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A Neutrosophic Study for the Transmission of Infection with Pathogenic Fungi from Males of Olive Fly Insects to Their Females

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Abstract: This paper presents the study of the effectiveness of horizontal transfer of local isolates of the pathogenic fungus Beauveria bassiana (Balsamo) on adults of olive fruit fly Bactrocera oleae (Rossi) at a concentration of 10^6 spores/ml in laboratory conditions (this work was carried out in specialized scientific laboratories). In addition, it is not possible to reach the desired results in such experiments effectively when the data and observations of the study are not clear and accurate. For this reason, in this paper, experimental data will be presented with inaccurate or uncertain observations using neutrosophic statistics. The purpose is to know the success of males contaminated with pathogenic isolates in the transmission of infection to females. In laboratory conditions through a neutrosophic reading of the study data. This proposed presentation provides greater accuracy, flexibility, and applicability than the classic experimental design in the case of uncertainty.

Keywords: Beauveria bassiana, bactrocera oleae, horizontal transmission, neutrosophic logic.

1. Introduction

Entomopathogenic fungi are the most common and easiest to distinguish insect pathogens. It is characterized by its superiority in terms of species, the wide range of its terrestrial and aquatic hosts, and its ability to form spores with which to resist unsuitable environmental conditions. In addition, it would have been possible, through these characteristics, to reach the epidemiological level if it were not for its close association with environmental conditions such as humidity and heat [9,15,18].

Early symptoms of infection begin with the host insect stopping feeding and losing balance with slow movement [13]. By penetrating the fungus' hyphal cells, we notice dark black spots resulting from the deposition of melanin at the hyphal penetration sites. The mycelium outside the insect is the most prominent manifestation of infection [18].

Infection of insects with fungal diseases goes through four successive steps:

1. Contact between the host and the sites of germination.
2. Adhesion and germination of the sporophyte tube.
3. Penetration and invasion of the fungus into the tissues and organs of the host under anaerobic conditions.
4. The death of the host Balsamo to the natural obstruction of the alimentary canal, bronchi, and circulatory systems, poisoning or physiological starvation) and the production of blastospores which are contagious, and the transition to the throwing state that ends with sporulation on the surface of the host's body [21].

The time required for the pathogenic fungus to kill the insect varies according to several factors, including the stage of the insect, humidity, and the pathogenic fungus itself. Most pathogenic fungi need 3-12 days from infection until the insect dies [18]. The fungus secretes a group of secondary metabolites and mycotoxins that are chemically diverse and vary according to the genetic strain of the fungus. These toxins are Beauvericin, Bas-sianin, Beauverolides, Bassianolide and Tenellin, which kill the host by destroying its tissues and degrading its cells, in addition, the growth of the fungus impedes the path of the blood fluid. In addition, by feeding the fungus, it depletes the nutrients present in the host's body, and thus the body organs of the insects infected with it die [11, 20].

These toxins can weaken and kill the insect before the mycelium fully developed inside the insect's body [20]. The pathogenic fungus can also kill the insect through its entry into the Gut of the larvae, killing them from starvation [12].

Many studies have proven the ability of pathogenic fungi to infect insects and cause death to them. Therefore, this research was conducted to study the possibility of transmission of infection from males treated with pathogenic fungi to females, from a neutrosophic point of view. This opens the way for dealing with issues surrounding study data that are not precisely defined.

Neutrosophic means the study of ideas and concepts that are neither right nor wrong, but between that, and this means (neutrality, indeterminacy, ambiguity, contradiction, and others), and that every field of knowledge and experience has its neutrosophic part, that part that contains

indeterminacy. The first to lay the foundations of the neutrosophic was the American philosopher and mathematician, "Florentin Smarandache", who presented neutrosophic logic in 1995 as a generalization of fuzzy logic [1,2]. As an extension of this, Ahmed Salama presented the theory of classical neutrosophic sets as a generalization of the theory of classical sets [3,4]. The neutrosophic has grown significantly in recent years. Many researchers have worked in the neutrosophic field of science around the world such as Huda E. Khalid et al [16,17]. Because it formed a real revolution in science through its application in many disciplines and scientific and practical fields [5-8]. In this research, we highlight the application of neutrosophic logic to the study data so that we have three cases (dead, indefinite, injured) instead of two cases as in the classical logic that does not recognize the existence of uncertain cases.

2. Research Materials and Methods:

1-Obtaining olive fruit fly adults: olive fruit fly larvae and pupae collected from the dissection of infected fruits collected from olive trees Taken from [14].

2-Isolation of the pathogenic fungus: An isolation of the fungus Beauveria bassiana approved, which follows the scientific classification. According to [19].

Kingdom of fungi, Department of Ascetic Fungi, Row: Sordariomycetes

Order: Hypocreales, Family: Clavicipitaceae, Genus: Beauveria

Genre: (Balsmo.criv.) (vuill,1912) B. bassiana.

3. Search Objective

Studying the role of male olive fruit fly in transmitting infection with the fungus Beauveria bassiana to females. Under laboratory conditions through a neutrosophic viewpoint. (That allows us to obtain incomplete or unclear information about the transmission of infection or the emergence of symptoms).

4. The Method of Work

The concentrations of 10^6 spores/ml of the sporophyte suspension of the pathogenic fungus B. bassiana were tested at a rate of 5 replications. 4 males were sprayed with the sporophyte suspension at a rate of 1 ml of the tested concentration at the age of 0-24 hours, after placing them in a glass tube and in the refrigerator at a temperature of 4ºC for a period 2-5 minutes to reduce the movement of

flies. Then the contaminated males were added to 4 females aged 0-24 hours in plastic containers with a diameter of 10 cm, and a height of 20 cm. 5 replicates were approved for each concentration. Males and females were monitored, and the possibility of pathogenic fungi transmitting to females by mating or attempting to mate in laboratory conditions was recorded, while the control males were treated with distilled water. The plastic containers were placed in the incubator at a temperature of 25±2ºC, a humidity of 60± 5%, and an illumination of 12:12 (dark: light). The death rates were recorded every 48 hours starting from the fourth day (when the insects had matured sexually and became able to mate) for 8 days after treatment.

5. Results and Discussion

The males contaminated with the pathogenic fungus by spraying the sporophyte suspension in the laboratory achieved success in transmitting the infection to the females. The death of females started on the sixth day of treatment, while the males started on the fourth day of treatment. In addition, the following study shows us in days (4-6-8) the Corrected death rates and infection rates, as well as the unspecified percentages that range between the healthy and the injured who have not yet shown symptoms.

On the fourth day. The death rate of males from the treatment was 45%, and 25% of the males had symptoms that ranged from simple to severe symptoms such as slow motion or even stopping movement and going up to the top of the breeding box. In addition, there are 30% (unspecified percentage) of Males did not show any symptoms. but this does not mean that these males are healthy, as they may be carriers of spores and are able to transmit them to females even if they are resistant to them. As for females, no death rate was recorded, and 20% of them showed some symptoms of the disease, such as slow movement and lack of nutrition. Therefore, 80% of females are not determined if they are healthy or infected, but they have not yet shown symptoms of the disease.

time (day)						*isolation B. bassiana*	*spore concentration/ml* 10^6
4							
Female			Male				
Undefined "He showed no symptoms"	A patient who has symptoms	Corrected death rate	undefined "He showed no symptoms"	A patient who has symptoms	Corrected death rate		
80%	20%	%0	30%	25%	45%		

Table (1): Corrected Death Rates and Infestation of Adult Olive Fruit Fly (When Males were Treated with an Isolate of the Pathogenic Fungus B. Bassiana in Vitro)

On the sixth day of treatment. The death rate of males reached 77.8%, as the fungus spores on it, and its secretion of toxic toxins affected the males greatly. In addition, 15% of the infected males showed symptoms ranging from mild to severe, and therefore 7.2% of the males were not determined whether they were healthy or infected and did not show symptoms yet.

As for females, the death rate was 20%, and 15% of the females' showed symptoms of infection ranging from mild to severe, and therefore 65% of females are not determined whether they are healthy or infected and have not shown symptoms yet.

time (day)						*Isolation B. bassiana*	spore concentration/ml 10^6
6							
Female			Male				
A patient who has symptoms	Corrected death rate	A patient who has symptoms	Corrected death rate	A patient who has symptoms	Corrected death rate		
65%	15%	%20	7.2%	15%	77.8%		

Table (2): Corrected Death Rates and Infestations for Adult Olive Fruit Flies (When Males were Treated with an Isolate of the Pathogenic Fungus B. Bassiana in the Laboratory).

On the eighth day of treatment. the death rate of males reached 90%, and 7% of the males showed symptoms ranging from mild to severe, and therefore 3% of the males were unspecified (if they were completely healthy or infected, the symptoms did not appear yet).

The death rate of females reached 35%, 20% of infected people showed symptoms ranging from mild to severe, and 45% were unspecified (healthy or injured, no symptoms appeared yet).

time (day)						***isolation B. bassiana***	**spore concentration/ml 10^6**
8							
Female			Male				
A patient who has symptoms	Corrected death rate	A patient who has symptoms	Corrected death rate	A patient who has symptoms	Corrected death rate		
45%	20%	%35	3%	7%	90%		

Table (3): Corrected Death Rates and Infestation for Adult Olive Fruit Fly (When Males were Treated with an Isolate of the Pathogenic Fungus B. Bassiana in the Laboratory).

6. Conclusion and Results

This paper concludes that studying the role of male olive fruit fly in transmitting infection with the fungus Beauveria bassiana to females under laboratory conditions through a neutrosophic point of view provides a more general and clear view (In the transmission of infection between insects). One of the well-known classic methods ends with the insect being infected or healthy only and eliminating the idea and the state of the existence of uncertainty. That is, it is possible that there is an unspecified case that appears healthy (and did not show any symptoms of infection), yet it is a carrier of the disease and causes infection. The results of the research either indicate that males are carriers of the disease, clearly and explicitly, or infected with no symptoms yet, but they can transmit it to females in both cases. Where the males are carriers of spores and transmit them to the females through mating or attempting to mate, and this is the aim of the study. Thus, according to our study, the chance of transmitting the disease from males to females becomes higher. This provides a correct view of the shortest possible time to achieve the goal, which is the largest possible infection rate and therefore the highest death rates, and we get rid of this insect and its damage to olive fruits as soon as possible. We look forward soon to generalizing this study to other types of insects.

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