Effect of insecticides on *Trichogramma chilonis* L., egg parasitoid of large cabbage moth, *Crocidolomia pavonana* F.

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ABSTRACT

The study was carried out to examine the effects of key insecticides against Trichogramma chilonis parasitism of large cabbage moth (LCM). Three days after spraying with AttackTM, OrtheneTM and EntrustTM (permethrin + pirimiphos-methyl, acephate and spinosad), no parasitism of LCM eggs occurred. After 3 days of Bacillus thuringiensis (Bt) treatment, parasitism of LCM egg mass was 100 %, which is the same as the control. No parasitism of the egg mass occurred after spraying with either AttackTM or OrtheneTM. The percentage of parasitised LCM eggs after Bt treatment was 13.48; the control showed the highest parasitism of LCM eggs (58.13 %). The mortality of T. chilonis adults (in descending order) due to the insecticides after 15 hours was Entrust, Attack, Orthene and Bt. The result suggests that Bt could be included in Integrated Pest Management Programmes that depend on T. chilonis parasitism of LCM eggs and T. chilonis activity.

Key words: Large cabbage moth, egg parasitoid, insecticide effect, *Trichogramma chilonis* and *Crocidolomia pavonana*.

INTRODUCTION

Large cabbage moth (LCM), *Crocidolomia pavonana* F. (Lepidoptera: Crambidae), is a major pest of brassica crops in tropical and subtropical regions of Africa, Asia and the Pacific Islands, including Samoa (Uelese et al., 2014). Insecticides are the only option to control LCM infestations by most farmers in Samoa and in other Pacific island countries where this pest is a problem (Uelese et al., 2014). This pest is relatively susceptible to most commonly used insecticides, and the widespread use of synthetic insecticides diminished LCM as the main principal pest of brassica crops in the tropical and subtropical regions (Ankersmit, 2009).

However, the use of insecticide is not always a viable and economical measure to control insect infestations due to their detrimental impact on the environment and non-target organisms (All and Treacy, 2006; Borgemeister et al., 1993; Heong et al., 1995; Hussain et al., 2012; Oka, 1995; Ooi and Shepard, 1994).

The natural enemies of LCM, Trichogrammatidae species particularly *T. chilonis* significantly suppressed its population (Uelese et al., 2014) but there are no records of the effect of insecticides on both LCM and its natural enemy. *T. chilonis* is present in some parts of the world such as Asia, Africa, North America, Central America and Caribbean, Europe and Oceania. It takes 3-5 days for the completion of its lifecycle. This parasitoid hosted in various insect pests but mostly on Lepidopterans. Bioassay studies of some insecticides have been carried out on *Trichogramma chilonis* but not with its host LCM (Boomathi et al., 2005; Hussain et al., 2012; Jalali et al., 2006; Mutambuki et al., 2003; Preetha et al., 2009; Sathish and Raghuraman, 2007).

The present study assessed the effects of four selected insecticides on *T. chilonis* parasitism of large cabbage moth eggs and the mortality of *T. chilonis* adults.

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MATERIALS AND METHODS

This study was carried out at University of the South Pacific (USP), School of Agriculture and Food Technology (SAFT), Alafua, Apia, Samoa between September and March 2015.

Rearing and maintaining the colony of large cabbage moth

The colony of LCM was raised and maintained from larvae collected from cabbage farms in Aleisa, Samoa. Emerged adults of LCM were then released into two oviposition cages (1 cage = 70 x 60 cm); the oviposition cages were covered with fine-sieve nylon net.

Five pot plants (4 weeks old cabbage plants) were placed in each cage and left undisturbed overnight to allow LCM oviposition to take place. Then, each pot plant was removed and replaced with fresh cabbage pot plants from the nursery. From the removed pot plants, oviposited egg masses attached to cabbage leaves were placed inside several Petri dishes for the continuous rearing of the LCM colony. The insect rearing was carried out under controlled conditions (26±3 oC, RH 65±5%, Photoperiod 12: 12) at the USP Entomology Laboratory.

Rearing and maintaining of *Trichogramma chilonis*

The colony of the egg parasitoid, *T. chilonis*, was maintained and monitored under controlled conditions (26±3 oC, RH 65±5%). Fresh day-old LCM egg masses (coloured light green) were removed from each leaf of the cabbage plants and placed on para-film using a fine brush. The para-film consisting of 6 egg masses were given to a population of *T. chilonis* to allow parasitism for three days. The parasitised egg masses were then removed and stored inside capsules for 4 days. The emerged adult wasps were used for parasitising new LCM egg masses in the oviposition cage. This work was done continuously until the end of the study.

Transferring and removal of cabbage pot plants from the oviposition cages

Twenty cabbage pot plants (4 weeks old), with an average of 4 leaves, were placed in the two oviposition cages, 10 pot plants per cage (same ages used for the oviposition experiment) in the morning.

Each cage consisted of more than 50 moths (male and female) of LCM adults; 10% of honey solution (a source of food for LCM adults) was placed in each oviposition cage. In both cages, cabbage plants and LCM adults were left undisturbed for 24 hours to provide enough time for the female moth's oviposition. After 24 hours, all pot plants from both oviposition cages plus the laid one day-old egg masses were removed and distributed into five treatments (four selected insecticides and the control) in separate labeled trays.

Four insecticides, permethrin + irimiphos-methyl, acephate, spinosad and *B. thuringiensis* were used for the bioassay trials.

Table 1: Description of insecticides and mixing rates used for the bioassay experiments.

Active Ingredient	Chemical Class	Recommended Rate
Permethrin + Pirimiphos-methyl	Organophosphate	4 ml/20 L
Acephate	Organophosphate	15 g/20 L
Spinosad	Microbial	3 ml/15 L
Bacillus thuringiensis	Microbial	20 g/20 L

Twelve plants were sprayed (3 plants per insecticide) and 3 plants were allocated as control. Each plant leaf was sprayed with 7 ml (mixed insecticides) for about 5 seconds using a spraying bottle. Sprayed and unsprayed (control) cabbage plants were placed in separate labeled trays and transferred inside the bioassay chamber, and left undisturbed for 1 hour.

Sprayed large cabbage moth egg mass with *Trichogramma chilonis*

After 1 hour of insecticide application, the sprayed and unsprayed LCM egg masses on cabbage plants were transferred into the bioassay chamber. From each sprayed and unsprayed cabbage plant per treatment, one leaf where LCM one day-old egg mass was laid, was removed using a scalpel and placed in labeled Petri dish. The 15 plastic Petri dishes (8.7 cm diameter per petri dish) were covered with round tissue paper and their lids had a ventilation hole (1.2 cm diameter) covered with very fine mosquito net.

Fifteen spherical cotton wools (diameter = 1 cm) were soaked in 10% of honey solution and placed in each Petri dish, 30 minutes before the placement of the wasps. One mated female wasp (2 days old) was also placed in each Petri dish, in which its gender was determined straight after emergence, one day before the experiment was carried out.

Sprayed and un-sprayed egg masses plus one pair of female *T. chilonis* in each Petri dish were laid for 3 days in CRD design. Observations were made on longevity of the parasitoid after 1, 3, 6, 9, 12 and 15 hours. *T. chilonis* parasitism of LCM eggs was also checked per treatment after 3 days. This was carried out by counting the number of eggs parasitised per egg mass in each replication per sprayed insecticide.

Statistical analysis

Effect of insecticides on *T. chilonis* mortality and parasitism was analysed by One way ANOVA, using Minitab 17 software.

RESULTS

Permethrin + pirimiphos-methyl**,** Acephate, Spinosad showed harmful effects on *T. chilonis* parasitism of LCM eggs; no eggs of the sprayed egg masses with these insecticides were parasitised. All of the LCM egg masses untreated (control) and Bt (Dipel) treatment were parasitized by *T. chilonis*. However, the number of parasitised eggs per individual LCM egg mass differed significantly between Dipel^{TM} (13.48 %) and control (58.13 %). Therefore, Dipel^{TM} reduced *T. chilonis* parasitism but it did not show the same harmful effect as the other three insecticides.

Table 2: Parasitism of large cabbage moth eggs per egg mass by *Trichogramma chilonis* 15 hours after spraying with Dipel.

Treatment	Trial	No. of Eggs/Egg Mass		No. of Parasitised Eggs/Egg Mass		% Parasitism
		Total	Mean	Total	Mean	
Control	1	82	27.33	45	15.00	54.88
	2	102	34.00	52	17.33	50.98
	3	112	37.33	69	23.00	61.61
	4	110	36.67	70	23.33	63.64
	Average	**101.5**		**59**		**58.13**
Dipel	1	124	41.3	18	6.0	14.52
	2	177	59.0	14	4.7	7.91
	3	96	32.0	17	5.7	17.71
	4	100	33.3	18	6.0	18.00
	Average	**124.25**		**16.75**		**13.48**

Mortality of adult *T. chilonis* was noted in all samples treated with insecticide and no mortality was observed in the Control.

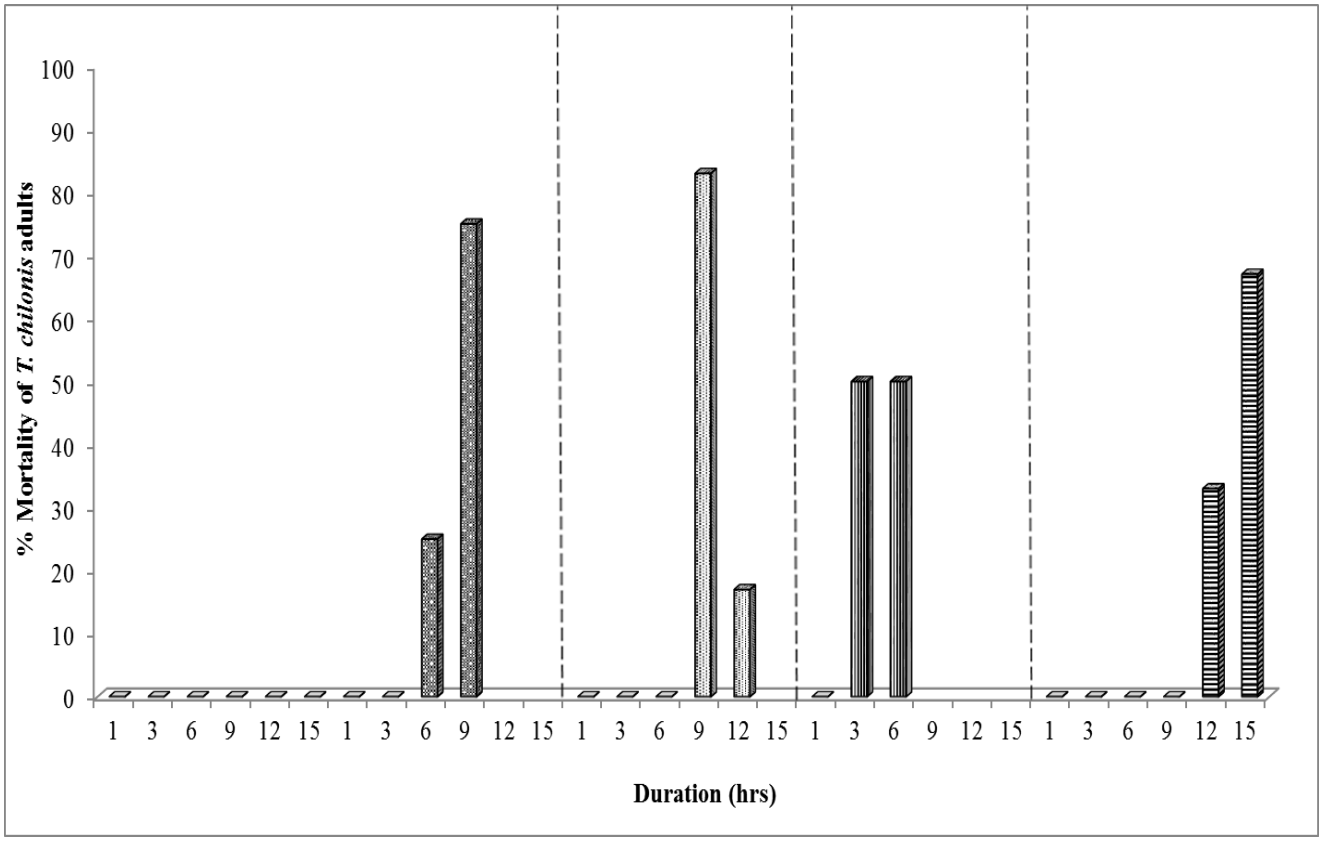

Figure 1: Percentage mortality of *Trichogramma chilonis* adults one day after spraying with insecticides and at various of observation ranging from one to 15.

However, 50 % of the *T. chilonis* adults died after 3 hours of Entrust™ treatment and the other 50 % died after 6 hours. Attack™ showed mortality of 25% after 6 hours of treatment and the other 75 % died 9 hours after treatment. About 83 % of the *T. chilonis* adults showed mortality after 9 hours of Orthene™ and then 17 % died 12 hours after. However, Dipel showed only 33 % mortality of the wasps 12 hours after treatment and 67 % were found dead after 15 hours of treatment.

The order of mortality against the insecticide used was Entrust™ > Attack™ > Orthene™ then Dipel™. Of all the tested insecticides on the mortality rate of *T. chilonis* adults, Dipel™ had lower toxicity to the parasitoid but not Entrust™, Attack™ and Orthene™.

DISCUSSION

Active ingredients play an important role in determining how harmful an insecticide can be to its target insect pest. Organophosphate insecticides, Attack™ and Orthene™, have both contact mode of action whereas biopesticides such as Entrust™ affect the nervous system and kill the insect within 1-2 days while Dipel™ damages the insect gut thereby preventing the insect to feed resulting in death within 2-3 days.

Attack™, Orthene™ and Entrust™ negatively affected *T. chilonis* parasitism by killing the parasitoid before any parasitism occurred. Wang *et al*. (2012) and Borgemeister *et al*. (1993) agreed with the effect showed by Attack™ after spray, wherein *T. chilonis* is highly susceptible to broad spectrum insecticides. The same conclusion was obtained by Saljoqi *et al.* (2012) and Jalali *et al*. (2006); Entrust™ (Spinosad) was found extremely toxic to *T. chilonis*. However, current findings also reported that Dipel™ is harmless to the parasitoid. The same conclusion was drawn by Pawar (1996) since Dipel™ (Bt) did not affect the survival of the parasitoid.

As the results revealed, *T. chilonis* adult mortality, in descending order, due to insecticides sprayed on LCM eggs after 15 hours of treatment, was Entrust™, Attack™, Orthene™ and Dipel™. Entrust™ was found to be more harmful against the egg parasitoid, *T. chilonis*, compared to other treatments, killing the majority of *T. chilonis* within 3 hours of exposure. Similar results were obtained by Jalali *et al*. (2006). Entrust™ also had toxic effects on other biological control agent *Trichogramma pretiosum* Ruberson and Tillman (1999).

CONCLUSION

T. chilonis parasitism of LCM eggs and egg parasitoid adult survival were affected by the insecticides. Attack™, Entrust™ and Orthene adversely affected *T. chilonis* parasitism rates of LCM eggs by killing the egg parasitoid before any parasitism took place. Entrust™ was more effective in killing the parasitoid followed by Attack™, Orthene™ and Dipel™. Of all the insecticides tested, Dipel™ reduced *T. chilonis* parasitism the least and parasitoid mortality was relatively less. Dipel™ could be considered for inclusion in IPM programmes that depend on *T. chilonis* parasitism of LCM.

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