American Journal of Environmental Sciences 6 (2): 168-176, 2010
ISSN 1553-345X

Study on the Effect of Allylisothiocyanate Formulations on Three Lepidopterous Insect Larvae, the Diamond Back Moth, *Plutella xylostella* (L.), the Small Cabbage White Butterfly, *Pieris rapae* (L.) and Tobacco Cut Worm, *Spodoptera litura* Fabr

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Abstract: Problem statement: The toxicity of seven of Allylisothiocyanate (AITC) formulations, AITC pure and in mixture with cypermethrin and chlorpyrifos, was evaluated using spray application and fumigation method against the diamondback moth, *Plutella xylostella* (L.), the small cabbage white butterfly, *Pieris rapae* (L.) and the tobacco cut worm, *Spodoptera litura* Fabr. **Approach:** Response varied according to insect species, specific formulation and AITC application rates. **Results:** Fumigant toxicity of the oil based on 9 h exposure was evaluated against the three insect larvae, the diamond back moth, *P. xylostella* larvae were more susceptible followed by tobacco cut worm, *S. litura* and cabbage white, *P. rapae* larvae. To *P. xylostella* larvae, LC_{50} value was 1.5 µL L^{-1} air, *S. litura* larvae ($LC_{50,}$ 1.8 µL L^{-1} air) and *P. rapae* larvae (LC_{50}, 2.0 µL L^{-1} air). These three insect larvae were killed after 9 h exposure to a dose of 4.3 µL L^{-1} air of the oil, showed no significance difference with that of phosphine (40 µg L^{-1} air) a recommended dose. On the other hand, on the spray application bioassays, mortality data counted 72h after treatment, the *P. rapae* larvae were most susceptible to AITC formulations followed by *S. litura* larvae and *P. xylostella* larvae. LC_{50} values, to *P. xylostella* larvae, HJ(A) and HJ(B) were the most potent toxicant (33 mg L^{-1}) and (46 mg L^{-1}) respectively, while the least toxic was HJ(C) (556 mg L^{-1}). To *S. litura* larvae, HJ(B) and HJ(A) were the most efficient (10 mg L^{-1}) and (22 mg L^{-1}) respectively, while HJ(D) ($LC_{50,}$ 385 mg L^{-1}) was found to be the least toxic. The both of HJ (B) and HJ (C) had similar insecticidal activity ($LC_{50,}$ 8 mg L^{-1}) to *Pieris rapae* larvae followed by HD (B) ($LC_{50,}$ 22 mg L^{-1}) and HD (A) ($LC_{50,}$ 82 mg L^{-1}). **Conclusion/Recommendations:** It was concluded that the AITC oil showed promise findings and may be a good alternative to old fumigant "phosphine". The others were found efficient on the spray application bioassays but the mixture of AITC with cypermethrin especially HJ (A) and HJ (B) were found to be better than AITC mixed with chlorpyrifos and AITC pure. So they can be used in the field of crop protection as safer and potential components of an integrated pest management strategy, especially allylisothiocyanate is considered fully biodegradable, less toxic and many cruciferous vegetables daily consumed by humans.

Key words: Allylisothiocyanate, fumigant activity, spray application bioassay, cruciferous insect larvae, *Spodoptera litura* larvae

INTRODUCTION

Crucifers are important vegetable crops and of high importance in the traditional diet and local economy in many Asian countries. The main insect pests of crucifer's crops are the caterpillars of the Diamondback Moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) and the small cabbage white butterfly, *Pieris rapae* (L.) (Lepidoptera: Pieridae). The caterpillars of these two species are considered as serious pests of cabbage crops. They damage the leaves by making holes and tears.

Talekar and Shelton (1993) reported that extensive problems arise because of the damage from agricultural

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insect pests, particularly from the Diamondback Moth (DBM), *Plutella xylostella* (Linaeus) (Lepidoptera: Yponomeutidae) and the small cabbage white butterfly, *Pieris rapae* (L.) (Lepidoptera: Pieridae), but the (DBM) is considered as a major and cosmopolitan insect pest of cruciferous plants. Control measures of these insect populations worldwide have been principally achieved through the use of conventional chemical insecticides and non-conventional insecticides such as *Bacillus thuringiensis*. This is exacerbated by the development of insecticides resistance in the former species. In addition, the acquisition of the pesticide resistance by diamondback moth is a well-known phenomenon and leads to pest control failures in cabbage crops through out the world. In North America, traditional farmers are used to manage these insects by using the synthetic insecticides (Liu *et al*., 2002). Although these insecticides are effective, their extensive use has disrupted natural biological control systems which resulted in resurgence of this insect and finally led to development of the insect resistance and had harmful effects on natural enemies and non-target organisms (Talekar and Shelton, 1993).

The tobacco army worm, *Spodoptera litura* Fabr. (Lepidoptera: Noctuidae) is a major agricultural insect pest, feeds on more than one hundred host plants. It considered as one of the most economically important insect in many countries especially India, Japan, China and other countries of Southeast Asia, it has been recorded as a cosmetic pest of sesame in Japan (Sintim *et al*., 2009). Synthetic pesticides are continuously used by traditional farmers to eliminate *Spodoptera litura* which leads to development of the resistance against almost all the commonly using pesticides in the specific area. Human health and environmental safety, as well as prevalence of insect resistance to synthetic insecticides have promoted a renewal of interest in biologically active plant-derived chemicals which can be expected to play an increasingly significant role in the commercial development of new products for regulating plant growth and for insect and weed control (Balandrin *et al*., 1985). Therefore, the development of safe and selective alternatives to control cabbage caterpillar and tobacco cut worms is needed, especially those with fumigant and/or contact activity. Furthermore, these alternatives should be easier and convenient to be applied in the field, greenhouses and in crops grown under plastic sheets in which the fumigation processes can be done under controlled conditions.

To our knowledge, a Little information exists with respect to managing *Plutella xylostella*, *Pieris rapae* and *Spodoptera litura* with naturally occurring substance, Allylisothiocyanate (AITC) although the bio-activity of this substance has been largely recorded. It was known that AITC is most toxic compound formed from allyl glucosinolate hydrolysis in *Brassica juncea* L. and possibly the most important for bio-fumigation, it could kill bacterial pathogens on plant seeds (Park *et al*., 2000), refrigerated beef meat (Chacon *et al*., 2006). The fungitoxicity of AITC vapor against *Penicillium expansum* on pears was also investigated, the best control to this species was obtained by exposing fruits for 24h in a 5mg L^{-1} AITC- enriched atmosphere (Mari *et al*., 2002). Shin *et al*. (2004) also showed that various biological effects of allylisothiocyanate have been reported including anti-oxidative, anti-bacterial and anti-fungal activities. Thus, it is reasonable to assess the insecticidal potentialities of seven formulations of Allylisothiocyanate (AITC), pure and mixed with cypermethrin or chlorpyrifos to be used as commercial insecticides. The formulations for oil and Hong Jing (D) were both AITC pure, whereas those for Hong Jing (A), Hong Jing (B) and Hong Jing (C) were mixed with cypermethrin. Hong Du (A) and Hong Du (B) were also mixed with chlorpyrifos. All these formulations were assessed against the Diamondback Moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), the small cabbage white butterfly, *Pieris rapae* (L.) (Lepidoptera: Pieridae) and the tobacco cut worm, *Spodoptera litura* Fabr. (Lepidoptera: Noctuidae) either by fumigation (for the oil only), or by spray application bioassays for the other formulations.

MATERIALS AND METHODS

Chemicals: The Allylisothiocyanate (AITC) formulations studied in this study are shown in Table 1. The oil and the other formulations were prepared and supplied, respectively by WUHUA a PhD student and Prof. SHI, from the Department of Plant Science, Huazhong Agricultural University, Wuhan/China.

Collection and culturing of insects: The cabbage white, *Pieris rapae* and diamond back moth, *Plutella xylustella* (Lepidoptera: Plutellidae, Yponomeutidae) larvae were collected from the field with fresh host plant, the cabbage, *Brassica* sp. leaves during summer season (May/June 2009) while the tobacco cut worm, *Spodoptera litura* larvae were collected during (Aug/Sept 2009). The cabbage white and tobacco cut worm collected from the field and directly used in the experiments because the insect density on the host plant was high during these periods. The other insect, diamond back moth larvae were collected and reared in the laboratory on young and fresh cabbage leaves in cylindrical cages (25×25×35 cm) and incubated under the following conditions: 25±2°C, 55±5% (RH) under a photoperiod of 12:12 (L:D). The adults were continuously supplied with honey diluted in distilled

Table 1: Allylisothiocyanate (AITC) pure and in mixture with cypermethrin or chlorpyrifos which were used in this study

Name	a.i.	Pure, mixture in ratio	Formulation type
Oil	(85%)	AITC, pure	EC
Hong Jing, HJ(A)	(12.7%)	AITC + Cypermethrin (1: 1)	Effervescent agent
Hong Jing, HJ(B)	(11.37%)	AITC + Cypermethrin (4: 1)	EA*
Hong Jing, HJ(C)	(10.2%)	AITC + Cypermethrin (2: 1)	Effervescent agent
Hong Jing, HJ(D)	(4.78%)	AITC, pure	Effervescent agent
Hong Du, HD(A)	(7.6%)	AITC + chlorpyrifos (2: 1)	WG
Hong Du, HD(B)	(7.1%)	AITC + chlorpyrifos (2: 1)	Effervescent agent

EC: Emulsifiable Concentrates; EA: Effervescent granule; WG: Wettable dispersible Granule; a.i.: Active ingredient

water (1:10, honey: water) in a piece of cotton hung in the central of the cage. Under these conditions, longevity of eggs, larvae, pupae and adults was counted. Approximately they were 3, 11, 6 and 8 days, respectively. The 3rd instar larvae of all three species were used in the experiments.

Insecticide experiments:

Fumigation toxicity on eppendorfmicro-test tubes: To test the fumigation toxicity of the AITC oil to diamond back moth, *Plutella xylustella* larvae, cabbage white, *Pieris rapae* and tobacco cut worm, *Spodoptera litura* larvae after 9 h exposure, a 1.5 L plastic container with screwed plastic caps was used as exposure chamber according to the method of Kim and Ahn (2001) with some modifications. A whatman No. 1 filter paper (9.0 cm in diameter) was placed on the bottom of the container, clean and young cabbage leaves were placed on the filter paper. Fifteen larvae were separately introduced in to containers through openings (made on the first quarter of the container) by using a fine brush. Then, the openings were closed by Para films. Eppendorf micro-test tube (1.5 mL) which used as a diffuser was hung at the geometrical centre of the vials. The vials were then covered with plastic caps. The concentrations of 0.9, 1.4, 2.0, 2.8 and 4.3 $\mu L L^{-1}$ air have been tested against the three insect larvae. Distilled water was used in untreated control (0). The bioassays were conducted under laboratory conditions as mentioned above (Collection and culturing of insects). All the treatments were replicated four times. Exposure time was 9 h after which, the plastic vials were opened and the tested insect larvae were removed and placed in clean Petri dishes with fresh food. The mortality was assessed 3 days over treatments. Larvae were considered dead if did not display any motion when they were prodded with fine pins.

Spray application toxicity on dishes: The effect of spray application of Allylisothiocyanate (AITC) formulations, HJ(A), HJ(B), HJ(C), HJ(D), HD(A) and HD(B), to diamond back moth, *Plutella xylostella* larvae, cabbage white, *Pieris rapae* larvae and tobacco cut worm, *Spodoptera litura* larvae were investigated on dishes. Freshly picked cabbage, *Brassica* sp. leaves were washed and dried on plate. The dish was prepared by using a whatman No.1 filter paper (9.0 cm in diameter) which was placed into labelled plastic Petri dishes (9.0 cm diameter×1.0 cm) and the dried cabbage leaves (5.0 cm in diameter) were then placed on the filter paper. Fifteen (15) larvae were individually introduced and placed on the cabbage leaves in the dishes. Different amounts of formulations were diluted in different quantities of water (1: 0.2, g: L) and a series of multiple concentrations were then set up between 8 and 2048 mg L^{-1}. Different concentrations were applied on the dishes by using the same volume (0.4 L) of different solutions in the hand spryer tank. The pressure in the sprayer was adjusted by pressing the air 60 times to achieve enough pressure to deliver the smallest possible droplet size for complete coverage of the treated dishes. Dishes in control series were sprayed with 0.4 L water. All experiments were conducted under laboratory conditions as mentioned before. Each treatment was replicated four times. The percentage mortality data was determined 72 h after treatment. The insects were considered dead only if they display no motion when they were touched with a fine brush.

Data analysis: The mortality data of adult insects were counted using Abbott (1925) and untransformed mortality data to each treatment were used for probit analysis to estimate LC_{50} and LC_{90} values of the insect species to different AITC formulations with their 95% confidal limits, the mortality means and Standard Error (SE) were estimated by one way ANOVA and Tukey test and then the relationship between the insect larval mortality rates and concentrations were modeled (SPSS, 1999).

RESULTS

Effect of the fumigant activity of AITC oil on the three insect larvae: The effect of the Fumigant activity of AITC oil to the three tested insect larvae is shown in Fig. 1 and Table 2. To the tested insect larvae, the

Table 2: LC_{50} and LC_{90} values of AITC oil [c] against the three tested insect larvae

Larval species	LC_{50} [a]	CL[b]	LC_{90} [a]	CL[b]
P. xylustella	1.5	1.05~1.66	2.3	1.96~3.02
S. litura	1.8	1.37~2.01	3.1	2.58~4.88
P. rapae	2.0	1.561~2.16	3.8	2.580~4.651

[a]: Units LC_{50} and LC_{90} = µL L^{-1} air; [b]: 95% lower and upper confidal limits are shown for both LC_{50} and LC_{90}; [c]: Allylisothiocyanate (85%, EC) pure

Table 3: LC_{50} and LC_{90} values of AITC formulations against the three tested insect larvae

Formulation Name	*P. xylustella* LC_{50} [a,b]	LC_{90} [a,b]	*P. rapae* LC_{50} [a,b]	LC_{90} [a,b]	*S. litura* LC_{50} [a,b]	LC_{90} [a,b]
HJ(A)	33 (16~49)	204 (105~723)	–	–	22 (1.9~41)	181 (101~968)
HJ(B)	46 (9~89)	317 (191~861)	8 (0.54~15)	42 (24~97)	10 (0.2~21)	165 (70~455)
HJ(C)	556 (260~1033)	5672 (2088~9329)	8 (0.46~16)	68 (37~285)	260 (127~431)	2106 (898~3145)
HJ(D)	146 (67~204)	655 (410~1901)	–	–	385 (228~502)	1312 (868~3150)
HD(A)	455 (233~839)	4016 (1555~7070)	82 (32~140)	861 (336~1521)	46 (28~63)	179 (109~541)
HD(B)	214 (43~373)	2934 (1105~5342)	22 (5~ 36)	190 (95~796)	31 (14~47)	170 (98~538)

[a]: Units LC_{50} and LC_{90} = mg L^{-1}; [b]: 95% lower and upper confidal limits are shown for both LC_{50} and LC_{90} in parentheses; –: Not tested

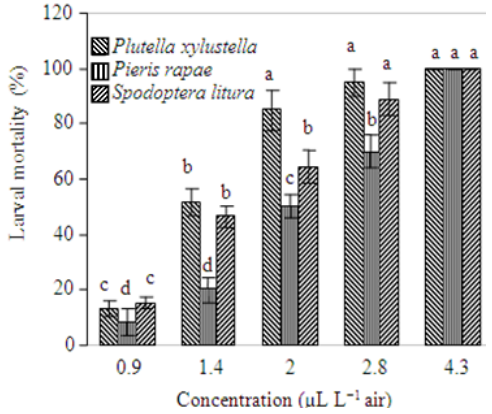

Fig. 1: Fumigant activity of AITC oil against diamond back moth, *Plutella xylostella*, small cabbage white, *Pieris rapae* and tobacco cut worm, *Spodoptera litura* on eppendorf micro-test tube in 1.5 L fumigation chamber 9 h exposure interval. Vertical bars indicate the standard error of means

mentality rates of mortality increased with increase in concentrations, the insect susceptibility to this oil was with a little difference. The *Plutella xylustella* larvae was most susceptible to the oil since the LC_{50} was 1.5 µL L^{-1} air followed by *Spodoptera litura* larvae ($LC_{50,}$ 1.8 µL L^{-1} air) and *Pieris rapae* larvae ($LC_{50,}$ 2.0 µL L^{-1} air) after 9h exposure in 1.5 L exposure chamber. The equivalent LC_{90} for the three insect species were 2.3, 3.1 and 3.8 µL L^{-1} air, respectively (Table 2). All insect larvae were killed after 9 h exposure to a dose of 4.3 µL L^{-1} air of the oil (Fig. 1).

Effect of spray application of AITC formulations on the three insect larvae: The effect of spray application of HJ (A), HJ (B), HJ(C), HJ (D), HD (A) and HD (B) against diamond back moth, *Plutella xylostella* larvae, tobacco cut worm, *Spodoptera litura* larvae and small cabbage white, *Pieris rapae* larvae 3 days after treatment, is shown in Fig. 2-4 and Table 3. The results showed that at the highest concentrations, almost all the formulations tested, able to induce 85% mortality or more to test insect larvae. The rates of mortality were dose dependant and the insect susceptibility was different.

For AITC pure, HJ (D), at high concentrations, 960 and 1920 mg L^{-1}, achieved 93 and 100% mortality to *P. xylustella* and *S. litura* larvae, respectively. The LC_{50} values were found to be 146 and 385 mg L^{-1} to both of the insect larvae. The corresponding LC_{90} were 655 and 1312 mg L^{-1} 3 days after treatment, respectively (Fig. 2, 3 and Table 3).

For AITC mixed with cypermethrin, HJ(A), HJ(B) and HJ(C), to *P. xylustella* larvae, at high concentrations, 160, 576 and 2048 mg L^{-1}, achieved 86, 96 and 88% mortality, respectively. HJ(A) was most potent since LC_{50} value was 33 mg L^{-1} followed by HJ(B) (LC_{50} = 46 mg L^{-1}) and HJ(C) LC_{50} = 556 mg L^{-1} while to *S. litura* larvae, at high concentrations, 320, 144 and 1024 mg L^{-1}, able to induce 93, 90 and 91% mortality, HJ(B)was most toxic (LC_{50}, 10 mg L^{-1}) followed by HJ(A) (LC50, 22 mg L^{-1}) and HJ(C) (LC50, 260 mg L^{-1}). Against *P. rapae* larvae, HJ(B) and HJ(C) had almost similar toxicity since 100 and 98% mortality was achieved at 144 and 128 mg L^{-1}, LC_{50} value was 8 mg L^{-1} for the both of HJ(B) and HJ(C) 3 days after treatment, respectively (Fig. 2-4 and Table 3).

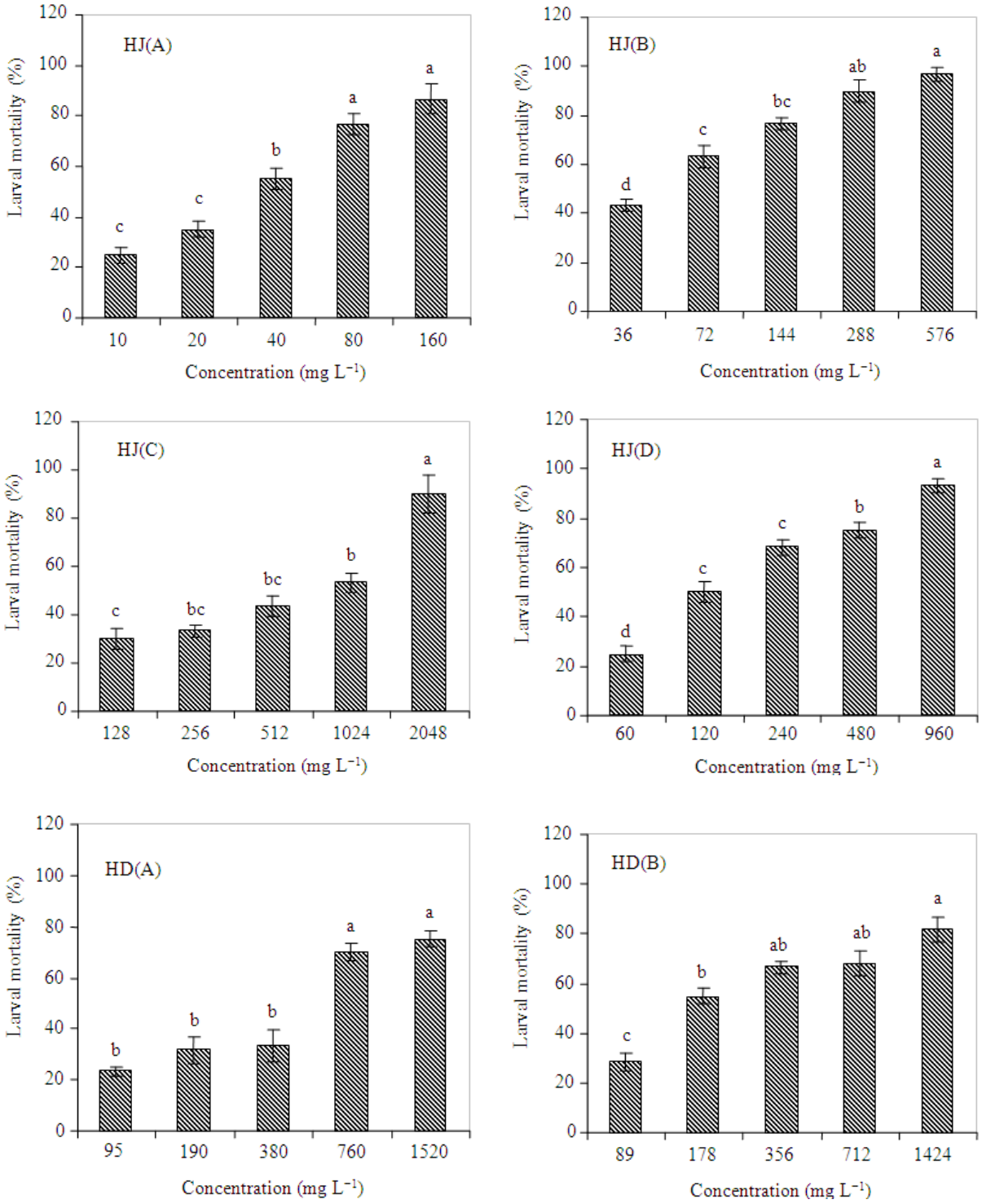

Fig. 2: Effect of spray application of HJ (A), HJ (B), HJ(C), HJ (D), HD (A) and HD (B) on the percentage mortality of *Plutella xylostella* larvae 3 days after treatment. Vertical bars indicate the standard error of means. Within each diagram, means followed by the same letter are not significantly different at ($p>0.05$) as determined by the Tukey HSD

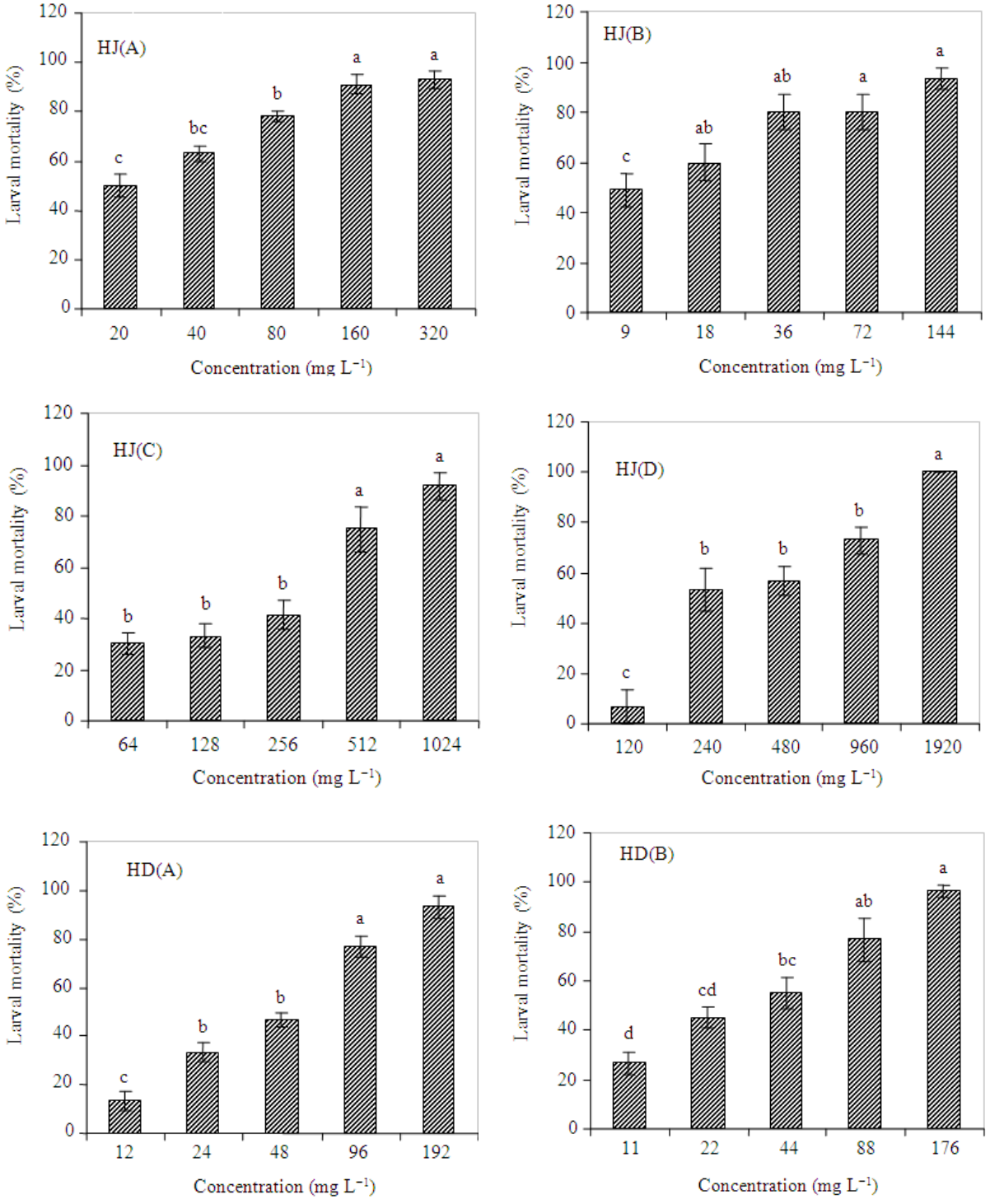

Fig. 3: Effect of spray application of HJ (A), HJ (B), HJ(C), HJ (D), HD (A) and HD (B) on the percentage mortality of tobacco cut worm, *Spodoptera litura* larvae 3 days after treatment. Vertical bars indicate the standard error of means. Within each diagram, means followed by the same letter are not significantly different at ($p>0.05$) as determined by the Tukey HSD

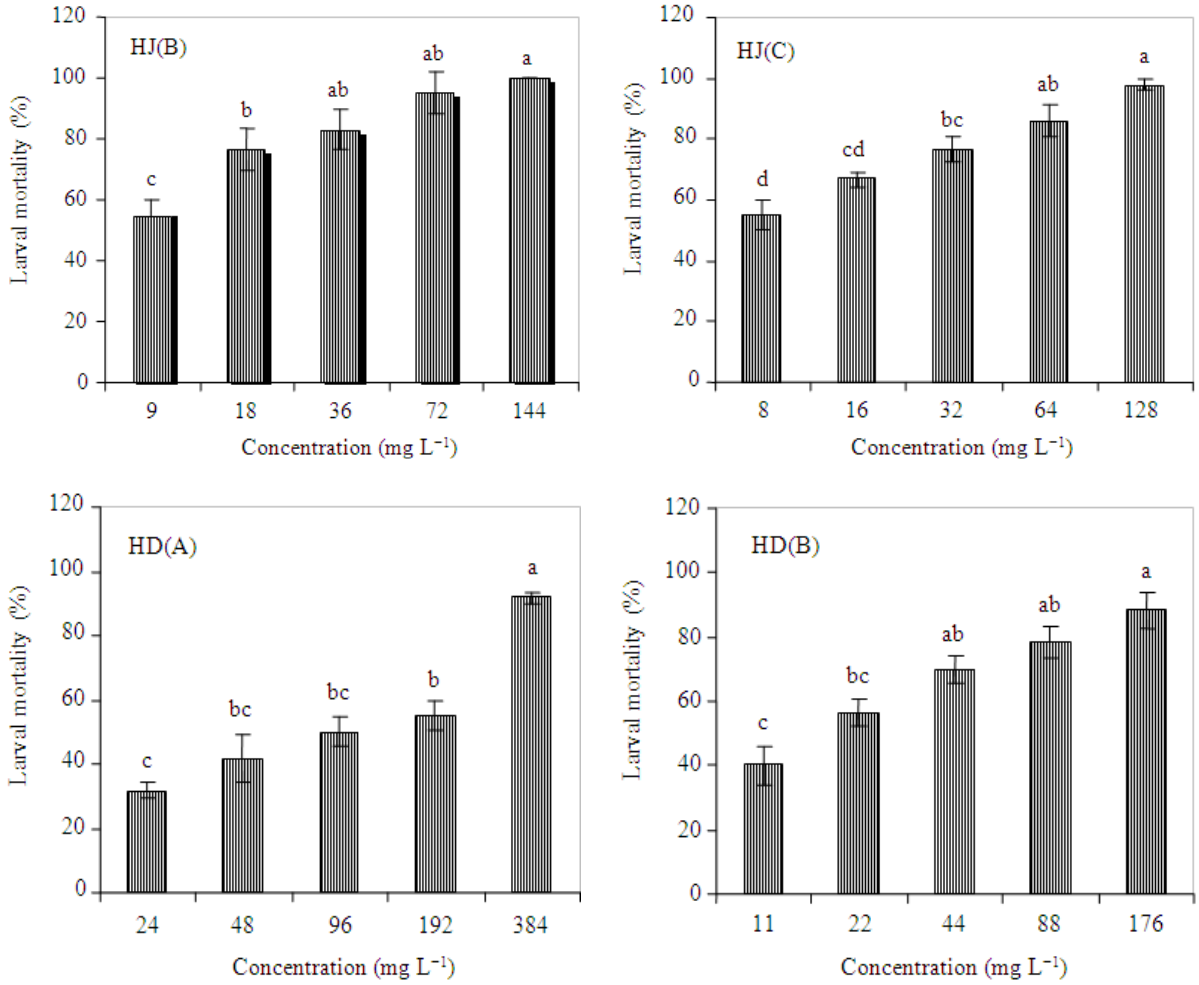

Fig. 4: Effect of spray application of HJ (B), HJ(C), HD (A) and HD (B) on the percentage mortality of *Pieris rapae* larvae 3 days after treatment. Vertical bars indicate the standard error of means. Within each diagram, means followed by the same letter are not significantly different at ($p>0.05$) as determined by the Tukey HSD

For AITC mixed with chlorpyrifos, HD (A) and HD (B), 76 and 81% mortality to *P. xylustella* were achieved at great concentrations, 1520 and 1424 mg L^{-1} of HD (A) and HD (B) respectively. HD (B) was found to be more toxic (LC50, 214 mg L^{-1}) than HD (A) (LC50, 455 mg L^{-1}). Similarly, To *S. litura* larvae, HD(B) was found better (LC50, 31 mg L^{-1}) than HD(A) (LC50, 46 mg L^{-1}), more than 90% mortality was induced at 192 and 176 mg L^{-1} for both of HD(A) and HD(B) respectively. To *P. rapae* larvae, HD(A) and HD(B) able to result in 91 and 88% mortality at high concentrations, 384 and 176 mg L^{-1}, HD(A) was found less toxic than HD(B) since LC50 values were 82 and 22 mg L^{-1} 3days after treatment, respectively (Fig. 2-4 and Table 3).

DISCUSSION

Our findings of the fumigant activity bioassays with pure Allylisothiocyanate (AITC) oil demonstrated that the oil showed a very strong insecticidal activity on three insect larvae in the vapor form. It is also evident that the efficacy of killing becomes stronger and faster when the applied concentration of the oil was increased at specified exposure interval. This is may be due to it's highly purity percentage and rapidly released its active ingredients in the atmospheric air and then easy penetrate the cuticle and/or enter through the spiracles to its target of sites which finally lead to faster kill. It is surprising to note that the three insect larvae tested have a little difference in their sensitivity to tested oil as indicated by LC_{50} and LC_{90}.The larval mortality was

recorded as 100% to the three insect larvae at 4.3 μL L^{-1} air after exposure of 9 h. This result indicates that AITC oil is comparable in activity with the commercial fumigant phosphine. In fact, against these insect larvae, the oil surpasses phosphine in efficacy, since a complete mortality was achieved at 4.3 μL L^{-1} air and 40 μg L^{-1} air after 9 and 24 h exposure to oil and phosphine respectively. The fumigant insecticidal activity which has been shown by the AITC oil in this study is in a good agreement with the reported data by Peterson *et al*. (1998). Apparently, this is also the case with fumigant activities of 66 plant essential oils which has been reported in *Plutella xylostella* larvae (Yi *et al*., 2007) and also AITC was lethally toxic to the generalist herbivore *Spodoptera* and *Plutella* (Li *et al*., 2000).

On the other side, our results indicated that AITC beside its fumigant action, is also act as a contact insecticide and stomach poisons because it was observed from the spray application bioassays that some of the treated insect larvae died soon or even after 24 h from the time of insecticide application while the others were died after 48 or 72 h, which proposed that the death may be due to the effect of feeding on the treated cabbage leaves. The results demonstrated that, the total of six AITC formulations which were tested against the three insect larvae were found insecticidal at different concentrations. The percentage mortalities which were induced by the highest concentrations of these formulations, ranging between 76 and 100%, showing the variation in their efficacy among these insect larvae. It is also evident from the results that, HJ (A) and HJ (B) among all formulations tested were almost the most potent to *P. xylostella* larvae as indicated by LC_{50} and LC_{90}. The order of toxicity was found to be HJ (A)>HJ (B)>HJ (D)>HD (B)>HD (A)> HJ(C). It is also clear from the results that, to both *Spodoptera litura* and *Pieris rapae* larvae, the most efficient formulations were HJ (B). The order of toxicity to both insects was found HJ (B)>HJ (A)>HD (B)>HD (A)>HJ(C)>HJ (D) and HJ (B) ≥HJ(C)>HD (B)>HD (A), respectively. These results suggest that this variation of the efficacy between AITC alone and in mixture, do not rely only on their toxicity, but may also some other factors influence their toxic action i.e., synergistic effect, insect species and cuticle, carrier and type of formulation. However, from these findings, AITC plus cypermethrin showed higher toxicity as compared to AITC plus chlorpyrifos and AITC pure in spray application bioassays. Thus, the mixture of AITC with cypermethrin and chlorpyrifos in controlling the three larval insect species were found better than the use of AITC alone.

Although the objectives set out for this research have been fulfilled, the scale up studies are required on the insect pests to understand the mechanism of action especially of allylisothiocyanate, because some observations on AITC insecticidal activity are still remain unknown. However, a publication by Chacon *et al*. (2006) showed that the AIT microencapsulated in acacia gum could be used in chopped refrigerated beef to reduce or eliminate large numbers of *Escherichia coli* O157:H7. Study on the insecticidal effect of this compound as a fumigant and monitoring the production of carbon dioxide (CO_2) by the insect were also conducted. It was demonstrated that the CO_2 expiration in insect exposed to the vapor of allylisothiocyanate and other compounds was significantly increased (Tsao *et al*., 2002).These indicated that AITC may also have inhibitory function on the insect respiratory system and mitochondrial complexes. Thus, more investigations for more details are needed.

CONCLUSION

In conclusion, the results of the present study indicate that the tested AITC formulations show promise results and may be useful as fumigants/contact insecticides for controlling the *Plutella xylostella* larvae, *Spodoptera litura* larvae and *Pieris rapae* larvae in the greenhouses or in the field or even in the field under the plastic sheets and may be a good alternatives to old fumigants and synthetic insecticides. Particularly this naturally occurring substance, allylisothiocyanate is known as safe, since these products occur in human food-stuffs (Fenwick *et al*., 1983) and no residual effects left on treated commodity (Wu *et al*., 2009). Thus is encouraging in the context of biorational insecticides. To the best employ the tested AITC formulations as potential insect control agents, further research and more details are needed on the morphological changes left on the treated host plant, effect on the natural enemies of the tested insect larvae and the mechanism of action to determine how this compound is acting and where its binding sites.

ACKNOWLEDGEMENT

The researcher is thankful to Dr. Zhang Guo An, Pro. Shi, Dr.Wang and PhD student, Wu Huo for many helpful discussions during the experiment.

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