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Study of the efficiency of Neuropteran predator, *Hemerobius indicus* Kimmins as potential biocontrol agent of notorious aphid, *Lipaphis erysimi* (Kaltenbuch) on *Brassica campestris* Linn. (cv. B-9)

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Abstract

The *Brassica* oil crops are the world's third most important source of edible oil. *Brassica campestris* Linn., an important oilseed crop of India is cultivated largely in Assam, Bihar, Orissa and West Bengal. The mustard aphid, *Lipaphis erysimi* (Kaltenbach) is a serious pest of cruciferous (*Brassica*) crops in India. The development of resistance to pesticides and toxicity to the non target organism is largely responsible for the attention on biological control. Natural predators can bring down pesticide use against pest in a proper IPM (Integrated Pest Management) strategy. Neuroptera (Insecta) are proven biological control agents against aphids. In the present investigation *Hemerobius indicus* Kimmins, a neuropteran predator, was reared on *Lipaphis erysimi* (Kaltenbuch), an important aphid pest of *Brassica campestris* (cv.B-9). Their development and aphid annihilation capability were assessed. Duration of egg, larval, pupal stages and the adult longevity were 6-7 day, 17-23 day, 10-12 day and 9-13 day respectively while feeding on *Lipaphis erysimi* (Kaltenbuch) at 21± 3.9ºC. Yield and morphological parameters of *Brassica campestris* Linn.(cv.B-9) were quantified and compared viz. Neuroptera and predator complex-controlled aphid infested plots were compared with that of uncontrolled and insecticide-controlled plot of *Brassica campestris* Linn. (cv.B-9). Results showed that during the early stages of plant development of *Brassica campestris* Linn. (cv.B-9) the predator *Hemerobius indicus* alone could be a safe replacement of insecticide in terms of yield of *Brassica campestris* Linn. (cv.B-9) only when the aphid *Lipaphis erysimi* (Kaltenbuch), population is relatively low.

Keywords: Biocontrol agent, Brassica, *Hemerobius, Lipaphis*, predator diversity.

1. Introduction

Insecticides destroy all insects irrespective of whether they are beneficial or not and contaminate the environment, threatning the well being of the other creatures (Hamilton, 2000). The value of predators in the biological control of insect pests in integrated pest management programs has been highlighted by several researchers (Hagen, 1962; Hagen and Van der Bosch, 1968; Hodek, 1966; Symondson et al., 2002; Dey, 2014e). The development of resistance to pesticides and toxicity to the non target organism is largely responsible for the attention on biological control (Burges & Hussey, 1971). Accessing adequate amounts of nutritious, safe, and culturally appropriate foods in an environmentally sustainable manner is important for a growing population (Carvalho, 2006). Natural predators can bring down pesticide use against pest in a proper IPM (Integrated Pest Management) strategy.

The angiosperm family Brassicaceae, also known as Cruciferae, contains about 3500 species and 350 genera, is one of the 10 most economically important plant families (Warwick et al., 2006). Crop *Brassica* encompass many diverse types of plants, which are grown as vegetables, fodder or sources of oils and condiments. The oleiferous *Brassica* species, commonly known as rapeseed-mustard, are one of the economically important agricultural commodities. The *Brassica* oil crops are the world's third most important source of edible oil. *Brassica campestris* Linn., an annual herb with erect stem, stout, simple of branched, 30-100 cm high, belongs to the family Brassicaceae. Leaves alternate, petiolate, large, more or less pinnatifid; inflorescence usually raceme; flowers yellow, pedicellate, tetramerous; fruit siliqua 3.7-7.5 cm, glabrous; seeds small, smooth. It is a very important oilseed crop and constitutes the major source of edible oil in India. The oil is one of the chief sources of erucic acid, a fatty acid of the oleic acid series and which has important applications in food and industry. The leaves are also eaten in many parts of India. It is often fed to cattle too. It is a *Rabi* (winter) crop that requires relatively cool temperature, a fair supply of soil moisture during the growing season and a dry harvest period (Banerjee et al., 2010). *Brassica campestris* Linn. is an important oilseed crop of India cultivated largely in Assam, Bihar, Orissa and West Bengal mainly as winter crop.

The mustard aphid, *Lipaphis erysimi* (Kaltenbach) is a serious pest of cruciferous (*Brassica*) crops in India (Brar et al., 1987; Bakhetia & Sekhon, 1989). Good control of mustard aphid can be obtained by spraying traditional organic insecticides (Khurana & Batra, 1989). However, some chemicals have posed some serious problems to health and environmental safety, because of their high toxicity and prolonged persistence (Kulkarni & Joshi, 1998). *Liphapis erysimi* (Kaltenbuch) infests the crop right from seedling stage to maturity. It ravages the crop during the reproductive phase and act as a limiting factor in the production. The losses in yield caused by mustard aphid ranged from 9 to 95% (Singh et al., 1980), 24.00 to 96.00 % (Phadke, 1985), 35.4 to 72.3 % (Bakhetia, 1986) up to 96% (Verma, 2000) at different places of India.

Insect pests, particularly aphids infesting crops of economic importance have been often controlled by their natural predators (Sanwar, 2013). Neuroptera is one of the smaller orders of insects (Winterton et al., 2010), but most larval neuropterans are predacious, often in agricultural systems, lending added importance to this group. In agricultural ecosystems some neuropteran species of the families Chrysopidae, Hemerobiidae, and Coniopterygidae are known as beneficial predators of plant-sucking insect pests. Neuropteran predators can be used to control aphids infesting plants of economic importance as found by several workers (Gautam & Tesfaye, 2002; Ashfaq, 2007; Dey, 2014b, 2014c).

Members of the family Hemerobiidae are known to be active aphid predators and early studies (Moznette, 1915; Curtright, 1923) clearly demonstrated their individual potential for the annihilation of aphid population, which resulted in successful introduction of these Neuropteran predators in various countries (Williams, 1927; Garland, 1978). Despite the availability of scattered information on early stages and life histories of Hemerobiidae, only a few species of these Neuropterans have been investigated for their potential as aphid predators (New, 1989). Information, specifically on the effectiveness of aphidophaga in commercial farming is scarce. In practice the biological control is acceptable when it keeps the aphid population below the economic threshold level, in terms of yield of the plants (Niemczyk, 1988; Tauber, 2000). An ideal natural enemy is one that consumes sufficient number of the preys at the right time to maintain a pest population below the economic injury threshold for the crop considered (Michaud & Belliure, 2000). The feeding rates of the predator was determined that reflects the aphid annihilation capability of the predator in the field (Bankowska et al., 1978). Further Dey and Bhattacharya (1997), Dey, (2014a) recorded the development and larval voracity of this endemic species predating on *Prociphilus himalayensis* Chakrabarti infesting *Lonicera sp* as well as predating on *Lipaphis erysimi* (Kaltenbuch) on *Brassica campestris*. The numerical response of the Neuropteran population can be determined in association with the other aphid predators in the open field following Readshaw (1973).

There is a long-standing, but unresolved, debate among biocontrol experts over whether introduction of multiple natural enemies leads to more efficient pest suppression than the release of single enemy species (Kakehashi et al., 1984; Denoth et al., 2002; Cardinale et al., 2003; Straub et al., 2008; Tylianakis & Romo, 2010). Assemblages of natural enemies are often engaged in a mixture of several direct and indirect interactions amongst species with both negative and positive effects on biological control (Roubinet et al., 2015). The predator biodiversity of the aphid pest, *Lipaphis erysimi* (Kaltenbuch) was studied (Dey, 2014d).

Assessment of the efficiency of the predator is a prime requisite before the incorporation of that predator to control the pests. At present, insecticide regulations are strict and the alternatives like biological control approaches are increasingly investigated worldwide. In this investigation an attempt was made to evaluate the efficiency of *Hemerobius indicus* Kimmins as a potential future bio-control agent against the notorious aphid pest, *Lipaphis erysimi* (Kaltenbuch) infesting *Brassica campestris* (cv.B-9). Evaluation of this species as a predator was made also in terms of the yield of the plant whose aphid was to be controlled and in terms of reproductive numerical response under natural condition. The effectiveness of *Hemerobius indicus* Kimmins in association with other natural predators of *Lipaphis erysimi* (Kaltenbuch) was also investigated in terms of plant yield of *Brassica campestris* (cv.B-9).

2. Materials and methods

2.1 Plant Material

The Indian field mustard cultivar, *Brassica campestris* (cv.B-9) was used for this investigation. This variety of mustard was cultivated under recommended dose (Directorate of Agriculture, Government of West Bengal, India) of chemical fertilizer @ N:P:K =100:50:50.

2.2 Study area

The experiment was set in the Bose Institute Experimental Farm, Madhyamgram, North 24-Parganas, West Bengal, India for two consecutive years (2000 – 2001, 2001 – 2002) and again at Berhampore Girls College, Murshidabad for three consecutive years

(2012 to 2014) during the months of November to February.

2.3 Study design and sampling

To evaluate the predatory efficiency of *Hemerobius indicus* Kimmins in controlling *Lipaphis erysimi* (Kaltenbuch), the yield and other morphological parameters of *Brassica campestris* (cv.B-9) was assessed by comparing four different set ups viz -

1) The Neuroptera-controlled plot in which only Neuroptera was used to control the aphids in absence of any insecticide.
2) The predator complex controlled plot where all the predators and parasites were allowed to infest upon aphids in absence of any insecticide.
3) The untreated control (-ve control or aphid infested control plot) where aphid infested plants were devoid of any predator, parasite or insecticide application.
4) Insecticide controlled plot (+ve control) in which the aphids were controlled using insecticides in absence of any predator or parasite.

Each plot contained 30 *Brassica campestris* Linn. (cv.B-9) plants with the spacing 50cm X 50cm between the plants. Normal agricultural practice was followed for the cultivation of *Brassica campestris* Linn. (cv.B-9). The experiment was carried out in 3 replications in different locations of the farm. Each plot was covered with fine mesh mosquito net [measuring 3.5m (L) X 3.0m (W) X 1.5m (H)]. The distance between the plants was maintained to facilitate their maximum growth and to prevent the movement of the predator larvae from one plant to another. The experiments were performed inside the mosquito net to prevent entry of other predators (coccinelidae or syrphidae) but the diameter of the mesh permitted movement of the alatae of the aphid to enter inside.

One day old, 1st instar larvae of *Hemerobius indicus* Kimmins were released at the rate of five larvae per plant on 15day-old *Brassica campestris* Linn. (cv.B-9) plants. The average ratio of the predator larvae: aphid was 1: 42.33 in the predator-controlled plot. The predator larvae were released for the second time after 21 days of the first release at the rate of five larvae per plant.

The insecticide-controlled plot was treated with the insecticide L- cyhalothrin, a synthetic pyrethroid, at 1ml./litre/21 days. The insecticide killed the aphids as well as the predators and parasites. Any larvae or adult of predator, adult parasite escaped death and all mummified aphids were removed by hand picking.

The untreated control, or aphid infested plot, was allowed to be infested by aphids. No insecticide was applied. All predator and parasite adult and larvae were removed.

In predator complex controlled plots no insecticide were applied and one day old 1st instar larvae of *Hemerobius indicus* Kimmins, *Coccinella septempunctata* L. and *Betasyrphus sp* were released at the rate of 5 larvae/plant, the parasites were expected to infest the aphid population automatically through the mesh of the net.

After maturation of pods, the plants were harvested and measured for plant height. The number of primary, secondary, tertiary branches and pods were counted. The total seed yield of the plants was measured. Analysis of variation for each trait was carried out using the statistical software SPSS-10. The agro-morphological data of the predator-controlled *Brassica campestris* plants were compared with those obtained from the insecticide-controlled and untreated control plots.

Freshly hatched larvae and the emerged adults of the predator were placed in seperate containers (7.2 x 7cm), at the rate of 1 individual per container in 10 containers, the mouth of the container covered with nylon net and counted number of aphids *Lipaphis erysimi* (Kaltenbuch), last instar and adults, were supplied as food for the predators. Each day, the surviving aphids were removed and freash aphids of the same stage were offered to the predator in the container. To arrive at the actual number of aphids consumed, dead aphids were removed from the container, examined under microscope for sign of larval or adult consumption and the number of aphids actually killed by the larvae or adult were recorded. Obeservations were made on the duration of each life stage of the predator (except the non feeding pupae) and the experiment were performed in seminatural condition at 21 ± 3.9^{0}C average temperature. The reproductive numerical response of the *Hemerobius indicus* Kimmins in comparision to the *Coccinella septempunctata* L. was determined by number of adults found in the *Brassica campestris* Linn. (cv.B-9) field infested by aphids in the farm by sweeping with a hand net and counting the numbers found in light traps once every 24 hours upto the harvest of the crop.

3. Results

The results reported in this paper are based on the assessment of the predatory efficiency of *Hemerobius indicus*, a Neuropteran predator, on *Lipaphis erysimi* (Kaltenbuch), an aphid infesting *Brassica campestris* Linn. (cv. B-9). The duration and aphid consumption of the different stages of the life cycle of the predator was determined from the experiment in closed container (Table 1). In the field, the predatory effect of the neuropteran was determined in terms of the yield of *Brassica campestris* Linn. (cv. B-9) obtained under different treatments.

3.1 Development of *Hemerobius indicus* Kimmins on *Lipaphis erysimi* (Kaltenbuch)

The total life stages of the predator *Hemerobius indicus* Kimmins lasted for 42-57 days when feeding on *Lipaphis erysimi* (Kaltenbuch) at 21 ± 3.9^{0}C. Duration of the egg, 1st instar, 2nd instar, 3rd instar, pupa and adult were 6-7 days, 5-6 days, 4-5 days, 8-12 days, 10-12 days and 9-13 days, respectively. Duration of each instar shows that the 2nd instar larvae develops fastest, while the 3rd instar larvae takes the maximum number of days for development. The pupal period is longer which is almost twice the duration of the 1st instar. *Hemerobius indicus* Kimmins takes 27-35 days approximately, for the completion of metamorphosis from larva into an adult. (Table 1).

3.2 Consumption of aphids by the predator larvae and adults

The results obtained from the experiment in closed containers showing the pattern of consumption of aphid, *Lipaphis erysimi* (Kaltenbuch) (nymphs and adults), by the larvae and adults of the predator, *Hemerobius indicus* Kimmins, are given in Table-1. Prey consumption rate was different at different developmental stages of the predator. The mean numbers of aphids consumed by the 1st instar, 2nd instar, 3rd instar larvae and adults of the predator were 61.85 ± 1.8 (range; 57-66 aphids/larva), 101.14 ± 9.09 (range 96-113 aphids/larva), 232 ± 12.23 (range 228-247 aphids/larva) and 254 ± 13.72 (range 271-367 aphids/larva) respectively.

3.3 Plant Mortality

Almost half of the plants, 47.74%, died in the untreated control plots due to heavy infestation of aphids before flowering, while there was no death of plants in the insecticide-controlled, Neuroptera-controlled and predator-controlled plots.

Table 1. Consumption of *Lipaphis erysimi* (Kaltenbuch) by different developmental stages of *Hemerobius indicus* Kimmins at 21 ± 3.9^{0}C.

***Hemerobius indicus* Kimmins Stage of development**	**Duration in days**	**Number of *Lipaphis erysimi* (Kaltenbuch) consumed by *Hemerobius indicus* Kimmins (Larva/Adult)**	**Mean ± SD *Lipaphis erysimi* (Kaltenbuch) consumed by *Hemerobius indicus* Kimmins (Larva/Adult)**
Egg	6-7	--	--
1st instar larva	5-6	57-66	61.85 ± 1.8
2nd instar larva	4-5	96-113	101.14 ± 9.09
3rd instar larva	8-12	228-247	232 ± 12.23
Pupa	10-12	--	--
Adult	9-13	271-367	254 ± 13.72
TOTAL	42-57	652-793	--

Table 2. Comparative average ± SD yield of *Brassica campestris* Linn. (cv. B-9) in different study plots.

***Brassica campestris* L. (cv. B-9)**	**Plant height (cm)**	**No. of primary branches/ Plant**	**No. of secondary branches/ Plant**	**No. of tertiary branches/ Plant**	**Pod number / plant**	**Yield/ plant (in gms)**
Only Neuroptera, *Hemerobius indicus* Kimmins Controlled plot	86.1 ± 1.97	1.00 ± 0.00	0 ± 0.00	6.70 ± 1.70	59.80 ± 10.62	7.20 ± 1.594
Predator complex Controlled plot	87.5 ± 11.1	1.00 ± 0.00	0 ± 0.29	6.74 ± 1.12	59.83 ± 17.61	8.60 ± 1.027
Untreated (Aphid infested plot)	102.20 ± 5.85	1.00 ± 0.00	3.40 ± 1.51	5.80 ± 1.40	23.20 ± 14.61	0.480 ± 0.365
Insecticide Controlled plot	89.70 ± 10.9	1.00 ± 0.00	0 ± 0.32	7.40 ± 1.07	60.40 ± 21.20	9.80 ± 1.033

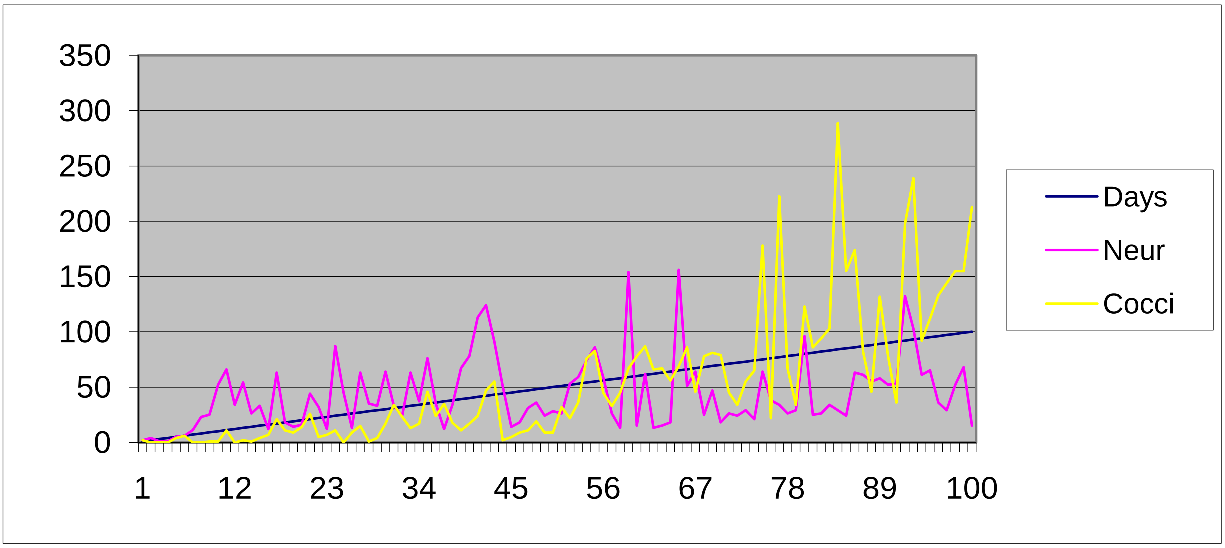

Fig 1. Comparative abundance of *Hemerobius indicus* Kimmins and *Coccinella septempunctata* in the Brassica (CV B9) field.

3.4 Effect on Plant Morphology

The plant height decreased by 12.05%, 15.58% and 14.38% in the insecticide-mparison to that of the aphid-infested plot. In comparison with the aphid-infested plot, the secondary branch number decreased by 67.64% and 70.58% in the insecticide- and Neuroptera-controlled plots, respectively, while the primary branch number remained the same in all three treatments. As compared to the insecticide-controlled plot, the tertiary branch number of the plants decreased by 9.45%, 8.91% and 21.62% in the Neuroptera-controlled, predator-controlled and aphid infested plots, respectively. The results are shown in Table 2.

3.5 Effect on Plant Yield

As compared to the insecticide-controlled plants, the pod number of the severely infested untreated control plot decreased by 61.58%, while the pod numbers of Neuroptera-controlled and the predator-controlled plot decreased only by 0.94% and 0.99%. Although, the seed yield (weight) reduction was significant in the Neuroptera-controlled (26.53%) and the predator-controlled (12.24%) plot as compared to that obtained from the insecticide-controlled plot, the yield reduction in aphid-infested plots were as high as 95.1%. The results are shown in Table 2.

3.6 Efficiency of Predator complex to control the pest

From the survey of the adult Neuroptera and the Coccinellid it is found that the Neuroptera are abundant in the aphid infested field early in the season when the aphid population density is quite low and coccinellids were not abundant. With the increase of the aphid population, density and temperature, the number of the coccinellids increased but the neuropterans failed to respond to the prey density growth (Fig 1).

controlled, Neuroptera- controlled plots and predator-controlled plot respectively, in co

4. Discussion

Neuropteran predators have been used biocontrol agents to control aphids as a safe replacement of the insecticide (Tauber et al., 2000; Gautam & Tesfaye, 2002; Ashfaq, 2007; Dey, 2014). A comparison of the yield of the insecticide-controlled, Neuroptera-controlled, predator-controlled and aphid-infested plots clearly show the following:

a) The best control of aphids is effected by insecticides,
b) The neuropteran predator, *Hemerobius indicus* Kimmins was partially, but significantly, effective in reducing yield loss due to aphid infestation.
c) Yield loss in the Neuroptera-controlled and predator-controlled plots were 26.53% and 12.24%, respectively, as compared to that obtained in the insecticide-controlled plots, while the yield loss in the untreated, aphid-infested plots was 95.1%.

In this study we have observed that the plant height and the secondary branch number increased in aphid-infested plants as compared to those of the plants in the predator- and insecticide-controlled plots. This is maybe due to the initial attempt of the plants to escape nutrient deficiency caused by severe sap sucking by the aphids, but at later stages, the plants succumbed to aphid attack resulting in the decrease in tertiary branch number. Decrease in pod number and yield are due to high infestation resulting heavy damage in the inflorescence.

A reduction in plant height and tertiary branches in the Neuroptera and predator-controlled plots are also notable. This is due to presence of some aphids and their sap sucking which causes loss of vigour in plants. But from the morphology it is evident that initially aphids could not pose a threat to the plant because of predation but later the rate of increase of aphid population overlapped predation. Build up of aphid colony in the inflorescence resulted in decreased seed weight, but the pod number was less effected due to prevention of early infestation by Neuroptera.

As the field was an isolated one and food material was abundant for the adults of both the predators, there was little chance of migration of the predator adults from the field. The reproductive numerical response of the coccinellids was better than the Neuropterans. The coccinellids population increased with the increase of the pest population growth, while the Neuropterans failed to keep up at the later stages of plant growth. From the agro-morphological and the population data it is evident that for the biological control of *Lipaphis erysimi* (Kaltenbuch) the *Hemerobius indicus* Kimmins can be used early in the season. However during the later stages of plant development, particularly during flowering, it is better if used together with other predators, especially the coccinellids.

Conclusion

From the above observations it is evident that predation by *Hemerobius indicus* Kimmins, as a sole control agent, is unable to control the aphid in later stages of plant development, particularly during flowering, even though both the larvae and the adults of the predator are aphidophagous in nature. Perhaps, the absence of other naturally occurring predators resulted in the loss of cumulative effect on the aphid population so there is a need to use all the predatory groups to share the prey population or to spray insecticide in later stages of plant development to effectively minimize yield loss. As there is no report of insecticide tolerance in Hemerobiidae it is very difficult to use both the insecticide and the predator at the same time. Increase in larval number of the predator at early stages of aphid infestation may lead to cannibalism amongst the predator larvae due to lack of sufficient food. Thus it is better to use a predatory complex to eradicate the aphid or suppress their population below economic the threshold level.

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