Study on bioactivity of lime, *Citrus aurantifolia* (Christm.) against larvae of Diamond back moth, *Plutella xylostella* (L.) on cabbage crop under laboratory condition.

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Abstract— High feeding activity of larval stages of *Plutella xylostella* leads to reduction in yield and causes economical loss. The uses of plant products are reported to be an alternative to synthetic pesticides for insect pest management. With this background, a laboratory study was carried out to evaluate the lethal, phagodetterent and post embryonic develomental effects of aqueous and methanol leaves extracts of *Citrus aurantifolia* on the third instar larvae of *P.xylostella* and compared with a synthetic pesticide Spinosad. Three concentrations of methanol leaf extract (MeLE) (0.05g/ml, 0.125g/ml and 0.2g/ml) , aqueous leaf extract (AqLE) (0.05g/ml, 0.15g/ml, and 0.2g/ml) and synthetic pesticide (SynP) (5g/l, 15g/l and 25 g/l) were tested along with solvent controls against 3rd instar larvae of *P.xylostella*. Larvae fed with cabbage leaves disc treated with MeLE at 0.2g/ml and AqLE at 0.15g/ml significantly ($P<0.05$) reduced the leaf consumption after 24 hours compared to controls. There was no significant difference ($P>0.05$) in leaf consumption between MeLE and AqLE. All the concentrations of MeLE and AqLE showed significantly ($P<0.05$) higher lethal effects after 48 h of exposure. LD_{50} values for MeLE and AqLE were found as 0.123 g/ml and 0.89 g/ml respectively after 48 h of treatment. All concentrations of synthetic pesticide except 5g/l showed 100% larval mortality after 24 hours of treatment. Pupal deformities and abnormal adult with curled wing also observed at 0.2g/mL of MeLE and 0.15 g/ml AqLE respectively. This study revealed that leaf extract of *C.aurantifolia* caused lethal, phagodetterent and growth inhibitory effect on larvae of *P. xylostella*..

Keywords—Citrus aurantifolia , synthetic pesticide ,*Plutella xylostella* , phagodetterent, lethal, growth inhibitory

I. INTRODUCTION

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Cabbage, *Brassica oleracea* L. Var.*capitata*is one of the important cruciferous market gardening vegetable grown in many parts of the country and generates income for the producers. However, it is subjected to various kinds of insect pests of which diamond back moth, *Plutella xylostella* is a most serious insect pest causing severe yield loss worldwide due to high feeding rate during the larval period. Indiscriminate and excessive application of chemical pesticides leads to serious effects on the environment, non target organisms and affecting the health of users and consumers and development of resistance to insecticide . To solve the resistance problem in *P.xylostella* to pesticides, farmers often increase the doses of pesticides (Selvaraj *et al*., 2017). Consumers and producers prefer organic compounds that are free of agro toxic chemicals therefore it leads to research to search the alternative control methods especially with plant products is being increasingly studied to minimize the chemical impact of pesticide. Citrus species have been reported as a source of botanical pesticides against several Coleopteran and Dipteran species (Abbassy *et al,* 1979; Nithiyagowry, 2015); Salvatore *et al*., 1983) in most of the studies Citrus plant parts peel, seed, fruit have been used against insect pests however a few studies were carried out on leaf of citrus (Abdullah, E. M., 2016; Thulashi & Nithiyagowry, 2010) against stored product pests. In this view, the present study attempts to evaluate the lethal, phagodetterent and growth inhibitory effects of aqueous and methanol leaves extracts of *C.aurantifolia* on third instar larvae of *P. xylostella* compared to synthetic pesticide Spinosad.

II. MATERIALS AND METHODOLOGY

A. Planting of cabbage, Brassica oleracea L. Var. capitata

Cabbage seedlings**,** *Brassica oleracea* L. Var. *capitata* containing 3 to 4 fully expanded leaves were obtained from nearby farmers field and were planted singly in plastic pot of 11 cm diameter and 13cm height containing red fertile soil, each potted seedlings kept in the laboratory in the pest free conditions (covered by mosquito net) and 28 + 3 ºC, 75 + 5 % RH and 12L: 12D photoperiod conditions.

B. Mass rearing of Plutella xylostella

Larvae and pupae of *P.xylostella* were collected from farmer's cabbage field. Pupal stages were introduced into vials ($29.28cm^3$) and larvae were introduced into rearing cage (31cm x 23.4 cm x 23.4cm) which consisting cabbage planted pots. Emerged adults of *P.xylostella* were collected from the cage and vials and were introduced into another setup cage which containing potted cabbage plant and were provided sugar solution (10 %) as feed for adults. Whole setup was covered by untreated mosquito net.

B.1. Rearing of Plutella xylostella for experiment

Five to ten adults emerged from the rearing cage were introduced into the cage containing the potted cabbage. Plants were checked daily for presence of eggs. Then, potted cabbage

with eggs was transferred into another cage allowed to hatching as larva. Nearly equal size (weight) of 3rdinstar larvae used for experiment.

C. Plant material used and extraction method

Leaves of *C. aurantifolia* collected from home garden were brought to the laboratory cleaned with water and air dried in shade. Dried leaves were ground in an electric grinder and resulting powder was passed through 250 µm mesh size sieve to obtain a powder.

C.1. Methanol leaf extract of Citrus aurantifolia

Fifty grams leaf powder of *C.aurantifolia* were mixed with 450 ml of methanol and the mixture was stirred about 3 hours in a magnetic stirrer and left to stand for 24 hours. Resulting mixture was filtered through filter paper (Whatman Schleicher and Schuell, 110 mm diameter). The solvent in the extract was completely evaporated in hot water bath (Temperature 70°C) until the constant weight of extract was gained from this stock solution a series of concentration (0.05g/ml, 0.125g/ml, and 0.2g/ml) of MeLE of *C.aurantifolia* was prepared by diluting the stock solution with distilled water.

C.2. *Aqueous leaf extract of Citrus aurantifolia*

The aqueous leaf extract (Aq.LE) was prepared from 10 g of fresh leaves of *C.aurantifolia* were taken from home garden and cut into small pieces and 40ml of distilled water was added into it. It was ground in an electric grinder. The suspension was strained through muslin cloth to obtain aqueous extract. A series of concentration of aqueous leaf extraction was prepared by diluting the stock solution with distilled water separately to get 0.05g/ml,0.1g/ml,0.15g/ml concentration of extracts.

C.3. Synthetic pesticide Success (Spinosad, 25g/ml (sp) (10ml-10 l))

Synthetic pesticide Spinosad (0.1ml) was mixed with 100ml distilled water. From this stock solution 5g/l, 15g/l, 25g/l concentrations of pesticide was prepared by diluting the stock solution with distilled water.

D. Bioassay

Same maturity of fresh cabbage leaves were taken from the cabbage crop grown in the insectary, Department of Zoology, University of Jaffna. Cabbage leaves were washed with distilled water and dried for about 10 mins. and cut into 44 discs of 6.4 cm diameter. The discs were separated into 11 batches of 4 discs each for the 11 treatments as given below:

Treatment 1. Methanol leaf extract @ 0.05g/ml
Treatment 2. Methanol leaf extract @ 0.125g/ml
Treatment 3. Methanol leaf extract @ 0.2g/ml
Treatment 4. Aqueous leaf extract @ 0.05g/ml
Treatment 5. Aqueous leaf extract @ 0.1g/ml
Treatment 6. Aqueous leaf extract @ 0.15g/ml
Treatment 7. Synthetic pesticide Spinosad @ 5g/l
Treatment 8. Synthetic pesticide Spinosad @ 15g/l
Treatment 9. Synthetic pesticide Spinosad @ 25g/l
Treatment 10.Methanol alone for control
Treatment11. Water alone for control

One set of leaf disc was dipped into the test solutions 0.05g/ml,0.125g/ml,0.2g/ml Me.LE of *C.aurantifolia* for 2 minutes separately. Similarly one set of leaf discs were dipped in to the 0.05g/ml,0.1g/ml,0.15g/ml Aq.LE of *C.aurantifolia*, and one set of leaf discs dipped into the 5g/l, 15g/l, 25g/l synthetic pesticide, methanol and water separately. All treated leaf disc were hold vertically to allow excess solution to drip off and allow to dry for 10 minutes. Then treated leaf discs were placed in the plastic petridish (8cm diameter) contained moistened filter paper to retard drying of the leaf discs and covered by a lid having 4cm diameter hole covered with fine nylon mesh to provide ventilation. The method of application was similar for all the treatments. Larvae of nearly equal size (0.005g±0.0005) (3rd instar) five in number were introduced into each petridish and for each replicate. The larvae were starved for 6 hrs before exposure. Moistened cotton wool block was placed inside the each petridish to retard drying of the discs. Petridishes were secured by rubber bands and kept under laboratory condition (temperature 30°C±2°C and 75%±5%RH) for 24 hours.

The area of leaf consumed, the percentage of mortality of the larvae was determined after every 24 hours of treatments for 3 days.

Four replication of each treatment with five larvae per replicates were maintained along with the solvent controls.

E. Determining the treatment effects

E.1. Phagodetterent effect

To study the phagodetterent effect of the extract on *P.xylostella* the progressive consumption of leaf area was measured at 24 h intervals for three days both in control and in treatments using graph paper method.

E.2. Lethal effect

The dead and survived larva was counted at 24 hours interval and dead larvae were removed from each treatment and counted separately and mortality data was corrected using Abbott's formula as follows (Abbott, 1925). After correcting mortality value the median lethal dose (LD_{50}) value were calculated by Probit analysis method (Finney, 1971).

E.3. Growth regulatory effect

The surviving larvae were allowed to develop for further observations such as pupation, adult emergence and developmental abnormalities.

F. Experimental conditions

This research was conducted in laboratory condition at University of Jaffna under prevailing environmental conditions of 27-30ºC and 70-75% RH during the day time .Larva of *P. xylostella* was selected by randomization. Control experiments were conducted concurrently along with experimental trials. Water control for aqueous leaf extraction of *C.aurantifolia* and synthetic pesticide Spinosad. Methanol control for methanol leaf extraction of *C.aurantifolia*.

G. Data analysis

Comparisons of mean numbers were made using one way analysis of variance (ANOVA).The mean values were separated using LSD through ANOVA.

Lethal dose (LD_{50}) value were calculated by Probit analysis method (Finney, 1971).

III. RESULTS AND DISCUSSION

A. Area of leaf consumed

The mean area of leaf consumed by larvae of *P.xylostella* differed significantly ($P<0.05$) among the treatments at different concentrations after 24h, 48h and 72 hours of exposure and was significantly lesser than the controls. For 24 h ($F=7.21$, $df=33$, $P<0.05$); after 48 h ($F=19.8$, $df=33$, $P<0.05$) and after 72 h ($F=27.44$, $df=33$, $P<0.05$).

The mean area of leaf fed upon by the larvae on the leaf discs treated with methanol and aqueous leaf extract of *C.aurantifolia* in all concentration tested were significantly ($P<0.05$) lesser than that of respective solvent controls after 24h,48h and 72h exposure. From the LSD test, it was observed that MeLE at 0.2 g/ml and Aq.LE at 0.15 g/ml shown significantly ($P< 0.05$) higher phagodetterent effect than the other treatments after 24 h of exposure (Figure 1). After 48 h of exposure there was no significant difference ($P>0.05$) between MeLE and AqLE in area of leaf consumed. Conventional pesticide Spinosad also exhibit significantly lesser leaf consumption area at 5g/l after 24 hour of exposure than control however no leaf consumption at 15g/ml and 25g/ml due to the lethal effect of pesticide on larvae.

This is probably due to phagodetterent activity of chemical compounds particularly Limonoids presence in the leaf extract of *C.aurantifolia* (Klocke and Kubo, 1982) reduced the consumption of leaf compared to solvents. Chemosensory responses to allelochemicals inhibit the taste cells (Loon van, 1996) therefore once larvae feed on the treated leaf susceptible larva cease feeding followed by starvation and finally result to death of larva.

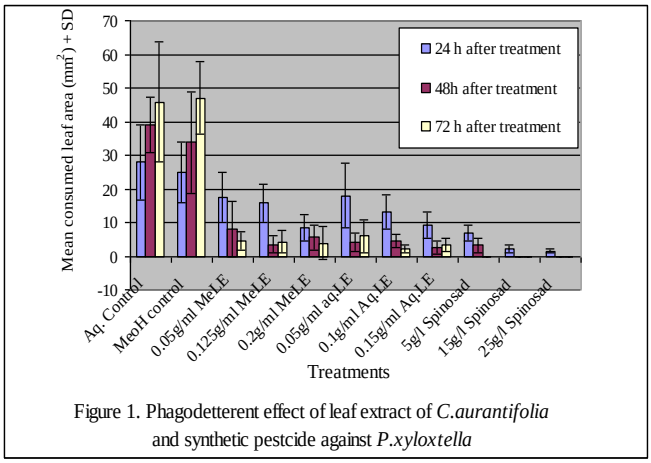

Figure 1. Phagodetterent effect of leaf extract of *C.aurantifolia* and synthetic pestcide against *P.xyloxtella*

B. Lethal effect

The experiment results revealed that when larvae exposed to both aqueous and methanol leaf extract the toxic effect on larvae of *P. xylostella* was significantly ($P<0.05$) higher with the increase of dose and with exposure time than the controls. There was a significant different in larval mortality among the treatments after 24 hours ($F=41.2.58$, $df=33$, $P<0.05$), after 48 hours ($F=33.1$,$df = 33$, $P<0.05$)and after 72 hours ($F=45.84$, $df=33$, $P<0.05$) of exposure. After 48 h of treatment , more than 40 % mortality was observed at 0.1 g/ml Aq. LE and 0.125 g/ml as 55 % and 75 % respectively. The maximum larval mortality was observed after 72 hrs as 95 % at 0.2 g/ml Me.LE and 85 % at 0.1 g/ml and 0.15 g/ml Aq.LE., however there was no significant difference ($P>0.05$) in larval mortality between aqueous and methanol leaf extract all concentrations tested after 72 hours of exposure and at 0.2g/ml after 48 hours. There was no larval mortality in solvent controls but when both leaf extract was compared with that of synthetic pesticide 100% mortality was observed all treatments of pesticide Spinosad (after 24hours of exposure except 5g/l concentration) (Figure 2). From the LSD test it was observed that after 24 h of treatment 0.125g/ml , 0.2 g/ml of MeLE 0.1 g/ml, 0.15 g/ml of AqLE shows higher lethal effect than the 0.05 g/ml of AqLE, 0.05g/ml of MeLE and solvent controls. But after 48 h of treatment all the treatments showed equally and significantky higher lethal effect than the controls.From the Probit analysis, LD_{50} values after 48 h of treatment was found as 0.123g/ml for Me.LE and as 0.89g/ml for Aq.LE.

This is due to active compounds particularly Limnoids group of terpenoidallelochemicals present in the leaf extract of *C.aurantifolia*act as phagodetternet (Klocke and Kubo,1982) against the larvae of DBM as a result larvae stop the feeding finally then leads to death. However larval mortality in synthetic pesticide Spinosad treatment is due to direct lethal effect.

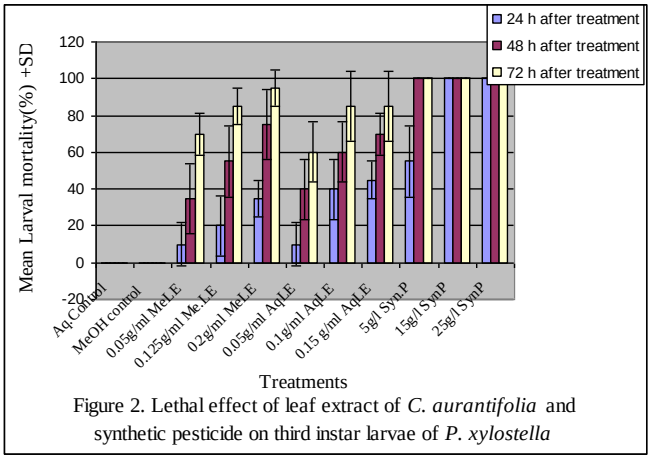

Figure 2. Lethal effect of leaf extract of *C. aurantifolia* and synthetic pesticide on third instar larvae of *P. xylostella*

C. Growth inhibitory effect

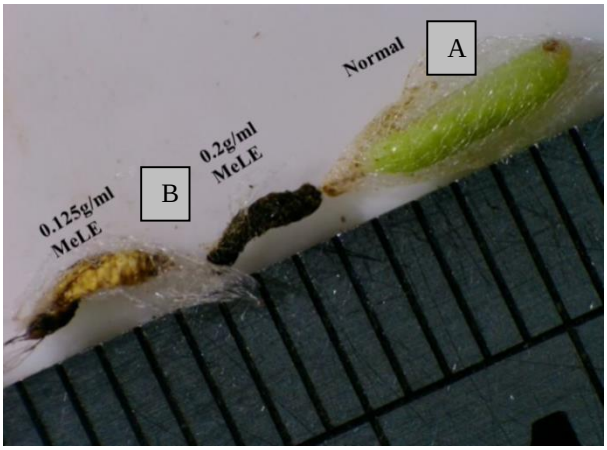

Plate 1.Developmental effect of leaf extract of *C. aurantifolia* on pupal stage of *P.xylostella*
A-Normal pupa, B- malformed Pupa

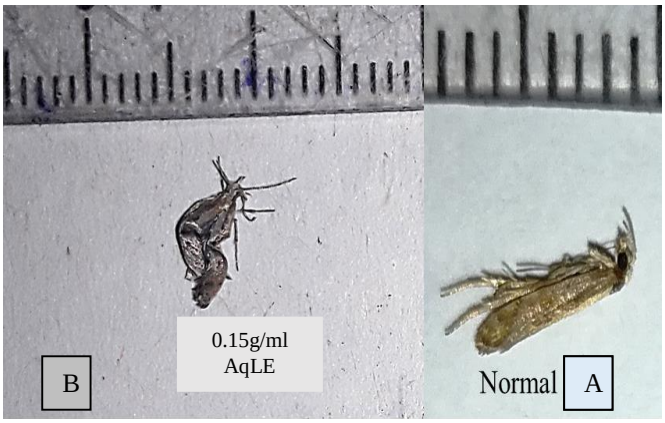

Plate 1.Developmental effect of leaf extract of *C. aurantifolia* on adult stage of *P.xylostella*
A-Normal adult, B- malformed winged adult

Treatment	survive larve (Mean)	Pupation (Mean)	Percentage of pupation	emergence of adult (Mean)	Percentage of adult emergence
0.05g/ml MeLE	1	0.75	75%	0.75	100%
0.125g/ml MeLE	0.75	0.5	66.7%	0.25	50%
0.2g/ml MeLE	0.25	0.25	100%	0	0%
0.05g/ml AqLE	2	1.5	75%	1.5	100%
0.1g/ml AqLE	0.75	0.5	66.7%	0.5	100%
0.15g/ml AqLE	0.75	0.5	66.7%	0.25	50%
5g/l SynP	0.25	0	0%	0	0%
15g/l SynP	0	0	0%	0	0%
25g/l SynP	0	0	0%	0	0%
Water control	5	5	100%	5	100%
Methanol control	5	5	100%	5	100%

In all treatments survive larva of *P.xylostella* became pupal stage but pupal stages were not emerge as adult in 0.2g/ml methanol leaf extract of *C.aurantifolia* (Table 1). Abnormal pupal stages with malformed adults were observed (Plate1). The regulation of larval development resulted in abnormal wing shape in some of the emerging adults when larvae treated with 0.15g/ml aqueous leaf extract of *C.aurantifolia* (Plate 2). This could be due to the presence of active compounds in leaf extract of *C. aurantifolia* such compound disrupting the moulting hormone (Vardhini *et al*., 1997). These effects are correlated with changes in haemolymph ecdysteroid and juvenile hormone titters due to blockage and or delay in the release of hormones from neurohormonal organs (Bamby and Klocke, 1990). The deformation of wings could also be due to the extensive cellular injuries causing cytotoxic effects that can alter the organism's physiology (Scudeler *et al*., 2014).

As a result of deformities of wing in emerging adults prevents the adult to fly there by reducing its movement pattern ultimately limit the reproduction and dispersal of *P.xylostella* (Mondedji, Kasseney and Nyamador, 2016). So this study clearly indicates that leaf of *C.aurantifolia* contains growth inhibitory substances.

IV. CONCLUSION

This study has showed that the leaf extract of *Citrus aurantifolia* exhibited significant lethal and phagodetterent effects on 3rd instar larvae of *Plutella xylostella*. Since the active phytochemical posses antifeedant activity, mortality and pupal adult abnormalities of this phyto pesticide in cruciferous crops can reduce crop damage as well as pest population. Moreover, both MeLE and AqLE exhibit similar lethal effect at .2 g/ml MeLE and 0.15 g/ml AqLE over 72 h exposure therefore the median lethal dosage exhibited by MeLE as 0.123g/ml and as 0.89g/ml for Aq.LE.

Table 1. Post treatment effect of leaf extract of *C.aurantifolia* on 3rd instar larvae of *Pleutella xylostella*.

The using AqLE at 0.15g/ml and MeLE at 0.2g/ml is an excellent alternative to prevent damage to crop caused by *P.xylostella*.

C.aurantifolia can be an excellent substitute for synthetic pesticides by reducing the hazards associated with synthetic pesticide Spinosad and other pesticides. Application of phytochemicals could be a promising approach to decrease the overall use of synthetic pesticide.

Leaf extract of *C.aurantifolia* could enhance the management of larva of *P.xylostella* by acting as phagodetterent, larvicide and by regulating is development from larva to adults and deforming wings. Therefore it can be considered as possible source of alternative insecticide in the management of *P.xylostella* and water is easily accessible solvents by farmers as such aqueous extract provide farmers with cheap pest control solution.

Further study needed to identify the active compound responsible for their effects and to do carryout experiment in field condition.

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