. harzianum inside biological control against insects still very limited. The increasing experimental guide about the topic this fungus looks promising, and puts it foreword development of Trichoderma strains combination active various targets [19]. However, the predictability and reproducibility of the effects of these beneficial fungi are still limited due to a lack of a thorough understanding of the

molecular mechanisms underlying the specificity of their interaction with different crop varieties, as well as how environmental factors modulate this interaction [20]. Mycoparasitism is a complicated physiological process that involves the generation of enzymes and secondary metabolites and should be regarded in the context of microbial competition. Trichoderma spp. have historically been regarded as necrotrophic mycoparasites; nevertheless, there is evidence that, in certain cases, they act as hemibiotrophs, producing modest damage to the host cell wall and residing intracellular in the host cell for an extended length of time [21]. Mycoparasitism, antibiosis, induced defence response (IDR), and competitive exclusion all play a role in Trichoderma-mediated illness suppression [22,23].

5- Conclusions:

Entomopathogenic fungi are thought to be a naturally occurring microbial control agent against many insects, helping to reduce host population in epizootics. The majority of them begin the infection process in the gut. Fungal enzymes aid in cuticle penetration, and toxin production induces the host's immune response, including activation/deactivation of host enzymes. The fungal propagation within the host body causes a significant depletion of host biomolecule availability, affecting variable parameters in the host life cycle, and eventually host death in a susceptible host. The difference in cuticular structure and chemical composition of the epicuticle influences the host-fungus relationship. Toxicity differences between selected fungal isolates.

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