ÇOMÜ Zir. Fak. Derg. (COMU J. Agric. Fac.)
2016: 4 (2): 95–103

Biological Control of Insect Pests Using *Trichogramma minutum* as Biological Control Agent in the Vicinity of BUITEMS

Imran Ali Sani[1*] Shahjahan Shabbir[1] Maryam Zafar[1] Nazeer Ahmed[1] Naeem Ahmed Shahwani[4] Asma Yousafzai[1] Shazia Irfan[3] Umair Ahmed[1] Salman Aziz[2] Arshad Ghani Khan[2] Mohammmad Nauman Irshad[2] Daniyal Jhon[1] Rida İbrar[1]

[1]Faculty of Life Sciences and Informatics Baluchistan University of Information Technology Engineering and Management Sciences, Quetta Pakistan
[2]Balochistan Agriculture College Quetta, Pakistan
[3]Sardar Bahadur Khan Women University Quetta, Pakistan
[4] Offices of Research Innovation and Commercialization, BUITEMS Pakistan
[*]Corresponding author: immesani@gmail.com

Recieved: 05.01.2017 Accepted: 15.03.2017

Abstract

In a natural environment, plants are continuously being attacked by different kinds of pests and pathogens such as bacteria, fungi and insects. On the other hand naturally plants can survive with the help of natural enemies of these unwanted pests and pathogens. In this way, population developments of all players in a natural environment are controlled. The objective of the study is to rear the *Trichogramma minutum* under laboratory conditions on eggs of *Sitotroga cerelalla*, collect their eggs on cards and then apply them in the vicinity of BUITEMS against aphids, jasids, borers and thrips. Trichogramma is a mini wasp belongs to family Trichogrammatidae which parasitized the eggs and adults of many pests, especially eggs of moths and butterflies. Various species and strains of *Trichogramma* tag and destroy different host eggs and prefer different crop habitats and have distinct searching abilities and strength to weather conditions. In this study *Trichogramma minutum* was reared in laboratory on eggs of Angoumois grain moth (*Sitotroga cerelalla*) under 25–32°C temperature and 55–70% relative humidity.

Keywords: Pest, Biocontrol, *Trichogramma minutum*, egg parasitoid, *Sitotroga cerelalla*

Zararlı Böceklerin Biyolojik Mücadelesinde *Trichogramma minutum*'un BUITEMS Kampüsün Civarında Biyolojik Mücadele Etmeni Olarak Kullanılması

Öz

Doğal bir ortamda bitkiler sürekli bakteriler, funguslar ve böcekler gibi çeşitli patojenler ve böcekler tarafından saldırıya uğramaktadır. Öte yandan doğal olarak bitkiler, istenmeyen zararlıların ve patojenlerin doğal düşmanlarının yardımıyla hayatta kalabilmektedirler. Böylece, bütün zararlıların populasyon gelişmeleri doğal bir ortamda kontrol altında tutulmaktadır. Bu çalışmanın esas amacı *Trichogramma minutum*'u laboratuar koşullarında *Sitotroga cerelalla*'nın yumurtaları üzerinde yetiştirilmek, daha sonra elde edilmiş yumurtaları kartonlarda toplamak ve BUITEMS kampüsün civarında bulunan yaprak bitleri, pireler, keseci kurtlar ve tripsler gibi zararlı böceklere karşı biyolojik mücadele etmeni olarak uygulanmaktı. Trichogrammatidae familyasına ait olan *Trichogramma* çok küçük parazit bir böceğidir ve birçok zararlıların yumurta ile erginlerini, özellikle güvelerin ve kelebeklerin yumurtaları, parazitlemektedir. *Trichogramma*'nın farklı tür ve suşları çeşitli konukçu yumurtaları etiketleyip yok eder ve hava koşullarına göre farklı bitki habitatlarını tercih ederek belirgin arama yetenekleri ve dayanıklılığına sahiptir. Bu çalışmada, *Trichogramma minutum*, 25–32 °C sıcaklık ve %55–70 oransal nem laboratuvar koşulların altında *Sitotroga cerelalla* yumurtalar üzerinde yetiştirilmiştir.

Anahtar Kelimeler: Zararlı, biyolojik mücadele, *Trichogramma minutum*, yumurta parazitoidi, *Sitotroga cerelalla*

Introduction

In recent decades, the awareness of harmful effects of chemical pesticides on plants and more importantly environment and human health have taken the attention of researchers to find other ways of pest control then using chemical agents. Moreover the resistance created in many pests has also resulted in the need to switch from chemical pesticides. Indicated by a report, submitted at the U.S Congress, Office of Test & Assessments (OTA), the use of biological agents could result in better control and overcoming the issues occurring in traditional pest control methods (Oliveira *et al*., 2003).Pests have been a problem since the evaluation of life. They have been a bigger trouble since the start of agriculture and farming. With the passing time, researchers have been in search of ways to control the pest's problem. From the use of fire to the use of chemicals and poisonous plants, the discovered methods have been made more and more effective. Chemical agents have been widely used and are still dominant but as we know, living species evolve themselves. Factors like increasing tolerance towards the chemical pesticides and the long term effects of such agents on environment, nature and human health (chemical pesticides making the farmed food unhealthy, and the chemicals reacting with air and generating harmful effects), have driven the attention towards a more liable and advantageous method, such as biological control method (Blibech *et al*.,2015). The biological methods are the methods that use other living organisms against pests with the provision of minimal harm to the health and environment. This method is an important component of the Integrated Pest Management (IPM) (Anonymous, 2016)

Keeping in mind that biological enemies of pests naturally occur and prey over them. This phenomenon is called Natural Pest Control. Biological pest control uses these biological agents are used with measured parameters and some human effort to eliminate the threat of pests. These biological agents are categorized as predators, parasites and pathogens and the agents of plant diseases are called antagonists. A beneficial biological agent should have:

1. A high reproductive rate.
2. Host specific
3. Flexible to different environmental conditions.
4. A good agent should reproduce and survive at low prey density.

A good biological agent would possess the above requirements thus making this method effective and beneficial as it would be a onetime implementation. There is no need for the reapplication of pesticide and the agents would establish themselves, giving a self–continuing form of control.

In our study *Trichogramma minutum* as biocontrol agent was used to control insect pests in vicinity of BUITEMS, reared on the eggs of *Sitotroga cerelalla*. Trichogramma are mini wasp belongs to family Trichogrammatidae about 0.5mm long (Laing *et al*., 1990). The adult female lay her eggs on other moth's eggs. An individual adult female can lay up to 300 eggs depending on specie of Trichogramma used and size of host's egg.

The larvae feed on the egg and then emerge as adults. The larvae take 10 days to develop within the pest moth egg, which turns brown or black as the larvae pupate. The adult wasps live anywhere from 7 to 14 days, depending on temperature and moisture (Pratissoli *et al*., 2004).

Sitotroga cerelalla an Angoumois grain moth used in our study to rear *Trichogramma minutum* in laboratory belongs to primary category of cereal grain pests that attack cereals both in field and storage. Major crops that are affected by the Angoumois grain moth are maize, oats, barley, rice, pearl millet, rye, sorghum and wheat. The adult moth is very small, have a wingspan of about 10–20 mm and 5–7 mm long with its wings folded (Cerealella and Steve Francia, 2015). Their whole life cycle completed in 4–5 weeks. Each Female lays 100–180 eggs on cereal seeds. The project was funded through Office of Research, Innovation and Commercialization (ORIC) with the purpose to prohibit the use of pesticides because of problems faced by the students and employees in BUITEMS ranging from minor problems like eye irritation, headache, nausea, dizziness and fatigue to severe cancer, reproductive and endocrine problems.

Materials and methods

The study was conducted at BUITEMS Quetta, Pakistan for mass production of *Trichogramma minutum* on the eggs of *Sitotroga cerelalla* under controlled conditions at 25–32°C temperature and 55–70% relative humidity during the period from July to October 2015. The study was based on four major steps described below. The control percentage of all insect pests in vicinity of BUITEMS after the releases of *Trichogramma minutum* was calculated by the formula:

Control rate = killed pests / total pests * 100

Rearing of *Sitotroga cerelalla*

The mass production of different species of *Trichogramma* on the eggs of their natural hosts is a common practice and started in 1930 in USA on the eggs of *Sitotroga cerelalla* an Angoumois grain moth (Flanders and Quednau, 1960). In this study the eggs of *Sitotroga cerelalla* is used for mass production of *Trichogramma minutum* for this purpose on 2 July,2015 wheat was taken after careful cleaning 800–1000 grains of wheat were kept in 60 glass jars along with eggs and adults of *Sitotroga cerelalla*. One large wheat grain is enough to provide sufficient food to one larva of *Sitotroga* (Hassan, S. and Gerding, P, 1994). After 28 days on 30 July, 2015 some adults were observed in two jars. Mating between newly emerged adults was started after 11 days on 10 august, then 3 days later all 60 jars have a growth. The relative humidity was maintained to 55–70% by spreading wet cotton on daily basis at 25–32°C temperature.

Rearing of *Trichogramma minutum*

On 18 September, 2015 four commercially prepared cards of eggs of *Trichogramma minutum* were shifted in four glass jars out of 60 jars the temperature and humidity were maintained same. There are approximately 100,000 eggs per card. After two weeks we found 100% control because all the *Sitotroga* were killed in four jars which means the wheat in these jars contain eggs of *Trichogramma minutum.*

Collection of eggs of *Trichogramma*

For collection of eggs of *Trichogramma* on 5 October a thick coat of glue was applied on 550 three by three sized cards and the eggs were sprinkled equally in a single layer with the help strainer. Each card have approximately 250 to 300 eggs. Allowed the cards to dry after drying the cards were placed in a polythene bag and refrigerated to delay the emergence of adults.

Releasing cards in the field

Adults and eggs of aphids, jasids, borers and thrips on roses (2000), geranium (500), jasmine (39), grapes (58), apricot (15) and pomegranate (55). On 9 October early in the morning the cards per stapled randomly under the side of leaves to avoid direct exposure of eggs from sun in the vicinity of BUITEMS. *Trichogramma* releases on cards in pupal stage. In two to three days convert into adults search out eggs of the pests and destroy them. Two observations were taken with the interval of 10 days. First observation on 19 October and second on 29 October.

Results

Release of *Trichogramma minutum* against aphids on pomegranate trees

Before applying any treatment the average of aphid count per pomegranate tree was 1500, in 55 trees of pomegranate aphid count estimate was 82500. After applying treatment, the control percentage was 80% calculated by the formula

Control rate = killed pests / total pests * 100

Because after 1st observation average of aphid count per pomegranate tree was reduced to 300. After 2nd observation 10 days later the control percentage was 90% calculated with same formula because the average of aphid count per pomegranate tree was more reduced to 30 per plant of pomegranate. The graphs below represents the total number of aphids in 55 trees of pomegranate, aphids dead and alive after 1st and 2nd observation.

Release of *Trichogramma minutum* against aphids on roses

Before applying any treatment the average of aphid count per rose plant was 1500, in 2000 plants of roses aphid count estimate was 1100000. After applying treatment, the control percentage was 80% calculated by the formula

Control rate = killed pests / total pests * 100

Because after 1st observation average of aphid count per rose plant was reduced to 110. After 2nd observation 10 days later the control percentage was 90% calculated with same formula because the average of aphid count per pomegranate tree was more reduced to 11 per plant of rose. The graphs below represents the total number of aphids in 2000 plants of roses, aphids dead and alive after 1st and 2nd observation.

Release of *Trichogramma minutum* against aphids on geranium

Before applying any treatment the average of aphid count per geranium plant was 225, in 500 trees of geranium aphid count estimate was 112500. After applying treatment, the control percentage was 80% calculated by the formula

Control rate = killed aphids / total aphids * 100

Because after 1st observation average of aphid count per geranium plant was reduced to 45. After 2nd observation 10 days later the control percentage was 90% calculated with same formula because the average of aphid count per geranium plant was more reduced to 1 per plant of geranium.

In grapes, apricot and jasmine there was no attack of aphids. The graphs below represents the total number of aphids in 500 plants of geranium, 55 plants of pomegranate and 2000 plants of roses aphids dead and alive after 1st and 2nd observation.

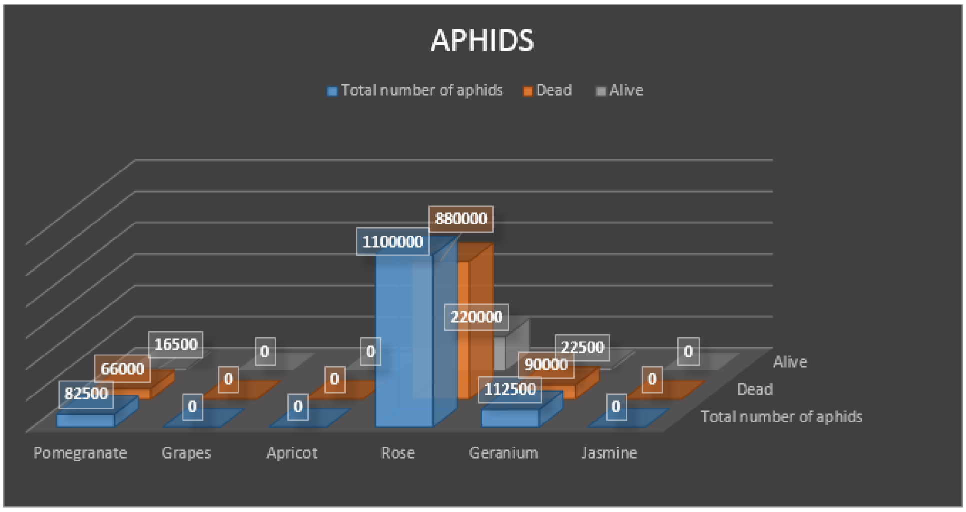

Figure 1. Effect of *Trichogramma minutum* on aphids on six different types of plants under study after 1st observation

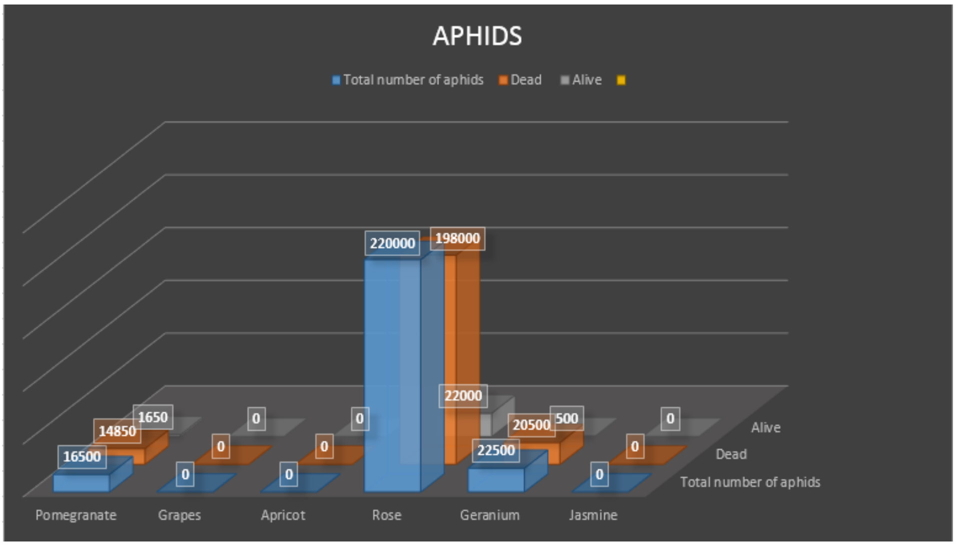

Figure 2. Effect of *Trichogramma minutum* on aphids on six different types of plants under study after 2nd observation

Release of *Trichogramma minutum* against jasids on pomegranate

Before applying any treatment the average of jasids count per pomegranate plant was 250, in 55 plants of pomegranate jasids count estimate was 13750. After applying treatment, the control percentage was 80% calculated by the formula

Control rate = killed jasids / total jasids * 100

Because after 1st observation average of jasids count per pomegranate plant was reduced to 50. After 2nd observation 10 days later the control percentage was 90% calculated with same formula because the average of jasids count per pomegranate tree was more reduced to 5 per plant of pomegranate. The graphs below represents the total number of jasids in 55 plants of pomegranate, jasids dead and alive after 1st and 2nd observation.

Release of *Trichogramma minutum* against jasids on roses

Before applying any treatment the average of jasids count per rose plant was 125, in 2000 plants of roses jasids count estimate was 250000. After applying treatment, the control percentage was 80% calculated by the formula

Control rate = killed jasids / total jasids * 100

Because after 1st observation average of jasids count per rose plant was reduced to 23. After 2nd observation 10 days later the control percentage was 90% calculated with same formula because the average of jasids count per rose was more reduced to 2 per plant of rose. The graphs below represents the total number of jasids in 2000 plants of roses, jasids dead and alive after 1st and 2nd observation.

Release of *Trichogramma minutum* against jasids on geranium

Before applying any treatment the average of jasids count per geranium plant was 65, in 500 trees of geranium jasids count estimate was 32500. After applying treatment, the control percentage was 80% calculated by the formula

Control rate = killed jasids / total jasids * 100

Because after 1st observation average of jasids count per geranium plant was reduced to 13. After 2nd observation 10 days later the control percentage was 90% calculated with same formula because the average of jasids count per geranium plant was more reduced to 1 per plant of geranium. The Figure 3 represents the total number of jasids in 500 plants of geranium, jasids dead and alive after first and second observation.

In grapes, apricot and jasmine there was no attack of jasids. The graphs below represents the total number of jasids in 500 plants of geranium, 55 plants of pomegranate and 2000 plants of roses jasids dead and alive after 1st and 2nd observation.

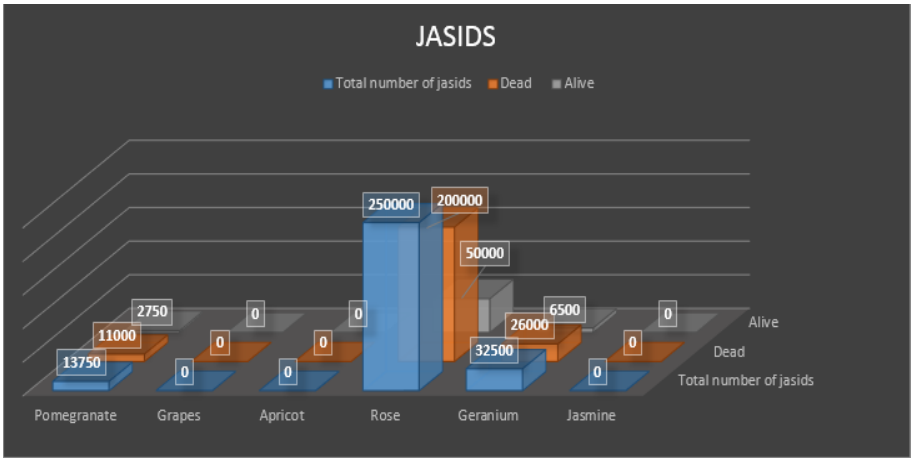

Figure 3. Effect of *Trichogramma minutum* on jasids on six different types of plants under study after 1st observation

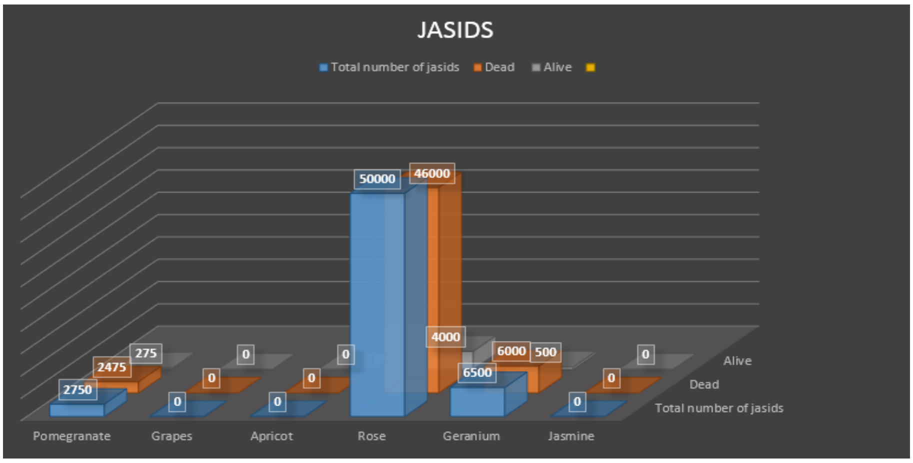

Figure 4. Effect of *Trichogramma minutum* on jasids on six different types of plants under study after 2nd observation

Release of *Trichogramma minutum* against thrips on roses

Before applying any treatment the average of thrips count per rose plant was 225, in 2000 plants of roses jasids count estimate was 450000. After applying treatment, the control percentage was 80% calculated by the formula

Control rate = killed thrips / total thrips * 100

Because after 1st observation average of thrips count per rose plant was reduced to 45. After 2nd observation 10 days later the control percentage was 90% calculated with same formula because the average of thrips count per rose was more reduced to 1 per plant of rose. The graphs below represents the total number of thrips in 2000 plants of roses, thrips dead and alive after 1st and 2ndobservation.

Release of *Trichogramma minutum* against thrips on geranium

Before applying any treatment the average of thrips count per geranium plant was 73, in 500 plants of geranium thrips count estimate was 36500. After applying treatment, the control percentage was 80% calculated by the formula

Control rate = killed thrips / total thrips * 100

Because after 1st observation average of thrips count per geranium plant was reduced to 15. After 2nd observation 10 days later the control percentage was 90% calculated with same formula because the average of thrips count per geranium plant was more reduced to 1 per plant of geranium. In pomegranate, grapes, apricot and jasmine there was no attack of thrips. The graphs below represents the total number of thrips in 500 plants of geranium and 2000 plants of roses thrips dead and alive after 1st and 2nd observation.

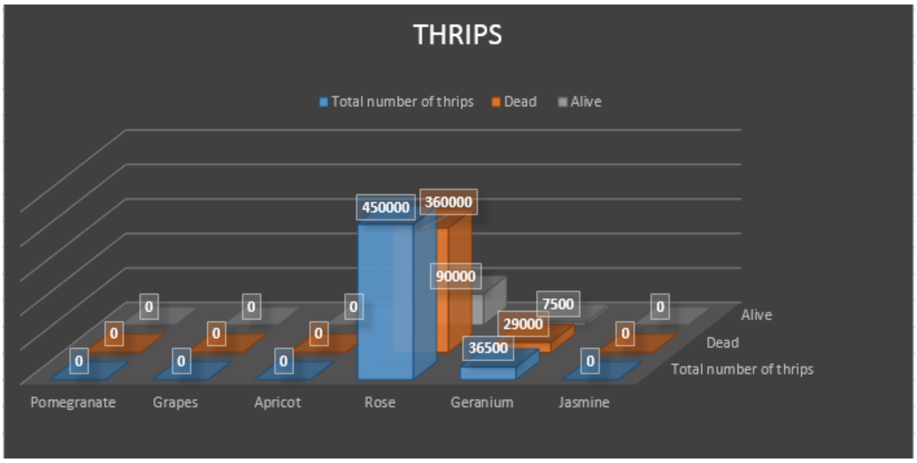

Figure 5. Effect of *Trichogramma minutum* on thrips on six different types of plants under study after 1st observation

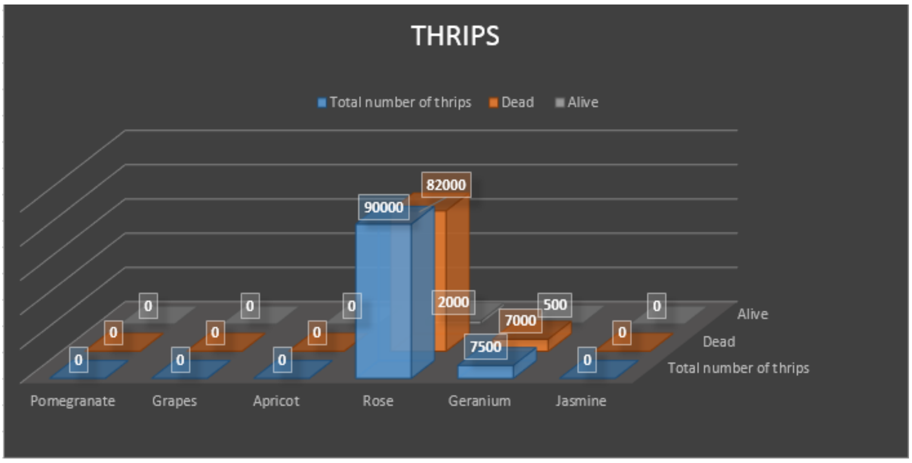

Figure 6. Effect of *Trichogramma minutum* on thrips on six different types of plants under study after 2nd observation

Release of *Trichogramma minutum* against borers on apricot

Before applying any treatment the average of borers count per apricot tree was 50, in 15 trees of geranium borers count estimate was 750. After applying treatment, the control percentage was 80% calculated by the formula

Control rate = killed borers / total borers * 100

Because after 1st observation average of borers count per apricot tree was reduced to 10. After 2nd observation 10 days later the control percentage was 90% calculated with same formula because the average of borers count per apricot tree was more reduced to 1 per tree of apricot.

Release of *Trichogramma minutum* against borers on jasmine

Before applying any treatment the average of borers count per jasmine tree was 50, in 39 trees of jasmine borers count estimate was 1950. After applying treatment, the control percentage was 80% calculated by the formula

Control rate = killed borers / total borers * 100

Because after 1st observation average of borers count per jasmine tree was reduced to 10. After 2nd observation 10 days later the control percentage was 90% calculated with same formula because the average of borers count per jasmine tree was more reduced to 1 per tree of apricot. In pomegranate, grapes, roses and geranium there was no attack of borers. The graphs below represents the total number of borers in 39 trees of jasmine and 15 trees of apricot dead and alive after 1st and 2nd observation.

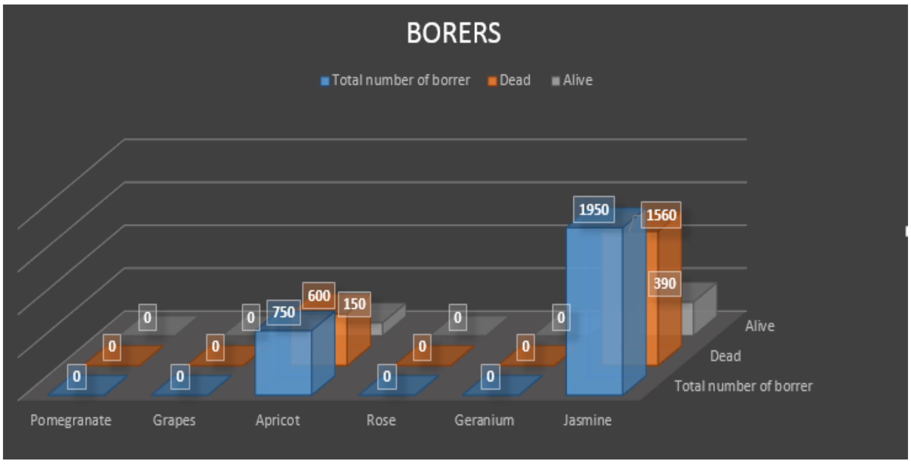

Figure 7. Effect of *Trichogramma minutum* on borers on six different types of plants under study after 1st observation

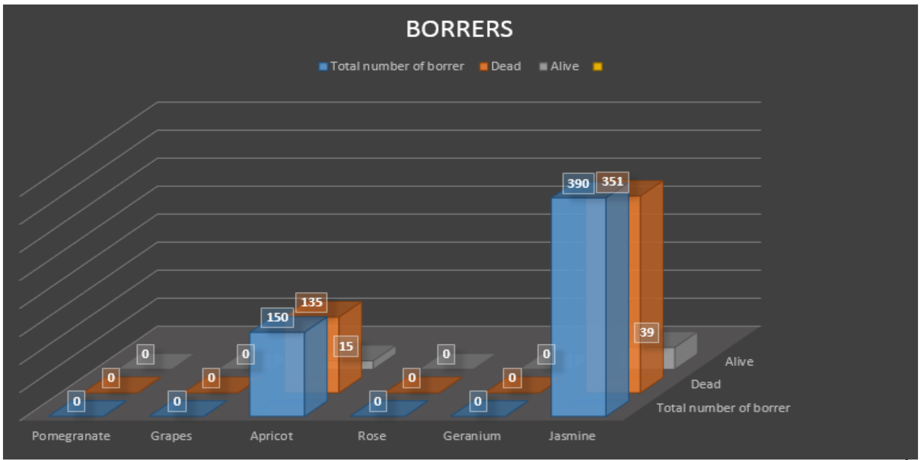

Figure 8. Effect of *Trichogramma minutum* on borers on six different types of plants under study after 2nd observation

Discussion

The use of pesticides to kill unwanted insects is a common practice all around us for example in gardens, institutes, parks and agricultural fields etc. These chemicals kills unwanted pests but also cause many environmental and health concerns. Similar health problems were faced by the students and employees in BUITEMS ranging from minor problems like eye irritation, headache, nausea, dizziness and fatigue to severe cancer, reproductive and endocrine problems. Because of such problems since one year the use of pesticides was prohibited. Now as the use of our biocontrol agent has given 90% control against observed insect pests. The project funded through ORIC provided significant results, so why not to use natural enemies of insect pests as all insects have natural enemies. The management of natural enemies of these insect pests is very important part of biocontrol and easily available when required. The future of biocontrol is auspicious but there are still many problems that need to be overcome. We must educate the farmers and people related to agriculture about importance of biocontrol instead of pesticides, identification of natural enemies, their conservation and their presence in the field etc.

Conclusion

However controlling insect pests especially moths and caterpillars using *Trichogramma* species is very effective and give significant results but there are still major obstacles and challenges that researchers face. Such challenges are:

- The genetic changes and modification often occur during mass production must be identified and understood in order to get more efficient results.
- Determine relation between released parasites and their effect on population of pests.
- Establish powerful processes of mass production of *Trichogramma*, storage, shipment and release in field.
- Proper management system that reduce or remove pesticides oppositions with natural enemies of insect pests.
- Choice of most suitable species of *Trichogramma* or other natural enemies.

References

Anonymous; 2016. Integrated Pest Management: Innovation–Development Process. 2009.Biological Control and Integrated Pest Management, p.207. https://www.citethisforme.com/cite/journal/autocite (Accessed 22 Jan. 2016).

Akhter, T., Jahan, M., Bhuiyan, M., 2013. Biology of the Angoumois Grain Moth, *Sitotroga Cerelalla* (Oliver) On Stored Rice Grain in Laboratory Condition. *J. Asiat. Soc. Bangladesh, Sci.* 39(1): 61–67.

Bai, B., Smith, S., 1993. Effect of host availability on reproduction and survival of the parasitoid wasp *Trichogramma minutum*. *Ecol. Entomol.* 18(4): 279–286.

Blibech, I., Ksantini, M., Jardak, T., Bouaziz, M., 2015. Effect of Insecticides on *Trichogramma Parasitoids* Used in Biological Control against *Prays oleae* Insect Pest. *ACES*. 5: 362–372.

Bryan, M.D., Dysart, R.J., Burger, T.L., 1993. Releases of introduced parasites of the alfalfa weevil in the United States, 1957–88. *Miscellaneous publication/United States Department of Agriculture. Animal and Plant Health Inspection Service (USA)*.

Bushra, S., Aslam, M., 2014. Management of *Sitotroga cerelalla* in stored cereal grains: a review. *Arch. Phytopathol. Pfl*, 47(19): 2365–2376.

CABI, 2007. *Sitotroga cerelalla* (Angoumois grain moth) datasheet. Crop Protection Compendium, 2010 Edition. CAB International l Publishing. Wallingford, UK.

Caltagirone, L., 1981. Landmark Examples in Classical Biological Control. *Annu. Rev. Entomol.* 26(1): 213–232.

Cerelalla, S., Francia, S., 2015. *Sitotroga cerelalla* Plant Pests of the Middle East. http://www.agri.huji.ac.il/mepests/pest/Sitotroga_cerealella/ (Accessed 20 Jan. 2016).

Consoli, F.L., Parra, J.R.P., Zucchi, R.A., 2010. Egg parasitoids in agroecosystems with emphasis on *Trichogramma*. Progress in Biological Control. Springer, Heidelberg, London.

Debach, P., Rosen, D., 1991. Biological Control by Natural Enemies. Cambridge Univ. Press, Cambridge, UK. 440 pp.

Flanders, S., Quednau, W., 1960. Taxonomy of the genus Trichogramma (Hymenoptera, Chalcidoidea, Trichogrammatidae). *Entomophaga*. 5(4): 285–294.

Hassan, S., Gerding, P., 1994. Production of the Angoumois grain moth Sitotroga cerelalla (Oliv.) as an alternative host for egg parasites Producción y utilización de Trichogramma para el control biológico de plagas. Chillán: Taller International, pp.20–26.

Hoffmann, M., Ode, P., Walker, D., Gardner, J., van Nouhuys, S., Shelton, A., 2001. Performance of *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae) Reared on Factitious Hosts, Including the Target Host, *Ostrinia nubilalis* (Lepidoptera: Crambidae). *Biol. Control*. 21(1): 1–10.

Hussey, N. W., Scopes, N., 1985. Biological pest control: the glasshouse experience. Cornell Univ. Press, Ithaca.

Laing, J.E., Eden, G.M., 1990. 2.0 Mass–production of Trichogramma *minutum* Riley on factitious host eggs. *Mem. Entomol. Soc. Can.* 122(S153):10–24.

Oliveira, H.N., Zanuncio, J.C., Pratissoli, D., Picanço, M.C., 2003. Biological characteristics of *Trichogramma maxacalii* (Hymenoptera: Trichogrammatidae) on eggs of *Anagasta kuehniella* (Lepidoptera: Pyralidae). *Braz. J. Biol. 63*(4): 647–653.

Pratissoli, D., Oliveira, H., Gonc¸alves, J., Zanuncio, J., Holtz, A., 2004. Changes in Biological Characteristics of *Trichogramma pretiosum* (Hym. Trichogrammatidae) Reared on Eggs of Anagasta kuehniella (Lep: Pyralidae) for 23 Generations. *Biocontrol. Sci.Tech.* 14(3):313–319.