

Biocontrol of *Ectomyelois ceratoniae* larva by bacterial strains obtained from pest infested gardens

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Abstract: Insect pest inflict lots of damage to agricultural products every years. Limitation of chemical toxins in pest controls made the application of microorganisms so interesting and important. In the current study in order to evaluate the biological control of insect pest by bacteria *Ectomyelois ceratoniae* were applied as test larva. The infested soils were collected from Khorasan-razavi gardens and kept in dark until analyzed in the laboratory. By culturing the samples in various conditions we obtained bacterial isolated. Of then a total of 10 different strains were selected to study their larvicidal activity. In each experiment we put 10 healthy third star larva in sterile plate containing 2 gram suitable food. An appropriate bacterial counts from mid log culture were added to their diets. The dead larva was counted and removed from plates up to one week. A total of 6 strains could kill *Ectomyelois ceratoniae* by the rate of 10-30% in comparison to the control.

Keyword: Biocontrol, Bacillus, *Ectomyelois ceratoniae*

INTRODUCTION

Pests are a group of living organisms including fungi, insects, nematodes, rodents and weeds, which, by attacking different parts of the plant, during various stages of their life, entail economic losses to the plant or product. In this case, the plant usually disappears or the performance and marketability of the product are significantly reduced. According to studies, 10 to 20 percent of the world's total food production is lost annually (25 to 30 percent) due to plant diseases and pests, caused by insect pests. [Kumari, et al., 2014]. Which leads to the imposition of a double economic burden on the international community. [Bhattacharjee et al., 2014]. Biological containment means the use of natural enemies, antagonists, or competing microorganisms to reduce the populations of a pest, so that the frequency of pests is reduced and damages less than what is possible. Insects, mites, weeds, pathogens, and vertebrates may all be targets of biological containment. Biological containment may take place through targeted human intervention, or the practical result of a self-burning natural force. Biological control may be used to reduce the population of farmland or forest pests or to adjust the inconsistencies caused by the entry of a new agent into a natural environment. Biological containment is a general term in which a biological agent prevents the exponential increase of the number of another living creature, and in this way reduces the population of an existing organism to a natural or even lower level. The widespread use of this method depends on the progress of the science of ecology. In the nineteenth century, biological control concepts and concepts were developed by recognizing the theoretical ecological principles, and using ecological formulas, biological control was projected. Therefore, it can be argued that biological control is derived from ecology, and is sometimes referred to as biological control, applied ecology. About 10,000 species of insects feed on plants and are considered as pest infections. Pest insects are important in that aspect, which are highly nutritious, highly migratory, have high fertility, have a long life span and are resistant to adverse environmental conditions. These features make them the most successful animal group. [Ansari, et al 2013]. For this reason, in the agricultural sector, there is an essential need to control the growth and proliferation of insect pests. Over the past decades, chemical insecticides have been the most widely used method for controlling insect pests in the agricultural and horticultural sector, and nowadays, despite the many ecological and medical problems caused by the excessive use of these toxins, they still have a high percentage of the market dedicated. This toxin has a long-term sustainability in the ecosystem. [Ansari, et al 2013]. And with the accumulation of plant tissues and its transmission along the food chain, it eventually enters the body of animals and humans and leads to the occurrence of acute and chronic diseases, including types of cancers, neurological damage, immune suppression

and congenital defects. [Gupta,et al.,2014] . The negative effects of these pesticides are not limited to the above, but they also create resistance to insect pests, water contamination [Brennan,et al.,2002] . And due to its non-specific effects, it eliminates other beneficial insects, such as predators and parasites, from these pests and many other insects. [Moir,et al., 2013,Hu,et al.,2000] .Taking into account the harmful effects of chemical pesticides, many efforts are being made to find wildlife-friendly and safe humans for managing insect pests. [Ansari,et al.,2013,Rui,et al.,2013] .Pest infestation in Iran's agricultural gardens has been widespread and has caused a lot of economic-social damage. Hence the identification of effective control methods for these insects is of great importance using less harmful methods. The pomegranate worm called *Ectomyelois ceratoniae* is the most important factor in reducing the quality and quantity of pomegranate and is one of the major obstacles to increasing pomegranate exports. This pest was first observed in Kashan in 1349 of the year in pomegranate gardens and is known for its early signs of pest infestation. On average, the percentage of pomegranate oil damage in the entire country and in different years is estimated at 30-25% of the product. The pomegranate worm spends the winter pomegranate in the form of larvae of different ages inside pomegranate and figs. Winter butterflies usually put their eggs on the bar and the flags and, rarely, on the inner surface of the skull. The larvae of the age of one after hatching fed on the inner surface of the casse and from the end of the second or early third century, by making a hole in the interior of the crown, the pomegranate fruit was introduced and, after entering, brought several factors from the group of fungi, bacteria and yeasts They pour into the pomegranate fruit. It covers the winter as a larva in infected pomegranates, and the larvae from the end of April to early June give it to the pomegranate corn, where they turn into pupa. Puppies are turned into a butterfly and come out of the pomegranate, depending on the weather conditions of the area and when the fruits are as large as lemons. Moths mate within 2-4 days, and the females spawn in the pomegranate crown and among the yellow flags. Each material contains about 25-20 eggs. After 8-10 days, the eggs are opened and turned into worms. The worms come from the same place. The period of larval activity is between 30 to 18 days, after which the larvae become pomegranate after the end of this period and become puppies. The pupil period lasts 8-7 days, and at the end of this period, the promise of the next generation will come out. [Shakeri et al.,2003] [Kashkuli et al ., 1975].Therefore, this insect is a major pest and is used as a model insect in this research. [Harding et al., 2015].In this study, the larval capacity of bacteria isolated from soil was evaluated on this insect.

MATERIALS AND METHODS

Breeding larvae of the insect *Ectomyelois ceratoniae*

In order to grow larvae, a number of puppies were obtained from the Department of Plant Protection, Faculty of Agriculture, Ferdowsi University of Mashhad, and their reproductive conditions were provided to make pupae into larvae and then to larvae. And the age required for biometric testing was used. [Kashkuli et al ., 1975]

Evaluation of the effect of 10 selected strains on insect larvae *Ectomyelois ceratoniae*

In order to investigate the effect of bacterial strains on larvae, 10×10^8 cfu / ml equivalents with sterile water were used as controls on 10 healthy larvae. From each strain, 500 μ l to 2 g of larvae was added and the results were evaluated daily for one week. [Rouhani et al., 2012] [Vijaya et al ., 2015].

RESULTS

The effects of larvae of 10 strains were investigated and the larvae survived in comparison to the control sample and dead larvae were determined through body movement and body color and weight loss (Fig. 1 and 2).



Figure 1: Dead larvae image and change their body color



Figure 2: The image of healthy larvae

As seen in Fig. 3, 5 strains destroyed at least 10% of the population of the *Ectomyelois ceratoniae* larvae. And one strain could destroy 30% of the larvae.

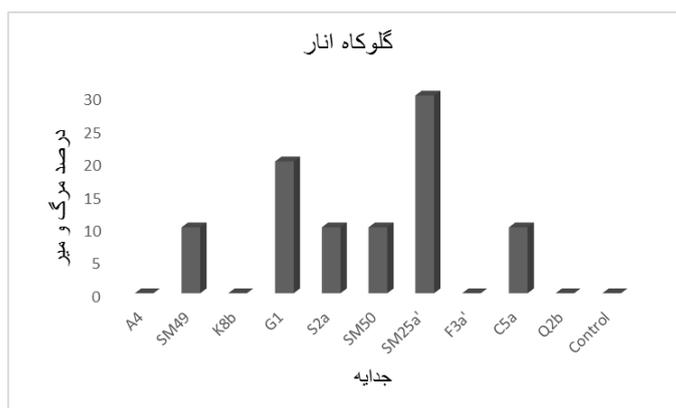


Figure 3: Study of different strains of bacteria on the *Ectomyelois ceratoniae* larvae

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