

## Effectiveness of neonicotinoids and organophosphate in the control of aphid and enhancement of pod formation in mustard crop in India

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### ABSTRACT

Mustard, *Brassica juncea*, is an economically important crop in India, the country annually produces 6-8 tons of mustard seeds and ranks third in the world production. Its production is, however infested by mustard aphid, *Lipachis erysimi*. The effectiveness of neonicotinoids and organophosphate insecticides was evaluated at mustard fields planted at Indian Agricultural Research Institute (IARI), New Delhi, India. The aim of this study was therefore to evaluate the efficacy of the two neonicotinoids (imidacloprid and thiomethoxam) and that of an organophosphate (malathion) in the management of mustard aphid and the enhancement of pod formation at different experimental sites. The insecticides were applied at the floral parts of randomly selected mustard crop plants. There was no significant difference in the mean numbers of mustard twig infected by aphid prior to spraying of both imidacloprid, thiamethoxam malathion and control at the mustard inflorescences in the two fields. However, following the application of the insecticides, the mean numbers of infected inflorescences generally continued to increase in the untreated plot throughout the experimental period but decreased significantly in all the treatments ( $F_{(3,599)} = 37.42$ ;  $P < 0.0001$ ). The mean numbers of pods developed per twig was not significantly different prior to the spraying but increases significantly in all the treatments after the spraying ( $F_{(3,599)} = 28.31$ ;  $P < 0.0001$ ).

The application of neonicotinoids and organophosphate can effectively control aphid and enhance pod formation in the mustard crop. The organophosphate (malathion) is more recommended insecticide to control mustard aphid as neonicotinoids are suspected to affect bee pollinators in agro-ecosystems in India.

**Key words:** Neonicotinoids, Imidacloprid, Thiamethoxam, Malathion, Mustard

### INTRODUCTION

Mustard, *Brassica juncea*, is an economically important crop in India, which is extensively grown traditionally as a pure crop as well as intercrop in marginal and sub-marginal soils in the eastern, northern and north western states<sup>1</sup>. The country annually produces 6-8 tons of

mustard seeds and ranks third in the world production<sup>1</sup>. Its production is, however constrained by several factors, mainly infestation by mustard aphid, *Lipachis erysimi* (Kalt) (Homoptera: Aphididae).

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The mustard aphid is the most serious and destructive pest of mustard, it was considered as a major limiting factor for successful cultivation of mustard including seed production in India<sup>13</sup> and Bangladesh<sup>20</sup>. A tremendous infestation due to this pest occurs during the massive flowering of mustard crop, especially between November and January. It causes crop losses through phloem feeding and studies showed that both nymphs and adults of the mustard aphid cause damage to the crop from seedling to maturity<sup>1,15,20</sup>.

Among the crop protection methods, insecticides are widely used for the control of harmful pests to enhance crop productivity<sup>5,7</sup>. Insecticides are globally used for crop protection to the extent of about two million tons per year, of which 24 percent is in the USA alone, 45 percent in Europe and 25 percent in the rest of the world including India<sup>4</sup>. In India, neonicotinoids such as imidacloprid, thiamethoxam and nitenpyram have been registered for pest control in different crops<sup>9</sup>. Neonicotinoids are systemic insecticides which are mainly applied as granules into the soil or as seed-dressings during crop planting<sup>9</sup>. These are frequently used for the control of various sucking insect pests in India<sup>8,12,15</sup>. The mode of transmission for these neonicotinoids are derived from neuron called an acetylcholine, which acts as agonistically to nicotinic acetylcholine receptor in the post-synapse during impulse transmission in an insect nerve, therefore influencing neural behaviour<sup>6,18,22</sup>. Various studies insecticidal activity, are implicated their adverse effects on the pollinators *viz.*, honeybees. The neonicotinoids have high contact toxicity to honey bees<sup>3,11,19</sup>.

Although studies have reported the use of thiamethoxam and malathion against mustard aphid and sawfly in India<sup>8,14</sup>, there is little information on the efficacy of the most frequently used neonicotinoids and organophosphate at the sublethal concentration in mustard crop under the field conditions in India. The present study therefore reports the efficacy of the two neonicotinoids *viz.*, imidacloprid and thiamethoxam and organophosphate, malathion on i) control of

mustard aphid infestation and ii) enhancement of mustard pod formation.

## MATERIALS AND METHODS

### Experimental Sites

Experiments were conducted in the two fields planted with mustard (*Brassica juncea*) at Indian Agricultural Research Institute (IARI), New Delhi, India. The mustard field A was located between latitude N 26°37.9' and longitude E 77° 09.3' and about 224.94m.a.s.l and the mustard field B between 28° 38.8' and longitude E 77° 08.2' and 207 m.a.s.l. The variation in attitude is due to the topography of IARI, field A was located at high land compared to field B. The mean temperature recorded at the IARI weather station ranged between minimum of 6.7-24.0°C and the maximum of 20.4-34.4°C from September to December, 2014. Sunrise time varied from 6:05 to 7:05 h Indian standard time (IST) and sunset varied from 17:30 to 18:26 h IST.

The neonicotinoid insecticides tested were imidacloprid 17.8% SL, Confidor® (Bayer CropScience Limited, manufactured by Saraswati Agro Chemicals Pvt. Ltd, Jammu and Kashmir, India) and thiamethoxam 25% WG, Tagxone™ (Tropical Agrosystem Pvt. Ltd, Chennai, India). Organophosphate insecticide, tested was malathion 50% EC, Suthion (manufactured by Super Ford Insecticide Limited, Secunderabad, India).

### Spraying of the three insecticides in the two fields

The study was conducted during the mass flowering of mustard (Pusa mustard-28, 2012) from the 20<sup>th</sup> November to the 10<sup>th</sup> December, 2014 and from 20<sup>th</sup> December, 2014 to the 20<sup>th</sup> January, 2015 in the field A and B, respectively. The mustard flowering period has been observed to coincide with high incidence of aphid infestation. Imidacloprid, thiamethoxam and malathion were applied at the selected inflorescences of the mustard crop in a randomized block design (RBD) as the most reliable method of creating homogenous treatment groups. The efficacies of the insecticides were determined on two parameters stated previously.

The experimental site was divided into five plots, having mustard inflorescences of similar in size and flowering intensity. In each plot, mustard crops (10 per treatment) were selected at random and subjected to the following treatments: i) with 5 ppm of imidacloprid at a rate of 200 ml per crop, ii) with 5 ppm of thiamethoxam at a rate of 200 ml per crop, iii) 5 ppm of malathion at a rate of 200 ml per crop and iv) with 1% emulsifier solution (Triton X-100), which acted as control. The control crops were covered by polythene plastic bags to avoid drift of insecticide treatment. The spraying was done by using a 1.5 l Pneumatic Hand Sprayer (ASPEE Agro Equipments Pvt. Ltd, Mumbai, India). The spraying of the three insecticides were done late in the evening from 05:45 h IST. Detailed information such as date, crop number and type of treatment was recorded on labels of different colour (i.e. red, blue, green and white) and tied to the crops.

#### **Evaluation of the three insecticides against aphid infestation**

The efficacies of the three insecticides were determined in each mustard field once per day for 20 days at an interval of three days, 10 days pre-treatment and another 10 days post-treatment, this makes three evaluations pre-treatment and another three evaluation post-treatment (1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> evaluation). Ten mustard crops from each treatment were assessed as follows: i) all main branches and their twigs per crop were counted, ii) five twigs from each crop were randomly selected and iii) numbers of twigs infested with aphid were also counted separately.

#### **Evaluation of the three insecticides against mustard pod formation**

The relationship between pod formation and the three insecticides tested was also determined in each field. The experimental protocol was similar to the previous study carried out to determine the efficacies of the insecticides against aphid infestation. Ten mustard crops from each treatment were assessed as follows: i) all main branches and their twigs per crop were counted, ii) five twigs from each crop were randomly selected and iii) pods produced from

the five twigs were also counted separately. Additional information such as numbers of pods withered was also recorded.

#### **Data Analysis**

Data was analyzed using SAS 9.3 software. Generalized linear model procedure (GLM) was used for the analysis. Analysis of variance (ANOVA) was used to compare the mean difference between treatments, day and time and their interactions. The parameters were also tested separately for the three treatments in each mustard field. Bonferroni correction was used to adjust for multiple mean comparisons. It is the most common way to control the family-wise error rate<sup>17</sup>.

## **RESULTS**

### **Mustard aphid damage**

In experimental field A, the mean numbers of mustard inflorescences infested by *L. erysimi* per twig recorded prior to the spraying of the three insecticides tested was not significantly different (Table 1) and ranged between 1.14 and 1.44. Following the application of the insecticides, the mean numbers of infected inflorescences generally continued to increase in the untreated plot throughout the experimental period but decreased significantly in all the treatments ( $F_{(3,599)} = 37.42$ ;  $P < 0.0001$ ). The mean numbers of infected inflorescences post-spraying ranged between 0.82 and 1.42 and that of untreated plot ranged between 2.04 and 3.34 (Table 1).

A similar trend was also observed in the experimental field B whereby the mean numbers of infested inflorescences recorded prior to the application of the tested insecticides was significantly different (Table 2). The level of infested inflorescences prior to the spraying ranged between 1.22 and 1.50 in all treatments including the control plot. Similarly, following the application of the insecticides, the mean numbers of infected inflorescences generally continued to increase in the untreated plot throughout the experimental period but decreased significantly in all the treatments ( $F_{(3,599)} = 79.75$ ;  $P < 0.0001$ ). The mean numbers of infected inflorescences post-spraying ranged between 0.78 and 1.46 and that of untreated plot ranged between 2.70 and 3.78 (Table 2).

**Mustard pod formation**

The efficacy of the tested insecticides was also determined against the numbers of mustard pod developed in the previous trial plots used for the assessment of aphid infestation. The mean numbers of mustard developed on experimental plot prior to the spraying of the three insecticides tested was not significantly different in the field A (Table 3). The mean numbers of pods developed per twig ranged between 18.72 and 19.84. However, after the application of the insecticides, the mean numbers of pods developed per twig generally remained constant in the untreated plot throughout the experimental period but increases significantly in all the treatments ( $F_{(3,599)} = 28.31$ ;  $P < 0.0001$ ). The mean numbers of pods developed per twig post-spraying ranged between 22.14 and 25.42 (Table 3). There were also significant differences

between treatments for all interactions ( $F_{(24,599)} = 1.60$ ;  $P = 0.04$ ).

A similar trend was also observed in the experimental field B. The mean numbers of mustard developed on experimental plot prior to the spraying of the three insecticides tested was not significantly different in the field A (Table 4). The mean numbers of pods developed per twig ranged between 17.28 and 19.70. However, after the application of the insecticides, the mean numbers of pods developed per twig generally remained constant in the untreated plot throughout the experimental period but increases significantly in all the treatments ( $F_{(3,599)} = 28.10$ ;  $P < 0.0001$ ). The mean numbers of pods developed per twig post-spraying ranged between 20.74 and 25.32 (Table 4). Unlike field A, there were no significant differences between treatments for all interactions.

**Table 1. Infested mustard inflorescences by *Lipachis erysimi* in the field A during production season of 2014/2015 at IARI, New Delhi**

| Treatment    | X±SE of infested inflorescences per twig |                            |                            | F&P-values: Treatment                |
|--------------|--|----------------------------|----------------------------|--------------------------------------|
|              | 1 <sup>st</sup> evaluation               | 2 <sup>nd</sup> evaluation | 3 <sup>rd</sup> evaluation |                                      |
|              | Pre-treatment                            |                            |                            |                                      |
| Control      | 1.24±0.14a                               | 1.36±0.15a                 | 1.38±0.16a                 | $F_{(3,599)} = 1.24$ ; $P = 0.29$    |
| Imidacloprid | 1.28±0.15a                               | 1.38±0.16a                 | 1.44±0.17a                 |                                      |
| Malathion    | 1.26±0.15a                               | 1.26±0.16a                 | 1.50±0.16a                 |                                      |
| Thiamethoxam | 1.30±0.16a                               | 1.18±0.17a                 | 1.14±0.16a                 |                                      |
|              | $F_{(24,599)} = 0.13$ ; $P = 1.0$        |                            |                            |                                      |
|              | Post-treatment                           |                            |                            |                                      |
| Control      | 2.40±0.21a                               | 2.80±0.20a                 | 3.34±0.23a                 | $F_{(3,599)} = 37.42$ ; $P < 0.0001$ |
| Imidacloprid | 1.26±0.14a                               | 1.42±0.15a                 | 1.30±0.14a                 |                                      |
| Malathion    | 0.96±0.12a                               | 1.04±0.15a                 | 1.04±0.13a                 |                                      |
| Thiamethoxam | 0.82±0.12a                               | 0.98±0.13a                 | 0.96±0.13a                 |                                      |
|              | $F_{(24,599)} = 0.11$ ; $P = 1.0$        |                            |                            |                                      |

<sup>a</sup>Means within a site followed by different letters are significantly different at  $P < 0.05$

**Table 2. Infested mustard inflorescences by *Lipachis erysimi* in the field B during production season of 2014/2015 at IARI, New Delhi**

| Treatment    | X±SE of infested inflorescences per twig |                            |                            | F&P-values: Treatment                |
|--------------|--|----------------------------|----------------------------|--------------------------------------|
|              | 1 <sup>st</sup> evaluation               | 2 <sup>nd</sup> evaluation | 3 <sup>rd</sup> evaluation |                                      |
|              | Pre-treatment                            |                            |                            |                                      |
| Control      | 1.28±0.16a                               | 1.50±0.13a                 | 1.40±0.15a                 | $F_{(3,599)} = 0.93$ ; $P = 0.42$    |
| Imidacloprid | 1.36±0.16a                               | 1.36±0.18a                 | 1.36±0.16a                 |                                      |
| Malathion    | 1.32±0.15a                               | 1.22±0.14a                 | 1.32±0.14a                 |                                      |
| Thiamethoxam | 1.46±0.15a                               | 1.34±0.15a                 | 1.48±0.18a                 |                                      |
|              | $F_{(24,599)} = 0.56$ ; $P = 0.96$       |                            |                            |                                      |
|              | Post-treatment                           |                            |                            |                                      |
| Control      | 2.70±0.22a                               | 3.18±0.21a                 | 3.72±0.20a                 | $F_{(3,599)} = 79.75$ ; $P < 0.0001$ |
| Imidacloprid | 1.28±0.13a                               | 1.46±0.15a                 | 1.14±0.14a                 |                                      |
| Malathion    | 0.96±0.12a                               | 0.96±0.11a                 | 1.02±0.13a                 |                                      |
| Thiamethoxam | 0.78±0.10a                               | 0.98±0.10a                 | 1.20±0.12a                 |                                      |
|              | $F_{(24,599)} = 0.67$ ; $P = 0.89$       |                            |                            |                                      |

<sup>a</sup>Means within a site followed by different letters are significantly different at  $P < 0.05$

**Table 3. Mustard pod formation per twig in the field A during production season of 2014/2015 at IARI, New Delhi**

| Treatment    | X±SE of pod formed per twig |                            |                            | F&P-values: Treatment        |
|--------------|-----------------------------|----------------------------|----------------------------|------------------------------|
|              | 1 <sup>st</sup> evaluation  | 2 <sup>nd</sup> evaluation | 3 <sup>rd</sup> evaluation |                              |
|              | Pre-treatment               |                            |                            |                              |
| Control      | 18.50±0.15a                 | 19.36±0.22a                | 20.48±0.15a                | F(3,599) = 2.1; P=0.09       |
| Imidacloprid | 19.72±0.15a                 | 19.56±0.14a                | 19.52±0.16a                |                              |
| Malathion    | 19.02±0.17a                 | 18.84±0.18a                | 20.66±0.16a                |                              |
| Thiamethoxam | 18.42±0.17a                 | 19.44±0.18a                | 19.50±0.15a                |                              |
|              | F(24,599) = 0.59; P=0.94    |                            |                            |                              |
|              | Post-treatment              |                            |                            |                              |
| Control      | 18.74±0.17a                 | 19.14±0.26a                | 19.84±0.19a                | F(3,599) = 28.31; P < 0.0001 |
| Imidacloprid | 22.76±0.38a                 | 23.32±0.33a                | 24.04±0.32a                |                              |
| Malathion    | 22.14±0.39a                 | 24.94±0.32a                | 25.42±0.29a                |                              |
| Thiamethoxam | 22.30±0.52a                 | 23.34±0.37a                | 24.20±0.41a                |                              |
|              | F(24,599) = 1.6; P=0.04     |                            |                            |                              |

<sup>a</sup>Means within a site followed by different letters are significantly different at P< 0.05

**Table 4. Mustard pod formation per twig in the field A during production season of 2014/2015 at IARI, New Delhi**

| Treatment    | X±SE of pod formed per twig |                            |                            | F&P-values: Treatment        |
|--------------|-----------------------------|----------------------------|----------------------------|------------------------------|
|              | 1 <sup>st</sup> evaluation  | 2 <sup>nd</sup> evaluation | 3 <sup>rd</sup> evaluation |                              |
|              | Pre-treatment               |                            |                            |                              |
| Control      | 18.48±0.18a                 | 18.72±0.17a                | 19.56±0.17a                | F(3,599) = 0.71; P=0.55      |
| Imidacloprid | 18.82±0.16a                 | 19.40±0.16a                | 19.72±0.15a                |                              |
| Malathion    | 18.56±0.17a                 | 18.02±0.18a                | 19.80±0.17a                |                              |
| Thiamethoxam | 17.28±0.16a                 | 18.46±0.18a                | 19.70±0.17a                |                              |
|              | F(24,599) = 0.66; P = 0.88  |                            |                            |                              |
|              | Post-treatment              |                            |                            |                              |
| Control      | 19.32±0.20a                 | 19.70±0.17a                | 20.10±0.19a                | F(3,599) = 28.10; P < 0.0001 |
| Imidacloprid | 22.52±0.35a                 | 23.76±0.34a                | 24.70±0.38a                |                              |
| Malathion    | 22.58±0.36a                 | 23.86±0.48a                | 24.02±0.31a                |                              |
| Thiamethoxam | 20.74±0.60a                 | 24.20±0.39a                | 25.32±0.51a                |                              |
|              | F(24,599) = 1.22; P = 0.22  |                            |                            |                              |

<sup>a</sup>Means within a site followed by different letters are significantly different at P< 0.05

## DISCUSSION

Mustard crop is largely cultivated in the States of Rajasthan, Gujarat, Uttar Pradesh and Haryana in about 6 m ha<sup>16</sup>. The neonicotinoids are highly recommended for the control of insect sucking pests in different crops in India, imidacloprid is widely used against shoot fly in pearl millet and Indian plum fruit fly, while thiamethoxam is used for the control of mustard aphids<sup>8</sup>. Beside this, an organophosphate (malathion) is often used for the control of aphid and sawfly in mustard as well as other closely related crops on the basis of crops group concept<sup>14,23</sup>. The present study was therefore

carried out on the efficacy of imidacloprid and that of malathion at the concentration which was eight times less than recommended foliar concentration of 40 ppm on related crops. Similarly, the efficacy of thiamethoxam was studied at the concentration which was five times less than on its foliar concentration of 25 ppm recommended on mustard crops<sup>23</sup>. The study showed decline in aphid infestations to the treated flowers/inflorescences in both experimental fields. Low mean numbers of infested mustard inflorescences recorded after the application of the three tested insecticides indicate its effectiveness in the management of

mustard aphid, *Lipachis erysimi*. Effective control of the two neonicotinoids viz., imidacloprid and thiamethoxam were also reported during the evaluation of new convectional insecticides for management of mustard aphid<sup>14</sup>. Similarly, imidacloprid and papaya leaf extract toxicity was also found effective against the mustard aphid<sup>21</sup>. In addition, malathion and neem extracts were found to be effective against mustard aphid in Bangladesh<sup>2</sup>.

On the other hands, the present study reports enhancement of mustard pod formations in both fields. The increase in the mean numbers of pods developed per twig show the effective of the tested inflorescences to improve pods/seed formation of the mustard plants. The results of this study are similar to other study conducted in Pakistan on pods/seed in mustard treated with some phosphorus insecticides including the malathion<sup>10</sup>.

### CONCLUSION

Although, present study indicates efficacy of the neonicotinoids and that of organophosphate in controlling the mustard aphid and increase pods/seeds formation, the latter is recommended for the control of mustard aphid due to its less adverse effect on pollinators especially honeybees. It is also essential to conduct a large scale ecosystem-wise analysis on various organophosphates registered in India. This will help to develop more sustainable integrated pest management (IPM) strategies for the mustard aphid in India.

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