Toxicity of insecticides and impact of gamma radiations on egg parasitoids

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Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir

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THESIS

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Faculty of Horticulture

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(Entomology)

2023

DEDICATION

Dedicated

to

My Beloved Parents

and

Research Guide

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Division of Entomology, Faculty of Horticulture

Certificate – I

This is to certify that the thesis entitled "**Toxicity of insecticides and impact of gamma radiations on egg parasitoids**" submitted in partial fulfilment of the requirements for the award of the degree of **Master of Science in Agriculture (Entomology),** to the **Faculty of Horticulture, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir** is a record of bonafide research work carried out by **Ms. Gayathri Kishore (Regd. No. MSH-2021-419)** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that any help or information received during the course of investigation has duly been acknowledged.

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We, the members of the Advisory Committee of **Ms. Gayathri Kishore (Regd. No. MSH-2021-419)** a candidate for the degree of **Master of Science in Agriculture (Entomology)** have gone through the manuscript of the thesis entitled, "**Toxicity of insecticides and impact of gamma radiations on egg parasitoids**" and recommend that it may be submitted by the student in partial fulfilment of the requirements for the award of the degree.

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This is to certify that the thesis entitled, "**Toxicity of insecticides and impact of gamma radiations on egg parasitoids**" submitted by **Ms. Gayathri Kishore (Regd. No. MSH-2021-419)** to the **Faculty of Horticulture, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir** in partial fulfilment of the requirements for the award of the degree of **Master of Science in Agriculture (Entomology)** was examined and approved by the Advisory Committee and External Examiner on …………….

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Title of the Thesis : **"Toxicity of insecticides and impact of gamma radiations on egg parasitoids"**

ABSTRACT

Among various parasitoids to manage various insect pests, *Trichogrammatids* are distributed all over Indian subcontinent to control important insect pests in various crops in India. Pesticides affect natural enemies by contact with treated plants during foraging, feeding on contaminated water or pollen available on the leaf surface. So the impact of pesticides and gamma radiations along with the suitability of eggs by *Trichogramma* species were studied. In the two bioassay methods dry residue method and filter paper method thiamethoxam 25% WG recorded the highest corrected mortality on adults of *Trichogramma chilonis*. Thiamethoxam 25% WG recorded the highest corrected mortality of 87.83 % followed by 72.71 % mortality with bifenthrin 10% EC while as, the lowest corrected mortality of 42.32 % was recorded in NSKE 5% @4ml/ L. The LC_{50} associated with the treatments revealed that the thiamethoxam 25% WG showed the effective results. The highest mortality was recorded @ 100Gy and 200Gy doses to the adults of *Trichogramma pretiosum* and the LC_{50} associated with the doses of gamma radiations revealed that 100Gy and 200 Gy showed highest mortality. At 5Gy dose of radiation, 5.00 % mortality was recorded while 25 Gy dose resulted in 13.33 % mortality while, 20 % mortality obtained @ 50 Gy. While, highest mortality of 98.33 and 100.00 % was recorded @ 100Gy and 200Gy doses to the adults of *Trichogramma pretiosum* . Aged eggs of *Corcyra cephalonica* are least preferred by the egg parasitoids. Washing of eggs of *Corcyra cephalonica* decrease the preference of egg parasitoids. The one day old eggs of *Corcyra* was preferred by both *T.pretiosum* and *T.bactrae* while two day old eggs were preferred by *T.chilonis* from the goodness of fit test. (*p*-value < 0.05).

Keywords: Bioassay, goodness of fit test, LC_{50} , mortality, parasitoids.

Signature of Student

Dated : ___________

Signature of Major Advisor

Dated: ________________

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Gayathri Kishore

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Chapter 1

INTRODUCTION

Biological control is an essential component in diverse ecosystems to avoid unwanted effects and impacts on non target organisms (Wang *et al*., 2019; Vincent *et al.,* 2001). Egg parasitoids are effective in comparison to chemical insecticides (Nicot 2011). *Trichogramma* spp. are important biological control agents (Van-Lenteren, 2003). *Trichogramma* spp. (Hymenoptera: Trichogrammatidae), are tiny wasps to parasitise especially eggs of lepidopteran pests across the globe (Zang *et al*., 2020; Pinto and Stouthamer 1994; Smith 1996; van lenteren 2000). In Canada, *Trichogramma* are mainly deployed manually on trichocards and are efficient means of releasing *Trichogramma* under field conditions (Dionne *et al.,* 2018). The tolerance to environmental changes, ability for mass rearing, the capacity to kill their hosts before damage and host specific that make *Trichogramma* spp. a valuable biological control agents (Orr *et al*.,2000; Parra 2019).

Trichogramma spp. are well organized insect nurseries across the globe and mass released by adopting novel methods in the incursion areas (Li *et al*., 2002; Song *et al*., 2015; Zhang *et al*.,2018) to manage various insect pests. They control more than 400 host species of different insect orders (Schindler *et al.,* 2022; Lingathurai *et al.,* 2015; Khan 2019). Different species of egg parasitoids sucessfully released in 30 countries on an estimated area of 80 million acres in diverse ecosystems (Li, 1994; Khan *et al.,* 2015) in corn, rice, cotton, sugar-beet, tomatoes, vegetables, and other fruit crops. (Hassan, 1993; Smith, 1996; Khan *et al.,* 2015*)*. The host species characteristics dramatically affect the fitness of *Trichogramma* parasitoids, in terms of their size, longevity, fecundity and host acceptance (Alsaedi *et al.,* 2017; Thiéry *et al.,* 2017; Li *et al*., 2019)

Synthetic insecticides are in use since last five decades to manage different insect pests. The side effects caused insecticides forced the pest managers, ecosystem planners to search for other sustainable alternatives to avoid deleterious impacts on environment, human health and other hazards. (Gerhardson, 2002). Though various biopesticides and bioagents are employed in comparison to chemical insecticides (Waheb, 2009). Among various parasitoids to manage various insect pests, *Trichogrammatids* are distributed all over Indian subcontinent (Manjunath *et al.,* 1985; Ananthakrishnan *et al.,* 1991; Khan *et al.,* 2014) to control important insect pests in various crops (Singh, 2001)in India. Pesticides affect natural enemies by contact with treated plants during foraging, feeding on contaminated water or pollen available on the leaf surface (Alcántara *et al*., 2017).

Undoubtedly., synthetic insecticides interfere feeding behaviour of insects and disrupt sex communications (Desneux *et al.,* 2007 ; Delpuech *et al.,* 1998) in *Trichogramma* males (Delpuech *et al.,* 1999). In most of the studies, insecticides cause mortality to egg parasitoids., while, sub lethal doses of insecticides, affect their development, behaviour and reproduction and performance (Desneux *et al.,* 2007; Hewa Kapuge *et al.,* 2003). Besides direct mortality caused by pesticides also affect their biology, life cycle, parasitism rate, longevity, sex ratio, adult emergence, intra-specific communication and mating (Khan *et al*., 2015; Zanuncio *et al*., 2016). The immature stages of egg parasitoids, live and feed within the host eggs and die along with them (Parreira *et al*., 2018a, Parreira *et al*., 2018b) due to massive application of pesticides. While, perusal of the literature revealed very scanty research work available on the lethal and sub lethal doses of insecticides on egg parasitoids (Ciancio *et al.,* 2007). It is very germane to evaluate the less effective insecticides against different egg parasitoids. Chemical control is very important in agriculture and efforts were made to assess the effectiveness of major insecticides (Janssen and van rijin 2021;Singh *et al*., 2012) in India for the management of various insects. The use of non-selective insecticides greatly reduce the beneficial potential of the biocontrol agents, especially *Trichogramma* spp., which are highly susceptible to insecticides than their hosts (King and Dunkin, 1986; Mahankuda and Sawai, 2020;Thangavel *et al*., 2018).

Various species of *Trichogramma* are raised to manage various insect pests (Hoffmann *et al*., 2001; Li, 1994) in various laboratories and insect nurseries. Among various egg parasitoids, *Trichogramma chilonis* is highly efficient with other compatible strategies (Schindler Bracha *et al.,* 2022 Muhammad *et al.* 2008). Insecticides reduce the efficiency of natural enemies, directly killing them or adverse effect on their growth, behaviour, foraging and mobility (Jepson 1989; Croft 1990; Mates *et al.,* 2012). Several insecticides are now banned in developing as well as in developed countries. While, various insecticides are considered as less dangerous to non-target organisms and natural enemies based on their residual toxicity to target as well as to non-target organisms. The inherent toxicity of insecticides, their formulations and, concentrations, timing, pattern of applications, and different climatic conditions (Temperate, tropical, subtropical, arid) influence different egg parasitoids, other parasitoids and insect predators exponentially (Lou *et al.,* 2014, Wajenberg *et al*., 1994; Hussain *et al*., 2015). Annually, egg parasitoids experimentally showed 50% parasitism against various lepidopteran pests (Davies *et al.,*2006a, Davies *et al*., 2009b) in more than 50 countries over to 32 million hectares., However, most of the crops are attacked by the number of insect pests and the egg parasitoids are not effective for other insect pests. So it is necessary to evaluate insecticides which might be safer to these egg parasitoids and simultaneously effective to other insect pests on the target crops. With an aim to provide relief to the growers, reduce pesticide pressure and overall safety to other natural enemies and environment, the present investigations "Toxicity of insecticides and impact of gamma radiations on egg parasitoids" was carried out with the following objectives:

1. To study the toxicity of insecticides on egg parasitoids.
2. To study the impact of doses of gamma radiations on egg parasitoids.
3. To study the preference and performance of different egg parasitoids on eggs of *Corcyra.*

Chapter 2

REVIEW OF LITERATURE

Perusal of the literature on the research problem titled as "Toxicity of insecticides and impact of gamma radiations on egg parasitoids" is detailed below:

2.1 Bioefficacy of insecticides

Asrar *et al.* (2022) evaluated neem seed extract and commonly used insecticides *viz*; buprofezin, lufenuron, methoxyfenozide, pyriproxyfen of which lufenuron and neem seed extract as least toxic, methoxyfenozide and buprofezin as moderately toxic and pyripoxyfen exhibited the highest toxicity after 4 hrs of exposure.

Khan (2022) applied selected pesticides to record acute mortality and parasitism to different stages of parasitoids *viz*; egg, larval and pupal stages. The parasitized host eggs dipped in formulated solutions of acetamiprid, fipronil and abamectin and spinetoram. All the treatments showed the acute mortality and parasitism as; acetamiprid (≤ 83.1 and ≤ 60.9%), fipronil (≤ 42.3and ≤ 72.7%) and abamectin (≤ 17.2 and ≤ 52.6%) spinetoram ≤ 33.9%, respectively. The adults of *Trichogramma chilonis at* the recommended field dosage of acetamiprid, spinetoram and fipronil after 24 hours showed that; fipronil induced > 95%, mortality followed by abamectin (71.3–83.5%) in four residual-age treatments. While, Khan (2019) evaluated selected pesticides to manage key insect pest complex of cotton and their cumulative effect on *T. chilonis mortality*.Besides,Similar studies revealed that chlorantraniliprole showed a mean emergence of *T. chilonis* (79--82% and 87–91%) at both 6.25x and 9.4x insecticides doses, both at larval and pupal stages of parasitoids, respectively. These findings are in agreement of Saour (2019) .

Duraimurugan and Lakshminarayana (2018) determined the toxicity of different insecticides *viz*; *Btk* formulations, flubendiamide, chlorantraniliprole, indoxacarb and novaluron, showed lesser effect on adult emergence and harmless (< 30% mortality) against *T. chilonis.*

Hussain *et al.* (2015) conducted field experiment to determine the effectiveness of insecticides *viz* a *viz* Proclaim 1.9EC, Match 50EC, Triflumuron 20SC and Imidacloprid 200SL against *T. Chilonis and recorded* adult emergence of 42.50-63.50% and is being considered safe to the adult parasitoid.

The experiments of Wang *et al. (*2012) evaluated the acute toxicity (membrane method of bioassay) of ten conventional insecticides to *T. chilonis* Ishii adults and their sublethal effects on the parasitoids in vitro. Besides, the sublethal concentration of the given insecticides revealed that the adult *T. chilonis* were most susceptible to chlorfenapyr, followed by fipronil, spinosad, avermectins, cypermethrin and cartap at LC_{30} values (0.3133, 0.3269, 1.5408, 3.2961, 6.1469 and 9.021) mg/litre, respectively after 8 hours of exposure.

Sattar *et al.* (2011) evaluated six insecticides *viz.*, emamectin benzoate, lufenuron, flubendiamide, spinosad, indoxacarb and neem oil against *T.chilonis* and concluded that flubendiamide is more safer as compared to neem oil, indoxacarb and lufenuron (mild toxic),and spinosad and emamectin found highly toxic against *T.chilonis.*

Preetha *et al.* (2009) evaluated nine insecticides, *viz*; imidacloprid, thiamethoxam, chlorantraniliprole, clothianidin, pymetrozine, ethofenprox, BPMC, endosulfan, acephate and (chlorantraniliprole 20% + thiamethoxam 20%), to determine their toxicity in insecticide-coated vials (scintillation) for *T. chilonis*. Based on risk quotient, which is the ratio between the field-recommended doses and the LC50 values, chlorantraniliprole found harmless to *T. chilonis* than other insecticides.

Khan *et al.* (2015) studied the interaction of *T. pretiosum* and nineteen pesticides (insecticides, miticides, fungicides and herbicides). The acute toxicity of nineteen pesticides against adult parasitoids and their behavioural effects on foraging parasitoid females *Viz*; (host antennation, stinging) and host feeding. The foraging behaviour of *T. pretiosum* was only affected by tolfenpyrad and concluded that the other pesticides are safer for the foraging of parasitoid among the tested pesticides.

Uma *et al.* (2014) tested eighteen insecticides under recommended protocol of International Organization for Biological Control (IOBC) (conventional and recent classes of insecticides) for their toxic effects on *Trichogramma japonicum* Ashmead at Kerala Agricultural University. Among the tested insecticides, acephate caused the highest mortality of (88.75 %) and chlorantraniliprole (21.25 %) to the adults of *T. japonicum*. The newer insecticides *viz*., emmemectin benzoate, fipronil, imidacloprid, indoxacarb, buprofezin and chlorantraniliprole, rated as harmless. Whereas spinosad, thiamethoxam and flubendiamide rated as slightly harmful to *T. japonicum* as per IOBC safety classification. Besides, the conventional organophosphates *viz*., acephate, chlorpyriphos and dichlorvos rated as moderately harmful. dimethoate, malathion, quinalphos carbaryl, fenvalerate and acetamiprid caused 30 – 79 % mortality rated them as slightly harmful to adults of *T. japonicum.*

Abbas *et al.* (2020) evaluated *Trichogramma chilonis* as well as five insecticides (Beta-cyfluthrin + Triazophos, 41.7% EC, acephate, 97%, profenofos + lambda – cyhalothrin, 61.5% EC, cypermethrin + profenofos, 440 EC, emamectin benzoate, 1.9% EC to find out the comparative bio efficacy of profenofos + lambda – cyhalothrin, 61.5% EC gave the best results followed by emamectin benzoate, 1.9% EC and cypermethrin + profenofos, 440 EC against *Helicoverpa armigera*. Besides, the cost benefit ratio showed the highest net return with *Trichograma chilonis* cards released plots followed by emamectin benzoate, 1.9% EC.

Shivankar *et al.* (2008) evaluated the efficacy of neonicotinoid, nereistoxin analogue, organophosphorus, carbamate insecticides, neem derivatives and an egg parasite, *Trichogramma chilonis* for the control of *Spodoptera litura* in sugar beet. *T. chilonis* and azadiractin 3000 ppm (5 ml/lit) recorded the larval reduction (89.7 and 89.3%) of *Spodoptera* and proved effective to *Spodoptera litura*.

Sehrawat *et al*. (2002) evaluated cartap hydrochloride 4G, monocrotophos 36 SL, *Bacillus thuringiensis* vaf. *Kurstaki*, endosulfan 35EC, *neem* 300 ppm (azadirachtin), ethofenprox 10EC and innundative release of egg parasitoid, *Trichogramma chilonis* Ishii against rice leaffolder, *Cnaphalocrocis medinalis*. Cartap hydrochloride 4G proved the most effective insecticide and reduced leaf folder's infestation and increase in grain yield (21.7%) over control.

Selvaraj *et al.* (2017) investigated the effect of newer insecticides *viz*., novaluron 10 EC, flubendiamide 20 WG, indoxacarb 4.5 SC + novaluron 5.25 SC, indoxacarb 14.5 SC, acephate 75 SP, cartap hydrochloride 50 SP and rynaxypyr 18.5 SC against *Plutella xylostella*. Cartap hydrochloride found most effective in reducing the larval population and also recorded highest yield among all the tested insecticides..Cartap hydrochloride showed highest acute toxicity to both (parasitoid & predator) with LC 50 values of (0.0099& 0.0043) for *Trichogramma chilonis* and *Chrysoperla zastrovi,* respectively.

Mandal (2012) evaluated the bioefficacy of cyazypyr 10% OD, imidacloprid 17.8% SL and fipronil 5% SC against the insect pest complex of tomato *vis-à-vis* its impact on natural enemies and crop health. Cyazypyr 10% OD @ 90 and 105 g a.i./ha was highly effective in controlling the fruit borer, *Helicoverpa armigera Hubn.*, aphid, *Aphis gossypii* Glov. and white fly, *Bemisia tabaci* Gen and also found safe to *T. pretiosum* pupae in vitro.

Wahenbam *et al.* (2018) investigated the toxic effect of modern insecticides (spiromesifen, rynaxypyr, cyazypyr, thiacloprid and Ttolfenpyrad) on pupal stage of hymenopteran parasitoid namely *Trichogramma chilonis* Ishii and *T. pertiosum* Riley. All the molecules *viz*, spiromesifen, rynaxypyr, cyaxypyr and thaicloprid were proved harmless to *Trichogramma*.

Qasim *et al.* (2018) revealed that six (Lufenuron, Carbosulfan, Abamectin, Bifenthrin, Indoxacarb, Chlorpyrifos) out of ten insecticides were extremely toxic to *T. chilonis* in the laboratory and other four insecticides (Triflumuron, Emamectin, Spinosad and Imidacloprid) were least toxic, causing only 10-24% and 12- 44% mortality under vitro after 24 and 48 hrs of application

Prema *et al.* (2016) evaluated the biosafety of fipronil 80 WG, chlorpyriphos 20 EC, dimethoate 30 EC and neem seed kernel extract (NSKE) to *Trichogramma chilonis* Ishii, *Telenomus* sp. and *Cyrtorhinus lividipennis* Reuter. The result revealed that, NSKE@ 5% was safe to *T. chilonis* recorded parasitisation of (92.6%) and emergence (94.1%) followed by fipronil 80 WG which recorded 80 per cent parasitisation of eggs and emergence of adult parasitoid. The adult emergence of *Telenomus* sp., was maximum in NSKE 5% followed by fipronil 80 WG.

Raghuraman *et al.* (1999) tested neem oil at different concentrations for oviposition deterrence, feeding deterrence, toxicity, sterility and insect growth regulator effects against *Trichogramma chilonis* Ishii. Neem seed oil @ 0.3% deterred oviposition (parasitisation), feeding and, no sterility effect was observed when the parasitoids were released.

2.2 Gamma irradiation on egg parasitoids and on their host

Gavriliţă *et al. (*2013) revealed that irradiated eggs of *Sitotroga cerealella* by gamma rays increased biological indices and the efficiency of *Trichogramma* in the field. Biological efficiency of *T. embryophagum* and the degree of damage on irradiated eggs and non-irradiated eggs of *Sitotroga cerealella* are significantly different.

Tuncbilek *et al. (*2012) studied the effect of previously irradiated host eggs of *Ephestia kuehniella* and *Sitotroga cerealella* with gamma radiations on the adults of *Trichogramma evanescens*. The effect of gamma radiations revealed no significant difference in parasitisation and in adult and female emergence *T.*

evanescens females as compared to untreated control up to 30 days. Besides, the storage time increased for up to 60 and 30 days for *E. kuehniella* and *S. cerealella* eggs, respectively.

Mansour (2010) showed that the radio-sensitivity of *E. kuehniella* eggs decreased with increasing age when irradiated with 25-48-h-old eggs, hatch was less than 5% at 100 Gy dose and eggs 49-72-h-old were more resistant. Also irradiation also negatively affected survival to the adult stage. When 25-48-h-old eggs were irradiated, survival to the adult stage was completely prevented at 75 Gy dose and no survival was observed beyond 100 Gy dose in 49-72-h-old eggs.

Hamed *et al.* (2009) showed that gamma radiations improve mass rearing capabilities of, *T. chilonis* Ishii as well as a predator, *Chrysoperla carnea* to achieve an area-wide control of cotton and sugarcane pests. The suitability (*S. cerealella*) eggs for parasitization by T. *chilonis* was prolonged with the application of gamma radiation in the range of 5-55 Gy from 3 to 7 days during pre-hatching and the parasitisation ranged from 78 to 94%, but decreased drastically at lower doses (5 and 15 Gy) in latter days. High doses were better than lower doses but 55 Gy successful decreased parasitization upto 45%.

Zhang & Cossentine (1995) in host-preference studies revealed that, *Trichogramma platneri adult* females treated with cobalt 60 reared on viable eggs of codling moth, *Cydia pomonella* parasitized significantly more on viable eggs than the nonviable eggs.

Harwalkar *et al. (*1987) successfully reared *Trichogramma brasiliensis* on the eggs laid by gamma-irradiated sterile female of, *Phthorimaea operculella and* no adverse effects on various developmental parameters even rearing after ten generations

Ebaldry (1965) experimented on the encyrtid *Copidosoma koehleri* Blanchard, reared on previously irradiated potato tuberworm (*Gnorimoschema operculella* (Zeller)) hosts, showed nutritional deficiency (lessened polyembryony, slower development) and higher mortality in the various stages of the parasitoid.

Carpenter *et al.* (2003) studied the acceptability and suitability of eggs of *Cryptophlebia leucotreta* (Meyrick) to parasitization by *Trichogrammatoidea cryptophlebiae* Nagaraja under choice/ no choice situations. Results indicated that *T. cryptophlebiae* successfully (accept, develop in and emerge) from false colding moth eggs present in the field under a sterile insect release programme (*N*♀ × *T*♂, *T*♀ × *N*♂ and *T*♀ × *T*♂) and suggested further evaluations.

Botto *et al*. (2010) studies the response of an Argentinean codling moth strain, *Cydia pomonella* exposed to sub-sterilizing radiation dose of 100 Gy to assess the acceptability and suitability of sterile codling moth eggs by the egg parasitoids, *Trichogramma cacoeciae* (Marchal) and *Trichogramma nerudai* (Pintureau and Gerding). Irradiated females survived better than irradiated male moths and also to non-irradiated (male and female) of codling moth adults. They further reported that the fecundity of irradiated female moths, reduced to more than 30% as compared to non-irradiated ones. Whereas, zero fertility was recorded. In addition, they stated that *Trichogramma nerudai* parasitized more eggs than *T. cacoeciae* by irradiated females.

Sarwar (2015) irradiated fruit-fly larvae at the dose of 15 Gy for enhancing the parasitism, development, and adult emergence of the larval parasitoid, *Trybliographa daci* (Weld) (Hymenoptera: Eucoilidae). Overall, parasitism by the parasitoid increased with age of the host larvae. However, higher parasitism occurred on 4 days old irradiated larvae of *B. zonata* compared to 5 days old. The female parasitoids preferred the irradiated larvae and significantly higher numbers of larvae were parasitized compared with non-irradiated larvae. A radiation dose of 80 Gy considered best to rear the pupal parasitoid, *Dirhinus giffardii* (Silvestri) (Hymenoptera: Chalcididae).

Cancino *et al*. (2012) proved that irradiated hosts of tephritid parasitoids represents in fruit fly augmentative biological control revealed positive outcomes for their mass rearing. Irradiation assures avoidance of fly emergence in non-parasitized hosts, while at the same time, it has no appreciable effect on parasitoid quality (fecundity, longevity and flight capability). Parasitoids of fruit fly (eggs, larvae and pupae) successfully developed on irradiated hosts for their easy shipment and storage.

Xu *et al.* (2016) in a no-choice test found out that, *T. japonicum* and *T. leucaniae* parasitized significantly more fertilized or UVF than unfertilized hosts and *T. chilonis* parasitized significantly more UVF than either fertilized or unfertilized hosts. In a choice test, all three *Trichogramma* parasitoids parasitized UVF hosts the most and unfertilized hosts the least.

Horrocks *et al.* (2016) used mated female *T. basalis* and gamma-irradiated at doses between 120 and 150 Gy and exposed to egg masses of their host *Nezara viridula* throughout their lifespans. This resulted in host mortality, despite a substantial reduction in developing parasitoid offspring, which followed a negative dose–response. There was no emergence of parasitoid offspring at 140 Gy and above.

2.3 Preference and performance of different egg parasitoids

Golbaghi *et al*. (2020) in the choice test proved *C. pomonella* eggs were preferred as hosts (vs. *S. cerealella* and *E. kuehniella*) during the first two days of the parasitism assay as well as during total parasitism assay overall. Furthermore, the production of females was higher in *C. pomonella* than in the other hosts.

Becker *et al.* (2023) proved that *T. cacoeciae* preferred larger *L. botrana* eggs when the larvae had fed on grapevine-containing diet but not when they fed on standard artificial diet. They furthermore suggested that the future efficiency of *L. botrana*-biocontrol by *T. cacoeciae* will not decrease under elevated CO_2 concentrations. The results highlight the importance of the host's diet for the parasitoid's preference and performance.

Van Atta *et al.* (2015) found that *Trichogramma* preferred to move toward higher intensities of UV-B radiation and parasitized more eggs in areas with higher UV-B radiation. However, higher UV-B radiation reduced the number of adult wasps emerging from host eggs.

Blackmer *et al*. (2004) investigated the response of naive and experienced fifth-instar and adult *L. hesperus* to odors associated with conspecifics and alfalfa, *Medicago sativa* L. Fifth-instar *L. hesperus* responded to all plant/insect combinations, whereas female *L. hesperus* only responded preferentially to vegetative and flowering alfalfa where conspecifics had fed for 24–72 hr and to vegetative alfalfa where conspecifics were added approximately 30 min before the test began. Males were not attracted to headspace volatiles from any of the alfalfa treatments

Belda and Jordy (2004) parasitoids with late-instar larvae and adults of *Sitophilus oryzae*, *Rhyzopertha dominica*, *Tribolium confusum* and *Lasioderma serricorne* (F.) and host food products and found that they preferred uninfested host food products (rice or flour) to empty controls and uninfested paddy rice to uninfested brown rice, while wheat flour was clearly more attractive to them than brown rice

Koschier *et al*. (2017) assessed the responses of *Frankliniella occidentalis* in the presence of background odours emanating from cucumber, capsicum, chrysanthemum, clove basil and lavender plants in a Y-tube olfactometer. Compared to clean-air conditions, a slightly lower percentage of thrips chose the Y-tube arm loaded with 10% *p*-anisaldehyde in the presence of cucumber leaf odour. With non-flowering clove basil plants in the background, *F. occidentalis* responses to 1% eugenol, a constituent of clove basil essential oil, were neutral and the same applied to responses to pure linalool, a constituent of lavender essential oil, in the presence of flowering lavender plants.

Shah and Rahman (2017) observed fecundity and adult longevity of *Bemisia tabaci* on four different host plants and compare its host plant attraction through a Y-tube olfactometer. Results showed that daily fecundity was different in the entire period of *B. tabaci* life and more number of total eggs laid on eggplant and medium on tomato and cucumber and lower on pepper. The olfactometer results confirmed that eggplant, cucumber and tomato are preferred hosts where pepper is the non-preferred host for *B. tabaci*.

Pizzol *et al.* (2012) studied the age effects of host eggs of *Lobesia botrana* on parasitism rate and the development of offsprings in vitro conditions on *Trichogramma cacoeciae.* They stated that the parasitization rate of (1,2, 3 and 4 days) old eggs varied from one to 4 days for *T. cacoeciae* adult females and post-emergence. The 3 to 4 days old eggs of *L. botrana* eggs were less parasitized by *T. cacoeciae* than 1 to 2 days old eggs. Besides, age effected parasitism, as one day old females produced fewer parasitized eggs than 2, 3 and 4days old females, respectively.

Pinturea *et al. (*2010) studied the emergence, mortality rates and fecundity of two strains of *T. cacoeciae*, France (Alsace) and another Tunisian (Degache), were set at constant temperatures of 15, 20, 25 and 30°C respectively. While as, both the strains showed the highest fecundity at 25°C..and the highest mortality recorded at 30°C. However, the emergence rates were relatively higher at all the temperatures, although the French strain did better at 15-25°C and the Tunisian one at 20-30°C.

Makee (2006) conducted laboratory experiments with infertile and fertile codling moth eggs to evaluate the potential parasitism and reproduction of *Trichogramma cacoeciae* and *T. principium.* The tendency of *T.*

cacoeciae females to attack infertile eggs as well as fertile eggs, whereas *T. principium* showed a greater preference for infertile eggs than fertile eggs.

Garg and Bhattacharyya (2005) fed the adult parasitoids with fine streaks of either 50% honey, 20% (fructose, sucrose, glucose, yeast hydrosylates), grape juice and also distilled water to the unfed parasitoids and also with freshly irradiated food for five successive generations. The results revealed that the fecundity of the parasitoids increased significantly at 2.5 and 5Gys irradiated honey in all generations as compared to the rest of the treatments.

Tunçbilek and Ayvaz (2003) reared *T. evanescens* on the eggs of *Ephestia kuehniella* and the adult emergence at 27 ± 1° C, 60–70 ± 5 % RH and L14:D10 fed on honey solution. Fresh (6–48 h) and old (72–96 h) The fresh host eggs (*Ephestia kuehniella)* offered to *T. evanescens* were more accepted than old eggs. and also the parasitization rate

Ozder (2002) observed that the parasitism rates of *Trichogramma cacoeciae* Marchall, *Trichogramma* evanescens Westwood and *T. brassicae* Bezdenco reared on dead embryos of *E. kuehniella* kept at -20°C during 1, 2 and 3 hrs. The lower parasitization rate was recorded on *E. kuehniella* eggs kept at -20°C for 3 hrs. Parasitization rates of 64%, 65.60% and 63.60% for *T. cacoeciae, T. evanescens and T. brassicae,* respectively, on *E. kuehniella* eggs kept at -20°C for 1 hr.

Zhang & Cossentine (1995) observed that the irradiated adults females of *Trichogramma platneri* Nagarkatti reared on viable *Cydia pomonella* eggs, parasitized more viable than nonviable eggs.

Schmidt (1970) demonstrated host development on the parasitism and mortality of *Pieris rapae* and *Trichoplusia ni* eggs attacked by *Trichogramma evanescens* Westwood in vitro revealed no significant difference in parasitisation rate on both the hosts.

Chapter-3

MATERIAL AND METHODS

The materials and methods adopted for carrying out the research proposal entitled as "Toxicity of insecticides and impact of gamma radiations on egg parasitoids" are as detailed below:-

Rearing of *Corcyra* culture for egg parasitoids:

The rice meal moth, *Corcyra cephalonica Stainton* (Lepidoptera: Pyralidae) ranks first in the mass culturing of entomophagous insects due to its amenability to mass production, adaptability to varied rearing conditions and its positive influence on the progeny of the natural enemies.

Sterilized the wooden boxes were kept in hot air oven at 100 degree centigrade for 1-2 hours. Sterilized crushed maize/sorghum/rice of 2.5 kg per box were poured. 50 grams of broken ground nut, 5 grams of yeast, 1 gram of wettable sulphur, 0.05 grams of streptomycin sulphate were added in each box. 1 cubic centimetre of *Corcyra* eggs per box were sprinkled on the top of culture medium and mixed with the medium thoroughly. The box was covered with lid and labelled with the date of inoculation. Favourable temperature for rearing is 28±2 degree centigrade and relative humidity, 75%±5%. The moth started to emerge within 45-50 days, the moths were collected inside the net by glass tubes and transferred to egg laying chamber. Cotton soaked 20% honey+ vitamin E solution was provided as adult food in the egg laying chamber. The eggs were collected daily.

Maintenance of Stock Cultures of egg parasitoids and preparation of Tricho cards

From the laboratory culture of *Corcyra cephalonica*, one cc eggs of *Corcyra cephalonica*, were uniformly spread and pasted on tricho cards, measuring 15 cm x 10 cm using gum arabica as pasting material. The cleaned eggs were uniformly sprinkled on the tricho cards with a tea strainer. The excess eggs pasted on the card were removed gently with a shoe brush. After that, the eggs of *C. cephalonica* were kept under UV lamp for 30 minutes to kill the embryo. The UV irradiated *Corcyra cephalonica* egg cards (tricho cards) were inserted into the polythene bags. These UV treated *C. cephalonica* egg cards and the nucleus culture of *Trichogramma chilonis* were kept in the polythene bags. Besides, in these polythene bags, 50% honey with vitamin E, soaked in cotton swab were provided as food to the adults of *Trichogramma chilonis*. The colour change in the irradiated *Corcyra cephalonica* egg cards from white to black indicated parasitization and the emerged adults of T*richogramma chilonis* were used for different experiments (Hassan and Sherif, 1993).

3.1 To study the toxicity of insecticides on egg parasitoid.

The experiment was carried in the AIRCP Biocontrol Laboratories, Division of Entomology, SKUAST-K. The laboratory culture of egg parasitoid, *Trichogramma chilonis* evaluated to study the bio efficacy of six insecticides at different concentrations (Table-1) by adopting different bioassays. Besides, the separate control was run for each treatment and the control treatment was sprayed with distilled water only. The details of the formulated insecticides, their trade names and manufacturers name are summarized as under:

Table 1: List of the formulated insecticides

S. No	Name of the Insecticide	Trade name	Manufacturers Name
1	Chlorantranilipole 18.5%SC	Coragen	Dupont
2	Bifenthrin 10% EC	Diamond	Gujarat pesticides Ltd.
3	Cypermethrin 25% EC	Super Killer25	Dhanuka
4	NSKE 5%	N.S.K.E	Virinchi Organics Pvt.Ltd
5	Malathion 25% WP	Malawon 25	Agri Bioche Pvt. Ltd
6	Thiamethoxam 25% WG	Taiyo	IFFCO-MC

Corcyra eggs + Crushed Maize 2.5 kg + other ingredients

Spreading of eggs on cards

Parasitising adults

Parasitised eggs of *Corcyra*

Plate 1: Rearing of *Trichogramma spp*.

Preparation and dilution of chemicals

Different insecticides (Table-2) purchased from authorized dealer's of various Pesticide Companies, India and diluted at desired concentrations for each insecticide *viz*; Chlorantraniliprole 18.5 SC; Bifenthrin 10 EC; Cypermethrin 25 EC; NSKE 5%; Malathion 25WP and Thiamethoxam 25 WG were prepared in the Formulation Laboratory, AICRP-Biocontrol, Srinagar. These formulated insecticides were then mixed with water at desired concentrations for bio-efficacy testing using different bioassays methods.

Table 2: List of the formulated insecticides diluted at different concentrations for bioassays

S.No.	Treatments	Concentrations
1	Chlorantraniliprole 18.5%SC	0.50ml/ litre of water
2	Chlorantraniliprole18.5%SC	0.40ml/litre of water.
3	Chlorantraniliprole 18.5%SC	0.30ml/litre of water.
4	Chlorantraniliprole 18.5%SC	0.20ml/litre of water.
5	Chlorantraniliprole 18.5%SC	0.10ml/litre of water.
6	Bifenthrin 10% EC	0.50ml/litre of water
7	Bifenthrin 10% EC	0.40ml/litre of water.
8	Bifenthrin 10% EC	0.30ml/litre of water.
9	Bifenthrin 10% EC	0.20ml/litre of water.
10	Bifenthrin 10% EC	0.10ml/litre of water.
11	NSKE 5%	4ml/litre of water.
12	NSKE 5%	3ml/litre of water
13	NSKE 5%	2ml/litre of water
14	NSKE 5%	1ml/litre of water.
15	NSKE 5%	0.50ml/litre of water.
16	Cypermethrin 25%EC	0.50ml/litre of water
17	Cypermethrin 25%EC	0.40ml/litre of water
18	Cypermethrin 25%EC	0.30ml/litre of water.
19	Cypermethrin 25%EC	0.20ml/litre of water
20	Cypermethrin 25%EC	0.10ml/litre of water.
21	Malathion 25%WP	0.50 ml/litre of water
22	Malathion 25%WP	0.40 ml/litre of water
23	Malathion 25%WP	0.30 ml/litre of water
24	Malathion 25%WP	0.20 ml/litre of water
25	Malathion 25%WP	0.10 ml/litre of water
26	Thiamethoxam 25%WG	0.50ml/litre of water
27	Thiamethoxam 25%WG	0.40ml/litre of water
28	Thiamethoxam 25%WG	0.30ml /litre of water
29	Thiamethoxam 25%WG	0.20ml /litre of water
30	Thiamethoxam 25%WG	0.10ml/litre of water
31	Control	Water @1ml spray

Bioassay Methods:

Different bioassays were performed using two different methods *viz*; Dry residual Method and Filter paper method.

3.1.1 Dry Residue Method

The insecticides were sprayed into the sterilized glass vials in laboratory conditions. One millilitre solution (01ml) of the test insecticides at different concentrations (Table-2) of all the test insecticides were sprayed into the glass vial using an atomiser on its inner side of the vials. These vials were allowed to dry at room temperature. The adults of *Trichogramma chilonis* were released into these vials. Fifteen adults of one day's old were kept in these vials. The adult mortality was calculated at an interval of 24, 48, 72 hours.

3.1.2 Filter Paper Method

In this technique,all the test insecticides (Table-2) at the desired concentrations were deposited on Whatman's Filter Paper (42) on the glass Petri dishes measuring (90mm x 15mm). In these Petri dishes, one milliliter (1ml) of the each test insecticide at the respective concentrations were sprayed with atomiser on rough side of these Filter paper in Petri dishes. All the test insecticide at the desired concentrations of each insecticide was allowed to dry in the Petri dishes for all the treatments at room temperature. Adults of the, *Trichogramma chilonis* were released onto the film of the toxicant in these Petri plates. Fifteen adults of uniform age were released for a period of 24, 48 and 72 hrs intervals to record the mortality and was calculated according to the Abbott's formula.

Abbotts Formula:

$$CM\ (\%) = \frac{T-C}{100-C} \times 100$$

Where, CM = Corrected mortality, C = mortality (%) in controls and T = mortality (%) in treatments.

Chemicals as per treatment details

Spraying of chemicals into the vials through atomiser

Addition of egg parasitoids into the vials under stable conditions (Temp: 28±2 degree Celsius, RH:75%)

Incubating in BOD for further observations

Plate 2: Bioassay Methods under laboratory conditions

3.1.3 Statistical analysis

The experiments were conducted in Complete Randomised Design (CRD) to run the laboratory experiments for testing bio-efficacy of different insecticides using different bioassays methods. Differences among means ± S.D were considered significant at $P \leq 0.05$. The percentage data was subjected to arc sine transformations for insect populations before Analysis of Variance. Mortality data analyses and LC_{50} values were conducted by Finney's probit analysis (PROC PROBIT, SPSS).

3.2 To study the impact of doses of gamma radiations on egg parasitoids.

Gamma Radiation Works

The different irradiation doses *viz*; 5Gy; 25Gy; 50Gy; 100Gy; 200Gy to the adults of the *Trichogramma pretiosum* were carried out in the Sher-e-Kashmir Institute of Medical Sciences, Soura, Kashmir. The gamma radiation at the given doses were given to the adults of *Trichogramma pretiosum* to study their mortality and survival. These adults were monitored at 26± 2 C^{o} and 75% RH to determine the survivability and mortality of *Trichogramma pretiosum*.

A gamma irradiation chamber (model Gamma Cell Elite-I) with a Cs-137 radioactive source was used. Glass vials containing adults were put into the irradiation chamber. The adults were returned to the BOD incubator and reared on diet medium. Within two to three days, some of these adults parasitized and turned the eggs of *Corcyra* black. Radiation doses, ranging from 10 to 200 Gy with an geometric progression were administered to adults of the same age group. For the following 10 days, the irradiated adults were watched for parasitising eggs. To help with the computation of the LC_{50} dose and LC_{90} dose, which kills 50% & 90% of the exposed adults within 24 hr, the irradiated adults were observed for the mortality. Twenty insects were tested for each dose.

Inoculation of egg parasitoids onto the testing vials under controlled conditions

Irradiation machine

Irradiated eggs of *Corcyra* kept for parasitisation by irradiated adults of *T.pretiosum*

Parasitising *T.pretiosum*

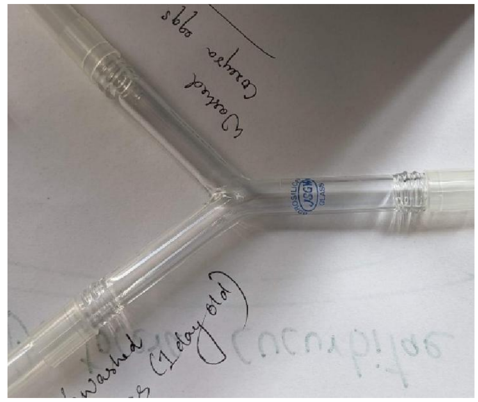
Plate 3: Gamma radiations Studies on *T.pretiosum* at SKIMS, Soura

3.3 To study preference and performance of different egg parasitoids on the eggs of *Corcyra.*

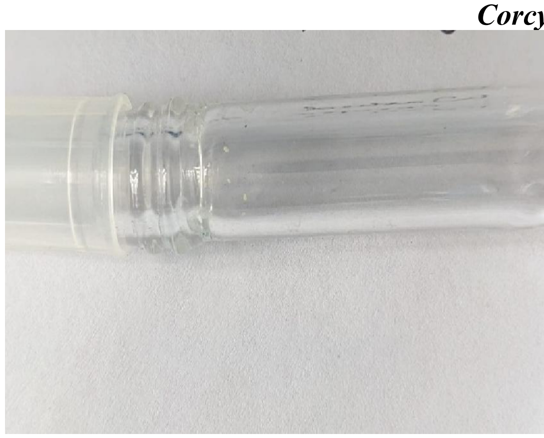
Preference and Performance of *Trichogramma* spp.

Three species of *Trichogramma Viz*; *T.chilonis, T.bactrae and T.pretiosum* were evaluated for their preference and performance on the *Corcyra* eggs. Two types of *Corcyra* eggs were used (washed and unwashed) for their parasitisation. The washed eggs of *Corcyra cephalonica* were rinsed in double distilled water for the removal stigmatic layer for five minutes. Besides, to study the attraction of egg parasitoids towards two types (washed & unwashed) Corcyra eggs of 1, 2 and 3 days old eggs. For this purpose, Y-tube olfactometer with stem (20-cm) and two arms (20-cm) at a 75° angle, with an internal diameter of 4 cm was used to run the preference test for the egg parasitoids. To pass the clean air into the Y-tube, it was filtered through activated charcoal into each arm of the olfactometer at the rate of 200 ml/min. All preference tests were conducted in a controlled room at 25°C, illuminated at 15 W yellow light.In each treatment, a group of ten adults of each species of egg parasitoid (*T. pretiosum, T.chilonis, T.bactrae)* were released into the Y-tube and to check their response for 180 seconds. Each treatment was repeated three times. When an adult walked up to the upwind end of an olfactometer arm, either within 4 cm of the end of the arm at the end of 180 seconds, it was recorded as making a choice for the odour of that arm..When an adult did not reach to any of the arms within 180 seconds, it was considered as no response. Each adult was used only once. The olfactometer tube and glass container were thoroughly washed with alcohol at the end of the observations for a given treatment and dried overnight in an oven at 200°C. Preference tests for all the egg parasitoids for their attraction towards different types of eggs of *Corcyra* of different age groups (1, 2 & to 3 days old) were run through the olfactometer separately for each egg parasitoid.

Unwashed eggs of *Corcyra* preferred by the egg parasitoids



Preference and performance test with washed and unwashed eggs of *Corcyra*



Y tube apparastus set up in laboratory conditions

Plate 4: Y tube apparatus for preference test on egg parasitoids

3.3.2 Statistical Data Analysis

Data on the mortality of irradiated adults were subjected to probit analysis using IBM's SPSS 29.0.1.0 (trial version) software. Differences among means ± S.D were considered significant at $P \leq 0.05$. The same software was used to perform Pearson's chi-square test to determine the significance of the relationship between dosage and mortality. To run the preference and performance tests, chi-square test was used to analyse the frequency distributions of the choices made by adults in each treatment. significant at $P \leq 0.05$. was measured by the differences among means ± S.D.

Chapter- 4

EXPERIMENTAL FINDINGS

The findings of research studies entitled **"Toxicity of insecticides and impact of gamma radiations on egg parasitoids"** are presented under the following headings:

4.1 To study the efficacy of formulated insecticides against adults of *Trichogramma chilonis* under laboratory conditions

Bioassay studies carried out on adults of *Trichogramma chilonis* to determine the bio efficacy of six insecticides at different dosage levels using different bioassays (Dry residue & Filter paper method) and check was run (water) as control treatment in a Completely Randomized Block Design (CRBD) under laboratory conditions in the All India Coordinated Research Project on Biological Control (AIRCP-BC). Division of Entomology, SKUAST-K.

4.1.1 Dry Residue Method

Corrected per cent mortality was recorded at different time intervals *viz*; 24, 48 and 72 hours at five different concentrations against *Trichogramma chilonis* in laboratory conditions. After 24 hours, at dosage of 0.5 g/L, Thiamethoxam 25% WG recorded the highest corrected mortality of 87.83 % followed by 72.71 % mortality with bifenthrin 10% EC while as, the lowest corrected mortality of 42.32 % was recorded in NSKE 5% @4ml/ L. At 0.4 g/ L, Thiamethoxam 25% WG recorded the highest corrected mortality of 81.8 % followed by 66.62 % mortality with bifenthrin 10% EC while as, the lowest corrected mortality of 42.34 % was recorded in NSKE 5% @3g/ L. At 0.3g/ L, Thiamethoxam 25% WG recorded the highest corrected mortality of 72.71% followed by 51.40 % mortality in bifenthrin 10% EC while as, the lowest corrected mortality of 30.24 % was recorded in NSKE 5%. At 0.2 ml/ L, Thiamethoxam 25% WG recorded the highest corrected mortality of 63.58 % followed by 51.40 % mortality with bifenthrin 10% EC while as, the lowest corrected mortality of 18.13 was recorded in NSKE 5% @1ml/ L. At 0.1 g/ L, Thiamethoxam 25% WG recorded the highest corrected mortality of 60.52 % followed by 42.38 % mortality with bifenthrin 10% EC while as, the lowest corrected mortality of 9.50 % was recorded in NSKE 5% @0.5ml/ L.

After 48 hours at dosage 0.5 g/L, Thiamethoxam 25% WG recorded the highest corrected mortality of 96.30 % followed by 92.59 % mortality with bifenthrin 10% EC while as, the lowest corrected mortality of 62.97 % was recorded in NSKE 5% @4ml/L. At 0.4 g/L, Thiamethoxam 25% WG recorded the highest corrected mortality of 92.59 % followed by 85.19 % mortality with bifenthrin 10% EC while as, the lowest corrected mortality of 55.56 % was recorded in NSKE 5% @3ml/L. At 0.3g/ L, Thiamethoxam 25% WG recorded the highest corrected mortality of 85.19 % followed by 77.78 % mortality in bifenthrin 10% EC while as, the lowest corrected mortality of 44.44 % was recorded in NSKE 5% at 2ml/L. At 0.2 g/ L, Thiamethoxam 25% WG recorded the highest corrected mortality of 81.48 % followed by 74.08 % mortality with bifenthrin 10% EC while as, the lowest corrected mortality of 33.33 % was recorded in NSKE 5% @1ml/ L. At 0.1 g/ L, Thiamethoxam 25% WG recorded the highest corrected mortality of 77.78 % followed by 70.37 % mortality with bifenthrin 10% EC while as, the lowest corrected mortality of 22.22 % was recorded in NSKE 5% @0.5ml/L

After 72 hours at a dosage of 0.5 g/ L, Thiamethoxam 25% WG, Bifenthrin 10% EC and Cypermethrin 25%EC recorded the highest corrected mortality of 100 % followed by 93.39 % mortality with malathion 25% WP while as, the lowest corrected mortality of 66.68 % was recorded in NSKE 5% @4ml/ L. At 0.4 ml/ L, Bifenthrin 10% EC recorded the highest corrected mortality of 100 % followed by Thiamethoxam 25% WG and Cypermethrin 25%EC recorded 93.39 % mortality while as, the lowest corrected mortality of 53.35 % was recorded in NSKE 5% @3ml/ L. At 0.3g/ L, Thiamethoxam 25% WG and Bifenthrin 10% EC recorded the highest corrected mortality of 93.39 % followed by 80.17 %

mortality in Cypermethrin 25% EC, while as, the lowest corrected mortality of 46.6 % was recorded in NSKE 5% at 2ml/L. At 0.2 g/L, Thiamethoxam 25% WG recorded the highest corrected mortality of 100 % followed by 80.17 % mortality with bifenthrin 10% EC while as, the lowest corrected mortality of 34.33 % was recorded in NSKE 5% @1ml/ L. At 0.1 g/L, Thiamethoxam 25% WG recorded the highest corrected mortality of 80.02 % followed by 73.41 % mortality with bifenthrin 10% EC while as, the lowest corrected mortality of 21 % was recorded in NSKE 5% @0.5g/ L. Among all the treatments, water as control treatment exhibited lowest corrected per cent mortality of 2.67 % at all the time intervals.

The order of efficacy of different insecticides against adults of *Trichogramma chilonis* with Dry Residue Method is as follows:-

Thiamethoxam 25% WG @0.5 ml/L > Thiamethoxam 25% WG @0.4 ml/L > Thiamethoxam 25% WG at 0.3 ml/L& Bifenthrin 10% EC at 0.5 ml/L> Bifenthrin 10% EC at 0.4 ml/L > Thiamethoxam 25% WG at 0.2 ml/L& Cypermethrin 25% EC at 0.5 ml/L > Thiamethoxam 25% WG at 0.1 ml/L> Bifenthrin 10% EC at 0.3 ml/L> Malathion 25% WP at 0.4 ml/L> Cypermethrin 25% EC at 0.4ml/L > Chlorantraniliprole 18.5% SC at 0.5 ml/L> Chlorantraniliprole 18.5% SC at 0.4 ml/L> Bifenthrin 10% EC at 0.2 ml/L> Malathion 25% WP at 0.3 ml/L> Cypermethrin 25% EC at 0.3 ml/L& Cypermethrin 25% EC at 0.2 ml/L> Chlorantraniliprole 18.5% SC at 0.3 ml/L> Malathion 25% WP at 0.5 ml/L> Bifenthrin 10% EC at 0.1 ml/L> Malathion 25% WP at 0.2 ml/L> N S K E 5% at 3 ml/L> N S K E 5% at 4 ml/L> Chlorantraniliprole 18.5% SC at 0.2 ml/L> Cypermethrin 25% EC at 0.1 ml/L> Malathion 25% WP at 0.1 ml/L > N S K E 5% at 2 ml/L> Chlorantraniliprole 18.5% SC at 0.1 ml/L> N S K E 5% at 1 ml/L> N S K E 5% at 0.5 ml/L

Table 3: Efficacy of different insecticides on the adults of *Trichogramma chilonis* through dry residue method

Insecticides	MORTALITY (%)														
	24 hrs					48 hrs					72 hrs				
	0.5ml/L	0.4ml/L	0.3ml/L	0.2ml/L	0.1ml/L	0.5ml/L	0.4ml/L	0.3ml/L	0.2ml/L	0.1ml/L	0.5ml/L	0.4ml/L	0.3ml/L	0.2ml/L	0.1ml/L
Chlorantranilipole 18.5% SC	53.33 (61.06)	53.31 (56.22)	48.05 (50.13)	39.77 (40.90)	29.26 (29.69)	74.07 (83.42)	70.37 (78.06)	66.67 (72.97)	59.26 (63.42)	44.44 (46.05)	86.78 (105.07)	80.02 (92.76)	73.41 (82.43)	59.9 (64.22)	53.26 (56.17)
Bifenthrin 10%EC	72.71 (81.40)	66.62 (72.91)	57.52 (61.29)	51.40 (53.99)	42.38 (43.76)	92.59 (118.34)	85.19 (101.95)	77.78 (89.11)	74.08 (83.42)	70.37 (78.06)	100 (157.07)	100 (157.07)	93.39 (120.51)	80.17 (93.01)	73.41 (82.43)
Cypermethrin 25%EC	63.58 (68.90)	54.4 (57.51)	48.41 (50.53)	48.41 (50.53)	36.31 (37.16)	81.48 (95.24)	77.78 (89.11)	70.37 (78.06)	62.97 (68.112)	62.97 (68.11)	100 (157.07)	93.39 (120.51)	80.17 (93.01)	66.65 (72.95)	59.9 (64.22)
Malathion 25%WP	45.37 (47.09)	57.46 (61.21)	48.41 (50.53)	42.38 (43.76)	33.27 (33.92)	74.08 (83.42)	70.37 (78.06)	62.97 (68.11)	59.26 (63.43)	51.85 (54.51)	93.39 (120.51)	80.17 (93.01)	73.41 (82.43)	66.65 (72.95)	53.26 (56.17)
Thiamethoxam 25%WG	**87.83** (97.07)	**81.8 (95.79)**	**72.71 (81.40)**	**63.58 (68.90)**	**60.52 (65.00)**	**96.30 (129.77)**	**92.59 (118.34)**	**85.19 (101.95)**	**81.48 (95.24)**	**77.78 (89.11)**	**100 (157.07)**	**93.39 (120.51)**	**93.39 (120.51)**	**100 (157.07)**	**80.02 (92.76)**
	4ml/L	**3ml/L**	**2ml/L**	**1ml/L**	**0.5ml/L**	**4ml/L**	**3ml/L**	**2ml/L**	**1ml/L**	**0.5ml/L**	**4ml/L**	**3ml/L**	**2ml/L**	**1ml/L**	**0.5ml/L**
NSKE 5%	**42.32 (43.70)**	**42.34 (43.71)**	**30.24 (30.72)**	**18.13 (18.23)**	**9.50 (9.51)**	**62.97 (68.11)**	**55.56 (58.90)**	**44.44 (46.05)**	**33.33 (33.98)**	**22.22 (22.40)**	**66.78 (73.12)**	**53.35 (56.28)**	**46.6 (48.47)**	**34.3 (35.01)**	**21 (21.15)**
Control	**2.67**					**4.00**					**6.66**				

Each values in column is a mean of three replications

*Values in parenthesis are arc sine transformed values

4.1.2 Filter Paper Method

The bio efficacy of different insecticides at different dosage levels and time intervals against adults of *Trichogramma chilonis* with particle film method under laboratory conditions revealed that after 24 hours adult exposure of *T. Chilonis* to the toxicant, at dosage of 0.5 g/ L, thiamethoxam 25% WG recorded the highest corrected mortality of 87.88 % followed by 69.70 % mortality with bifenthrin 10% EC and cypermethrin 25% EC while as, the lowest corrected mortality of 48.49 % was recorded in NSKE 5% @4ml/ L. At 0.4 g/ L, thiamethoxam 25% WG recorded the highest corrected mortality of 81.82 % followed by 63.64 % mortality with bifenthrin 10% EC while as, the lowest corrected mortality of 51.51% was recorded in NSKE 5% @3ml/L. At 0.3g/ L, thiamethoxam 25% WG recorded the highest corrected mortality of 72.73 % followed by 57.58 % mortality in bifenthrin 10% EC while as, the lowest corrected mortality of 30.30 % was recorded in NSKE 5% at 2ml/ L. At 0.2 g/ L, thiamethoxam 25% WG recorded the highest corrected mortality of 66.67 % followed by 48.48 % mortality with bifenthrin 10% EC, cypermethrin 25%EC and malathion 25% WP while as, the lowest corrected mortality of 18.18 % was recorded in NSKE 5% @1ml/ L. At 0.1 g/L, Thiamethoxam 25% WG recorded the highest corrected mortality of 60.61% followed by 42.42 % mortality with bifenthrin 10% EC and cypermethrin 25% EC while as, the lowest corrected mortality of 6.06 % was recorded in NSKE 5% @0.5ml/ L.

After 48 hours at dosage 0.5 g/ L, thiamethoxam 25% WG recorded the highest corrected mortality of 96.30% followed by 92.59% mortality with bifenthrin 10% EC while as, the lowest corrected mortality of 66.97 %was recorded in NSKE 5% @4ml/ L. At 0.4 g/L, Thiamethoxam 25% WG recorded the highest corrected mortality of 92.59% followed by 85.19 % mortality with bifenthrin 10% EC while as, the lowest corrected mortality of 59.26 % was recorded in NSKE 5% @3ml/ L. At 0.3g/L, thiamethoxam 25% WG recorded the highest corrected mortality of 85.19 % followed by 77.78 % mortality in bifenthrin 10% EC while as, the lowest corrected mortality of 48.15% was recorded in NSKE 5% at 2ml/L. At 0.2 g/L, thiamethoxam 25% WG recorded the highest corrected mortality of 81.48% followed by 74.08 % mortality with bifenthrin 10% EC while as, the lowest corrected mortality of 33.33% was recorded in NSKE 5% @1ml/ L. At 0.1 g/L, thiamethoxam 25% WG recorded the highest corrected mortality of 77.78 % followed by 70.37 % mortality with bifenthrin 10% EC while as, the lowest corrected mortality of 22.22 % was recorded in NSKE 5% @0.5ml/ L.

After 72 hours at a dosage of 0.5 g/ L, thiamethoxam 25% WG, bifenthrin 10% EC and cypermethrin 25%EC recorded the highest corrected mortality of 100 % followed by 83.33 % mortality with chlorantraniliprole 18.5% SC while as, the lowest corrected mortality of 58.33 % was recorded in NSKE 5% @4ml/L. At 0.4 ml/ L, bifenthrin 10% EC recorded the highest corrected mortality of 100 % followed by thiamethoxam 25% WG and cypermethrin 25%EC recorded 91.67% mortality while as, the lowest corrected mortality of 50 was recorded in NSKE 5% @3ml/ L. At 0.3g/ L, thiamethoxam 25% WG and bifenthrin 10% EC recorded the highest corrected mortality of 91.69 % followed by 83.33 % mortality in cypermethrin 25% EC while as, the lowest corrected mortality of 41.67% was recorded in NSKE 5% at 2ml/ L. At 0.2 g/ L, thiamethoxam 25% WG recorded the highest corrected mortality of 91.67 % followed by 75 % mortality with bifenthrin 10% EC while as, the lowest corrected mortality of 33.33% was recorded in NSKE 5% @1ml/ L. At 0.1 g/L, thiamethoxam 25% WG and bifenthrin 10% EC recorded the highest corrected mortality of 66.67 % followed by 50 % mortality with cypermethrin 25% EC while as, the lowest corrected mortality of 25% was recorded in NSKE 5% @0.5ml/L. Among all the treatments, water as control treatment exhibited lowest corrected mortality of 10.33 % at all the time intervals.

The order of efficacy of different insecticides against adults of *Trichogramma chilonis* with Filter Paper Method is as follows: -

Thiamethoxam 25% WG at 0.5 ml/L> Thiamethoxam 25% WG at 0.4 ml/L> Thiamethoxam 25% WG at 0.3 ml/L> Bifenthrin 10% EC at 0.5 ml/L& Cypermethrin 25% EC at 0.5 ml/L> Thiamethoxam 25% WG at 0.2 ml/L& Bifenthrin 10% EC at 0.4 ml/L> Thiamethoxam 25% WG at 0.1 ml/L& Cypermethrin 25% EC at 0.4 ml/L> Bifenthrin 10% EC at 0.3 ml/L& Malathion 25% WP at 0.5 ml/L& Malathion 25% WP at 0.4 ml/L> Chlorantraniliprole 18.5% SC at 0.5 ml/L> Cypermethrin 25% EC at 0.3 ml/L& N S K E 5% at 3 ml/L> N S K E 5% at 4 ml/L> Chlorantraniliprole 18.5% SC at 0.4 ml/L& Bifenthrin 10% EC at 0.2 ml/L& Cypermethrin 25% EC at 0.2 ml/L& Malathion 25% WP at 0.3 ml/L& Malathion 25% WP at 0.2 ml/L> Chlorantraniliprole 18.5% SC at 0.3 ml/L& Bifenthrin 10% EC at 0.1 ml/L& Cypermethrin 25% EC at 0.1 ml/L> Malathion 25% WP at 0.1 ml/L> Chlorantraniliprole 18.5% SC at 0.2 ml/L& N S K E 5% at 2 ml/L> Chlorantraniliprole 18.5% SC at 0.1 ml/L& N S K E 5% at 1 ml/L> N S K E 5% at 0.5 ml/L.

Table 4: Efficacy of different insecticides on the adults of *Trichogramma chilonis* through filter paper method.

Insecticides	MORTALITY (%)														
	24 hrs					48 hrs					72 hrs				
	0.5ml/L	0.4ml/L	0.3ml/L	0.2ml/L	0.1ml/L	0.5ml/L	0.4ml/L	0.3ml/L	0.2ml/L	0.1ml/L	0.5ml/L	0.4ml/L	0.3ml/L	0.2ml/L	0.1ml/L
Chlorantranilipro le 18.5% SC	54.55 (57.69)	48.48 (50.61)	42.42 (43.80)	30.3 (30.78)	18.18 (18.28)	74.07 (83.42)	74.07 (83.41)	66.67 (72.97)	59.26 (63.42)	55.556 (58.90)	83.33 (98.51)	91.67 (115.96)	66.67 (72.97)	50.00 (52.35)	41.67 (42.97)
Bifenthrin 10%EC	69.70 (77.12)	63.64 (68.98)	57.58 (61.35)	48.48 (50.61)	42.42 (43.81)	92.59 (118.3)	85.19 (101.95)	77.78 (89.11)	74.08 (83.42)	70.37 (78.06)	100.00 (157.07)	100.00 (157.07)	91.67 (115.96)	75.00 (84.80)	66.67 (72.97)
Cypermethrin 25%EC	69.70 (77.12)	60.61 (65.11)	51.51 (54.11)	48.48 (50.61)	42.42 (43.81)	81.48 (95.24)	77.78 (89.11)	70.37 (78.06)	66.6 (72.97)	55.56 (58.90)	100.00 (157.07)	91.6 (115.96)	83.33 (98.51)	58.33 (62.28)	50.00 (52.35)
Malathion 25%WP	57.58 (61.35)	57.58 (61.35)	48.48 (50.61)	48.48 (50.61)	39.39 (40.49)	70.37 (78.06)	74.08 (83.42)	70.37 (78.06)	62.97 (68.11)	55.56 (58.90)	75.00 (84.80)	75.00 (84.80)	66.67 (72.97)	58.33 (62.28)	41.67 (42.97)
Thiamethoxam 25%WG	**87.88 (107.33)**	**81.82 (95.82)**	**72.73 (81.43)**	**66.67 (72.97)**	**60.61 (65.11)**	**96.30 (129.77)**	**92.59 (118.34)**	**85.19 (101.95)**	**81.48 (95.24)**	**77.78 (89.11)**	**100.00 (157.07)**	**91.67 (115.96)**	**91.67 (115.96)**	**91.67 (115.96)**	**66.67 (72.97)**
	4ml/L	**3ml/L**	**2ml/L**	**1ml/L**	**0.5ml/L**	**4ml/L**	**3ml/L**	**2ml/L**	**1ml/L**	**0.5ml/L**	**4ml/L**	**3ml/L**	**2ml/L**	**1ml/L**	**0.5ml/L**
NSKE 5%	**48.49 (50.62)**	**51.51 (54.11)**	**30.30 (30.78)**	**18.18 (18.28)**	**13.06 (13.09)**	**66.67 (72.97)**	**59.26 (63.42)**	**48.15 (50.23**	**33.33 (33.98)**	**22.22 (22.40)**	**58.33 (62.28)**	**50.00 (52.35)**	**41.67 (42.97)**	**33.33 (33.98)**	**25.00 (25.26)**
Control	**2.66**					**4.00**					**7.33**				

Each values in column is a mean of three replications
*Values in parenthesis are arc sine transformed values

4.1.4 LC_{50} value of different insecticide formulations against adults of *Trichogramma chilonis*

Two insect bioassay studies (Dry residue and filter paper method) were carried out to determine the order of toxicity of six insecticides *viz*; chlorantraniliprole, bifenthrin, cypermethrin, NSKE, Malathion, thiamethoxam against adults of *Trichogramma chilonis* to calculate the LC_{50} values for test insecticides.

4.1.4.1 Dry residue method

In this method, six insecticides at five different concentrations were evaluated to calculate the values of LC_{50} values against adults of *Trichogramma chilonis*. The date presented in the Table indicated that after 24 hours, the lowest LC_{50} was calculated in Thiamethoxam 25% WG (0.148 ±0.261) followed by bifenthrin 10% EC (0.695 ±0.912) and cypermethrin 25% EC (1.010±0.222). The highest LC_{50} was calculated in NSKE 5%EC (2.368±1.012). The LC_{50} values for all the test insecticides at five different concentrations ranged from 0.148 ±0.261 to 2.368±1.012. In dose response bioassay, all insecticide concentrations of test were found to be highly efficacious against insecticides against adults of *Trichogramma chilonis* (df = 3, p=0.05). The highly significant difference was observed in Thiamethoxam 25% WG (0.007) at all the concentrations at 5% level of significance.

After 48 hours, the lowest LC_{50} was calculated in Thiamethoxam 25% WG (0.047 ±0.506) followed by bifenthrin 10% EC (0.079 ±0.434) and cypermethrin 25% EC (0.498±0.395). The highest LC_{50} was calculated in NSKE 5%EC (1.879±0.265). The LC_{50} values for all the test insecticides at five different concentrations ranged from 0.047 ±0.506 to 1.879±0.265. In dose response bioassay, all insecticide concentrations of test were found to be highly efficacious against insecticides against adults of *Trichogramma chilonis* (df = 3, p=0.05). The highly significant difference was observed in NSEE 5% (0.006) at all the concentrations at 5% level of significance.

After 72 hours, the lowest LC_{50} was calculated in Thiamethoxam 25% WG (0.0754 ±0.747) followed by bifenthrin 10% EC (0.097 ±0.713) and chlorantraniliprole 18.5% SC (0.182±0.539). The highest LC_{50} was calculated in NSKE 5%EC (1.455±0.294). The LC_{50} values for all the test insecticides at five different concentrations ranged from 0.0754 ±0.747 to1.455±0.294. In dose response bioassay, all insecticide concentrations of test were found to be highly efficacious against insecticides against adults of *Trichogramma chilonis* (df = 3, p=0.05). The highly significant difference was observed in cypermethrin 25% EC (0.005) at all the concentrations at 5% level of significance.

Table 5: LC_{50} value of different insecticide formulations on the adults of *Trichogramma chilonis* after 24 hrs using dry residue method

Insecticides	LC_{50}	Intercept	χ^2	95% Confidence Interval		Relative toxicity
				Lower Bound	Upper Bound	
Chlorantraniliprole 18.5% SC	1.242	0.715	0.163	0.320	5.410	**1.9**
Bifenthrin 10%EC	0.695	1.032	0.404	0.105	5.517	**3.4**
NSKE 5%	**2.368**	0.223	0.153	1.509	6.320	**1**
Cypermethrin 25%EC	1.010	0.741	0.716	0.342	19.628	**2.3**
Malathion 25%WP	1.165	0.776	0.465	0.311	18.766	**2.0**
Thiamethoxam 25%WG	**0.148**	1.491	1.465	0.108	2.274	**16**

Table 6: LC_{50} value of different insecticide formulations on the adults of *Trichogramma chilonis* after 48 hrs using dry residue method

Insecticides	LC_{50}	Intercept	χ^2	95% Confidence Interval		Relative toxicity
				Lower Bound	Upper Bound	
Chlorantraniliprole 18.5% SC	0.460	1.264	.007	0.068	8.370	**4.06**
Bifenthrin 10%EC	0.079	1.714	1.270	0.408	19.168	**23**
NSKE 5%	**1.879**	0.272	0.203	1.156	6.029	**1**
Cypermethrin 25%EC	0.498	1.326	0.696	0.260	9.334	**3.7**
Malathion 25%WP	0.778	1.156	0.176	0.324	8.435	**2.41**
Thiamethoxam 25%WG	**0.0471**	2.229	2.945	0.0373	0.448	**39.9**

Table 7: LC_{50} value of different insecticide formulations on the adults of *Trichogramma chilonis* after 72 hrs using dry residue method

Insecticides	LC_{50}	Intercept	χ^2	95% Confidence Interval		Relative toxicity
				Lower Bound	Upper Bound	
Chlorantraniliprole 18.5% SC	0.182	1.191	0.273	0.0682	3.370	**7.9**
Bifenthrin 10%EC	0.0977	3.092	2.091	0.0520	1.064	**14**
NSKE 5%	**1.455**	0.779	0.203	0.783	9.626	**1**
Cypermethrin 25%EC	0.561	2.496	1.082	0.737	1.122	**2.5**
Malathion 25%WP	0.346	2.008	0.944	0.580	2.013	**4.1**
Thiamethoxam 25%WG	**0.0754**	2.856	1.201	0.0634	2.889	**19.3**

4.1.4.2. Filter Paper method

In this method, six insecticides at five different concentrations were evaluated to calculate the values of LC_{50} values against adults of *Trichogramma chilonis*. The data presented in the Table indicated that after 24 hours, the lowest LC_{50} was calculated in Thiamethoxam 25% WG (0.147 ±0.388) followed by bifenthrin 10% EC (0.923 ±0.354) and cypermethrin 25% EC (1.086±0.350). The highest LC_{50} was calculated in NSKE 5%EC (2.028±0.265). The LC_{50} values for all the test insecticides at five different concentrations ranged from 0.148 ±0.261 to 2.368±1.012. In dose response bioassay, all insecticide concentrations of test were found to be highly efficacious against insecticides against adults of *Trichogramma chilonis* (df = 3, p=0.05). The highly significant difference was observed in chlorantranilipole 18.5 % SC (0.004) at all the concentrations at 5% level of significance.

After 48 hours, the lowest LC_{50} was calculated in thiamethoxam 25% WG (0.039 ±0.488) followed by bifenthrin 10% EC (0.079 ±0.434) and cypermethrin 25% EC (0.271±0.388). The highest LC_{50} was calculated in NSKE 5%EC (1.634±0.267). The LC_{50} values for all the test insecticides at five different concentrations ranged from 0.039 ±0.488 to 1.634±0.267. In dose response bioassay, all insecticide concentrations of test were found to be highly efficacious against insecticides against adults of *Trichogramma chilonis* (df = 3, p=0.05). The highly significant difference was observed in NSKE 5% (0.002) at all the concentrations at 5% level of significance.

After 72 hours, the lowest LC_{50} was calculated in Thiamethoxam 25% WG (0.167 ±0.658) followed by bifenthrin 10% EC (0.097 ±0.591) and cypermethrin 25% EC (0.202±0.558). The highest LC_{50} was calculated in NSKE 5%EC (1.367±0.299). The LC_{50} values for all the test insecticides at five different concentrations ranged from 0.167 ±0.658 to 1.367±0.299. In dose response bioassay, all insecticide concentrations of test were found to be highly efficacious against insecticides against adults of *Trichogramma chilonis* (df = 3, p=0.05). The highly significant difference was observed in cypermethrin 25% EC (0.003) at all the concentrations at 5% level of significance.

Table 8: **LC_{50} value of different insecticide formulations on the adults of *Trichogramma chilonis* after 24 hrs using filter paper method**

Insecticides	**LC_{50}**	**Intercept**	χ^2	**95% Confidence Interval**		**Relative toxicity**
				Lower Bound	**Upper Bound**	
Chlorantraniliprole 18.5% SC	1.963	0.707	0.090	0.312	4.311	**1.03**
Bifenthrin 10%EC	0.923	0.834	1.025	0.205	6.517	**2.1**
NSKE 5%	**2.028**	0.283	0.271	1.331	3.781	**1**
Cypermethrin 25%EC	1.086	0.860	0.841	0.249	22.309	**1.8**
Malathion 25%WP	1.428	0.648	0.347	0.324	19.767	**1.4**
Thiamethoxam 25%WG	**0.147**	1.496	1.058	0.0214	2.452	**13.7**

Table 9: LC_{50} value of different insecticide formulations on the adults of *Trichogramma chilonis* after 48 hrs using filter paper method.

Insecticides	LC_{50}	Intercept	χ^2	95% Confidence Interval		Relative toxicity
				Lower Bound	Upper Bound	
Chlorantraniliprole 18.5% SC	0.182	1.802	0.786	0.682	3.370	**8.9**
Bifenthrin 10%EC	0.079	1.714	1.270	0.408	19.168	**20**
NSKE 5%	**1.634**	0.293	0.210	1.050	4.144	**1**
Cypermethrin 25%EC	0.271	1.412	0.606	0.178	7.146	**6**
Malathion 25%WP	1.640	1.032	1.083	0.424	6.435	**0.9**
Thiamethoxam 25%WG	**0.0398**	2.057	1.201	0.0234	2.889	**41**

Table 10: LC_{50} value of different insecticide formulations on the adults of *Trichogramma chilonis* after 72 hrs using filter paper method.

Insecticides	LC50	Intercept	χ^2	95% Confidence Interval		Relative toxicity
				Lower Bound	Upper Bound	
Chlorantraniliprole 18.5% SC	0.707	2.190	1.253	0.269	1.617	**1.9**
Bifenthrin 10%EC	0.202	2.463	2.164	0.004	3.623	**6.7**
NSKE 5%	**1.367**	0.830	0.158	0.728	7.682	**1**
Cypermethrin 25%EC	0.252	2.626	2.282	0.171	0.574	**5.4**
Malathion 25%WP	0.725	1.748	0.039	0.580	2.013	**1.8**
Thiamethoxam 25%WG	**0.167**	2.797	0.721	0.038	0.352	**8.1**

4.2 To study the impact of Gamma Radiations on egg parasitoids

The gamma radiation at the given doses were given to the adults of *Trichogramma pretiosum* to study their mortality at Sher-e-Kashmir Institute of Medical Sciences, Soura, Kashmir

4.2.1 Mortality with various doses of Gamma Radiations

The different irradiation doses *viz*; 5Gy; 25Gy; 50Gy; 100Gy; 200Gy were given to the adults of the *Trichogramma pretiosum* to obtain the per cent mortality as detailed.

At 5Gy dose of radiation, 5.00 % mortality was recorded while 25 Gy dose resulted in 13.33 % mortality while, 20 % mortality obtained @ 50 Gy. While, highest mortality of 98.33 and 100.00 % was recorded @ 100Gy and 200Gy doses to the adults of *Trichogramma pretiosum*.

The mortality percent followed the trend; 200 Gy > 100 Gy > 50 Gy > 25 Gy > 5 Gy (All emitted by Gamma cell elite 1, Cs -137 with 0.662 Mev at a dose rate of 6.7 Gy/min)

Characteristics of the beams used for irradiation of *Trichogramma pretiosum under laboratory conditions during 2021-2022*

Machine	Type of Beam	Energy of the beam	Dose rate at the calibration point
Gamma Cell Elite 1	Gamma	0.662mev	6.7Gy/min

Table 11: Dose dependent impact of gamma radiations in the mortality (%), survival ability (%) and parasitization rates of *Trichogramma pretiosum*

Doses Of Gamma Radiations (Gy)	Survivability (%)	Parasitization rate (%)	Mortality (%)
0	**100.00** (157.07)	45.00 (46.67)	**0.00** (0)
5	**95** (125.32)	70.17 (77.78)	5.00 (5.002)
25	86.66 (104.84)	**71.15** (79.16)	13.33 (13.37)
50	80.00 (92.72)	68.75 (75.80)	20 (20.13)
100	1.66 (1.67)	0.00 (0)	98.33 (138.79)
200	0.00 (0)	0.00 (0)	**100.00** (157.07)

Each values in column is a mean of three replications
*Values in parenthesis are arc sine transformed values

4.2.2 LC_{20}, LC_{50}, LC_{90} values of different doses of Gamma Radiations irradiated on the adults of *Trichogramma pretiosum*

One day old *Trichogramma pretiosum* adults were exposed to the doses of gamma radiations *viz*: 5GY, 25Gy, 50Gy, 100Gy, 200Gy to obtain the LC_{20}, LC_{50}, LC_{90} values. The data recorded and revelead that at the lower Gys recorded the lowest moratlities for *Trichogramma pretiosum,* while, at the higher Gys, the highest mortality was recored for the one day old adults of *Trichogramma pretiosum.* At 5 Gy level, the mortality of 5.00 % was recorded and the highest mortalities of (98.33 and 100.00) were recorded at 100 and 200 Gy doses for the adults of *Trichogramma pretiosum*.There is significant variations at all the Gy doses in response to their mortalities.

Table 12: LC_{20}, LC_{50}, LC_{90}, values of different doses of gamma radiations irradiated on the adults of *Trichogramma pretiosum* under laboratory conditions in 2021-2022.

Doses	Values	95% Confidence limits (lower bound)	95% Confidence limits (upper bound)	Intercept
LC_{20}	2.384	1.641	5.674	5.157
LC_{50}	2.249	1.441	4.998	
LC_{90}	2.148	1.345	5.554	

4.3 Preference and performance of egg parasitoids

Three species of *Trichogramma Viz*; *T.chilonis, T.bactrae and T.pretiosum* were evaluated for their preference and performance on the *Corcyra* eggs using a Y- tube olfactometer at Division of Entomology, SKUAST-K, Shalimar.

Chi-square analysis or the Goodness of fit was conducted to evaluate and assess the preference and performance tests. Eggs of *Corcyra cephalonica* when left for parasitisation by *Trichogramma bactrae* gave χ^2 value 6.84 when analysis was conducted between washed and unwashed eggs. The highly significant difference was observed in one day old eggs of *Corcyra cephalonica* (0.0089) of all the day old eggs at 5% level of significance (d.f = 1)

Different age old eggs of *Corcyra cephalonica* when left for parasitisation by *Trichogramma chilonis* gave χ^2 Value 12.16 when analysis was conducted between washed and unwashed eggs. The highly significant difference was observed in 2 day old eggs of *Corcyra cephalonica* (0.0005) of all the day old eggs at 5% level of significance (d.f = 1)

Eggs of *Corcyra cephalonica* when left for parasitisation by *Trichogramma pretiosum* gave χ^2 Value 6.84 when analysis was conducted between washed and unwashed eggs. The highly significant difference was observed in 1 day old eggs of *Corcyra cephalonica* (0.00089) of all the day old eggs at 5% level of significance (d.f = 1).

Table 13: The preference test conducted on the different age groups (eggs) of *Corcyra cephalonica*.

Species	1, 2 and 3 age-old eggs	
	χ^2	*p* - value
T.bactrae	6.84	0.0089
T.chilonis	12.16	0.0051
T.pretiosum	6.84	0.0089

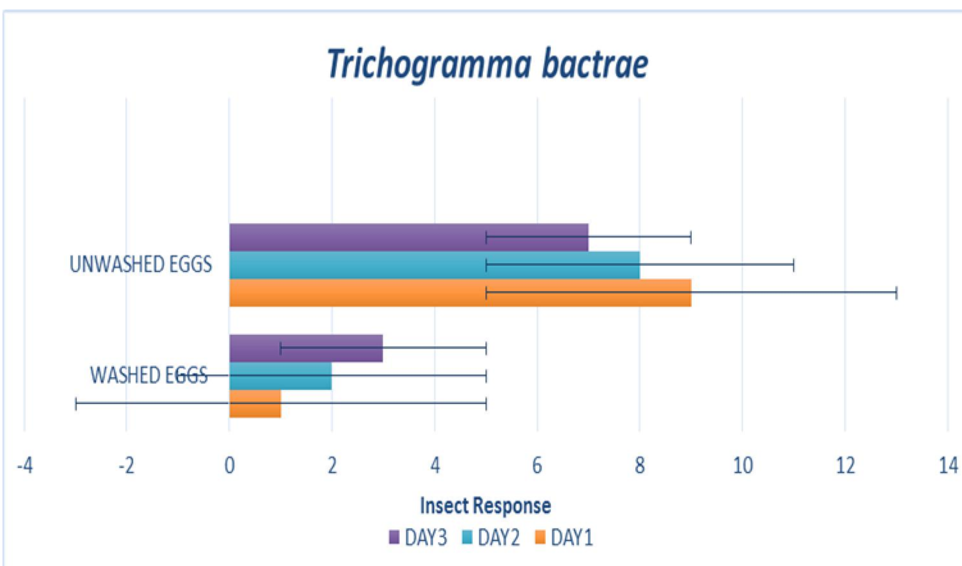


Fig. 1: Insect response observed when unwashed eggs and washed eggs were plotted against preference of *Trichogramma bactrae* under laboratory conditions 2021-2022

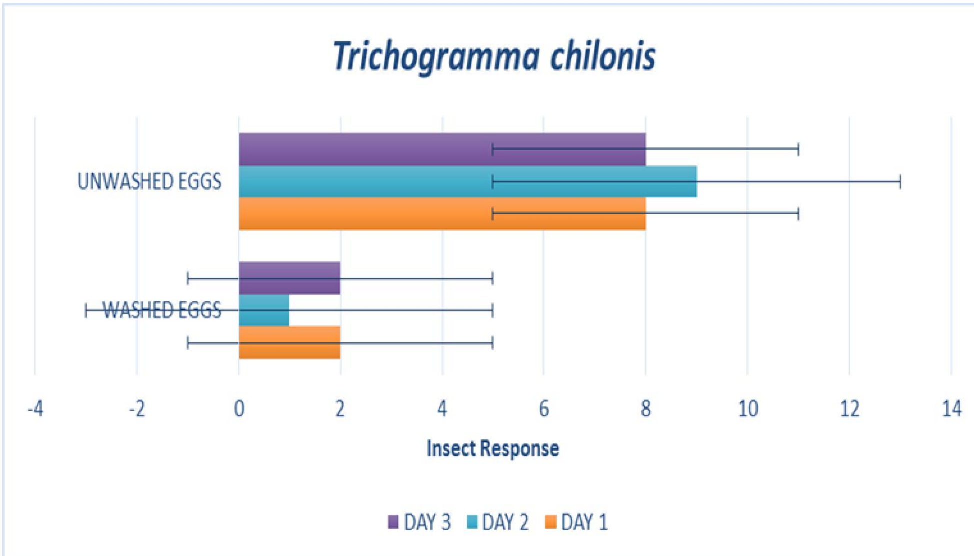


Fig 2: Insect response observed when unwashed eggs and washed eggs were plotted against preference of *Trichogramma chilonis* under laboratory conditions 2021-2022

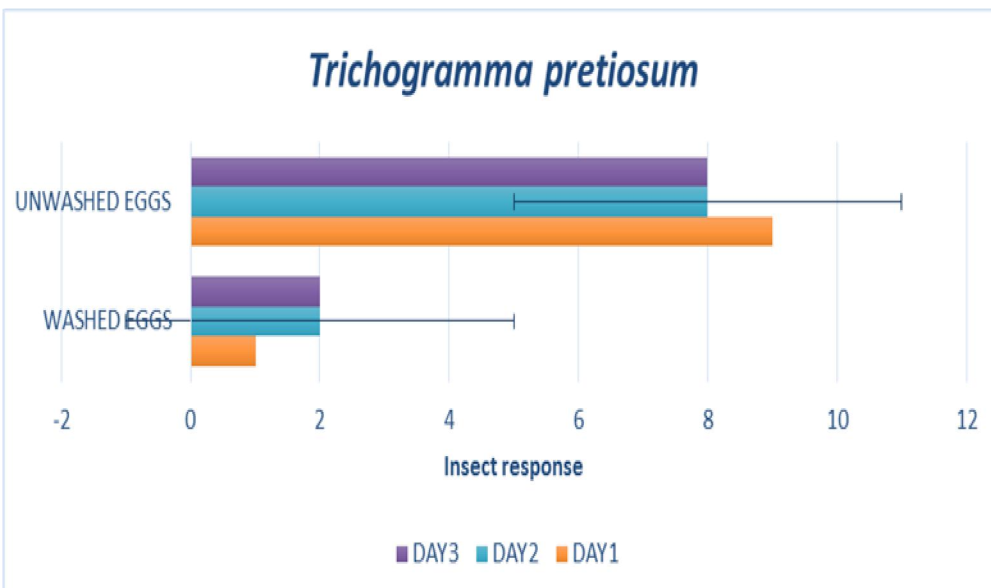


Fig. 3: Insect response observed when unwashed eggs and washed eggs were plotted against preference of *Trichogramma pretiosum* under laboratory conditions 2021-2022

Chapter 5

DISCUSSION

5.1 To study the toxicity of insecticides on egg parasitoids;

To evaluate the effectiveness of different insecticides *viz*; chlorantranilipole 18.5 SC; Bifenthrin 10 EC; Cypermethrin 25 EC; NSKE 5; Malathion 25WP and Thiamethoxam 25 WG at three different dosage levels against the *Trichogramma chilonis* adults in vitro. Evaluation of these tested insecticides against *T. chilonis* were quantified using dry residue and filter paper methods. These two bioassays methods were considered relevant, though lot of bioassays are being adopted to determine the efficacy and effectiveness of these insecticides (Sattar *et al*. 2011, Prema *et al*. 2016) against different parasitoids. The evaluation of insecticides for egg parasitoids is very important because of their smaller size and other peculiar attributes. Therefore, the mortality of all the test insecticides against *T. chilonis* has been evaluated at different time intervals. Besides, the LC_{50} values quantified for each insecticide from the above bioassay methods to calculate the selective toxicity of these insecticides against *T. chilonis*. These values will aid in revealing the highest toxic and least toxic insecticides against this egg parasitoids. The best and safe insecticides which cause significant mortality to *T. chilonis* may further be evaluated under field conditions, as there are lot of factors which significantly hampers their preference and performance due to stress of biotic and abiotic factors. Different levels of toxicities found in different insecticides, in vitro revealed significant mortalities against *T. chilonis*. The efficacy of the formulated insecticides against *T.chilonis* under laboratory conditions are briefly summarized as detailed below:-

5.1.1 Dry Residue Method:

Dry Residue Method allows for the assessment of insecticides to check the particular dose or doses are effective for the egg parasitoids or other insect pests under vitro. The efficacy of the insecticides (Chlorantranilipole 18.5 SC;

Bifenthrin 10 EC; Cypermethrin 25 EC; NSKE 5; Malathion 25WP and Thiamethoxam 25 WG) against *T. chilonis* at five different concentrations *viz*; 0.5, 0.4, 0.3, 0.2, 0.1 and 5 ml/L and 4, 3, 2, 1 and 0.5 ml/L of water, respectively for NSKE. The data on the corrected mortality showed significant difference at higher doses as compared to control treatment. All the treatments are highly significant at 5 % level of significance. While the mortality % of *T. chilonis* proportionately increased at higher doses as compared to the lower doses. Our findings are in agreement with Varghese (2013) who reported higher mortality at higher concentration of insecticides against egg parasitoids. The present study reported that thiamethoxam 25% WG and bifenthrin 10% EC recorded the highest mortality % and the LC_{50} value was found in accordance to the present findings. While, Bikash & Shinde (2021) documented that the thiamethoxam recorded the most lethal against *T.chilonis.* whereas, Qasim *et al.* (2018) reported that bifenthrin in terms of toxicity, revealed the most effective insecticide. Preetha *et al.* (2009) reported that chlorantranilipole 18.5% EC as the least toxic. Prema *et al.* (2016) stated that NSKE 5% is the safer insecticides and could be explored for future reach programmes, which showed significant mortality for the *T. chilonis* at $P \leq 0.05$. as compared to other treatments. Though, it showed lowest mortality as compared to other treatments and LC_{50} values against *T. chilonis*.

5.1.2 Filter paper method:

Filter paper allows for the assessment of insecticides to check the particular dose or doses are effective for the egg parasitoids or other insect pests under vitro. The efficacy of the insecticides (Chlorantranilipole 18.5 SC; Bifenthrin 10 EC; Cypermethrin 25 EC; NSKE 5; Malathion 25WP and Thiamethoxam 25 WG) against *T. chilonis* at five different concentrations *viz*; 0.5, 0.4, 0.3, 0.2, 0.1 and 5 ml/L and 4, 3, 2, 1 and 0.5 ml/L of water, respectively for NSKE. The data on the corrected mortality showed significant difference at higher doses as compared to control treatment. All the treatments are highly significant at 5 % level of significance. While the mortality % of *T. chilonis* proportionately increased at higher doses as compared to the lower doses. Our findings are in agreement with Varghese (2013) who reported higher mortality at higher concentration of insecticides against egg parasitoids. The present study reported that thiamethoxam 25% WG and bifenthrin 10% EC recorded the highest mortality % and the LC_{50} value was found in accordance to the present findings. While, Bikash & Shinde (2021) documented that the thiamethoxam recorded the most lethal against *T.chilonis.* whereas, Qasim *et al.* (2018) reported that bifenthrin in terms of toxicity, revealed the most effective insecticide. Preetha *et al.* (2009) reported that chlorantranilipole 18.5% EC as the least toxic. Prema *et al.* (2016) stated that NSKE 5% is the safer insecticides and could be explored for future reach programmes, which showed significant mortality for the *T. chilonis* at $P \leq 0.05$. as compared to other treatments. Though, it showed lowest mortality as compared to other treatments and LC_{50} values against *T. chilonis*.

5.2 To study the impact of doses of gamma radiations on egg parasitoids

Different irradiation doses *viz*; 5Gy; 25Gy; 50Gy; 100Gy; 200Gy were applied to *Trichogramma pretiosum* adults, which is considered as a potential egg parasitoid for the *Helicoverpa armigera.* The adults of *Trichogramma pretiosum* were exposed to different irradiation doses to quantify their mortality and survivalbility % so that the traits if developed may be explored for future research programmes due to mutations. Though, the ionisation radiations in the form of Gy rays are highly lethal as they induce lot of heat and are lethal to organisms (Hallman *et al.,* 2010).The higher ionisation radiations induce high heat to the test organisms (Bakri *et al.,* 2005).

The results of our study indicate a clear dependence of the response of the *Trichogramma pretiosum* adults using different gamma radiation doses. The data on mortality % significantly revealed that at higher doses, the mortality of the adults proportionately increased than the lower doses. (Hamed *et al*., 2009). The present study recorded 5 to 50 Gy doses which cause lower mortality% for *T.*

pretiosum. Besides, the LC_{50} value was also low (2.267) from 5 and 50 Gy doses, respectively. The exposure to *T. pretiosum* adults at 100 Gy and 200 Gy doses cause significant mortality of 98.33 and 100 for *Trichogramma pretiosum*. Hamed *et al.* (2009) proved that 50Gy dose of radiations are least lethal against a *T.chilonis*. However, our findings are first to record mortalities and survivability at different radiation doses and could be exploited for future research programmes. Our data is also near the values of doses reported by Mansour M. Y (2003, 2010, 2015, 2016).

Further, the trichocards on which the host eggs of *Corcyra cephalonica* were glued were exposed to exposed to 10 Gy irradiation doses. The other batch of *Corcyra cephalonica* eggs, were not exposed to irradiation as served as control. When the *T. pretiosum* adults were released on irradiated and un-irradiated eggs.

Our results revealed the quick adult emergence rates of *T. pretiosum* on irradiated eggs of *C. cephalonica* and less as compared to control (un-irradiated eggs of *C. cephalonica*. While as the irradiated eggs of *C. cephalonica* were more parasitised than the unirradiated eggs of *C. cephalonica*. Our findings are are in agreement with Xu *et al*. (2016) who reported that UV radiated *C. cephalonica* eggs were more parasitized than the un- irradiated eggs. While, Tuncibelek *et al*. (2009) observed no adult emergence and parasitisation at the irradaition doses of 200Gy and 150Gy for *T. evanesecens,* which was reared on both irradiated and un-irradiated host eggs of *Sitrotroga cereallella.*

5.3 To study the preference and performance of different egg parasitoids on eggs of *Corcyra*

Three species of *Trichogramma Viz*; *T.chilonis, T.bactrae and T.pretiosum* were evaluated for their preference and performance on the *Corcyra* eggs using a Y- tube olfactometer, Pheromone Technology Laboratory, Division of Entomology, SKUAST-K. For studying the preference of three egg parasitoids, in relation to the different age groups of *C. cephalonica* eggs revealed that young host eggs of *C. cephalonica* are more preferred as compared to older eggs. It indicated that earlier host eggs of *C. cephalonica* are more preferred by all the egg parasitoids (*T.pretiosum, T.chilonis, T.bactrae*) just to complete their egg, larval, pupal development stages. The viability of the host eggs are very important for the parasitoid survival as they compete inside the host eggs of *C.cephalonica* for their resources on which they live and survive. Our results revealed that *T. chilonis* significantly influenced by the different age regimes (1 day, 2 day and 3 day old eggs of *C.cephalonica*). While, *T bactrae* and *T. pretiosum* showed strong preference for the one day old eggs of *C.cephalonica*. In contrary to that, *T. chilonis* exhibited strong preference to 2 days old eggs of *C. cephalonica*. All the egg parasitoids are least preferred to the 3 days old eggs of *C. cephalonica*. Myint *et al*. (2022) demonstrated under no-choice conditions, eggs of different ages of hosts, accepted by *Trichogramma* however, the *T. dendrolimi* parasitised significantly fewer eggs with increasing host age.

Beisdes, under no- choice conditions, all the egg parasitoids (*T.pretiosum, T.chilonis, T.bactrae*) showed preference towards the unwashed eggs as compared to the un-washed eggs of *C.cephalonica*. The reason for such attraction is due the presence of surface chemicals on the insect surface which acts as cues for egg parasitoids for parasitisation of their host (Howard and Blomquist, 2005; Menzel et al., 2019).The results were in close association with the findings of It was demonstrated that egg parasitoids while landing on the eggs of hosts perceive the host with their legs and antenna to ensure it is not being parasitised. So both the chemical and visual cues are important for taking the decision for the parasitisation of the host (Blomquist and Bagneres, 2010). In addition, the eggs of the insects are glued with lot of cuticular hydrocarbons which play role as pheromones (mate finding, kin recognition, sexual communication and pre-copulatory courtship behavior, Chung and Carroll, 2015). CHCs have been shown to be crucial in eliciting appropriate behavioral responses in several species (Howard, 1998; Ruther et al., 2000, 2011; Krokos et al., 2001; Sullivan, 2002; Steiner et al., 2005; Ablard et al., 2012; Sullivan and Erbilgin, 2015; Weiss et al., 2015; Pfeiffer et al., 2018; Böttinger et al., 2019).

Chapter-6

SUMMARY AND CONCLUSION

The investigation on "Toxicity of insecticides and impact of gamma radiations on egg parasitoids" was carried out in the AIRCP Biocontrol Laboratories, Division of Entomology, SKUAST-K and SKIMS Soura, Srinagar during the year of 2021 and 2022. The results obtained during the course of study are summarized as under:

6.1 To study the toxicity of insecticides on egg parasitoid.

In the two bioassay methods dry residue method and filter paper method thiamethoxam 25% WG recorded the highest corrected mortality on adults of *Trichogramma chilonis*. The LC_{50} associated with the treatments revealed that the thiamethoxam 25% WG showed the effective results.

6.2 To study the impact of doses of gamma radiations on egg parasitoids.

The highest mortality was recorded @ 100Gy and 200Gy doses to the adults of *Trichogramma pretiosum* and the LC_{50} associated with the doses of gamma radiations revealed that 100Gy and 200 Gy showed highest mortality. At 5Gy, 25Gy and 50 Gy doses of radiation high emergence rates were observed than the unirradiated adults of *Trichogramma pretiosum*.

6.3 To study preference and performance of different egg parasitoids on the eggs of *Corcyra.*

Aged eggs of *Corcyra cephalonica* are least preferred by the egg parasitoids. Washing of eggs of *Corcyra cephalonica* decrease the preference of egg parasitoids. The one day old eggs of *Corcyra* was preferred by both *T.pretiosum* and *T.bactrae* while two day old eggs were preferred by *T.chilonis* from the goodness of fit test. (p- value ≤ 0.05).

To summarize the experimental findings, it was concluded that:

- All the bioassay results revealed that thiamethoxam 25% WG cause the highest mortality at all the test concentrations on *Trichogramma chilonis*.
- High parasitization rates by *T. pretiosum* was observed by applying the sub lethal doses (5Gy, 25Gy) of gamma radiations.
- *T. chilonis* is significantly influenced by the different age regimes while, *T.bactrae* and *T. pretiosum* showed strong preference for the one day old eggs of *C. cephalonica*. In contrary to that, *T. chilonis* exhibited strong preference to 2 days old eggs of *C. cephalonica*. All the egg parasitoids are least preferred to the 3 days old eggs of *C. cephalonica*
- The preference and performance tests revealed that all the species preferred unwashed eggs over washed eggs of *Corcyra cephalonica*.

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CERTIFICATE

Certified that all the corrections/amendments as suggested by External Examiner **Dr. T. A. Shusha,** Associate Professor, Department of Zoology, University of Kashmir, Hazratbal during Viva-Voce examination held on **25-08-2023** have been incorporated in the manuscript entitled "**Toxicity of insecticides and impact of gamma radiations on egg parasitoids**" submitted by **Ms. Gayathri Kishore (Regd. No. MSH-2021-419).**

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