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# Selection of chemical pesticide to minimize toxic effect to ladybird beetle [*Coccinella septempunctata* Linn (Coleoptera: Coccinellidae)]while managing black bean aphid [*Aphis fabae* Scop. (Hemiptera: Aphididae)]

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**Abstract**

Faba bean (*Vica faba* L.) is important crop of Nepal which is grown in all suitable climatic zone of the country. Different insect pests attack faba bean but black bean aphid, *Aphis fabae* Scop. (Hemiptera: Aphididae) is of more concern. Most of the farmers shifted to another crop due to *A. fabae* problem. Different insecticides have been sprayed to manage this aphid but most of the farmer were unable to control it. However, very limited research were conducted for its ecofriendly management. Thus, we evaluate different insecticides such as nitenpyram, flonicamid, imidacloprid, dimethoate, azadirachtin, and neem oil on laboratory. Scintillating glass vial test and filter paper test were employed. Higher number of aphid mortality were found on dimethoate with $LT_{50}$ value of 15.93 hour followed by nitenpyram, and imidacloprid with 18.61 and 32.87 hour, respectively on scintillating glass vial test. On filter paper test, $LT_{50}$ of dimethoate was 27.34 hour followed by imidacloprid and nitenpyram with 49.51 and 53.44, respectively. Similarly, higher lady bird beetle *Coccinella septempunctata* Linn (Coleoptera: Coccinellidae) mortality were also caused by dimethoate with $LT_{50}$ value of 63.38 hour followed by imidacloprid and nitenpyram with 153.21 188.42 hour. Our result suggested that nitenpyram or imidacloprid could be used for ecofriendly management of *A. fabae* with low mortality of its predator *C. septempunctata*. However precautionary measure has to be taken before applying pesticides and waiting period has to be maintained for harvesting.

**Keywords:** Black bean aphid, insecticides, seven spotted ladybird beetle

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## INTRODUCTION

Faba bean, *Vica faba* L., (also known as fava or broad bean) is one of the primitive legume crops and fifth most important pulse crop of world production (Merga et al 2019). It is used for human food as well as feed source for livestock (El-Wakeil and Talaat 2009). However,

there are many economically important faba bean insect pests which harm the production every year. Some of the harmful insects are black bean aphid, *Aphis fabae* Scop. (Hemiptera: Aphididae), *A. craccivora* Koch, *A. gossypii* Glover), thrips, leaf mining fly, fruit borer, moth larvae, leaf hoppers, seedling beetle and weevils (Nuessly et al 2004) etc. Among them aphid (*A. fabae, A. craccivora, A. gossypii*) were found to be serious damaging pest in faba bean plant of Nepal (Basukala and Subedi 2014). *Aphis fabae* is one of the most important pests attacking many cultivated crops, including faba beans, tomatoes, potatoes, and tobacco, as well as numerous wild and ornamental plant species (Volkl and Stechmann 1998, Akca et al 2015). *Aphis fabae* generally infest on top of the plant and underside of young leaves in the center of the crown which causes leaves curl and become severely distorted. It also transmits plant viruses (Patriquin et al 1988, Raymond et al 2000, Tosh et al 2002). Hansen et al (2008) found that yield losses can exceed more than 50% due to infestation from the *A. fabae. Aphis fabae* has been found associated with many natural enemies such as lady beetles, green lacewing larvae, and minute pirate bugs (Hance 1987, Han 1997). Among the natural enemies, the parasitoid braconid wasp (*Aphidius* ssp.), predator seven spotted ladybird beetle *Coccinella septempunctata* Linn., pirate bugs *Orius* spp, and hover flies are known to be the most abundant natural enemies of *A. fabae* found in Nepal.

In Nepal, the conventional management of *A. fabae* include several insecticides. However, using non-selective insecticide may also harm beneficial arthropods (Echegaray and Cloyd 2012, Villanueva and Walgenbach 2005). Selective insecticide are known to have greater specificity towards pests and are less harmful and compatible with beneficial organisms than conventional pesticides (Lefebvre et al 2012, Martinou et al 2014, Stara et al 2011). Flonicamid is insecticide that have a novel mode of action which reduce the risk to beneficial insects. The use of selective insecticides that are less toxic to natural enemies is a pre-requisite for the environmentally friendly management of *A. fabae*. Natural enemies having excellent prey searching capabilities, swift prey handling, and specific in nature make them an effective component of integrated pest management (IPM) programs (Bozsik 2006, Stark et al 2007, Volkl et al 2007); lady bird beetle is one of the natural enemies with such characteristics.

For the management *A. fabae* in compatible manner with biological control agents, some new neonicotinoid insecticides were evaluated with organophosphate and botanical insecticides. Use of neonicotinoids insecticides are common in crop production and suppress target herbivores effectively (Regan 2017). Combining the use of biological controls with selective pesticides may increase the adoption of integrated pest management (IPM) strategies, and ensure that such strategies are more generally available and more effective (Fishel 2013). Information on the acute toxicity of insecticides on natural enemies is important for the successful implementation of conservational biological control and the development of IPM strategies (Dangi and Lim 2017). Therefore, in present study we aimed to develop ecofriendly management strategy by determining the toxic effect of different insecticide on *A. fabae* and its predator, *C. septempunctata*.

## MATERIALS AND METHODS

### Laboratory conditions

Lab experiment was conducted at National Horticulture Research Centre (NHRC), Khumaltar, Lalitpur ($27.6485^0$ N, $85.3253^0$ E and 1332 masl), Nepal Agriculture Research Council, Nepal in the year 2022. Maximum and minimum room temperature were $25.32\pm0.43$ and $22.03\pm0.23$ $^0$C whereas maximum and minimum relative humidity (RH) were

61.21±2.33%, and 43.05±2.93 respectively during the experiment period. For the experiment, nymph of *Aphis fabae* were collected from *Vica faba* and tested with different concentrations of various insecticides (Table 1) in two different method of bioassay i.e. glass scintillation vials method and filter paper method.

**Glass scintillation vials method**

In this method, experiments were conducted without providing food to aphids on 15 ml glass scintillation vials (Figure 1 a). Different insecticides with 50 microliter each (Table 1) were placed in each vials. Distilled water were use as control. The solution were then allowed to be coated on the inner surface of the vial including lid, and let it dry on room temperature. Five *Aphis fabae* were placed in each glass vial, then the lid was closed. The mortality data was recorded in every 12 hour interval until 96 hour of the duration. Ten repetition were made for each insecticides. Aphid mortality was confirmed when no movement observed after prodded with brush.

**Filter paper method**

Whatman Filter paper (8.5 mm Ǿ, 125mm thickness) were placed on plastic Petridis (Omark, 99 mm Ǿ) (Figure 1 b). After four hours of starvation, 5 *Aphis fabae* were placed on the filter paper treated with 1 microliter of each insecticide (Table 1). Insecticides were allowed to dry before placement of *Aphis fabae*. Distilled water were used as control. Mortality were recorded at 12 hour interval up to 96 hour. Ten replication were conducted.

**Feeding method for *Coccinella septempunctata***

Unknown age of adult *Coccinella septempunctata* were collected from NHRC field and evaluated in feeding method (Figure 1 c). Collected *C. septempunctata* was placed individually on 50 ml falcon tube (Polulab centrifuge tube) for 24 hour for starvation. Cotton (Absorbent cotton wool I.p) were cut on 1.5x0.5 $cm^2$ and were contaminated with 1 microliter of each insecticides (Table 1) after placing them on petri dishes. Single *C. septempunctata* was transferred to each petri dish and mortality were recorded at 24 hour interval up to 240 hour of insect exposure. Each treatment were replicated 50 times.

**Table 1.** Insecticide trade name, active ingredient, and its dose used in experiment.

| S.N. | Trade name | Active ingredient | Dose (ml/L) |
|---|---|---|---|
| 1 | King Guard | Nitenpyram 10% SL | 0.3ml/L |
| 2 | Ulala | Flonicamid 50% WG | 0.3gm/L |
| 3 | Allmida | Imidacloprid 17.8% SL | 0.3ml/L |
| 4 | Tagore | Dimethoate 30% EC | 0.3ml/L |
| 5 | Nimbecidine | Azadirachtin 300 PPM | 5ml/L |
| 6 | Neem oil | Neem oil *Azadirachta indica* 100% | 5ml/L |
| 7 | Control | Distilled water | |

SL: Soluble (liquid) concentrate, WG: water-dispersible granule, EC: Emulsifiable concentrate . PPM: Parts per million

**Figure 1.** Experiments method. a Glass scintillation vial bioassay method of testing aphid, b Filter paper bioassay method of testing aphid, and c Feeding method for *Coccinella septempunctata*

### Statistical analysis

Corrected mortality data within each treatment were subjected to test probit regression analysis to calculate the lethal median time ($LT_{50}$) with SPSS 16. Significant differences among treatments were determined based on the 95% CI (Confidence Intervals). Each hour after exposure dead and live insect were analyzed using proportion $X^2$-test (Zar, 2010). Comparison between two method of bioassays were analyzed using *t-test* with SPSS 16

## RESULTS

### Median lethal time of *Aphis fabae* and *Coccinella septempunctata*

### Scintillating vial bioassay on *Aphis fabae*

The time needed for different treatments to cause 50% mortality ($LT_{50}$) was found different. $LT_{50}$ values for dimethoate, nitenpyram and imidacloprid were 15.9 hour ($CL_{95}$% 5.1-32.1)], 18.6 hour ($CL_{95}$% 8.2-26.6) and 32.9 ($CL_{95}$% 24.5-40.1) which were less than azadirachtin 75.0 hour ($CL_{95}$% 68.3-83.6), Neem oil 79.4 hour ($CL_{95}$% 63.8-113.2) and flonicamid 91.0 hour ($CL_{95}$% 69.9-169.7) respectively (Table 2). Based on $LT_{50}$ values, dimethoate, nitenpyram and imidacloprid was found effective than the other tested insecticide (Table 2).

**Table 2.** Lethal median time ($LT_{50}$) of *Aphis fabae* exposed in insecticides at glass scintillation vials method (n=10)

| Treatment | $LT_{50}$ | 95% CI (Lower-Upper) | Df | $\chi 2$ | Slope | Intercept | P Value |
|---|---|---|---|---|---|---|---|
| Nitenpyram | $18.61^{a}$ | 8.16-26.56 | 5 | 19.63 | 0.06 | -1.20 | 0.001 |
| Flonicamid | $91.03^{b}$ | 69.85-169.72 | 7 | 34.06 | 0.02 | -1.52 | 0.001 |
| Imidacloprid | $32.87^{a}$ | 24.45-40.14 | 7 | 16.93 | 0.04 | -1.24 | 0.018 |
| Dimethoate | $15.93^{a}$ | 5.05-32.07 | 3 | 17.84 | 0.13 | -2.07 | 0.0001 |
| Azadirachtin | $74.99^{b}$ | 68.29-83.61 | 7 | 8.51 | 0.02 | -1.59 | 0.290 |
| Neem oil | $79.35^{b}$ | 63.82-113.18 | 7 | 30.87 | 0.02 | -1.76 | 0.0001 |

$LT_{50}$ value followed by different lower case superscript letters are significantly different among treatment.

### Filter paper bioassay on *Aphis fabae*

Our result showed that the time for 50% mortality ($LT_{50}$) of different treatments are different. $LT_{50}$ values of dimethoate, imidacloprid and nitenpyram was 27.3 hour ($CL_{95}$% 12.0-51.8), 49.5 hour ($CL_{95}$% 44.1-55.0) and 53.4 hour ($CL_{95}$% 50.2-56.8) respectively (Table 3). However, nitenpyram, imidacloprid and dimethoate were more effective than azadirachtin, neem oil, and flonicamid with 97.0 hour ($CL_{95}$% 88.5-114.7), 97.2.0 hour ($CL_{95}$% 88.5-114.7) and 106 hour ($CL_{95}$% 85.4-180.0) of $LT_{50}$ (Table 3). Based on $LT_{50}$ values, nitenpyram imidacloprid and dimethoate were found to be more effective than azadirachtin neem oil and flonicamid

**Table 3.** Lethal median time ($LT_{50}$) of *Aphis fabae* exposed in insecticides at filter paper method (n=10)

| Treatment | $LT_{50}$ | 95% CI (Lower-Upper) | Df | $\chi 2$ | Slope | Intercept | P Value |
|---|---|---|---|---|---|---|---|
| Nitenpyram | 53.44[a] | 50.15-56.78 | 7 | 7.23 | 0.05 | -2.58 | 0.405 |
| Flonicamid | 106.65[b] | 85.43-180.01 | 6 | 19.13 | 0.02 | -2.46 | 0.040 |
| Imidacloprid | 49.51[a] | 44.05-55.01 | 7 | 12.11 | 0.045 | -2.28 | 0.097 |
| Dimethoate | 27.34[a] | 12.04-51.81 | 3 | 17.28 | 0.15 | -4.03 | 0.001 |
| Azadirachtin | 97.03[b] | 88.53-114.71 | 7 | 12.61 | 0.04 | .043 | 0.082 |
| Neem oil | 97.22[b] | 90.51-107.39 | 7 | 9.41 | 0.03 | -3.35 | 0.225 |

$LT_{50}$ value followed by different lower case superscript letters are significantly different among treatment.

## Feeding bioassay of *Coccinella septempunctata*

Our result showed that the time for 50% mortality of *C. septempunctata* ($LT_{50}$) on different treatments demonstrated varyingly. $LT_{50}$ values on dimethoate, 36.4 hour (CL $_{95}$% 31.6-41.0) was found significantly different than all the treatments (Table 4). However, nitenpyram, and imidacloprid $LT_{50}$ value of 153.2 h (CL $_{95}$% 132.5-178.2) and 188.4 hour (CL $_{95}$% 171.5-210.5) were not significantly different. But, nitenpyram were found different with neem oil 195.1 h (CL $_{95}$% 181.2-212.6), azadirachtin 295.9 (CL $_{95}$% 255.6-368.4), and flonicamid 287.7 h (CL $_{95}$% 253.1-347) (Table 4). Based on $LT_{50}$ values dimethoate were found to be more harmful followed by nitenpyram, imidacloprid, azadirachtin, neem oil and flonicamid (Table 4).

**Table 4.** Lethal median time ($LT_{50}$) of *Coccinella septempunctata* exposed in insecticides at petri dish (n=50)

| Treatment | $LT_{50}$ | 95% CI (Lower-Upper) | Df | $\chi 2$ | Slope | Intercept | P Value |
|---|---|---|---|---|---|---|---|
| Nitenpyram | 153.21b | 132.48-178.16 | 9 | 16.12 | 0.01 | -1.298 | 0.013 |
| Flonicamid | 287.73d | 253.08-346.97 | 9 | 8.22 | 0.01 | -2.040 | 0.022 |
| Imidacloprid | 188.42bc | 171.47-210.48 | 9 | 10.51 | 0.08 | -1.434 | 0.138 |
| Dimethoate | 36.38a | 31.60-41.03 | 3 | 4.441 | 0.05 | -1.77 | 0.209 |
| Azadirachtin | 295.85d | 255.63-368.43 | 9 | 13.14 | 0.01 | -1.80 | 0.005 |
| Neem oil | 195.13c | 181.20-212.59 | 9 | 9.62 | 0.01 | -1.94 | 0.231 |

$LT_{50}$ value followed by different lower case superscript letters are significantly different among treatment.

## Proportional Chi square on mortality of pest and predator

### Vial bioassay on *Aphis fabae*

Mortality was higher in nitenpyram dimethoate and, imidacloprid ($\chi^2$ = 15.37, df 5, P = 0.009) on 12 hour after exposure (Figure 2). Mortality were higher in nitenpyram and dimethoate followed by imidacloprid after 24 hour of expose ($\chi^2$ = 170, df = 5, P < 0.001) (Figure 2). Similarly, mortality was higher in dimethoate and nitenpyram followed by imidacloprid and azadirachtin ($\chi^2$ = 159, df = 5, P < 0.001) after 36 hour of exposure (Figure 2). Mortality was higher in dimethoate and nitenpyram followed by imidacloprid and azadirachtin after 48 hour of exposure ($\chi 2$ = 161, df = 5, P < 0.001). Tested all insect were dead on dimethoate (Figure 2). Mortality was also higher in dimethoate and nitenpyram followed by imidacloprid and azadirachtin after 60 hour of exposure ($\chi 2$ = 146, df = 5, P < 0.001) (Figure 2). Similaryly, Mortality was higher in dimethoate and nitenpyram followed by imidacloprid and azadirachtin after 72 hour of exposure ($\chi^2$ = 113, df = 5, P < 0.001) (Figure 2).

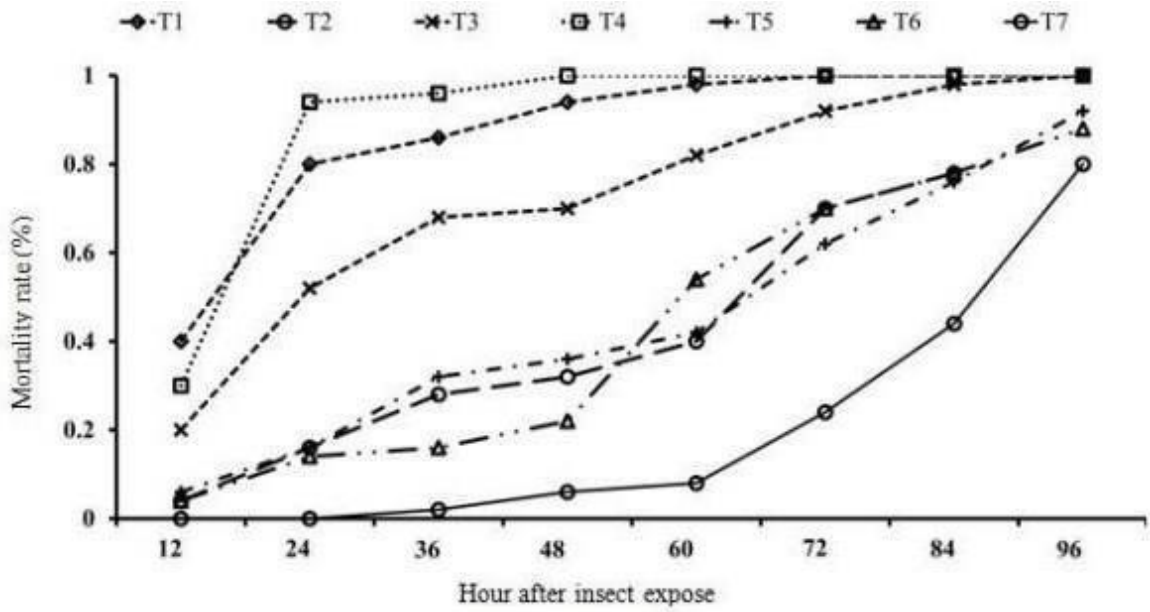


**Figure 2.** Mortality rate of *Aphis fabae* after exposed to different treatments at 12 hours interval unto 96 hours of exposure on glass scintillation vial bioassay n = 50. T1= Nitenpyram, T2 = Flonicamid, T3= Imidacloprid, T4 = Dimethoate, T5 = Azadirachtin, T6 = Neem oil, T7 = control

### Filter paper bioassay of *Aphis fabae*

Mortality was higher in dimethoate and nitenpyram followed by imidacloprid and azadirachtin ($\chi 2 = 26$, df = 5, $P < 0.001$) after 12 hour of exposure (Figure 3). Mortality was higher in dimethoate, imidacloprid followed by nitenpyram and azadirachtin ($\chi 2 = 194$, df = 5, $P < 0.001$) after 24 hour of exposure (Figure 3). Mortality was higher in dimethoate followed by nitenpyram and imidacloprid ($\chi 2 = 180$, df = 5, $P < 0.001$) after 36 hour of exposure (Figure 3). Mortality was higher in dimethoate followed by nitenpyram and imidacloprid ($\chi 2 = 118$, df = 5, $P < 0.001$). All aphids were dead on dimethoate after 48 hour of exposure (Figure 3). Mortality was higher in dimethoate followed by nitenpyram and imidacloprid ($\chi 2 = 140$, df = 5, $P < 0.001$) after 60 hour of exposure (Figure 3). Mortality was higher in dimethoate and nitenpyram followed by imidacloprid and azadirachtin ($\chi 2 = 77$, df = 5, $P < 0.001$) after 72 hour of exposure (Figure 3).

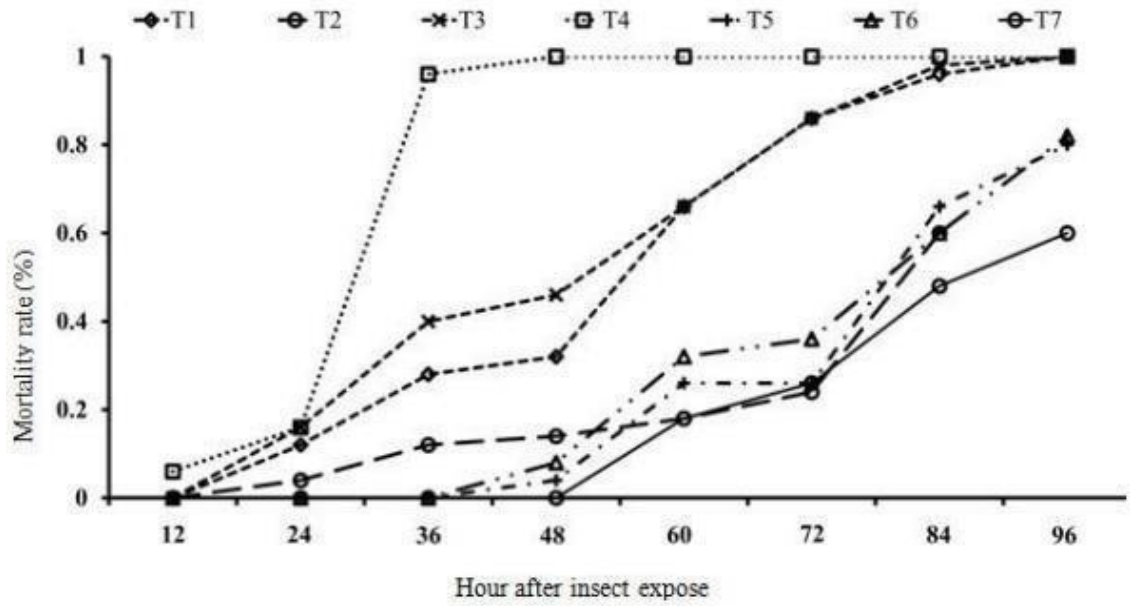


**Figure 3.** Mortality rate of *Aphis fabae* after exposed to different treatments from 12 hours interval to 96 hours of exposure on filter paper bioassay n = 50. T1= Nitenpyram, T2 = Flonicamid, T3= Imidacloprid, T4 = Dimethoate, T5 = Azadirachtin, T6 = Neem oil, T7 = control

### Comparison between bioassay methods: Vial bioassay and Filter paper bioassay on *Aphis fabae*

While comparing the effectiveness to two different methods of bioassay, result showed that mortality was higher in nitenpyram (P = 0.007), azadirachtin (P = 0.003), imidacloprid (P = 0.010) and neem oil (P = 0.042) in glass scintillating vial over filter paper bioassay, however there was not any significant difference between two methods for flonicamid (P = 0.060) and dimethoate (P = 0.249) pesticide effectiveness (Table 5).

**Table 5.** Comparison between two method of bioassay i.e. scintillation vials and filter paper method for *Aphis fabae*

| Treatment | Correlation | Std. Error Mean | T value | Df | P value |
|---|---|---|---|---|---|
| Nitenpyram | 0.774 | 4.473 | -3.641 | 8 | 0.007 |
| Flonicamid | 0.207 | 4.068 | -2.188 | 8 | 0.060 |
| Imidacloprid | 0.940 | 2.226 | -3.389 | 8 | 0.010 |
| Dimethoate | 0.755 | 7.566 | -1.348 | 4 | 0.249 |
| Azadirachtin | 0.725 | 2.524 | -4.191 | 8 | 0.003 |
| Neem oil | 0.588 | 3.303 | -2.422 | 8 | 0.042 |

### Feeding bioassay of *Coccinella septempunctata*

Mortality rate was higher on dimethoate at 24 hour ($\chi^2$ = 73, df = 5, P < 0.001), 48 hour ($\chi^2$ = 120, df = 5, P < 0.001) and 72 hour ($\chi^2$ = 135, df = 5, P < 0.001) of exposure. However, mortality rate was found higher on nitenpyram ($\chi^2$ = 120, df = 5, P < 0.001) than control on 48 hour of exposure (Figure 3 and Figure 4). Mortality rate was also higher after 72 hour of exposure on nitenpyram ($\chi^2$ = 72, df = 5, P < 0.001) than flonicamid and control (Figure 4). Similar result was found where dimethoate mortality rate was higher ($\chi^2$ = 116, df = 5, P < 0.001) than other tested insecticide but nitenpyram mortality rate was also higher than control after 96 hour of *C. septempunctata* exposure (Figure 4). All tested ladybird beetle were dead on dimethoate after 96 h of exposure (Figure 4). Nitenpyram mortality was higher than the control ($\chi^2$ = 110, df = 5, P < 0.001) even after 120 h of exposure (Figure 4). However,

mortality rate was higher on nitenpyram and imidacloprid ($\chi^2$ = 96, df 5, P < 0.001) than the control after 144 hour, ($\chi^2$ = 85, df = 5, P < 0.001) 168 hour, ($\chi^2$ = 70, df = 5, P < 0.001) 192 hour, ($\chi^2$ = 63, df = 5, P < 0.001) 216 hour and ($\chi^2$ = 55, df = 5, P < 0.001) 240 hour of exposure (Figure 4). Similarly, mortality rate of nitenpyram was higher than flonicamid ($\chi^2$ = 97, df = 5, P < 0.001) after 144 hour, ($\chi^2$ = 85, df = 5, P < 0.001) 168 hour, ($\chi^2$ = 70, df = 5, P < 0.001) 192 hour, ($\chi^2$ = 63, df = 5, P < 0.001) 216 hour and ($\chi^2$ = 55, df = 5, P < 0.001) 240 hour of exposure (Figure 4). Flonicamid was found safe to ladybird beetle than tested other insecticide.

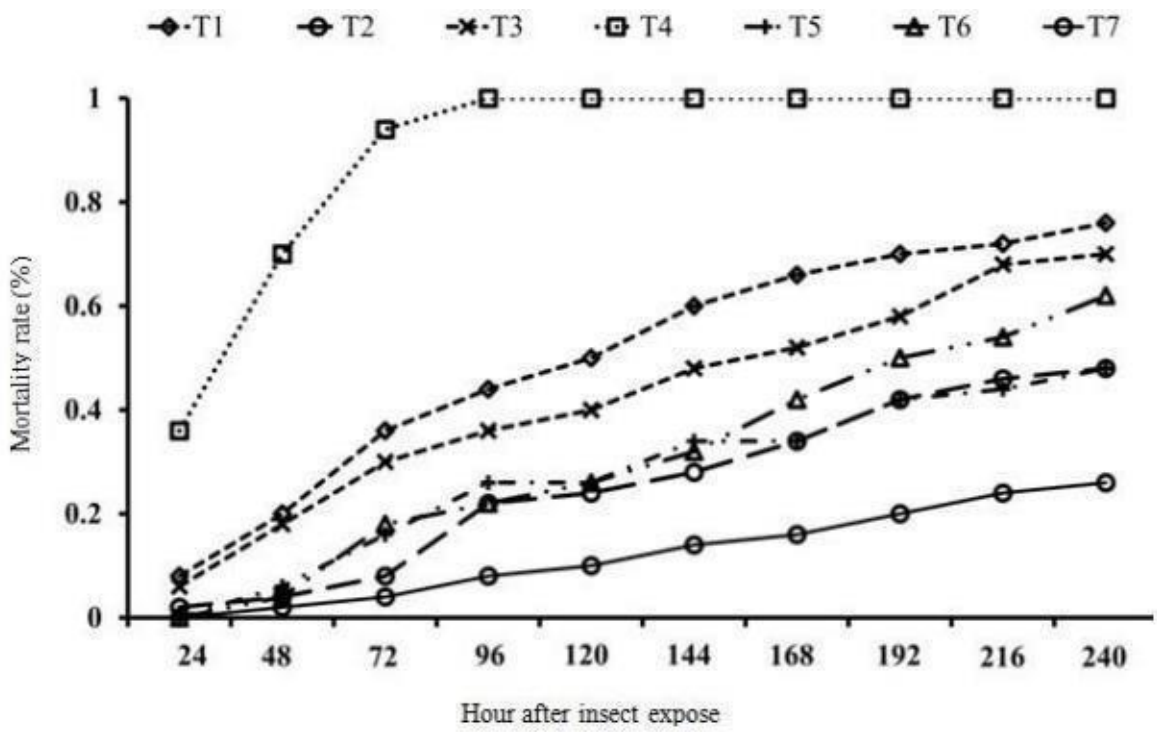


**Figure 4.** Mortality rate of *Coccinella septempunctata* after exposed to different treatments from 12 to 240 hours of exposure on petri dish n = 50. T1= Nitenpyram, T2 = Flonicamid, T3= Imidacloprid, T4 = Dimethoate, T5 = Azadirachtin, T6 = Neem oil, T7 = control

## DISCUSSION

Glass scintillating vial bioassay method was found more reliable than the other tested methods when different insecticides were evaluated against *A. Fabae* for its toxicity (Table 5) Supporting the present findings, Snodgrass et al (2005) also showed similar results against brown stink bugs. Our results showed that dimethoate was toxic to aphid by direct contact and feeding methods (Table 2, Table 3). Holland et al (2000) showed that dimethoate is more hazardous to its natural enemies including seven spotted ladybird beetle *Coccinella septempunctata* L (Table 4, Figure 4). Direct contact of lady bird beetle to dimethoate adversely effect its growth (Sattar et al 2018) while indirect effect was described by Singh et al (2004) which showed that aphid treated with dimethoate were less preyed upon by *C. septempunctata*. The use of insecticides to control the outbreaks of *A. fabae* also affects large proportion of its natural enemies including ladybird beetle locomotion and respiratory function (Xiao et al 2017). Despite to known hazard to beneficial species, broad-spectrum organophosphate dimethoate is still commonly used in the Nepal due to easy accessibility and low cost (Aryal et al 2021, Kovalkovicova 2015).

It has been suggested that, for ecofriendly pest management of faba bean, alternative of dimethoate should be applied (Mousa et al 2013). It has also been suggested that now selective insecticide such as neonicotinoids group could be the alternate for sucking insect pest management of faba bean (Nauen and Denholm 2005). The neonicotinoids, the newest major class of insecticides, have outstanding potency and systemic action for crop protection against piercing-sucking pests (Tomizawa and John 2005). Neonicotinoids are among the most effective insecticides for the control of sucking insect pests such as aphids, whiteflies, leaf and planthoppers, thrips, some micro lepidoptera and a number of coleopteran pests (Elbert et al 2008) but are less toxic to natural enemies (Akhtar et al 2021). Nitenpyram and imidacloprid was found to be effective against *A. fabae* (Table 2, Table 3). Similar result was reported with nitenpyram effectiveness against sucking insect jassid on brinjal (Saleem et al 2018) and brown plant hopper on rice (Zhang et al 2010) and imidacloprid against *Myzus persicae* (Sulzer) Aphididae Hemiptera) by Akbar et al (2014). The variable toxic effects against *A. fabae,* might be due to different characteristics of neonicotinoids which influence the movement in plant tissues such as solubility of the water that greatly affects the toxicity in sucking and piercing type insects like *A. fabae* (Cloyd and James 2011). These findings in the present study (Table 2, Table 3, Figure 2, Figure 3) have many similarities as those demonstrated by the Wei and Liu (1999), who reported that hydrolysis of neonicotinoids insecticides increases with the increased surrounding temperature, which affects the toxicity level of insecticides. Imidacloprid was found safe for *C. septempunctata* in laboratory (Table 4, Figure 4) which was supported by result of Bozsik (2006). Yu et al (2014) also reported that imidacloprid did not affect the pupation rate or adult emergence of *C. septempunctata* at sublethal concentrations. Imidacloprid or nitenpyram can be applicable for managing piercing-sucking pests allowing lady beetles to survive and develop (Figure 2, Figure 3 and Figure 4). Nitenpyram was safe for *C. septempunctata* egg, larva as well as adult throughout the residual contact when applied at recommended dose (Jiang et al 2018).

Both neonicotinoid (imidacloprid and nitenpyram) were found less toxic to natural enemies *C. septempunctata* (Table 4 Figure 4) and high toxic to *A fabae* (Table 3, Table 3, Figure 3, Figure 4). Lin (2020) found these insecticide less toxic to natural enemies *Orius sauteri* but highly toxic to pest *Frankliniella occidentalis*. Imidacloprid is non-toxic to a predatory mite, *Agistemus fleschneri* (Summers) used in apple orchard IPM program, so these natural enemies may be better suited for programs utilizing neonicotinoids (Bostanian and Larocque 2001). Combinations of neonicotinoids and the entomopathogenic nematodes *Heterorhabditis bacteriophora* Poinar and *H. newzealandica* Poinar acted synergistically to cause increased mortality than either alone in white grubs (Coleoptera: Scarabaeidae) (Koppenhofer and Fuzy 2008).

Because of the relatively low risk to non-target organisms and environment, high target specificity and their versatility in application methods, these important classes of new insecticides will certainly play a greater role in the present context of environmental safety, their consequent uses in integrated pest management and insect resistance management program (Kodandaram and Halder 2010). Neonicotinoids are increasingly used for systemic control of plant-sucking insects, replacing the organophosphorus compounds and methyl carbamate, which have decreased effectiveness because of resistance or increased restrictions due to toxicological considerations (Tomizawa and John 2003). Nitenpyram and imidacloprid can be used for *A. fabae* which is comparatively less toxic to lady bird beetle than dimethoate. However, precautionary measure has to be taken before applying pesticides and waiting period has to be maintained for harvesting.

## CONCLUSION

The mortality rate of *Aphis fabae* and *Coccinella septempunctata* were found different among tested insecticides. Mortality rate of *A. fabae* was found higher on dimethoate followed by imidacloprid and nitenpyram on both tested methods.However, mortality rate was also higher on dimethoate to *Coccinella septempunctata*. Our result suggested that imidacloprid or nitenpyram could be used to manage *A fabae* to conserve its predator *Coccinella septempunctata.* Furthermore, glass scintillating vial bioassay method could be more reliable to find out toxicity of the tested insecticide evaluated against *A. Fabae*.


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### Authors)' Contributions

Bikash Bhusal conduct the experiments described in the text. Bikash Bhusal and Sunil Aryal were involve in data analysis, interpretation, and drafting of the manuscript. Ishwori Prasad Gautam was was involved in critical revision of the manuscript. All authors listed have made a substantial, direct and intellectual contribution to the study, and approved it for publication.

### Conflicts of Interest

The authors have no relevant financial or non-financial interests to disclose.

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