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IMPACT OF INSECTICIDES ON TRICHOGRAMMA CHILONIS PARASITISM AND IN THE OVIPOSITION PATTERN OF LARGE CABBAGE MOTH EGGS

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This study determined the effects of key insecticides on *Trichogramma chilonis* parasitism in field trials. It showed that the number of large cabbage moth (LCM) eggs in Chinese cabbage was significantly reduced after application of Attack™ (p: 0.001) and Entrust™ (p: 0.00) but not after spraying with Orthene™ (p: 0.09) and Dipel™ (p: 0.485). Negative effects of insecticides were observed on the parasitism rate of *T. chilonis* and LCM egg masses, which were more likely to be only partially parasitised after an insecticide application. Attack™ and Entrust™ adversely affected *T. chilonis* parasitism rates of LCM eggs. However, the effect of Orthene™ and Dipel™ did not significantly differ from each other and could be safer to the parasitoid since both reduced *T. chilonis* parasitism the least. The distribution of LCM eggs in the cabbage leaves was also affected by the insecticides; before insecticide application the majority of egg masses were concentrated in the upper surface of the foliage but after insecticide sprays most of the egg masses were found in the lower surface of the foliage. This study suggests that Orthene™ and Dipel™ have no effect on the parasitism but Dipel™ was found safer to the parasitoid and it could be considered for inclusion in IPM programmes that depend on *T. chilonis* parasitism of LCM.

Keywords: large cabbage moth; oviposition pattern; egg parasitoid; impact of insecticide; *Trichogramma chilonis*

The use of insecticides is a powerful tactic that can quickly and economically suppress a pest population. However, insecticides are not always economically viable due to their negative effect on non-target organisms, especially parasitoids and other beneficial insects that help in reducing some pest infestations. The parasitoids are highly susceptible to broad-spectrum insecticides and this type of insecticides can quickly reduce the infestation of the target pest before reaching the economic threshold (Borgemeister et al., 1993; Wang et al., 2012).

Several common insecticides used to control insect pests of cabbage in South Africa and in the Pacific islands such as Samoa include pyrethroids, insect growth regulators and microbial pesticides. Some of these insecticides could be harmful to both the pests and their natural enemies.

In Samoa, LCM is the main constraint faced by cabbage farmers. It is a major pest of Brassica crops in the sub-tropical regions of Africa, Asia and some Pacific island countries (Morillo-Rejesus and Navasero-Ward, 2003; Ueese et al., 2014; Varela et al., 2003) and it attacks crops from transplanting through harvest (Ebenebe et al., 2006). Insecticides are used by most cabbage farmers in Samoa to control LCM infestations but their effects on LCM and its natural enemy, *T. chilonis*, have not been documented.

Thus, this study was carried out in Samoa to determine the impact of insecticides on completely and partially parasitised large cabbage moth egg masses and to examine

the effect of insecticides in the oviposition patterns of large cabbage moth egg masses on cabbage leaves.

Material and methods

Experimental field sites and insecticides used

This study was carried out at the USP, SAFT, Alafua in Samoa in five different locations (100–200 m apart) and using four insecticides (Attack™, Orthene™, Dipel™ and Entrust™). Attack™ is a broad-spectrum insecticide while Orthene™ is a systemic insecticide; Dipel™ and Entrust™ are bio-pesticides. The insecticides used have different active ingredients; Attack™ (Permethrin + Pirimiphos-methyl), Orthene™ (Acephate), Entrust™ (Spinosad) and Dipel™ (*Bacillus thuringiensis*).

Preparation of cabbage seedlings in the nursery and field

Kwang Moon, variety of Pak Choi, was raised on trays that contained sterilized top soil and potting mix at the USP greenhouse nursery. Two weeks after sowing, seedlings were thinned and watered regularly. Trays of cabbage seedlings were placed outside the greenhouse exposed to sunlight for hardening before transplanting.

The preparation of each plot (50 m²) was done by ploughing. The soil was softened and four beds (1 bed =

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9 m²) were prepared per plot. Each plot was added with NPK one week before transplanting.

Cabbage plot layout and seedlings transplanting

Two hundred cabbage seedlings were sown per plot (50 per bed), with a spacing of 50 × 50 cm within plants and between rows. The spacing between each bed per plot was 1 meter. Four weeks old seedlings were used and the transplanting was done in the afternoon. Plants were watered and weeded regularly.

Large cabbage moth and *Trichogramma chilonis* sampling before and after insecticide sprays

The abundance of LCM and its natural enemy *T. chilonis* was monitored on cabbage plots in September – October, 2014 and March – April, 2015 at USP, SAFT, Alafua campus. After 2 weeks from transplanting of the cabbage seedlings into each plot, fifty percent of the plants (100 plants) were randomly selected from each plot for observation.

Of the 100 selected plants, 25 plants were randomly selected from each bed per plot. The selected plants were marked with labels 1–100 one week prior to the sampling of LCM and its natural enemy, *T. chilonis*. The sampling of the LCM egg masses and *T. chilonis* was carried out after 2 weeks from transplanting. The sampling followed two intervals, before insecticide sprays and after insecticide sprays.

Before insecticide sprays, the sampled plant in each plot was observed for LCM egg mass in 2 weeks, 2 days per week. The selected plants were observed for LCM egg masses using Lumagny 4 Xilluminated magnifying glass. The egg masses were counted and recorded as unparasitised and parasitised on each plant. The parasitised egg masses were further categorised as full and partially parasitised egg masses. Photos of the parasitised egg masses were taken using a Microscope Digital Camera DP21 (Olympus SZX10). Photos were later analysed to confirm the actual number of eggs. The location of each egg mass on the cabbage leaf was also

recorded. The locations of egg masses before and after insecticide spray were monitored. The total number of cabbage leaves per sampled plant and leaf or leaves where the egg mass was laid was counted and recorded.

The positions of the oviposited egg mass on the leaf of the cabbage plant were categorised as top of leaf towards edge (left & right – TELr), top middle towards midrib (TMM), bottom of leaf towards edge (left & right – BELr), bottom middle towards midrib (BMM) and leaf stalk (Stk). The oviposition patterns or the distribution of the oviposited egg masses before and after insecticide spray were monitored and recorded. Each insecticide was applied to each plot after one day from completing the last observation in the first interval (before insecticide sprays). All insecticides were sprayed on the same day in the morning using knapsack sprayers (20 L capacity). The mixing rates of each insecticide followed the manufacturer's recommendation. Four observations were carried out every alternate day after insecticide application (1, 3, 5 and 7 days after spraying).

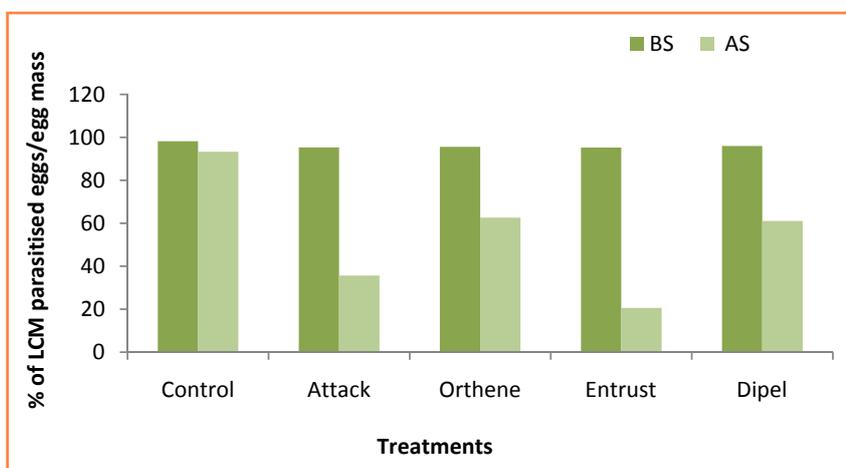


Figure 1 Percentage of large cabbage moth (LCM) parasitised eggs per egg mass before (BS) and after insecticide spray (AS)

Table 1 Number of parasitised eggs of large cabbage moth after insecticide spray (AS) based on Fisher Pairwise Comparison

Treatments	Number of parasitised eggs after treatment
Control	92.4 ^a
Dipel TM	53.1 ^b
Orthene TM	45.2 ^b
Attack TM	12.3 ^c
Entrust TM	3.5 ^c

means that do not share a letter are significantly different

Results and discussion

Trichogramma chilonis parasitism of large cabbage moth eggs before and after insecticide spray

The number of LCM eggs was significantly reduced after spraying with either AttackTM ($F_{1,31} = 12.29$, $p = 0.001$) or EntrustTM ($F_{1,31} = 17.53$, $p = 0.000$). On the other hand, the number of parasitised eggs did not show any significant difference for the Control ($F_{1,31} = 0.05$, $p = 0.819$) and after spraying with either OrtheneTM ($F_{1,31} = 3.06$, $p = 0.09$) or DipelTM ($F_{1,31} = 0.5$, $p = 0.485$). The reductions of *T. chilonis* parasitism of LCM parasitised eggs due to the effect of insecticides are clearly shown in Figure 1. AttackTM and EntrustTM had adverse effects on the egg parasitoid, *T. chilonis*, but OrtheneTM and DipelTM were not harmful to the parasitoid (Table 1).

Safety of bio-pesticides to non-target organisms over conventional pesticides has been explained by various scientists. EntrustTM (bio-pesticide Spinosad) is one of the two

insecticides found potent against the *T. chilonis* parasitism of LCM eggs in the current study and even to *Trichogramma* species in general. Entrust™ kills susceptible species such as Trichogrammatidae by causing rapid excitation of the insect nervous system. Due to this unique mode of action, Entrust™ is valued in resistance management programs. It has both contact and stomach activity; fast acting wherein the insect dies within 1–2 days after ingesting the active ingredient (Saljoqi et al., 2012). Boomathi et al. (2005) reported that Entrust™ showed harmful effects to *T. chilonis* due to its contact toxicity under laboratory condition. The results of the current study also agreed with the findings of Ruberson and Tillman (1999) who reported Entrust™ to have toxic effects on another *Trichogramma* species, *T. pretiosum*. According to Suh et al. (2000) and Tillman and Mulrooney (2000), fresh spray of Entrust™ kills *Trichogramma* and other parasitoids and honey bees. The same conclusion was drawn by Jalali et al. (2006) and Saljoqi et al. (2012). Entrust™ was found extremely toxic to *T. chilonis*, which is in accordance with the current findings.

The target pests and their biological control agents such as predators and parasitoids are susceptible to broad-spectrum insecticides. The target pests are usually killed or poisoned by most broad-spectrum insecticides and it could be the same impact on the biological agents. As for predators, *Aphidius gifuensis* after treatment with Attack™ showed harmful effect (Kobori and Amano, 2004). Attack™ was also found harmful to *Phytoseiulus persimilis* (Kavousi and Talebi, 2003). The *Trichogramma* wasps were also highly susceptible to most broad-spectrum insecticides. This is the reason why various attempts to suppress pest population by biological control measures have often failed due to the detrimental impact of chemicals on beneficial insects (Borgemeister et al., 1993). The current study found that Attack™, a broad-spectrum insecticide composed of permethrin and pirimiphos-methyl as active ingredients, affected the parasitism rate, which is in accordance to Borgemeister et al. (1993). Wang et al. (2012) also drew the same conclusion, wherein Attack™ has affected the parasitoid. Entrust™ had harmful effect on *Trichogramma exiguum* (Suh et al., 2000), which is in conformity with the findings of the current study wherein *T. chilonis* parasitism was affected after Attack™ treatment.

Moreover, Orthene™ a systemic insecticide with Acephate as an active ingredient, did not affect the parasitism. Acephate is considered toxic to beneficial organisms such as bees (Kidd and James, 1991). A study found that Acephate had the longest residual activity toward *Aphytis melinus*. This beneficial insect died in a short period of time after treatment of Acephate. This chemical also showed to be very toxic to adult *Microplitis croceipes* parasitoids, and caused 100% mortality at the lowest recommended field rates (Desneux et al., 2007). The findings of the current study are inconsistent with previous researchers as explained above by Acephate having no effect on the egg parasitoid, *T. chilonis*, parasitism rate.

Dipel™ (Bt) was also found to have no effect on *T. chilonis* parasitism rate similar to Acephate. The current finding agrees with that of Pawar (1996) who reported that Bt was found to have not affected the survival of the egg parasitoid, *T. chilonis*.

Effect of insecticides on the amount of completely and partially parasitised egg masses of large cabbage moth

There is a real significance on the LCM egg mass level of parasitism (completely or partially) before and after insecticide application (Table 2). Most of the LCM egg masses were found completely parasitised by the egg parasitoid, *T. chilonis*, before insecticide spray but not after treatment ($F_{3, 159} = 30.16, p = 0.000$). It also showed that most of the egg masses found after insecticide sprays were partially parasitised ($F_{3, 159} = 20.04, p = 0.000$). The level of parasitism (completely or partially) was affected after insecticide treatments. The findings of the current study revealed that more LCM egg masses recorded after insecticide application were partially parasitised compared to most LCM egg masses that were fully parasitised before insecticides treatment. Thus, the insecticides affected the number of completely and partially parasitised egg masses.

Table 2 Number of partially and completely parasitised egg masses before (BS) and after insecticide spray (AS)

Number of completely (Cpe) and partially parasitised eggs (Ppe) before (BS) and after treatment (AS): Tukey Method 95% Confidence			
Treatments	Time	Ppe	Cpe
Control	BS	0.25 ^b	2.81 ^a
	AS	0.81 ^a	2.56 ^a
Attack™	BS	0.94 ^a	3.19 ^a
	AS	1.38 ^a	0.19 ^b
Orthene™	BS	0.56 ^b	2.13 ^a
	AS	1.75 ^a	0.5 ^b
Entrust™	BS	0.63 ^a	3.38 ^a
	AS	0.63 ^a	0.06 ^b
Dipel™	BS	0.44 ^b	1.44 ^a
	AS	2.38 ^a	0.44 ^b

Large cabbage moth egg mass distribution (oviposition patterns) before and after insecticide spray

Before the insecticide spray, most egg masses were oviposited in the top part of the cabbage leaves, including the top part of the leaf towards the edge (left and right – TEI_r) and top part of the leaf towards the midrib (TMM). However, after insecticide spray majority of the oviposited egg masses concentrated in the bottom parts of the foliage, the bottom part towards the edge (left and right – BEI_r), bottom part towards the midrib (BMM) and leaf stalk (Stk) (Table 3). The distribution (oviposition patterns) of LCM egg masses in cabbage leaves was also affected after insecticide application. More egg masses concentrated in the upper (top) part of the leaves towards the edge and the midrib (TEI_r and TMM) before insecticide spray but after treatment most LCM egg masses were concentrated in the bottom part of the cabbage leaves towards the edge, mid-rib (BEI_r and BMM) and leaf stalk (Stk). The current study result is

Table 3 Mean number of oviposited egg masses in different locations of cabbage leaves

Time (before/after)	Treatments	Location of LCM egg mass on cabbage leaves				
		TElr	TEMM	BElr	BMM	Stk
Before insecticide spray	control	3.75 ±0.57	4.25 ±0.57	0.44 ±0.16	0.31 ±0.12	0.00 ±0.00
	attack	5.44 ±1.28	6.25 ±1.61	1.00 ±0.27	1.43 ±0.55	0.19 ±0.14
	orthene	3.94 ±0.57	3.94 ±0.82	0.38 ±0.18	0.75 ±0.17	0.06 ±0.06
	entrust	4.63 ±0.75	7.69 ±1.17	1.13 ±0.20	0.88 ±0.27	0.69 ±0.30
	dipel	2.81 ±0.54	3.88 ±0.83	0.25 ±0.11	0.38 ±0.13	0.19 ±0.10
After insecticide spray	control	4.50 ±1.05	9.63 ±1.59	0.94 ±0.25	0.94 ±0.40	1.44 ±0.46
	attack	1.31 ±0.45	1.50 ±0.90	6.06 ±0.91	9.31 ±1.47	2.44 ±0.76
	orthene	1.75 ±0.47	0.94 ±0.69	4.88 ±1.10	8.06 ±2.03	2.56 ±1.03
	entrust	0.94 ±0.25	1.38 ±0.60	4.19 ±1.08	6.50 ±1.38	1.75 ±0.80
	dipel	1.19 ±0.43	1.31 ±0.55	4.06 ±0.94	8.19 ±1.62	2.25 ±0.58

TElr – top towards edge-left and right; TEMM – top towards midrib; BElr – bottom towards edge-left and right; BMM – bottom towards midrib; Stk – leaf stalk before and after insecticide spray

inconsistent with the findings reported by Uelese et al. (2014) wherein most egg masses were concentrated in the bottom part of head cabbage the leaves without using insecticides.

Conclusions

T. chilonis parasitism of LCM eggs was significantly affected by Attack™ and Entrust™ treatment. However, Orthene™ and Dipel™, on the other hand, did not affect *T. chilonis* parasitism rate and are considered safe to use for the egg parasitoid. The insecticides used also affected the level of parasitism of completely and partially parasitised egg masses where more egg masses were found partially parasitised after insecticide treatment. Insecticides also affected the distribution of LCM egg mass on the cabbage leaves. The egg masses were mostly concentrated in the top part of the cabbage leaves especially towards the midrib followed by the edge before insecticide treatment. However, egg masses were concentrated in the bottom part of the cabbage leaves after insecticide application. Orthene™ or Dipel™ did not affect the parasitism but Dipel™ was found safer for the parasitoid.

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