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Insecticides and Parasitoids

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1. Introduction

More than 1,000,000 of insect species live on the earth with close association to each other. The population density of living organisms is regulated by abiotic and biotic factors during growth and development processes of each organism within some fluctuation, depressing the outbreak of some species. Abiotic factors like Flood or Dry involves in fluctuation of population. Biotic-regulation factors like deficiency of foods, predation and parasitism are important to depress the outbreak of population. In insects, parasitoids live over 200,000 species (Askew, 1971). Especially in agro-environment, planting monopolized by single crop in a wide area makes suitable condition for multiplication of some pest insect species and their outbreaks. Chemical control using pesticides for depression of pest population had been considered as one of better choice because of its immediate efficacy when outbreak happened. It has already clarified, however, that use of non-selective insecticides makes resurgence of insect pests caused by rapid decreasing of natural enemies. Agro-chemicals with selective toxicity have recently been developed, but it is not enough to examine their effects on natural enemies yet. To obtain agro-crops with secure and low price, we have to understand both specificities of natural enemies like parasitoids and of insecticides. Many parasitoid species works well to regulate the population density of pest insect in a well-conditioned cultivated space. Effective utilization of parasitoids and pesticides based on the various characteristics on each local region produce low density of pest population constantly. Simplified interaction between pest insects and natural enemies had made many unfortunate consequence of pest control like case of introduced natural enemies to invasion pests. Banker plants (Trap crops) are used for keeping the population of natural enemies permanently in constant density when natural enemies are multiplied and released artificially. It is developed as useful methods that 'companion plants' for supplying the foods like nectar to natural enemies or 'refuge' as hiding place to prevent them from leaving and so on. However, devotion only to biological control is not adequate for regulation of pest population density corresponding to climate change year after year. When population of some pest insect breaks out to high density unexpected, natural enemies including parasitoid will lose to control for the population density of the pests. We will be forced to use chemical control temporarily. However, exclusive devotion to biological control with no pesticides or to chemical control ignoring biotic regulation seems not to produce the good results.

Although pest-control by IPM has been recommended recently, susceptibility of insecticides to parasitoids is not examined enough from various viewpoints. It is well known that parasitoids are one of important natural enemies to many pest species and are used

	Pest species	parasitoid	effects	references
Bt-toxin	<i>Choristoneura fumiferana</i>	<i>Apanteles fumiferanae</i>	high % of parasitism to aged host	Nealis, VG et al., 1992
	<i>Prays olease</i>	<i>Three species</i>	growth effect	Varlez, S et al. 1993
	<i>Choristoneura fumiferana</i>	<i>A. fumiferanae</i>	spray-timing	Cadogan, BL et al. 1995
	<i>Plutella xylostella</i>	<i>Diadegma insulare</i>	no effect	Ulpah, S et al. 1996
	<i>P. xylostella</i>	<i>D. insulare</i>	no effect	Xingeng, W & Shusheng, L. 1998
	<i>P. xylostella</i>	<i>Cotesia plutellae</i>	no effect on oviposition	Chilcutt, CF & Tabashnik, BE 1999
	<i>Lymantria dispar</i>	<i>Compsilura concinnata</i>	reduced parasitism	Erb, SL et al., 2001
	<i>Liriomyza trifolii</i>	<i>Diglyphus isaea</i> , <i>Dacnusa sibirica</i>	no effect	Ozawa A et al., 2001
	<i>Mamestra brassicae</i>	<i>Trichogramma dendrolimi</i>	no effect	Takada, Y et al., 2001
	<i>Cnaphalocrocis medinalis</i>	<i>Trichogramma chilonis</i>	no effect	Sehrawat S et al., 2002
	<i>Pseudaletia unipuncta</i>	<i>Cryptoapanteles militaris</i>	no effect	Schuler, TH et al., 2003
	rice pests	parasitoid complex	small effect	Schoenly, KG et al., 2003
	<i>Helicoverpa armigera</i>	<i>Microplitis mediator</i>	negative effect	Xu, YY et al., 2004
	<i>Plutella xylostella</i>	<i>C. plutellae</i>	small effect	Amend, J & Basedow, T 1997
	<i>Plutella xylostella</i>	<i>Diadegma insulare</i>	small effect	Jiang, YH et al., 2004
	<i>Plutella xylostella</i>	<i>Cotesia plutellae</i>	small effect	Sisterson, MS & Tabashnik, BE 2005
	<i>Eldana saccharina</i> , <i>Busseola fusca</i> , <i>Sesamia calamistis</i>	<i>Cotesia sesamiae</i>	positive effect	Bhatti, MA et al., 2005
	<i>Plutella xylostella</i>	<i>Diadegma fenestralis</i> , <i>Cotesia plutellae</i>	less effective	Bhardwaj, V et al., 2005
	<i>Plutella xylostella</i>	<i>Cotesia plutellae</i> , <i>Macromalon orientale</i> , <i>Diadromus collaris</i> , <i>Brachymeria excarinata</i>	small impact	Reyes, SG et al. 2005
	<i>Tuta absoluta</i>	<i>Trichogrammatoidea bactrae</i>	harmless	Riquelme Virgala, MB et al. 2006
	<i>Anagasta kuehniella</i>	<i>Thichogramma pratissolii</i>	no effect on wasp	Pratissoli, D. etal. 2006
	<i>Anagasta kuehniella</i>	<i>Thichogramma pratissolii</i> , <i>T. pretiosum</i>	no effect on wasp	Polanczyk, RA et al. 2006
	<i>Chilo partellus</i>	<i>Trichogramma chilonis</i>	no effect	Jalali, SK & Singh, SP 2006
	<i>Anagasta kuehniella</i>	<i>Thichogramma chilonis</i> , <i>Cheilomenes sexmaculata</i>	no effect	Basappa, H 2007
	<i>Helicoverpa armigera</i>	<i>Campoletis chlorideae</i>	significant reduction	Sharma, HC et al. 2008
	<i>Plutella xylostella</i>	<i>Diadegma semiclausum</i> , <i>Diadromus collaris</i>	better than chemicals	Nga, LT et al 2008
	<i>Plutella xylostella</i> , <i>Pieris rapae</i>	<i>Cotesia vestalis</i> , <i>Oomyzus sokolowskii</i> <i>Diadromus collaris</i> <i>C. glomeratus</i> , <i>C. rubecula</i> , <i>Pteromalus puparium</i>	weak impact	Furlong, MJ et al 2008
	<i>Helicoverpa armigera</i>	<i>Campoletis chlorideae</i>	high mortality	Mohan, M et al. 2008
	<i>Plodia interpunctella</i>	<i>Habrobracon hebetor</i>	fewer parasitoid emerged	Oluwafemi, AR et al. 2009
	<i>Thaumetopoea pityocampa</i>	<i>Exorista larvarum</i>	few effect	Marchetti E. et al. 2009
	<i>Plutella xylostella</i>	<i>Trichogrammatoidea bactrae</i>	no effect	Wang, D-S et al. 2010

Table 1-1. Effect of Bt-toxin on parasitoid species.

Bt-crops	Pest species	parasitoid	effect	ref
Bt-tobacco	<i>Heliothis virescens</i>	<i>Campoletis sonorensis</i> , <i>Cardiochiles nigriceps</i>	affect parasitoid larva of <i>C. sonorensis</i>	Johnson, MT, 1997
Bt-corn	<i>Ostrinia nubilalis</i>	<i>Erborus terebrans</i> , <i>Macrocentrus grandii</i> <i>Goidanich</i>	no difference	Orr, DB & Landis, DA, 1997
Bt-corn	<i>Ostrinia nubilalis</i>	parasitoid community	no difference	Lozzia, GC, 1999
Bt-potato	<i>Macrosiphum euphorbiae</i>	<i>Aphidius nigripes</i>	small effect	Ashouri, A et al. 2001
Bt-brassica	<i>Diaretella rapae</i>	<i>Myzus persicae</i>	no effect	Schuler, TH et al. 2001
Bt-corn	<i>Eoreuma loftini</i>	<i>Parallorhogas pyralophagus</i>	harmful	Bernal, JS et al. 2002
Bt-corn	<i>Ostrinia nubilalis</i>	<i>Lydella thompsoni</i> , <i>Pseudoperichaeta nigrolineata</i>	no difference	Bourguet, D et al. 2002
Bt-brassica	<i>Plutella xylostella</i>	<i>Cotesia plutellae</i>	no difference	Schuler, TH et al. 2003
Bt-corn	Aphids	<i>Aphidius ervi</i> , <i>A. rhopalosiph</i> , <i>A. uzbekistanicus</i> , <i>Lysiphlebus</i> <i>testaceipes</i> , <i>Praon volucre</i>	no effect	Pons, X & Stary, O. 2003
Bt-cotton	<i>Pseudoplusia includens</i>	<i>Cotesia marginiventris</i> , <i>Copidosoma floridanum</i>	reduced longevity	Baur, ME & Boethel, DJ 2003
Bt-corn	<i>Ostrinia nubilalis</i>	<i>Lydella thompsoni</i>	no difference	Manachini, B 2003
Bt-rice	rice pests	paddy arthropod community	no effect	Liu, Z-C et al. 2003
Bt-brassica	<i>P. xylostella</i>	<i>C. plutellae</i>	no effect	Schuler, TH et al. 2004
Bt-corn	<i>Chilo partellus</i>	<i>C. flavipes</i>	harmful	Pruez, G & Dettner, K. 2004
Bt-rice	<i>Chilo suppressalis</i>	<i>Apanteles chilonis</i>	harmful	Jiang, Y-H et al. 2004
Bt-cotton	<i>Helicoverpa armigera</i>	<i>Trichogrammatoidea lutea</i> , <i>Telenomus ullyetti</i>	no influence	Mellet, MA et al.2004
Bt-corn	<i>Chilo partellus</i> , its primary parasitoid <i>Cotesia flavipes</i>	<i>Tetrastichus howardi</i> (hyperparasitoid)	harmful	Prueitz, G et al.2004
Bt-corn	<i>Micraspis discolor</i>	<i>Trichomma cnaphalocrosis</i>	no effect on behavior	Reyes, SG 2005
Bt-corn	european corn borer	arthropod community	less community disturbance	Dively, GP 2005
Bt-cotton	<i>Helicoverpa armigera</i>	<i>Microplitis mediator</i>	minus effect	Liu, X-X et al. 2005
Bt-corn	<i>Spodoptera littoralis</i>	<i>Cotesia marginiventris</i>	negative effect	Vojtech, E et al. 2005
Bt-corn	<i>Diabrotica virgifera</i> , <i>Chaetocnema pulicaria</i> , <i>Rhopalosiphum maidis</i>	ladybird beetles, damsel bugs, pirate bugs, flower flies, green lacewings, spiders <i>Macrocentrus cingulum</i>	no adverse impact	Bhatti, MA et al. 2005
Bt-corn	<i>Ostrinia nubilalis</i>	<i>Coleomegilla maculata</i> , <i>Cycloneda munda</i> , <i>Orius</i> <i>insidiosus</i> , <i>Chrysoperla</i> <i>carnea</i> , <i>Macrocentrus</i> <i>cingulum</i>	some significantly affected	Pilcher, CD et al. 2005
Bt-cotton	<i>Helicoverpa armigera</i>	parasitoid and pollinator	parasitoid population decreased	Hofs, JL et al. 2005
Bt-corn	<i>Monolepta bifasciata</i>	predatory beetles, lacewings, <i>Trichogramma</i> sp	no effect	Reyes, SG & Jovillano- Mostoles, MDA 2005
Bt-corn	<i>Micraspis discolor</i>	<i>Trichomma cnaphalocrosis</i>	no difference	Reyes, SG. 2005
Bt potato	<i>Phthorimaea operculella</i>	<i>Apanteles subandinus</i> , <i>Micromus tasmaniae</i>	no effect	Davidson, MM et al. 2006
Bt-corn	<i>Spodoptera frugiperda</i>	<i>Cotesia marginiventris</i>	Bt was detected in parasitoid	Ramirez-Romero, R et al. 2007
Bt-potato, Bt-brassica	<i>Spodoptera litura</i> , <i>Helicoverpa armigera</i>	<i>Cotesia kazak</i> , <i>Meteorus</i> <i>pulchricornis</i>	a few impact	Walker, GP et al. 2007
Bt-corn	<i>Rhopalosiphum maidis</i>	<i>Cotesia marginiventris</i>	positive effect on performance of wasp	Faria, CA et al. 2007
Bt-cotton		parasitoid and predator	population density decreased	Han, L-Z et al. 2007
Bt-corn	<i>Spodoptera frugiperda</i>	<i>Campoletis sonorensis</i>	no effect on development	Sanders, CJ et al.2007
Bt-rice	<i>Sogatella furcifera</i> , <i>Nilaparvata lugens</i> , <i>Laodelphax striatellus</i>	<i>Cyrtorhinus lividipennis</i>	negative effect	Chen, M et al. 2007
Bt-cotton	<i>Helicoverpa armigera</i>	predatory spiders, coccinellid, chrysopid	significant reduction	Sharma, HC et al. 2007
Bt-cotton	<i>Helicoverpa armigera</i>	arthropod community	no detrimental effect	Whitehouse, MEA et al. 2007
Bt-brassica	<i>Plutella xylostella</i>	<i>Diadegma insulare</i>	hazardless	Chen, M et al. 2008
Bt-brassica	<i>Pieris rapae</i>	<i>Pteromalus puparum</i>	significantly affected	Chen, M et al. 2008
Bt-brassica	<i>Plutella xylostella</i>	<i>Cotesia vestalis</i> , <i>Chrysoperla</i> <i>carnea</i>	trace of Bt	Wei, W et al. 2008
transgenic pine	<i>Pseudocoremia suavis</i>	<i>Meteorus pulchricornis</i>	adverse effect	Barracough, EI et al. 2009
Bt-chickpea	<i>Helicoverpa armigera</i>	<i>Campoletis chlorideae</i>	adverse effect	Dhillon, MK & Sharma, HC 2010
Bt-corn	<i>Spodoptera frugiperda</i>	<i>Cotesia marginiventris</i>	weak attractance	Desneux, N et al. 2010
Bt-corn		rove beetles as predator	no difference	Balog, A et al. 2010
Bt-cotton	<i>Spodoptera exigua</i> , <i>Heliothis armigera</i>	<i>Microplitis tuberculifer</i>	no effect on behavior	Zhang, N et al. 2010

Table 1-2. Effect of Bt-crops on parasitoids.

extensively in biological and integrated pest control. More than 810 research papers related with insecticide and parasitoid in IPM have been accumulated during this decade from 2000 to 2010 for examining the impact of insecticides on introduced or native parasitoids and/ or predators in laboratory condition or agro-fields, resulting that parasitoids are very high susceptibility to non-selective insecticides like pyrethroids, organophosphates, and carbamates except Bt toxin.

Recently Bt-toxin or Bt-transgenic crops have been developed and the susceptibility to parasitoids and/ or predators (32 of Bt-toxin spray and 42 of Bt-crops in total 74 research papers, Table 1) was examined, resulting small impact on parasitoid and predator or on their communities.

Many reviews have already discussed about the side effect or the risk assessment of transgenic plants on non-target insects (Schuler et al. 1999; Groot & Dicke 2002; Dutton et al. 2003; Lövei & Arpaia 2005; Sisterson & Tabashnik 2005; Wolfenbarger et al., 2008; Lövei et al. 2009; Grzywacs et al. 2010; Gurr et al. 2010). However, severe problems have occurred also in Bt-transgenic crops that pest insects had gained the resistance to Bt-toxin just like development of the insecticide-resistance to many chemical insecticides. Approach like 'high dose/ refuge strategy' (Chilcut & Tabashnik, 2004) or pyramid by expression of two genes have been tried to prolong the effectiveness of Bt-crops (Kumar et al. 2008, Ives et al., 2011). Although many chemical insecticides produced until present are toxic to natural enemies, we may be able to use them effectively by knowing the risk of chemical insecticides to maintain the predator and parasitoid communities sustainably.

In natural fields including agro-fields, parasitoids grow and develop mostly as eggs or larvae in/ on their hosts and a few adult wasps stay with searching the hosts. Examination only on adult stage is insufficient for clarifying the susceptibility of parasitoid to insecticides. It is one of important points to examine the effect of chemical insecticides on the parasitized hosts in the developmental stages from oviposition to adult-emergence for evaluating the critical dosage to parasitoids. Effective usage of natural enemies like parasitoids in the agro-fields controlled by pesticides causes a decrease in the dosage of insecticides and brings agricultural crops with safety for human. Both ecological and physiological researches will be required for control of pest-population density. In this chapter, first as example, we tested effect of neonicotinoids on parasitoid along with the growth and development, for considering the characteristics of parasitoids.

1.1 Effect of insecticide on parasitoid

1.1.1 Direct and indirect effects of neonicotinoids on endoparasitoid along with the development

Recently although there are some researches published about the effect of neonicotinoids on egg parasitoids, there are a few papers on larval parasitoids (Table 2). These results showed variety from harmless to toxic impact.

However, the different results should be rearranged by difference of nutritional strategy between egg and larval parasitoids. Egg parasitoid ingests egg-yolk of the host soon after hatch as nutritional resource for growth, resulting become distended larval shape (Takada et al., 2000; Jarjees et al., 1998; Hutchison et al., 1990). Egg parasitoids are able to avoid the toxic effect of insecticides through the chorion of the host, using a protective role that is essential to normal development of the host embryo, and can circumvent the accumulation of toxic substance by sucking almost all egg-yolk from host egg at once after hatch. On the other hand, larval parasitoids have many chances to be exposed to insecticides during

category of parasitoid	parasitoid	target pest insect	Chemicals	effects	references
species richness	37 species of predators and parasitoids	leafhoppers, aphids, thrips and whiteflies	imidacloprid	no significant impact	Marquini, F. et al., 2002
egg parasitoid	<i>Aphytis melinus</i> , <i>Eretmocerus eremicus</i> , <i>Encarsia formosa</i> , <i>Gonatocerus ashmeadi</i> <i>Trissolcus nigripedius</i>	California red scale and sweetpotato whitefly, glassy-winged sharpshooter, <i>Dolycoris baccarum</i>	acetamiprid thiamethoxam	less toxic less susceptible	Prabhaker, N., et al., 2007 Paine, ID, et al., 2011
	<i>Aphytis melinus</i> , <i>Comperiella bifasciata</i> , <i>Rodolia cardinalis</i>	<i>Aonidiella aurantii</i> , <i>Idcerya purchasi</i> , <i>Panonychus citri</i>	imidacloprid	a little disruptive	Grafon-Cardwell, EE, et al., 2008
	aphlinid parasitoid	<i>Bemisia tabaci</i>	imidacloprid thiamethoxam	parasitism reduction	Naveed, M. et al., 2010
	<i>Trichogramma pretiosum</i>	<i>Anagasta kuehniella</i>	acetamiprid, imidacloprid	harmless	Carvalho, GA, et al., 2010
	<i>Trichogramma minutum</i>	<i>Acrobasis vaccinii</i>	thiacloprid acetamiprid	toxic	Wise, JC., et al., 2010
egg-, larval parasitoids	<i>Avetianella longoi</i> , <i>Syngaster lepidus</i>	-	imidacloprid	significantly lower survival	Lim, UT, 2008
egg-, egg-larval, larval parasitoids	<i>Trichogramma chilonis</i> , <i>Chelonus blackburni</i> , <i>Bracon hebetor</i>	<i>Gossypium hirsutum</i>	imidacloprid	moderate impact	Preetna, E et al., 2010
larval parasitoid	<i>Iphtia vernalis</i>	<i>Popillia japonica</i>	imidacloprid, thiamethoxam	adverse impact	Oliver, JB et al., 2005
	<i>Diachasma alloeum</i>	<i>Rhagoletis mendax</i> , <i>H. pomonella</i>	imidacloprid	adverse impact	Stelinski, LL et al., 2006
	<i>Diadegma insulare</i> , <i>Oomyzus sokolowskii</i>	<i>Plutella xylostella</i>	acetamiprid	less toxic	Cordero et al.,2007

Table 2. Effect of neonicotinoids on parasitoids.

developmental period (from egg to larval stages) and are dead together with the host when it is killed by insecticides, because Neonicotinoid have a strong effect on lepidopteran larvae. So in this chapter, to consider how to regulate the use of insecticide like neonicotinoid to larval parasitoid, it is necessary to examine the susceptibility of larval parasitoid to insecticide along with development. We use oriental armyworm *Mythimna separata* (Walker) as host and its endoparasitoid *Cotesia kariyai* (Watanabe) as a model system. *Mythimna separata* is a big pest for Poaceae plants and sometime make a big surge of population density. However, ecological population of *M. separata* is regulated with many kinds of parasitoids, major 5 species of endoparasitoids, *Campoletis chlorideae* Uchida, *Microplitis* sp., *C. kariyai*, *C. ruficrus* (Haliday), *Meteorus pulchricornis* (Wesmael), and an ectoparasitoid *Euplectrus separatae* Kamijo, and was normally kept low density under a local stable condition. *Cotesia kariyai* is a major gregarious endoparasitoid to oviposit from 30 to over 100 eggs in a *M. separata* host at once and can parasitize 2nd to 6th (last) host instar successfully (Tanaka et al., 1987).

1.1.2 Parasitoid wasps attack and oviposit the host *M. separata* treated previously with insecticides

To determine the sub-lethal dose activity of various neonicotinoids to unparasitized control, the unparasitized hosts 1, 2, 3 d after last ecdysis (D1L6, D2L6, D3L6, 6th larval stage is last instar) were used. Unparasitized hosts reach at the maximum weight 3 d after last ecdysis and become wandering stage to prepare pupation at D5L6. D2L6 larvae showed a low susceptibility to neonicotinoids, especially Thiamethoxam (Thm) and Dinotefran (Dnt), but high susceptibility to pyrethroid Permethrin (Per), organophosphate Fenitrothion (MEP), and Pyridalyl (Pyr) was observed, comparing to label rate (Table 3).

Insecticide	D1L6	D2L6	D3L6	Concentration used in each chemicals (ppm)	Label rate (ppm)
Acetamiprid (Act)	309.3	391.1	505.6	320	100
Thiamethoxam (Thm)	1163.8	1185.3	1158.3	400	50
Dinotefuran (Dnt)	1695.6	3116.6	1508.6	1200	100
Clothianidin (Clt)	204.6	1930.6	384.0	960	80
Thiacloprid (Thc)	702.5	640.2	780.3	640	150
Permethrin (Per)	160.7	57.5	247.2	24	100
fenitrothion (MEP)	192.0	169.2	131.7	24	500
Pyridalyl (Pyr)	0.7	4.0	0.3	0.8	100

Table 3. LC50 value (ppm) of various insecticide to unparasitized host *Mythimna separata*.

From these results, concentration of each insecticide treatment was determined. These values means different susceptibility of *M. separata* even on the same instar at sub-lethal dose, and it is hard to be generalized.

The emergence rate of parasitoid from host parasitized after insecticide treatment (post-treatment) informs us if parasitoids oviposit the host larvae treated by insecticides. Oviposition was performed within 2 hrs post-treatment of insecticide. Stinging behavior for oviposition was assured in every case. For example, Acetamiprid (Act), Thiacloprid (Thc) and Pyr treatments produced high larval emergence rate of parasitoid when parasitized post-treatment compared to pre-treatment, suggesting that oviposition was not disturbed by insecticide treatment (Fig. 1).

On the other hands, high pupation rate of hosts after Imidacloprid (Imd) treatment shows the possibility that parasitoid wasps may hesitate to inject the eggs though they stung the hosts. On the other hands, high emergence rate in insecticide treatment post-parasitization at sub-lethal dose means that the host or the parasitoid larvae possessed the detoxification ability to each insecticide and acquired some degree of tolerance to insecticides.

Chemicals ^{*1}	LC50 (ppm)					
	D1L6 ^{*2}	D1L6P ^{*3}	D3L6	D3L6P	D5L6P	D 7 L6P
Act	309.3	608.4	505.6	176.1	786.9	377
Thm	1163.8	139.9	1158.3	40	49.9	436.3
Dnt	1695.6	1199	1508.6	1194.7	1102.6	1043.7
Clt	204.6	962	384.0	96	205.5	20.2
Thc	702.5	639.2	780.3	120	483.9	8563.3
Per	160.7	24	247.2	40.8	78.8	62.4
MEP	192.0	456	131.7	132	134.5	49.6
Pyr	0.7	3.5	0.3	4.4	3.9	2.5
^{*1} Act:Acetamiprid, Thm: Thiamethoxam, Dnt: Dinotefuran, Clt: Clothianidin, Thc: Thiacloprid, Per: Permethrin, MEP: fenitrothion, Pyr: Pyridalyl						
^{*2} : day1- 6th instar of unparasitized control						
^{*3} : day1-6th instar of parasitized host <i>Mythimna separata</i>						

Table 4. LC50 value of various neonicotinoids to unparasitized and parasitized hosts 1, 3, 5, 7 days after parasitization.

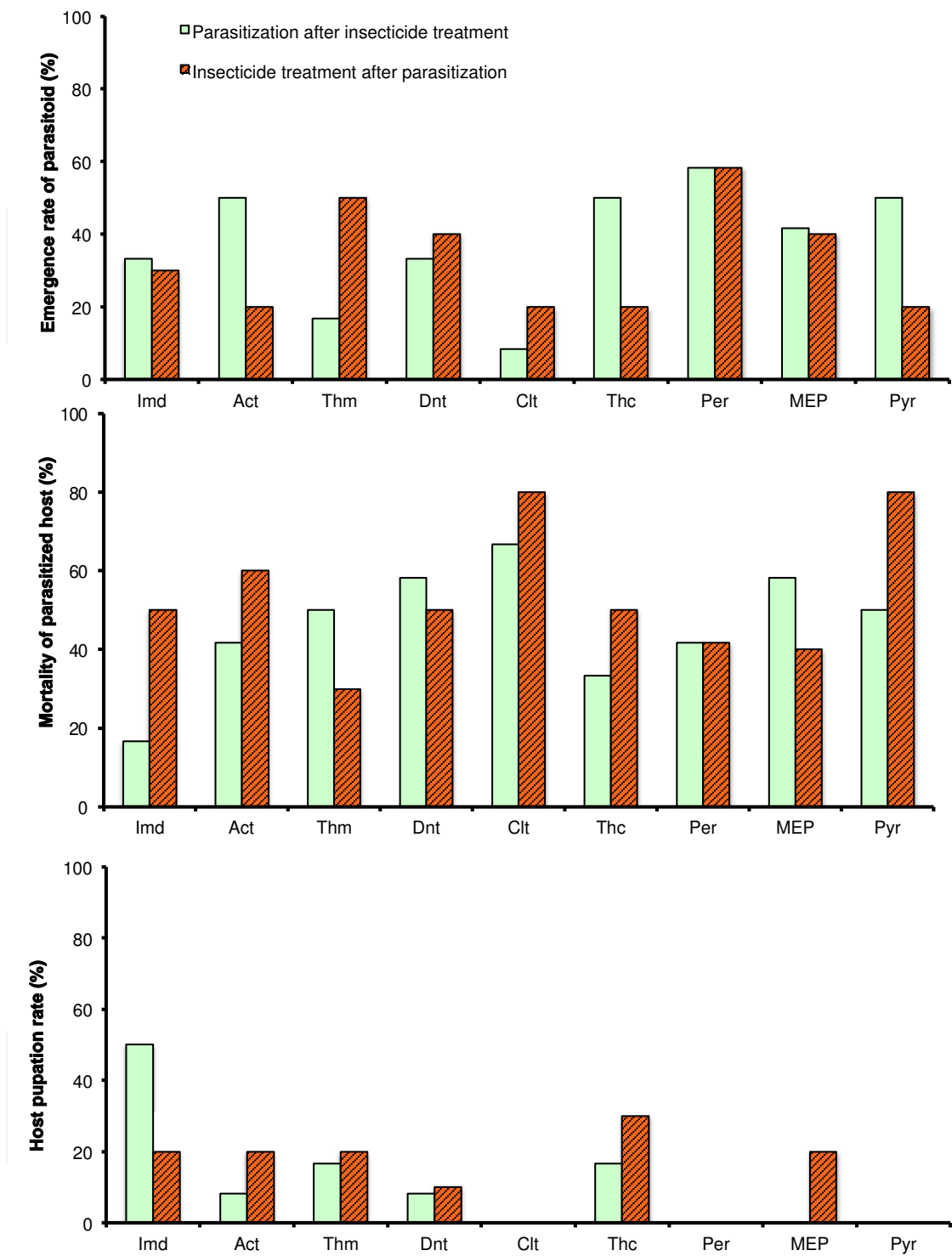


Fig. 1 Emergence rate of parasitoid larvae from the host treated by various insecticides at sub-lethal dose. Neonicotinoid insecticide treatment before parasitization made no impact on oviposition of parasitoid wasp. The parasitoid larvae emerged from the host treated successfully when the parasitized hosts were not killed by insecticide treatment. Total number of hosts treated with each insecticide was about 30 [ten for each, 3 replicates]. lmd: Imidacloprid, Act: Acetamipriel, Thm: Thiamethoxam, Dnt: Dinotefuran, Clt: Clothianidin, Thc: Thiachloprid, Per: Permethrin, MEP: fenitrothion, Pyr: Pyridalyl.

However, Clothianidin (Clt) treatment made low emergence rate of parasitoid causing by high mortality of host. Especially no emergence of parasitoid from parasitized hosts was observed also after day 3 post-parasitization (Fig. 2). Eggs of *C. kariyai* hatch and become 1st instar at 3.5 days after oviposition, and become 2nd instar on 5-6 days after oviposition. Insecticide treatment after parasitization was performed on each developmental point, on egg stage (day 1; D1), just before hatch (day 3; D3), on first instar (day 5; D5), on 2nd instar of parasitoid (day 7; D7). Parasitized hosts showed high susceptibility (meaning lower LC50 value) than that of unparasitized control, in treatment on every developmental days (Table 4), especially Clt treatment affect the larval emergence, meaning that parasitoid larva has no tolerance to Clt during larval stages. Further, Clt affect the adult eclosion heavily (Table 5).

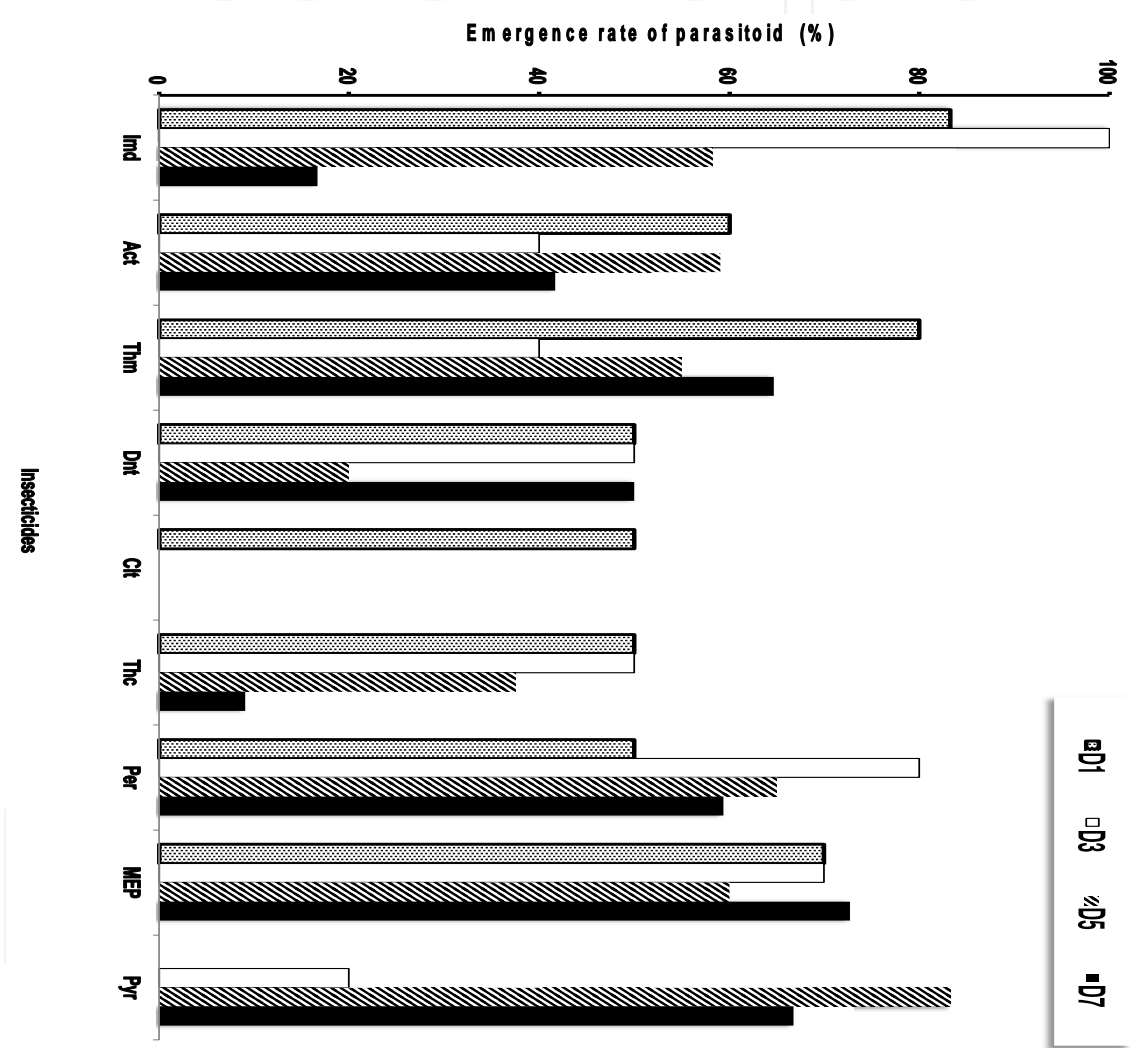


Fig. 2. Effect of treatment of insecticides along with development of the parasitoid on the parasitoid emergence rate sub-lethal dose. *Cotesia kariyai* hatches at 3.5 days post oviposition, 1st instar ecdyses to 2nd instar at 5 to 6 days, finally 3rd instars emerge from the host after ecdysis to 10 days after oviposition at 25 ± 1°C. Insecticide treatment was performed on 1, 3, 5, 7 days after parasitization. Total number of hosts treated with each insecticide was about 30 [ten per each, 3 replicates]. Imidacoprid (Imd). Acetamiprid (Act), Thiamethoxam (Thm), Dinotefuran (Dnt). Clothianidin (Clt), Thiacloprid (The), Permethrin (Per), Fenitrothion (MEP), Pyridalyl (Pyr).

Insecticide	No of insects tested per each days	adult eclosion (%)			
		D1	D3	D5	D7
Imid	12	100	100	85.7	100
Act	12	100	100	100	80
Thm	12	100	75	81.8	59.1
Dnt	12	100	100	100	80
Clf	12	0	0	0	0
Thc	12	75	80	22.2	0
Per	12	100	100	100	90.9
MEP	12	100	100	83.3	100
Pyr	12	100	87.5	83.3	100

*1 Imd: Imidacloprid, Act:Acetamiprid, Thm: Thiamethoxam, Dnt: Dinotefuran, Clf: Clothianidin, Thc: Thiacloprid, Per: Permethrin, MEP: fenitrothion, Pyr: Pyridalyl

Table 5. Adult eclosion rate from cocoon of parasitoid emerged from parasitized host treated by various insecticides.

For susceptibility of adult wasp to insecticide, ten female wasps was released in a 15 ml grass tube inside coated with active ingredients of various insecticide diluted in various concentration for 24 hrs with two replication (Table 6), resulting that parasitoid female wasps showed very high susceptibility to all insecticides. Even insecticides diluted than commercial label killed almost all wasps (Table 6).

Insecticide*1	Commercial Label recommended conc (ppm)	Concentration used (ppm)	Mortality of wasp (%)*2
Imd	100	60	80
Act	100	3.2	40
Thm	50	4	85
Dnt	100	12	80
Clf	80	9.6	100
Thc	150	12	95
Per	100	0.24	59
MEP	500	2.4	100
Pyr	100	0.08	20

*1 Imd: imidacloprid, Act:Acetamiprid, Thm: Thiamethoxam, Dnt: Dinotefuran, Clf: Clothianidin, Thc: Thiacloprid, Per: Permethrin, MEP: fenitrothion, Pyr: Pyridalyl

*2 Susceptible test for each insecticide was perofomed releasing 10 females in a 15 ml glass tube coated with active ingredients of each insecticide and dead wasps were counted 24 hrs later

Table 6. Susceptibility of parasitoid female wasp to neonicotinoid insecticides.

However, about 10 times diluted neonicotinoids like Thm, Clt, and Thc to LC50 value on D3L6 parasitized hosts made 80-100% mortality, in contrast to Permethrin diluted 50-100 times showed similar mortality. These results suggest that the neonicotinoids made slightly severe effect on larval parasitoid responsible for strong insecticidal potency to the death of lepidopteran hosts although they are less toxic than pyrethroids or organophosphates to parasitoid.

1.2 Parasitoid

Parasitoids are grouped in two categories as idiobiont and koinobiont based on nutritional strategy (Haeselbarth, 1979, Askew & Shaw, 1986). Parasitoids categorized as idiobiont that attack egg, pupal adult host stages, and paralyze or kill the hosts by venom preceding oviposition, thus develop in non-growing hosts and utilize the host resource existed at the time of parasitization for the growth and development. On the other hand, koinobiont can exploit the host resource increased after parasitization, because the parasitized hosts continue to grow and metamorphose during at least the initial stage of parasitism (Fig. 3). These include egg-larval and larval-pupal parasitoids or larval parasitoids that do not permanently paralyze their hosts at oviposition (Godfray, 1994).

1.2.1 Egg parasitoids as idiobiont

Idiobionts include many ectoparasitoids and egg or pupal endoparasitoids, and their venoms have characteristics to paralyze or kill the hosts and contain many kinds of enzymes to digestive most of host tissues with many variety (Moreau and Guillot, 2005). Venom of idiobionts as larval ectoparasitoids like *Bracon* spp. shows permanently paralyzing activity to the host (Beard, 1978, Quicke, 1997, Weaver et al. 1997). Venom is virulent and toxic potency to the host. Pupal ectoparasitoids also have to paralyze and fix the host to avoid consumption of food resource by growth of the host after parasitization with venom. On the other hands, *Nasonia vitripennis* as pupal endoparasitoid has non-paralysing venom that causes developmental arrest by 13 to 200.5 kDa proteins (Rivers et al., 2006), but venom shows PO (Phenol oxidase) activity and may induce apoptosis in host tissues (Abt & Rivers, 2007). *Mellitobia* wasp shows different mode of action in developmental arrest to different host species (Deyrup et al., 2006). These means that apoptotic tissues induced by venom are used for parasitoid development with time lag, with condition that their available resource is kept by developmental arrest. Idiobiont venom acts to arrest the host development and to ensure the food resource while preventing the unregulated decomposition by bacteria. Many kinds of venom in *Pimpla hypocondriaca* has already been reported and well reviewed by Moreau & Guillot (2005). In pupal endoparasitoid *Pimpla hypochondriaca*, many functional proteins in venom have been analysed; 28 k and 30 kDa proteins as serine protease (Parkinson et al. 2002a), 22 kDa as pimplin of paralytic peptide (Parkinson et al. 2002b), 39.9 kDa as reprotysin type metalloprotease (Parkinson et al. 2002c), 74 kDa with antibacterial and proteolytic activity (Dani et al., 2003).

Venom components of egg parasitoids is not clarified although a few case is analyzed; *Telenomus heliothidis* (Strand et al., 1983, 1986, Strand, 1986), *Trichogramma pretiosum* (Strand, 1986), *T. dendrolimi* (Takada et al., 2000), *T. australicum* (Jarjees & Merritt, 2004). Venoms (female-derived factor or acid gland) are injected into the host eggs with parasitoid egg and arrest the host development. Jarjees & Merritt (2004) suggested that venom was responsible for host die and degeneration of host tissues using sterile female.

Egg parasitoid ingests the host contents like yolk at once after hatch for growth and development (Takada et al., 2000) and consumes the contents of the host killed or decomposed by venom as nutritional resource for growth and development in the host. Rapid ingestion of the host yolk absorbed insecticides enhances the possibility of disturbance to the growth and development of the parasitoid. The difference of susceptibility of egg parasitoids to insecticides may be attributed from direct effect on the larval and pupal development and from the difference of food intake speed of the host contents, though it is further possibility that the residual insecticides outside of egg-shell disturb the emergence from host egg.

1.2.2 Larval endoparasitoid as koinobiont

Larval endoparasitoids have many chances to be affected during a long larval period indirectly through the host physiological action by insecticide. Koinobionts let the hosts survive and exploit nutrient from hosts during development of parasitoid associating with invasion in host hemocoel and are demanded both avoidance of host immune response and acquisition of nutrition from the host with minimal damage. Severe damage of the host during early development may lead to precocious death of the parasitoid. If they give severe damage to the hosts to get nutrients from hosts, parasitoids are exposed to risk for death.

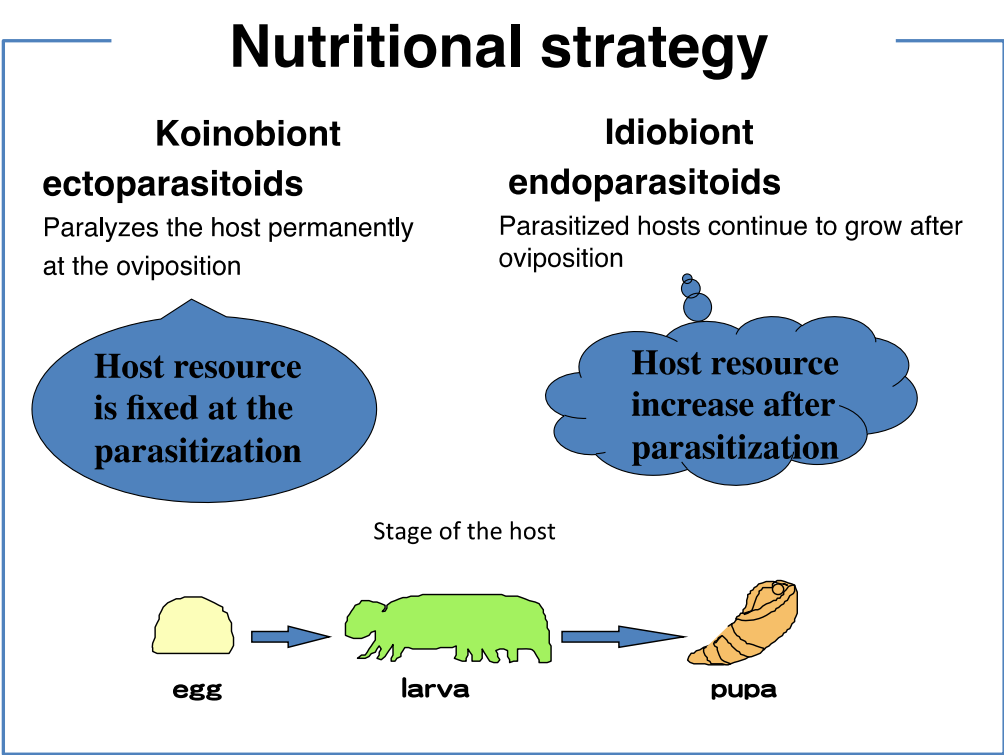


Fig. 3. Nutritional strategy of parasitoids.

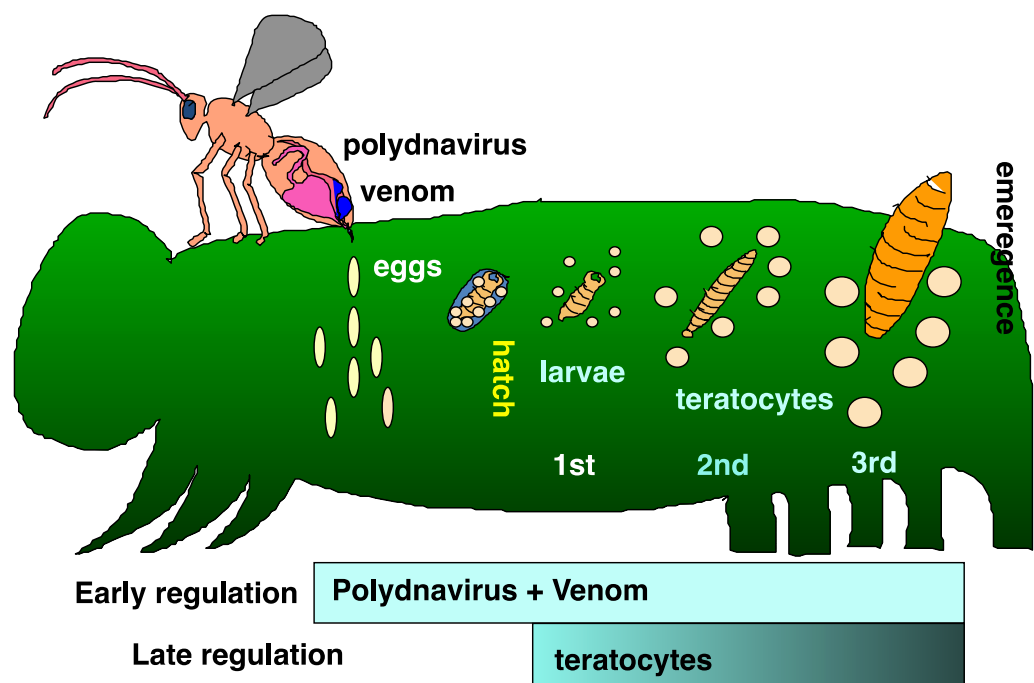


Fig. 4. Development of endoparasitoid and regulation factor of endoparasitoid on physiological state of the host. Polydnavirus (PDV) plus venom are injected into the host and regulate the physiological condition. Braconid endoparasitoids release their teratocytes which derived from the serosal cells of the egg after hatch and regulate the physiological condition during later stage.

Endoparasitoids develop the convenient tools like polydnaviruses (PDVs) plus venom and/ or teratocytes to get nutrients without severe damage in evolutionary process. Mutualistic relationship between PDVs and the endoparasitoids are estimated before about $70-73 \pm 10$ million years ago by calibration using fossil data (Whitfield, 2002, Drezen et al., 2003). Endoparasitoids seem to incorporate a nudivirus-related gene from ancestral Nudivirus and enable to produce the particles delivering in the host tissue cells for successful parasitism (B  zier et al., 2009a, b). PDV enables to regulate the physiological state of the host by penetration into each host tissue heterogeneously, especially in hemocytes and fat body. Viral genes expression alters the immune system and development of the host (Drezen et al., 2003, Beckage & Gelman, 2004, Kroemer & Webb, 2005, Webb & Strand, 2005, Gill et al., 2006, Asgari, 2006, Pennacchio & Strand, 2006, Kim et al., 2007). Hemocytes penetrated by PDV may lose ability to recognize and to encapsulate the foreign substances like eggs. Peptides or small proteins expressed from genes encoded in PDV play a role in physiological suppression of host immune response. Many suppression factors as PDV gene products are found. For example, protein tyrosine phosphatase (PTP) which known to play a critical role in the control of cellular events like proliferation, differentiation, and metabolism, and are a group of enzymes that remove phosphate groups from phosphorylated tyrosine residues on protein, then its expression affects the cellular PTP activity of the host (Espagne et al., 2004, Provost et al., 2004, Falabella et al., 2006, Gundersen-Rindal & Pedroni 2006, Ibrahim et al., 2007, Pruijssers & Strand, 2007, Ibrahim & Kim, 2008, Suderman et al., 2008, Shi et al., 2008), Cystatin which has inhibitory activity to cysteine proteases (Serbielle et al., 2008, Espagne et al., 2005), I  B-like (vankyrin) genes play a role in suppressing NF-  B activity in immune response (Kroemer & Webb, 2005, Bae

&Kim, 2009), and more Cysteine-rich domain products (Strand et al., 1997, Barandoc & Kim, 2009) and EP-1 like gene (Harwood & Beckage, 1994, Harwood et al., 1994, Kwon & Kim, 2008) including numerous hypothetical genes (Kroemer & Webb, 2004) may suppress the host immune response.

Venoms seem to change with evolution from ectoparasitoids to endoparasitoids (Whitfield, 2003), because venom may change from virulent action like killing the hosts to temperate action to lose toxic potency (Sclenke et al., 2007). Venoms of endoparasitoids contain many proteins in large molecular weight (Leluk et al., 1989) that lose the permanent paralytic function and promote of PDV expression in the host cells (Asgari, 2006).

Teratocytes are released and developed from serosal cell of parasitoid egg and produce some kind of regulatory protein along with the development (Fig.4). Endoparasitoids, on evolutionary process of having invaded from outside to inside, are required both to depress the host immune response specifically mentioned above and to get enough food and duration for growth and development at minimum damage to the host. Teratocytes play a role for extending larval stage of the host for getting enough nutrient required for their own growth and development. In case of Braconidae or Chalcidoidea, teratocytes function as one of factors to maintain the larval state (Dahlman et al., 2003). Elongation of larval state in parasitized hosts may increase the chance of contact with insecticides under natural condition. However, there is no information about detoxifying ability of teratocytes during late parasitism.

On the other hands, braconid endoparasitoids use teratocytes to take nutrients from host for avoiding severe damage to the host (Fig. 4). The most endoparasitoids seem to be assumed as hemolymph feeders (Thompson et al., 2001, 2002, Kaeslin et al., 2005), but In *C. kariyai-M. separata* association, second instars began to take fat body of host as food with help of teratocytes to ensure the big growth during 2nd instar stage (Nakamatsu et al, 2002, Tanaka et al. 2006). *Cotesia kariyai* also fed the host hemolymph as nutrient during first instar. Teratocytes attached on the surface and removed the outer membrane like cell matrix of the fat body with enzyme digestion locally, resulting that the second parasitoid larvae were easy to take the contents of the fat body as food. However, it is essential that the actin filaments in the fat body cells were broken previously by function of PDV plus venom (Tanaka et al., 2006). Although amount of consumption of the host fat body depend on the number of parasitoid larvae in a host, more than 100 parasitoid larvae consume almost all fat bodies (Nakamatsu & Tanaka, 2004). It was predicted that the larval endoparasitoids like *C. kariyai* might lower the susceptibility to insecticide during later parasitism by losing the fat body of the host.

1.2.3 Physiological regulation of endoparasitoid to insecticide

Koinobiont parasitoids that leave the host to continue growing after parasitization similar to unparasitized one are protected negatively through physiological action of the hosts from direct effect. Physiological milieu of the parasitized host is altered by PDV plus venom function from immediately after parasitization. Immune depression made us predict the lowering of resistance activity against the foreign substances penetrated into the body including xenobiotics and the detoxification ability of the host decreased with progressive ingestion of host fat body. However, in *Plutella xylostella-Cotesia vestalis* (= *plutellae*), Glutathion-s-transferase (GST) was enhanced the activity by PDV plus venom stimulation, because GST activity in egg stage was enhanced by oviposition or artificial injection of PDV

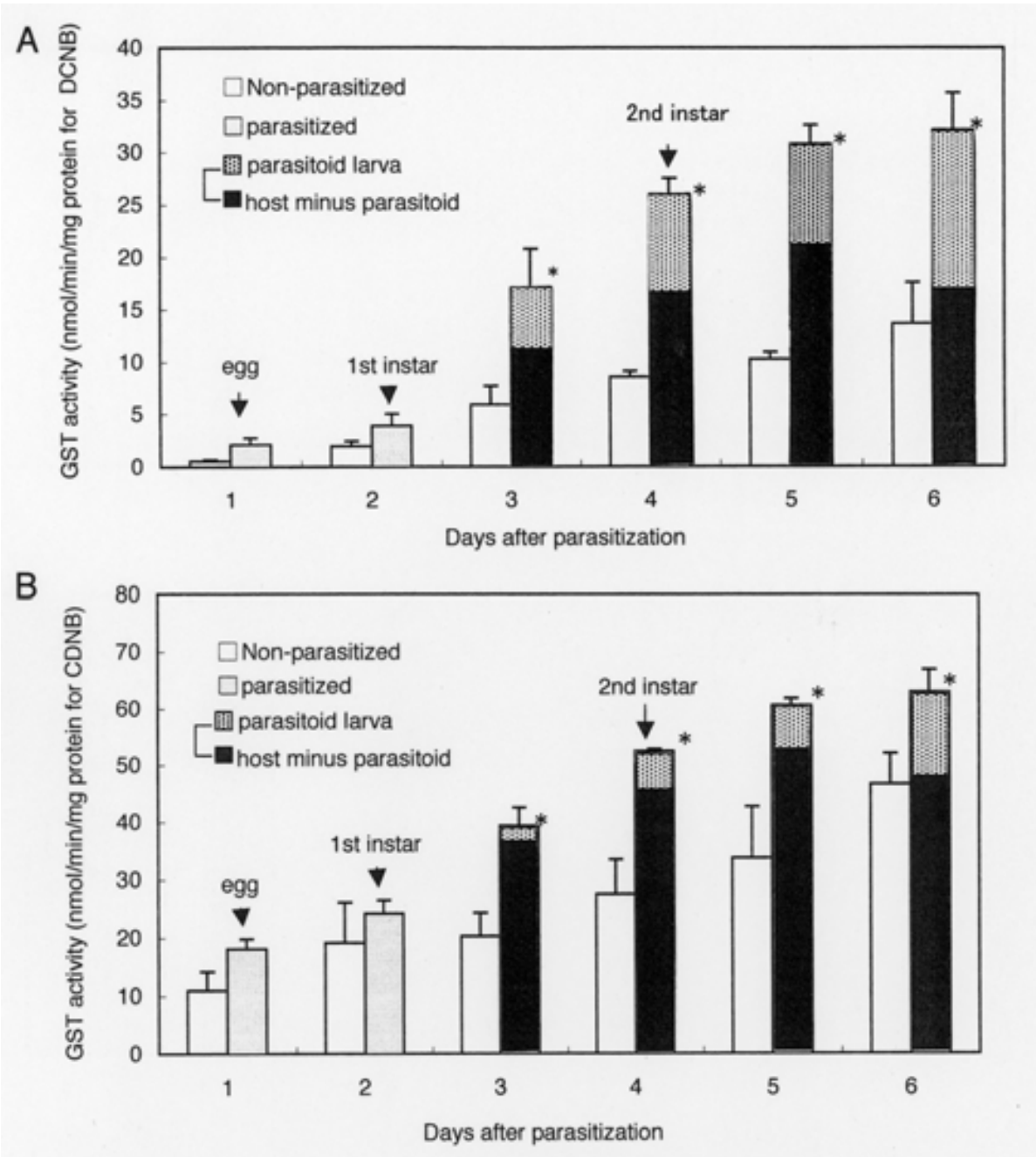


Fig. 5. Glutathion-s-transferase (GST) activity of the *Plutella xylostella* enhanced by parasitization of *Cotesia vestalis* (= *plutellae*). Data from Takeda et al. (2006). GST activity was measured with two enzyme substrates, individually (DCNB and CDNB). High GST activity of the hosts containing parasitoid larva was observed in later stage of parasitism.

plus venom (Takeda et al. 2006). Especially during late stage of parasitization while parasitoid larva consumed the host fat body, a low susceptibility to organophosphate (diazinon and fenitrothion) was detected. It was clarified that enhancement of CYP and GST enzymes of both parasitoid larva in parasitized hosts and the host itself causes the low susceptibility to insecticides with high enzyme activity (Fig. 5 from Takeda et al., 2006). *Cotesia vestalis*, solitary endoparasitoid did not consume absolutely and remained the host fat body of the host. Further, endoparasitoid larva contributed to the detoxification of the

host after treatment of insecticide. Amount of fat body remained in the host after parasitization seemed to be determined by two factors, the degree of inhibition to the host growth after parasitization and amount of fat body consumed by the parasitoid larva. These suggested that the parasitized hosts are able to acquire the resistance to insecticides when parasitoids do not consume all the host fat body. The spraying of organophosphates may make small impact on the surviving of parasitoids under agro-fields though the difference in susceptibility of parasitoids is not examined.

2. Conclusion

Parasitoids have different nutritional strategy. This difference seems to affect the susceptibility to insecticide. Idiobiont like egg parasitoid can utilize the dead host as nutritional resource. Normally idiobiont parasitoids kill or paralyze the host and stop the development of the host using venom. Many reports inform us a little effect of insecticides on the egg parasitoids. If insecticide hard to penetrate inside the host egg, parasitoid wasps can emerge from the parasitized eggs except that residual effect on the egg-shell kill the wasps at the emergence. On the other hands, koinobiont parasitoids utilize the host that continues to grow after parasitization, and are kept under physiological depression, especially in immune response by PDV plus venom. These mean the high susceptibility to insecticides during larval development. After all, larval parasitoids cannot develop in and emerge from hosts killed by insecticide treatment during their development even if the parasitoid larvae have resistance against the pesticide chemicals. Sub-lethal dose did not make severe effect on emergence rate of parasitoid even when insecticide treatment was performed during late parasitism except some neonicotinoids, though the susceptibility of the hosts treated with insecticides before parasitization or of the hosts treated with insecticides after parasitization along with growth and development was different between insecticides. On the other hands, parasitoid wasps had a high susceptibility to insecticides. When the insecticide spray in the agro-fields should be performed using place to escape for wasps like refuge, companion or banker plants. If transgenic crops will be used with methods or techniques that constrain the development of resistance strain, it may be valid and useful to depress the pest insect population. The parasitoid larvae were successfully emerged from the parasitized hosts at sub-lethal dose anytime during larval development, though the emergence rate is low. The parasitoids emerged from the hosts may lead to the potential to regulate the population density of pest insect.

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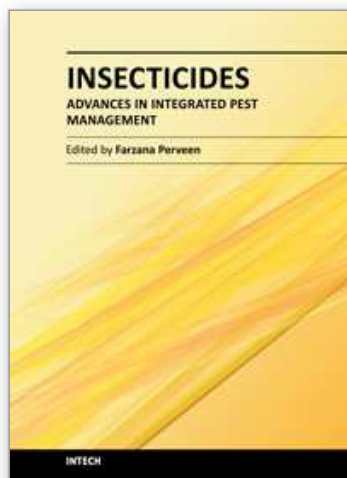
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This book contains 30 Chapters divided into 5 Sections. Section A covers integrated pest management, alternative insect control strategies, ecological impact of insecticides as well as pesticides and drugs of forensic interest. Section B is dedicated to chemical control and health risks, applications for insecticides, metabolism of pesticides by human cytochrome p450, etc. Section C provides biochemical analyses of action of chlorfluazuron, pest control effects on seed yield, chemical ecology, quality control, development of ideal insecticide, insecticide resistance, etc. Section D reviews current analytical methods, electroanalysis of insecticides, insecticide activity and secondary metabolites. Section E provides data contributing to better understanding of biological control through *Bacillus sphaericus* and *B. thuringiensis*, entomopathogenic nematodes insecticides, vector-borne disease, etc. The subject matter in this book should attract the reader's concern to support rational decisions regarding the use of pesticides.

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