

## PREPARATION OF WETTABLE POWDER FORMULATION OF *BACILLUS THURINGIENSIS* KD2

 Rasoul Marzban<sup>1\*</sup>,  Jaber Babaei<sup>2</sup>,  Maryam Kalantari<sup>1</sup>,  Fatemeh Saberi<sup>3</sup>

<sup>1</sup>*Iranian Research Institute of Plant Protection, Education and Extension Organization (AREEO), Tehran, Iran*

<sup>2</sup>*Department of Chemistry, University of Tehran, Tehran, Iran*

<sup>3</sup>*Department of Chemical Engineering, Science and Research Branch, Islamic Azad University, Tehran, Iran*

*\*Corresponding Author:*

*E-mail: r.marzban@areeo.ac.ir*

*(Received 11<sup>th</sup> November 2020; accepted 05<sup>th</sup> March 2021)*

**ABSTRACT.** Formulation has always been regarded as the most important part of production in the microbial pesticides industry and one of the main concerns of producers. In this research, methods of preparation of wettable powder (WP) formulation from a *Bacillus thuringiensis* (Bt) isolate (KD2) were presented in which the active material (spore-crystal mixture) was increased in a liquid culture medium. The spore-crystal mixture was separated from the culture medium. Then, the mixture was dried in a drying agent with addition of 3% w/v of Triton X100, Tensiofix LX, Tensiofix BCZ in three separate treatments. The resulted technical powder was converted to wettable powder formulation by adding moisturizers, spreaders and filler materials and protective materials against ultraviolet light. Biological and physico-chemical tests including suspensibility, wettability and bioassay of the formulations as well as their phytotoxicity were done in comparison with two commercial products i.e. Biolep and Belthirul. The results of all formulations showed that the amount of suspension were 20% to 67% and the required time of wetting were 11 to 19 seconds. The results of the biological tests of formulations showed that the highest mortality rate on *Helicoverpa armigera* larvae was belonged to Belthirul with 78% and the lowest was recorded for Biolep with 53%. Among nine formulations, Tritonx100 + kaolin and kaolin + Lx formulations represented the better results. According to the results of LD<sub>50</sub> and the fact that the kaolin + Lx formulation is more cost effective, this formulation is suggested for commercializing.

**Keywords:** *Bacillus thuringiensis*, formulation, emulsifier, wettable powder

## INTRODUCTION

The formulation of pesticides in practice means that a small amount of the active ingredient spread at a high level in the environment and the formulation itself is commercially regarded as the last step in the preparation of pesticides in the factory. Formulation is a set of operations performed on the active ingredient to improve its properties of pesticide for storage, transportation, and application, effectiveness of penetration, sustainability and health. Formulation determines how a pesticide should be used and in order to protect the health of the community and food safety, human societies tend to replace conventional chemical pesticides with harmless compounds such as microbial pesticides. Therefore, research institutes and universities are trying to focus on identifying and introducing new and effective biological control factors while fulfill their commitment for maintaining food security and health of community. Business companies

also like using the researcher's findings to develop new commercial compounds that are acceptable for community [15]. Their goal is to make new technologies, which are more predictable and more competitive with chemical pesticides. A biological microbial pesticide is a combination of biological control agent (BCA) and associated substance for improving and influencing on the BCA. The purpose of formulation of BCAs is the sustainability of them during production, distribution, storage, transportation facilitation, application and protection of BCAs on environmental factors and increasing the efficiency of them. In order to have an effective formulation, it is necessary to understand the biological control factor, the host or target pest, the environment (ecosystem) and its interaction with other organisms (such as host plants, soil microorganisms or other natural enemies of pests). From the point of view of the final user there is a need for common understanding of method and tools and it is important to consider the customer demands in formulation of BCAs.

The work should be started with the proper understanding of biological factor and the target pest. One of the key aspects of a pesticide formulation research program is the safety and acceptability of materials associated with regulatory agencies in all areas where pesticide will be used. The experiences of a wide range of specialists can be consulted for determining the ingredient of a formulation and helping to develop an appropriate formulation. The formulation of BCAs cannot be separated from their production process, as the production process can be very effective in their efficiency and sustainability.

For proper use of microbial agents, preparation of a suitable formulation is important. In fact, formulation is an important link between the production of pesticides and their application.

A formulation that can provide durability performance and resistance to environmental conditions will be more acceptable in the market [1]. The formulation of microbial pesticides is a vital bridge between production and use and it determines the production economy, long-term storage, user friendly and improved field efficiency [13, 19]. The formulation of a microbial pesticide is one of the rings of the process chain that is needed to produce effective pesticides. Other rings of this chain include discovering BCAs, production and fixation, as well as considerations on its cost. The taken decisions at each stage have positive or negative effects in the success at the other states. Some useful organisms can be very effective in the lab, while they may not be effective in some market stages. The general reason of low instability of these products is during storage to application. The amount of the active ingredients of these products are low and decreased rapidly in the environment.

One of the most important issues regarding formulation is the relationship among microorganisms, their environment and their mode of action. Some organisms such as insect pathogens bacteria and viruses should be eaten by insects pest to have an effect, while others like fungi to control plant disease and weeds should be sprayed on them and insect pathogenic nematodes should have the ability of searching for pests. Therefore, a formulation must can cause pests to feed on these organisms or can increase the condition of dealing with pests and as well as the power of the search for organisms to be able to feed fast in the new environment. In addition, other factors such as the type of product (plant), target pests and how to use should be considered.

Formulations of BCAs seem to be harder than chemical pesticides. Active substances, which are usually a living organism, should be kept in proper condition to produce the desired result; these materials should also be protected from chemical or physical damage at the time of formulation. Since these organisms are nature-friendly, the substances

added to them during the formulation should be also in a way that preserves this property. Nowadays, it is tried to increase the effectiveness, durability and extent of the spread of organism (active agent) by using genetic engineering.

Formulation can promote the use of microbial pesticides. For example, granular formulation with slow release granule from *Bacillus thuringiensis israelensis* is used for mosquitoes in water. For application in the foliage, advances in foliage application technology such as droplet size optimization have been used to fully integrate the live agent [16, 20]. The natural degradation of microorganisms in the environment and the reduction of their populations eliminate the problems and concerns of residual pesticides, and the effect on non-target organisms. On the other hand, the durability and survival of the microbial factor is very important in controlling the pest. The durability of up to 14 days on the foliage is somewhat real and natural. These formulations are provided for the fungi that are on the plants aerial parts [11]. In the formulation of biological insecticides, the wettable powder is the most common formulation method that is used. Wettable powder formulation is a dry powder that is used after suspension in water. Wettable powder is a combination of a biological agent or an active substance with surfactant, wetting agent, a suspending agent and a filler material that is prepared after grinding with particles of about 5 micrometers [10]. Among the solid formulations of biological pesticides, a lot of attention has been paid to powders, which are due to stability during storage, water absorption suspension and the use of commonly used pesticides at the time of use [4]. The purpose of this study is to develop a new wettable powder formulation from local *Bt* strain for controlling *Helicoverpa armigera* and the other members of Noctuidae family.

## MATERIALS AND METHODS

### *Fermentation of the bacterium*

*B. thuringiensis* strain KD2 was used from culture collection of Biological Control Research Department, IRIPP [14]. Batch fermentation was carried out in an agitator equipped 6 l Fermenter (FCU/PU05, Medorex, Germany) with a working volume of 5 l. Circulator Co (Model; VS-190 CS) and Millipore manufactured the related devices including a control system, a circulator, and an air pump respectively. The fermentation was performed under completely aseptic conditions to prevent contamination during the process. The 10% (v/v) inoculation was transferred from the Erlenmeyer flask to the fermenter, which contained 3 liters of culture medium. The operational parameters was pH of 7.5 (adjusted by 1 N H<sub>2</sub>SO<sub>4</sub> and 1 N NaOH), temperature of 30 centigrade and mixer speed of 200 rpm. Air flow rate was set at 1 vvm and foam production was controlled by automatic addition of sterile antifoam solution for 72 h. The fermentation medium was composed of corn extract (5% w/v) and sugarcane molasses (2% w/v) and mineral salts: iron sulphate 0.003% wt:vol, zinc sulphate 0.02% wt:vol, magnesium sulphate 0.02% wt:vol and manganese sulphate 0.02% wt:vol [5]. The spores and crystals of *Bt* were collected via centrifugation at 6000×g [17]. Consequently, Triton X-100, Tensiofix LX, and Tensiofix BCZ emulsifiers were added to fermentation liquids in three separate treatments at a rate of 3% by weight, dried by industrial desiccator (Nature biotechnology company, Iran), and maintained at 4° C.

### **Formulation of wettable powder of Bt spore and crystal**

To formulate KD2 strain of *Bt*, 25% of the biomass of the strain was used with 75% of the additive. About 60% from 75% of additives were allocated to the filler, talc powder (Merck, Germany), kaolin (Kimia Sabzavar, Iran) and *diatomaceous earth* (IMERYS, USA) and 3% to one of the suspension materials as mentioned earlier, in final, 12% moisturizer, sodium lauryl sulfate (Merck, Germany) and sorbic acid (Merck, Germany) and titanium dioxide (Merck, Germany) were added. In this way, nine formulations were made for physically, chemically and biologically evaluation.

### **Biological, physical and chemical evaluation of formulations**

In the final stage, which is the stage of biological and physicochemical tests, the final formulations were compared with the two formulations, Belthirul® *B. thuringiensis* var *kurstaki* strain PB-54 (Probelte Co, Spain) and Biolep® *B. thuringiensis* var *kurstaki* strain Z-52 (Sibbiopharm Co., Russia).

### **Wettability of formulations**

100 mL of the distilled water was poured into the Erlenmeyer flask, and then 0.1 g of wettable powder was added to the surface of the water, and then the required time for complete wetting was recorded. This experiment was repeated four times and then mean time was calculated [12, 2].

### **Suspensibility of formulations**

The formulation suspensibility is determined according to the CIPAC method, 0.75 g from each final formulation were added to 250 mL of distilled water (with a hardness of 342 ppm, pH 6 to 7) and 60 times in head and bottom and then half an hour in a stationary position. From the top of the mixture, 225 ml was removed and the residue was smoothed and dried and its weight was recorded. The percentage of suspensibility was measured and based on the recorded weight of each treatment compared to the original weight in three replicates.

### **Biological properties of formulations**

The biocontrol potency of the formulations was evaluated based on the percentage mortality of different formulation on the four 4-days larvae of *Helicoverpa armigera*. For this purpose, nine formulations were prepared with two commercial formulations of Belthirul and Biolep at a concentration of 0.5/1000 (recommended concentration) and one control in 12 treatments in three replicates on the age of two larvae (4 days) of the bollworm were evaluated. The pieces of artificial food [21] there harvested in grams and each sample was poured into 10 mL samples at a concentration of 0.5/1000 prepared for each formulation. Then, placed inside the test tubes. It should be noted that impregnation of food levels from control treatment started higher concentration and was applied to reduce the error. For each concentration, 45 tubes were prepared in 15 replicates. In each tube, a 4 days larva that simultaneously hatched and uniform in color and size were placed by brush on treated food. After 48 hours, the larvae fed infected food to the bacteria; they were transferred to the same test tubes with *Bt* free food. Larval mortality was recorded up to seven days. The larval mortality rate was based on blackness and lack of response of the larva to needle [9].

### ***Determination of median lethal concentration (LC<sub>50</sub>) & international toxic unit (ITU) for formulation***

First, a primary bioassay was performed on the age of two (4-days) larva of the cotton bollworm for minimum and maximum mortality. Then, seven concentrations for the volume metric test were determined to calculate LD<sub>50</sub> [9]. In this method the final formulations and formulations of Belthirul and Biolep, using artificial food on four-day larvae of cotton bollworm and their pathogenicity are investigated. The pieces of artificial food were cut to one gram and poured 10 microliters of the prepared concentrations on each section of the food using sampler, and then they were placed inside the test tube. For each concentration 45 test tubes were ranged in three replicate, in each test tube a four-days larva was placed in the test tube with a brush on the treatment food.

After 48 hours, the larval fed infected food to the bacteria then they were transferred to the same test tubes with healthy food and *Bt* free. Mortality was recorded up to seven days after the start of the test. The larval mortality rate was based on blackness and lack of response of the larva to needle. Bioassay experiments were performed as completely randomized design and the data was analyzed by SAS software (SAS®, USA). ITU formula was calculated based on the following formula, it should be noted that the commercial formulation of Belthirul was considered as standard.

$$\text{wetable powder formulated ITU} = \frac{\text{ITU standard} \times \text{LD}_{50} \text{ standard}}{\text{wetable powder formulated LD}_{50}}$$

### ***Phytotoxicity test***

The amount of phytotoxicity damage of the formulates on their buds and leaves of the tomato plants was measured by spraying of 4% concentration for 7 days in three replicates [9]. Tomato plants (*Lycopersicon esculentum* L.) (Seminis tomato f 1 8320) were grown in 10-cm plastic pots in sterile soil mix (peat-perlite-vermiculite, 55-20-25). Plants were grown in a greenhouse under natural lighting with day and night temperatures varying between 21°C and 33°C. Plants were irrigated daily. After 6 weeks the amount of phytotoxicity damage of the formulates on their buds and leaves of the tomato plants as leaf speckling, leaf margin necrosis (browning) or chlorosis (yellowing), brown or yellow leaf spots or patches, leaf cupping or twisting, plant stunting or plant death was measured by spraying of 4% concentration for 7 days in three replicates [9].

## **RESULTS AND DISCUSSION**

### ***Preparation of formulation***

At this stage, Tensiofix BCZ, Tensiofix LX, Triton X100 emulsifiers in three separate treatments 3% (weight) were added to the broth fermented and the spore-crystalline powder-drying agent was combined with the above-mentioned emulsifiers and was kept in 4° C.

In the next step other formulation materials such as bulking agent, filler and distributor materials, in three treatment of talc powder, kaolin and *diatomaceous earth*, sodium laurel sulfate was added and mixed to final formulated and to be evaluated physically, chemically and biologically in competition with commercial formulation.

**Physical and chemical test on formulation**

The tests result of suspensibility, wettability and formulation mortality (efficacy) compared with Belthirul® (Probelte Co.) was displayed in Table 1.

**Table 1.** *Suspensibility, wettability and insecticidal potential of formulations*

| Formulations                                                                               | Suspensibility<br>(% ± SE) | Wettability<br>(sec ± SE) | % mortality on<br><i>H. armigera</i> |
|--------------------------------------------------------------------------------------------|----------------------------|---------------------------|--------------------------------------|
| <i>Bt</i> + Triton X100 + Diatum + sorbic acid + sulfat loryl sodium (Formulation No. 1)   | 40 ± 1.1                   | 12 ± 0.58                 | 72 ± 1.15                            |
| <i>Bt</i> + Triton X100 + Talc + sorbic acid + sulfat loryl sodium (Formulation No. 2)     | 33 ± 1.2                   | 16 ± 1.15                 | 75.3 ± 0.58                          |
| <i>Bt</i> + Triton X100 + Kaolin + sorbic acid + sulfat loryl sodium (Formulation No. 3)   | 67 ± 0.58                  | 13 ± 1.0                  | 59.7 ± 1.55                          |
| <i>Bt</i> + Tensiofix LX + Diatum + sorbic acid + sulfat loryl sodium (Formulation No. 4)  | 34 ± 0.58                  | 15 ± 0.58                 | 57.3 ± 1.45                          |
| <i>Bt</i> + Tensiofix LX + Talc + sorbic acid + sulfat loryl sodium (Formulation No. 5)    | 20 ± 1.53                  | 19 ± 0.58                 | 75.7 ± 0.67                          |
| <i>Bt</i> + Tensiofix LX + Kaolin + sorbic acid + sulfat loryl sodium (Formulation No. 6)  | 60 ± 1.16                  | 17 ± 1.53                 | 62 ± 1.15                            |
| <i>Bt</i> + Tensiofix BCZ + Diatum + sorbic acid + sulfat loryl sodium (Formulation No. 7) | 34 ± 0.58                  | 16 ± 0.58                 | 57.3 ± 0.88                          |
| <i>Bt</i> + Tensiofix BCZ + Diatum + sorbic acid + sulfat loryl sodium (Formulation No. 8) | 20 ± 0.58                  | 15 ± 1.53                 | 63.7 ± 0.33                          |
| <i>Bt</i> + Tensiofix BCZ + Kaolin + sorbic acid + sulfat loryl sodium (Formulation No. 9) | 47 ± 0.58                  | 12 ± 0.58                 | 71.3 ± 1.2                           |
| Belthirul                                                                                  | 67 ± 0.58                  | 18 ± 0.58                 | 78 ± 1.5                             |

**Determination of LD<sub>50</sub> and ITU of formulation**

The CFU of kaolin + Triton X100 (formulation No. 3), kaolin + Lx (formulation No. 6) and Belthirol was obtained as  $5.3 \times 10^{10}$ ,  $7.7 \times 10^{10}$  and  $8.2 \times 10^{10}$ , respectively. The bioassay results after seven days were analyzed by SAS softwares and LD<sub>50</sub> of each formulation was obtained and shown in table 2.

After determining the LD<sub>50</sub> for each formulation and regarding to obtain the results, the formulations were compared with each other. LD<sub>50</sub> values for the formulation of kaolin + Triton X100 (formulation No. 3), kaolin + Lx (formulation No. 6), Belthirol were estimated as 0.28, 0.32, and 0.38, respectively. The lowest LD<sub>50</sub> in competition with other formulations was kaolin + Tritonx100 (formulation No. 3), which can be used as a superior formulation. However, But economically, considering the cost of preparing Triton X100, the kaolin + Lx formulation (formulation No. 6) is more economical and

cost-effective considering the cost price. The ITU of the kaolin + Lx formulation was found: 38000 IU mg<sup>-1</sup>.

### Phytotoxicity

In this experiment, the damage to the tomato buds and sensitive leaves measured with 4% concentration in three replicates. In sprouts, as well as young leaves of tomato plants sprayed on them for 7 days, no phytotoxicity effects as leaf speckling, leaf margin necrosis (browning) or chlorosis (yellowing), brown or yellow leaf spots or patches, leaf cupping or twisting, plant stunting or plant death were observed in any formulation.

**Table 2.** Bioassay of formulations on second larvae of cotton-boll worm.

| Pest                        | Formulation         | The number of larvae in 3 rep. and for 7 conc. | LD <sub>50</sub> mg larve <sup>-1</sup> | Confidence Interval (%95)<br>lower and upper limit | Slope       | Intercept   | df | Pr>chisq |
|-----------------------------|---------------------|------------------------------------------------|-----------------------------------------|----------------------------------------------------|-------------|-------------|----|----------|
| <i>Helicoverpa armigera</i> | Lx + Kaolin         | 315                                            | 0.32                                    | 0.22-0.39                                          | 2.92 ± 0.62 | 1.43 ± 0.24 | 16 | 0.90     |
|                             | Tritonx100 + Kaolin | 315                                            | 0.28                                    | 0.19-0.36                                          | 2.67 ± 0.52 | 1.44 ± 0.23 | 16 | 0.24     |
|                             | Belthirol           | 315                                            | 0.28                                    | 0.28-0.48                                          | 2.26 ± 0.44 | 0.95 ± 0.19 | 16 | 0.97     |

The results of the current study showed that the amount of suspensibility were 20 to 67% and the required time of wettability were 11 to 19 seconds. The formulations that contained kaolin as a filler material had the highest suspensibility. The results of the biological tests showed that the highest and lowest mortality was related to the commercial formulations of Belthirol and Biolep, respectively. Our formulations were placed between these two commercial formulations in terms of bio-efficacy. The formulations using talc powder as a filler material had a higher mortality rate on the four-day larvae of cotton bollworm. In general, out of nine formulations that have been made, formulations of kaolin + Triton X-100 (formulation No. 3), kaolin + Tensiofix Lx (formulation No. 6) (Table 1) had better result. According to Table 2 and cost-effective economically the kaolin + Tensiofix Lx formulation (formulation No. 6) was recommended as a commercially viable finding. The results of kaolin + Tensiofix Lx formulation (formulation No. 6) showed that suspensibility, wettability, LD<sub>50</sub> and its %mortality on *H. armigera* were 60%, 17 seconds, 0.32 mg larve<sup>-1</sup> and 62%, respectively.

Eski et al. determined the moisture content, suspensibility and wettability of a new wettable powder biopesticide as 8.3, 86% and 21 s, respectively [7]. The results of some wettable powder formulation of Bt have shown that the superior formulation, had 73% suspensibility and wetting time of 25 S, the results of laboratory bioassays showed that this formulation had the highest mortality rate of about 95 % [20]. The results of microencapsulation of Bt by spray drying have shown that the number of viable spores, wetting time, percentage of suspensibility and moisture content of the product produced under optimum conditions were determined as  $8.1 \times 10^{11}$  cfu g<sup>-1</sup>, 25.22 S, 77.66% and

7.29%, respectively. The  $LC_{50}$  was determined as  $1.6 \times 10^4$  cfu ml<sup>-1</sup> on *Spodoptera exigua* (Hübner) in the laboratory conditions [6]. The results of optimization of a wettable powder formulation for two native Bt strains have shown that maximum suspensibility (73 and 71%) for YD5 and KH4 strains, respectively, and 6-7% moisture content, in both formulations were stable without any changes during one year. The  $LC_{50}$  of the selected formulation for YD5 and KH4 strains against cotton bollworm larvae and elm leaf beetle was 550 and 510 ng/cm<sup>2</sup> leaf, respectively [18]. The results of spray-dried wettable powder formulations of Bt have shown that suspensibility, wettability and  $LC_{50}$  value against *Spodoptera exigua* larvae of the formulation were measured as 55%, 24 s and  $5.69 \times 10^4$  CFU/ml [22]. García Rodríguez et al. reported that Bt encapsulated with starches derived from amaranth showed a high level of insecticidal activity when tested on *Manduca sexta* larvae [8].

## CONCLUSION

In this study, the wettable powder formulation of a native *Bacillus thuringiensis* strain (KD2) was prepared in a laboratory scale fermenter. The culture medium was concentrated and spray dried. The concentrated culture medium was formulated with natural ingredients. The formulation was also optimized based on physical and biological properties of the formulated Btk-KD2. According to results, Btk-KD2 based biopesticide can be used for cotton bollworm management and can be tested against other Lepidoptera. Therefore, it reduces the use of chemical insecticides, thus reducing the resistance of pests to chemical insecticides and reducing the environmental and health risks of chemical pesticides, which is one of the concerns of farmers and human society.

**Acknowledgement.** This research was funded by Iranian National Science Foundation (INSF) Project (93028668).

## REFERENCES

- [1] Andrew, M., Whitford, F., Jordan, T. (1995): Pesticides and formulation Technology.
- [2] Arunsiri, A., Supphantharika, M., Ketunuti, U., (2003): Preparation of Spray-Dried Wettable Powder Formulations of *Bacillus thuringiensis*-Based Biopesticides. J. Econ Entomolo. 96, 292-299.
- [3] Behle, R. W. (2003): Discovery and development of biological agents to control crop pests. J. Entomol. 32, 183-195.
- [4] Brar, S. K., Verma, M., Tyagi, R. D., Valero, J. R. (2006): Recent advances in downstream processing and formulations of *Bacillus thuringiensis* based biopesticides. Process Biochemistry 41(2), 323–342.
- [5] Dastpak, V. (2012): Optimization of *Bacillus thuringiensis* biopesticide production using agricultural by-products as nutrient sources in lab. (Master's science). Science and research branch Islamic Azad university. (In Persian).
- [6] Eski, A., Demirbağ Z., Demir, İ. (2019): Microencapsulation of an indigenous isolate of *Bacillus thuringiensis* by spray drying. Journal of Microencapsulation 36(1), 1-22.
- [7] Eski, A., Demir, İ., Sezen. K., Demirbağ, Z. (2017): A new biopesticide from a local *Bacillus thuringiensis* var. tenebrionis (Xd3) against alder leaf beetle (Coleoptera: Chrysomelidae). World Journal of Microbiology and Biotechnology 33(5), 95.
- [8] García Rodríguez Gaytán Martínez, M., Barrera-Cortés, J., Ibarra, J. E., Martínez Bustos, F. (2015): Bio-insecticide *Bacillus thuringiensis* spores encapsulated with amaranth



- derivatized starches: studies on the propagation “in vitro”. *Bioprocess and Biosystems Engineering*, 38, 329–339.
- [9] Kalantari, M., Marzban, R., Magollifard, Z., Abbasipour, H. (2013): Study of virulence and molecular characteristics of some *Bacillus thuringiensis* isolates on cotton bollworm and diamondback moth. *Journal of BioControl in Plant Protection* 2(1), 17–26. (In Persian).
  - [10] Knowles, D. A. (2005): New developments in crop production, product formulation. *Agrow reports*. 349p.
  - [11] Köhl, J., Gerlagh, M., De Haas, B. H., Krijger, M. C. (1998): Biological control of *Botrytis cinerea* in cyclamen with *Ulocladium atrum* and *Gliocladium roseum* under commercial growing conditions. *J. Phytopathol.* 88, 568–575.
  - [12] Lisansky, S. G., Quinlan, R. J., Tassoni, G. (1993): The *Bacillus thuringiensis* production handbook. Newbury: CPL Press, pp. 124.
  - [13] Marzban, R., Askari, H. (2010): Microbial Pest Control Achievements, Challenges and Vision. *Proceedings of the Congress on half a century of the pesticide usage in Iran*. P. 9. (In Persian).
  - [14] Marzban, R., Salehi, J. G. (2006): Distribution of *Bacillus thuringiensis* in the Agricultural Soils of Iran. *Biotechnology, Agriculture and the Food Industry*, pp. 95-100. (In Persian).
  - [15] Marzban, R., Tajbakhsh, M. (2004): Comparison of several methods for detection and quantification of  $\beta$ -exotoxin in commercial *Bacillus thuringiensis* products. *Appl. J. Entomol. Phytopathol.* 71, 141–149. (In Persian).
  - [16] Peng, G., Wolf, T. M. (2011): Improving spray deposition on vertical structures: the role of nozzle angle, boom height, travel speed, and spray quality. *Pest Technology* 5 (Special Issue 1), 67–72.
  - [17] Saberi, F., Marzban, R., Ardjmand, M. (2014): Optimization of *Bacillus thuringiensis* production process in lab Fermenter. *Biol. Control Pests Plant Disease* 3(2): 165–172. (In Persian).
  - [18] Salehi Jouzani, Gh., Moaven, E., Morsali, H. (2014): Optimization of a wettable powder formulation for two native *Bacillus thuringiensis* strains. *Biological control of pests and plant diseases* 3 (1), 7–15.
  - [19] Satinder, K., Brar, M., Verma, M., Tyragi, R. D., Valero, J. R. (2006): Recent advances in downstream processing and formulations of *Bacillus thuringiensis* based biopesticides. *Proce. Biochemist* 41, 323-342.
  - [20] Shazdeh Ahmadi Sajjadi, A., Salehi Jouzani Gh.R., Assemi H., Shahadati Moghadam, Z. (2020): Production of wettable powder biopesticide formulation from two superior *Bacillus thuringiensis* strains native to Northern Iran. *CORESTA Congress*, Online, 2020, Agronomy/Phytopathology Groups, APPOST 02.
  - [21] Tamez-Guerra, P., et al., (2000): Assessment of microencapsulated formulations for improved residual activity of *Bacillus thuringiensis*. *Journal of Economic Entomology* 93 (2), 219–225.
  - [22] Teakle, R.E., Jensen, J.M. (1991): *Heliothis armigera*, pp. 313-332. In: Singh, p. & Moore, R.F. (eds.), *Hand Book of Insect Rearing*. Elsevier, Amsterdam.
  - [23] Teera-Arunsiri, A., Supphantharika, M., Ketunuti U. (2003): Preparation of spray-dried wettable powder formulations of *Bacillus thuringiensis*-based biopesticides. *Journal of Economic Entomology* 96 (2), 292–299.