



Natural predators of the mustard aphid *Lipaphis erysimi* show differential predation preferences on the aphids feeding on different cole crops

M. Shaikh² • M. Pawar² • S. Pandit² • S. Mitra¹

¹Department of Botany, Savitribai Phule Pune University (formerly, University of Pune), Pune, Maharashtra;

²Department of Biology, Indian Institute of Science Education and Research, Pune, Maharashtra

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ABSTRACT

Smart use of predators considering their adaptations to their prey's host plant's chemical defense composition is a chemical ecology-based pest control strategy which is rapidly gaining popularity. Protection programs of cole crops, which are severely damaged by various pests, mainly specialist aphids, often desire generalist natural enemies of the insect pests. Since cole crops drastically differ in their compositions of glucosinolates, the primary defense compounds of Brassicaceae, finding natural enemies that are not affected by the glucosinolate composition ingested by their preys is important. For this, we screened various natural enemies of the devastating Brassicaceae-specialist mustard aphid *Lipaphis erysimi* feeding on six different cole crop hosts broccoli, cabbage, cauliflower, knol khol, mustard and radish. We found that lacewings, ladybird beetle larvae and two spider species can effectively prey upon aphids feeding on hosts of different glucosinolate compositions. Moreover, we found two spider species that showed differential predation associated with the aphids varying glucosinolate ingestion. Ladybird beetle (*Coccinella septempunctata*) and jumping spider (*Plexippus paykulli*) were found to be the most efficient *L. erysimi* predators on all the tested crops. These results are important for the management of cole crop pests as all the predators used in the study are generalists and can be used against a wide range of pests. More importantly, since *L. erysimi* sequesters its host's glucosinolates and myrosinase and itself acts as a mustard oil bomb when attacked by the natural enemies, high predation efficiency of the screened predators is highly promising.

1. Introduction

Brassicaceae is an economically important plant family that contributes to the world's most commonly cultivated vegetables like cabbage, cauliflower, kale, arugula, cress, broccoli, Brussels sprouts, radish, turnips, knol khol, etc. and oil seeds like mustard, rapeseed, etc. This group of plants have high culinary importance as they are rich in carotenoids, vitamins A and C, iron, calcium, magnesium, and dietary fibre (Guerena 2006). Moreover, they are also a source of dietary protein (Colon and Toor 2016). Cole crops are grown both in hilly regions as well as plains where, low temperatures (15°C– 20°C) and moist soils, which can provide continuous water throughout the season are

available. They are also popularly grown in polyhouses and other indoor farming setups (Singh, 2000; Spaldon *et al.*, 2018; Vashisth *et al.*, 2013). In India, cole crops are cultivated in all seasons, except extreme winters (Thakur *et al.*, 2012). Variety of insect pests severely damage cole crops, hamper their productivity, and drastically reduce their market value. These crops are mainly attacked by aphids, (*Brevicoryne brassicae*, *Lipaphis erysimi*, *Myzus persicae*), cabbage butterflies (*Pieris spp.*), diamondback moth (*Plutella xylostella*), cabbage head borer (*Hellula undalis*), semilooper (*Plusia orichalcea*), cabbage semilooper (*Plusia orichalcea*, *Trichoplusia ni*), cutworms (*Agrotis flammatra* and *A. ipsilon*), flea beetle (*Phyllotreta cruciferae*), painted bug (*Bagrada cruciferarum*), leaf webber (*Crosidolomia binotalis*) and sawfly

(*Athalia lugens proxima*). According to Thakur *et al.*, 2012, aphids and cabbage butterflies are the most important pests on cole crops. Among the aphids, *B. brassicae* and *L. erysimi* are Brassicaceae specialists whereas *M. persicae*, the peach aphid is a generalist. Since aphids are parthenogenic and have a short life cycle, they are highly difficult to control. Specialist aphids are more challenging as they are well-adapted to their host plant's chemical defense dynamics. Prevention of colonization is the most commonly used strategy to control aphid populations. Generally, pesticides are used to control aphid infestation and reduce the economic loss. However, pesticide can be hazardous in case of vegetables like arugula, cabbage, kale, radish, etc., which are generally eaten raw as a salad. Use of pesticides causes ill effects on human health and also increases crop production costs (Parween *et al.*, 2016). Therefore, bio-control strategy is frequently used to obtain chemical pesticide free harvests.

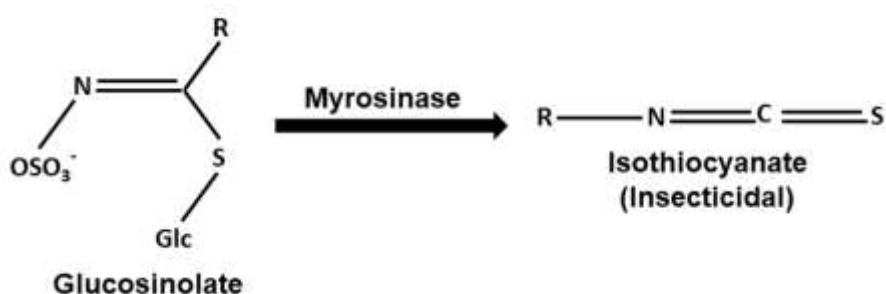
Brassicaceous plants contain several defense compounds such as benzenoids, flavonoids, phenolics, terpenoids and indole-sulfur compounds. However, glucosinolate-myrosinase system is their characteristic defense.

Glucosinolates are nitrogen- and sulfur-containing anionic pretoxins (Fig. 1). Myrosinase is a thioglucosidase family enzyme, which deglucosylates glucosinolates to release the toxic isothiocyanates (Fig.1) (Halkier and Gershenzon, 2006).

Figure Legends

Figure 1. Brassicaceae's glucosinolate-myrosinase defense system. In cole crops, upon tissue damage by pest insects, the separately compartmentalized enzyme myrosinase hydrolyses stored glucosinolates and produces isothiocyanates, which is toxic to insects. Aphid *L. erysimi* acquires glucosinolates and myrosinase from the host plant without mixing them and sequesters them in separate body compartments. Upon predator attack, these compartments burst, separately sequestered glucosinolates and myrosinase mix to form isothiocyanates; thus an aphid individual acts as a mustard oil bomb.

Figure 1.



Myrosinase is stored in idioblasts and guard cells to avoid its contact from the glucosinolates, which are stored in specialized sulfur-rich cells close to the phloem and thus avoid the self-damage by isothiocyanate formation. They come in contact with each other to form isothiocyanates only after the herbivore ruptures the compartments while chewing the plant tissue. This process is popularly known as the detonation of mustard oil bomb, since isothiocyanates are commonly known as mustard oils (Andreasson and Jorgensen, 2003; Koroleva *et al.*, 2000; Luthy and Matile, 1984; Zhao *et al.*, 2008). Isothiocyanate ingestion causes retardation of growth and development, pupal mass decrease and mortality in herbivores (Gols *et al.*, 2008a; Gols *et al.*, 2008b; Gols *et al.*, 2008c). Toxicity of isothiocyanates can be partly attributed to the reaction between electrophilic isothiocyanate and amino acid residues of proteins, which is thought to cause the disulfide bond cleavage (Kawakishi and Kaneko, 1987).

While sucking on plants, some aphids can avoid the disruption of cells and the mixing of glucosinolate and myrosinase to form toxic isothiocyanate thereof. Interestingly, specialist aphids of cole crops like *B. brassicae* and *L. erysimi* can compartmentalize and sequester the ingested glucosinolates and myrosinases similar to their host to avoid the production of toxic isothiocyanate (Bridges *et al.*, 2002). When predators attack these aphids, the compartments in their body get disrupted to mix glucosinolates and myrosinase to form isothiocyanates.

This, predator-mediated disruption of tissue releases isothiocyanate which can be detrimental for the predators. Thus, these aphids co-opt their host plant's principle defense mechanism to deter their natural enemies. Biological control of such aphids becomes challenging and requires isothiocyanate-insensitive natural enemies, to add to this complexity, different cole crops possess different repertoires of glucosinolates. In such case, natural enemies which will be able to control these aphid populations irrespective of their host plants are desired. Here, we report various generalist predators of the glucosinolate-myrosinase co-opting Brassicaceae specialist aphid *L. erysimi* and demonstrate their sensitivities when this aphid feeds on different cole crops.

2. Materials and Methods

Field survey

Cole crop fields in the plain and hilly regions in and around Pune (Maharashtra, India) were surveyed in November 2019 and different *L. erysimi* predators were identified. Predators from this initial record, which were naturally found in our experimental field in the Indian Institute of Science Education and Research, Pune were used in the further study.

Experimental field

Radish (*var.* Ivory white; Syngenta), cauliflower (*var.* Kimaya; Syngenta), knol khol (*var.* Neo; Acsen Hy Veg Pvt. Ltd.), cabbage (*var.* Royal star BC-86; Syngenta), broccoli (*var.* Green magic; Sakata Ltd.) and mustard (*var.* Black Bombay -76; Bombay Super Hybrid Seeds Ltd.) were planted in a completely randomized block design. All the plants were regularly irrigated and were provided with 15 kg/ha N, 15 kg/ha P and 5 kg/ha K every 15 days. No pesticides were sprayed.

L. erysimi aphid and predator counts were recorded in November 2019. Aphids/ plant of every host were counted. Generalist predators of *L. erysimi* namely ladybird beetle larvae (*Coccinella septempunctata*), lacewing (*Chrysoperla carnea*), jumping spider (*Plexippus paykulli*), Adanson's house jumper (*Hasarius adansonii*), wolf spider (*Wadicosa fidelis*) and crab spider (*Thomisus projectus*) on the *L. erysimi*-infested plants were also counted.

Predation bioassays

To find the aphid predation rates of different predators and their preferences to aphids feeding on different cole crops, predation assays were set up. One predator individual was provided with 100 aphids in a 200 cc polypropylene

container. For each predator species, 10 such individuals (n= 10) were provided with 100 aphids feeding on one cole crop and this setup of 10 individuals was used for the aphids feeding on all the cole crops used in this study. Predators were allowed to prey upon the given aphids for 24 hours after which the remaining aphids were counted. Predator individuals that were found dead after this 24 hour assays were also counted to calculate the predator mortality.

Statistical analyses

Frequencies (mean± SE) of the occurrences of aphids and different predator species on different host plants and the predation rates were analyzed using one-way ANOVA and the significant differences between means were determined using Fisher's LSD *post hoc* test ($p \leq 0.05$). Significant differences in predator mortalities (%) were determined using the 2×2 contingency tables in Fisher's exact test ($p \leq 0.05$).

3. Results

When planted in the completely randomized block design, *L. erysimi* showed differential occurrences on different cole crops (Fig. 2a). Highest frequency of these aphids was observed on cauliflower (596.04± 31.48), followed by cabbage (495.45± 34.25), knol khol (307.37± 32.15), mustard (228.08± 33.57), broccoli (58.91± 7.48) and the lowest was found on radish (9.79± 2.04) (Fig. 2b).

In the initial field surveys in and around Pune, various predators of *L. erysimi* were found. These included *C. septempunctata*, *Chilomenes sexmaculatus* (Six-spotted zigzag ladybird beetle), *C. carnea*, *Chrysoperla zastrowi* (lacewing), *Geocoris ochropterus* (big-eyed bug), *Crematogaster wroughtonii* (fire ant), *Pheidole indica* (big-headed ant), *Componatus compressus* (carpenter ant), *P. paykulli*, *H. adansonii*, *W. fidelis*, *T. projectus* and *Lycosa pseudoannulata* (wolf spider). Of these, *C. septempunctata*, *C. carnea*, *P. paykulli*, *H. adansonii*, *W. fidelis* and *T. projectus* were regularly found to be preying upon *L. erysimi* in our experimental field. *C. septempunctata* and *C. carnea* could be found throughout the day. Spiders were mainly found to be preying during the morning (7 am to 10 am) and evening (5 pm to 8 pm) hours. Occurrences of various *L. erysimi* predators is given in Fig. 3. *C. carnea* occurred at highest frequency on cauliflower (4.87± 0.72) followed by cabbage (2.95± 0.58) and broccoli (2.62± 0.53) and the lowest on knol khol (1.00± 0.22), mustard (1.50± 0.19) and radish (0.25± 0.13) (Fig 3a). *C. septempunctata* was most abundant on mustard (1.66± 0.19) followed by cauliflower (0.95± 0.16) and least abundant on knol khol (0.16± 0.09)

Figure 2. The specialist sucking pest *L. erysimi* showed differential occurrences on different cole crops. (a) A schematic showing the completely randomized block design wherein six cole crops namely, broccoli, cabbage, cauliflower, knol khol, mustard, and radish were planted. **(b)** Occurrence of the specialist sucking pest *L. erysimi* showing that it prefers cauliflower the most and radish the least (One way ANOVA, $F_{5,138} = 74.3$; $p < 0.0001$). Values are means (\pm SE) of frequencies on 24 plants. Significant differences were determined by One-way ANOVA with Fisher's LSD *post hoc* test; different letters indicate significant difference at $p \leq 0.05$.

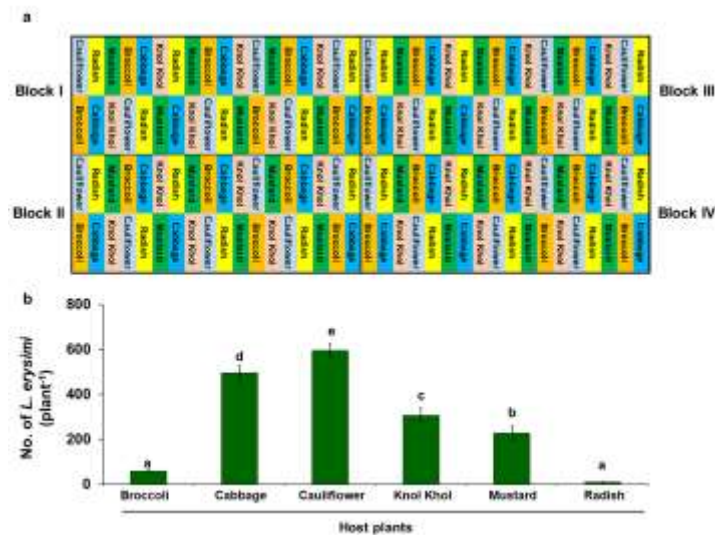


Figure 2

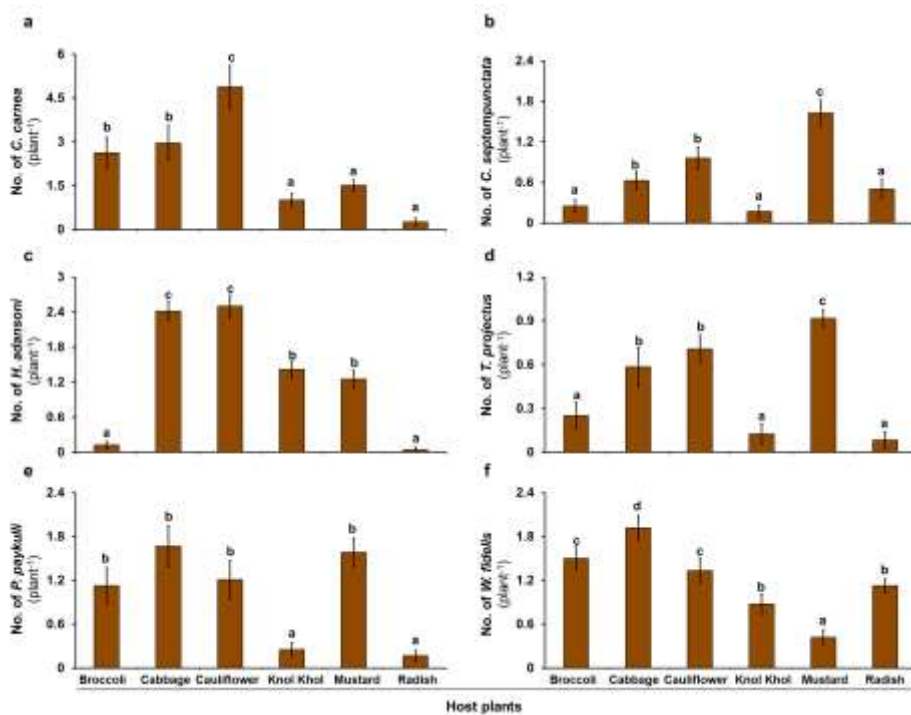


Figure 3

Figure 3. Occurrence of natural predators of *L. erysimi* on different cole crops. Occurrence of **(a) *C. carnea*** (One way ANOVA, $F_{5,138} = 13.06$; $p < 0.0001$) **(b) *C. septempunctata*** (One way ANOVA, $F_{5,138} = 14.58$; $p < 0.0001$) **(c) *H. adansonii*** (One way ANOVA, $F_{5,138} = 58.88$; $p < 0.0001$) **(d) *T. projectus*** (One way ANOVA, $F_{5,138} = 15.07$; $p < 0.0001$) **(e) *P. paykulli*** (One way ANOVA, $F_{5,138} = 9.55$; $p < 0.0001$) **(f) *W. fidelis*** (One way ANOVA, $F_{5,138} = 13.45$; $p < 0.0001$) on different cole crops. Values are means (\pm SE) of predator's numbers on 24 plants. Significant differences were determined by One-way ANOVA with Fisher's LSD *post hoc* test; different letters indicate significant difference at $p \leq 0.05$.

(Fig 3b). *H. adansoni* showed highest occurrence on cabbage (2.41 ± 0.15) and cauliflower (2.50 ± 0.19) and lowest on radish (0.04 ± 0.04) (Fig 3c). *T. projectus* mainly occurred on mustard (0.91 ± 0.05) and cauliflower (0.70 ± 0.09) inflorescences and inside the cabbage whorls (0.58 ± 0.13); on other hosts, *T. projectus* was relatively rare (Fig 3d). Occurrence frequencies of *P. paykulli* on different cole crops were as follows: broccoli (1.12 ± 0.25), cabbage (1.66 ± 0.28), cauliflower (1.20 ± 0.26), knol khol (0.25 ± 0.09), mustard (1.58 ± 0.19) and radish (0.16 ± 0.07) (Fig 3e). Abundance of *W. fidelis* on different host plants was as follows: broccoli (1.5 ± 0.15), cabbage (1.91 ± 0.16), cauliflower (1.33 ± 0.16), knol khol (0.87 ± 0.13), mustard (0.41 ± 0.10) and radish (1.12 ± 0.09) (Fig 3f). *H. adansoni* and *T. projectus* clearly showed differential preferences towards *L. erysimi* fed on different cole crops (Fig. 4). Four predators showed quite uniform predation on aphids irrespective of their hostplant, with predation of *C. carnea* between 10.09 ± 1.98 and 14.70 ± 1.84 (Fig. 4a), *C. septempunctata* between 47.70 ± 3.54 and 57.20 ± 4.66 (Fig. 4b), *P. paykulli* between 76.00 ± 6.64 and 85.60 ± 7.49 (Fig. 4e) and *W. fidelis* between 20.30 ± 2.06 and 26.60 ± 2.55 (Fig. 4f). *H. adansoni* showed high predation frequencies on mustard (51.00 ± 3.59), cauliflower (45.70 ± 6.93) and knol khol (46.20 ± 5.80) fed aphids, followed by 24.20 ± 3.30 on cabbage and lowest on radish (18.90 ± 2.91) and broccoli (18.30 ± 2.55) fed ones (Fig. 4c). *T. projectus* also showed a similar pattern; it showed high predation on mustard (20.30 ± 2.08) and cauliflower (18.10 ± 2.07) fed aphids, followed by knol khol (12.80 ± 5.78), cabbage (12.50 ± 1.78) and lowest on radish (7.70 ± 1.17) and broccoli (7.40 ± 0.93) fed ones (Fig. 4d).

H. adansoni and *T. projectus* showed mortality after preying upon the aphids that they showed low predation frequency on (Fig. 5). *H. adansoni* showed 40%, 30% and 10% mortality on radish-, broccoli- and cabbage-fed aphids, respectively (Fig. 5a). Similarly, *T. projectus* showed 50%, 30% and 10% mortality on radish-, broccoli- and cabbage-fed aphids, respectively (Fig. 5b). Other two spiders also showed mortality after feeding on aphids. *P. paykulli* showed 10% mortality after preying upon cauliflower-fed aphids (Fig. 5c) and *W. fidelis* showed 10% mortality after preying upon broccoli-, knol khol- and radish-fed aphids (Fig. 5d). *C. carnea* and *C. septempunctata* did not show mortality.

4. Discussion

In the recent years, use of natural enemies in pest management has phenomenally increased because of the hazards of synthetic pesticides. As mentioned before, a

biocontrol agent that can be used irrespective of the pest's host crop can be the most useful broad spectrum agent. We found that the predation frequencies of *C. carnea*, *C. septempunctata*, *P. paykulli* and *W. fidelis* remained unaltered for all the hosts of *L. erysimi*. Highest aphid predation rates were shown by *P. paykulli* followed by *C. septempunctata*. However, *P. paykulli* showed mortality while preying upon cauliflower-fed aphids. Since occurrence frequencies of the studied predators did not drastically differ from each other, we infer that both *C. septempunctata* and *P. paykulli* can be effective biocontrol agents against *L. erysimi*. *C. septempunctata* can be used for wide range of cole crops whereas *P. paykulli* may show mortality with some of *L. erysimi*'s hosts.

Mishra and Singh, 2019 found negative correlation between glucosinolate content of host plants and aphid abundance. Although glucosinolate profiles of cole crops starkly differ, major type of glucosinolate in them is aliphatic one (Bhandari *et al.*, 2015; Carrea *et al.* 2008; Kim *et al.*, 2010; Kushad *et al.*, 1999; Park *et al.*, 2012). Broccoli mainly contains glucoraphanin (Bhandari *et al.*, 2015; Carlson *et al.*, 1987). sinigrin, glucobrassicin and progoitrin are the major components of cabbage glucosinolate repertoire (Newton *et al.*, 2009; Rungapamestry *et al.*, 2006). Glucoraphenin and glucoiberberin are principle glucosinolates of cauliflower (Bhandari *et al.* 2015; Carlson *et al.*, 1987). Knol khol mainly contains glucoiberberin and glucoerucin (Carlson *et al.*, 1987; Reichelt *et al.*, 2002). Gluconapin and gluconasturtiin constitute about 97% of mustard's glucosinolate pool (Carlson *et al.* 1987). White radish on the other hand, contains indolic 4-hydroxy-glucoerucin in considerable amounts along with the major aliphatic glucosinolates glucoraphanin and glucohydroerucin (Ciska *et al.*, 2008; Force *et al.*, 2007). Indeed, the profiles of occurrence of aphids and predators and predation and mortalities of *H. adansoni* and *T. projectus* associated with radish were indicative of this difference. It can be inferred that even a little addition of indolic glucosinolate in the aliphatic pool can starkly alter the performance of insects and arachnids associated with the host plants.

We recently showed that host plant's glucosinolates and their metabolism by the herbivore prey *Plutella xylostella* do not affect *C. carnea* (Sun *et al.*, 2019). Our current results on *L. erysimi* feeding *C. carnea* are congruent with those earlier findings. Apart from thwarting the herbivore growth, some volatile isothiocyanates play a role of parasitoid attractants. Various isothiocyanates have been observed to attract the

Figure 4. *In vitro* predation assay with natural predators and *L. erysimi* fed on different cole crops. Predation efficiencies of different natural predators, namely (a) *C. carnea* (One way ANOVA, $F_{5,54} = 0.69$; $p = 0.63$) (b) *C. septempunctata* (One way ANOVA, $F_{5,54} = 0.84$; $p = 0.52$) (c) *H. adansonii* (One way ANOVA, $F_{5,54} = 11.38$; $p < 0.0001$) (d) *T. projectus* (One way ANOVA, $F_{5,54} = 9.56$; $p < 0.0001$) (e) *P. paykulli* (One way ANOVA, $F_{5,54} = 0.28$; $p = 0.91$) (f) *W. fidelis* (One way ANOVA, $F_{5,54} = 0.43$; $p = 0.82$) were assessed in an *in vitro* assay. Values are means (\pm SE) of frequencies of aphids preyed upon by the respective predators ($n = 10$). Significant differences were determined by One-way ANOVA with Fisher's LSD *post hoc* test; different letters indicate significant difference at $p \leq 0.05$

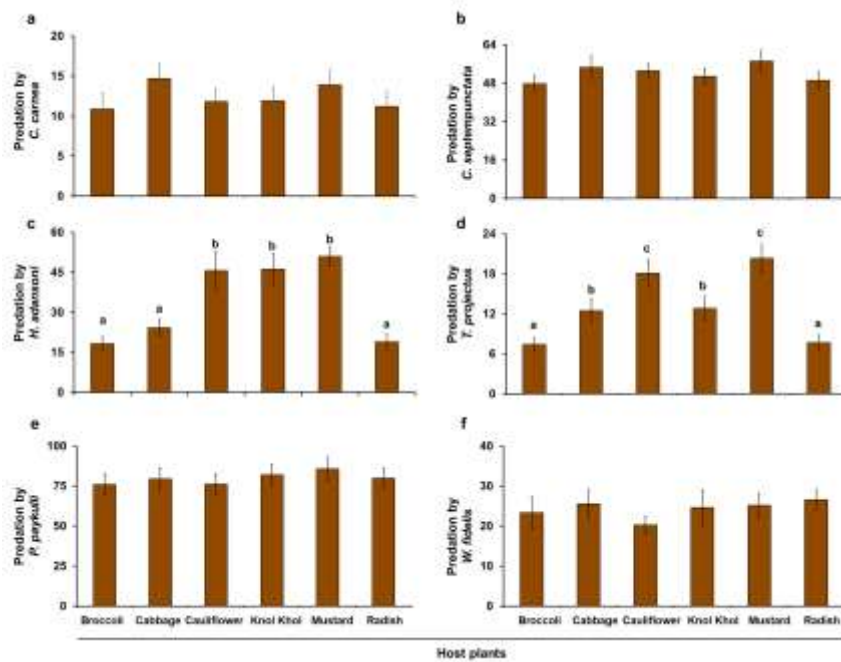


Figure 4

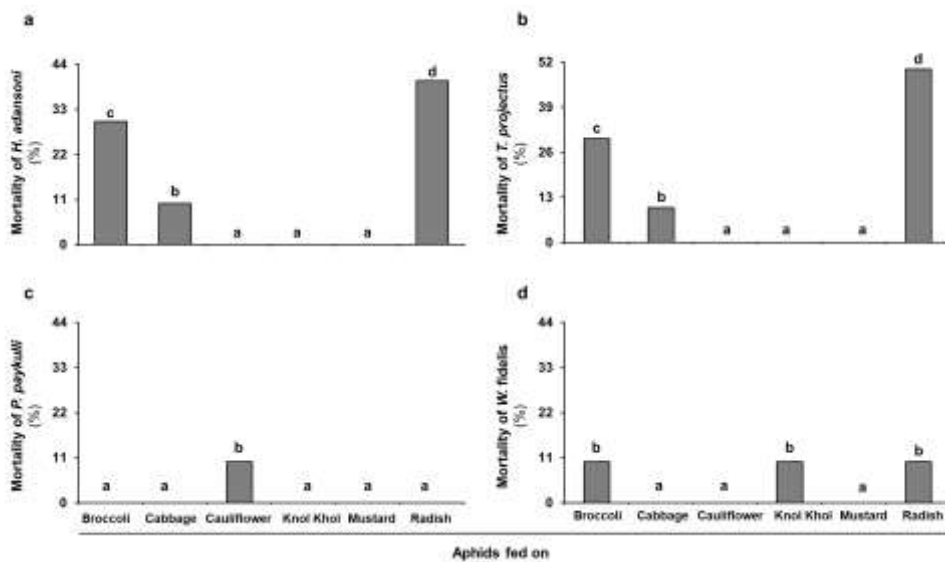


Figure 5.

Figure 5. *H. adansonii* and *T. projectus* showed higher mortality when preying upon the radish and broccoli feeding aphids than the other predators. Percentage mortality of all the natural predators was determined after preying upon aphids. (a) *H. adansonii* (Fisher's exact test; $p < 0.0001$) (b) *T. projectus* (Fisher's exact test; $p < 0.001$) (c) *P. paykulli* (Fisher's exact test $p \leq .001$) (d) *W. fidelis* (Fisher's exact test; $p \leq .001$) showed mortality upon feeding on aphids. However, *C. carnea* and *C. septempunctata* did not show any mortality. Values are binary data of 10 predators fed on aphids. Significant differences were determined by Fisher's exact test; different letters indicate significant difference at $p \leq 0.05$.

parasitoids of aphids, beetles and lepidopteran herbivores (Bruce 2014; Jonsson and Anderson 2007; Pope *et al.*, 2008; Smid *et al.*, 2002; Van Poecke *et al.* 2001). Pope *et al.*, 2008) found that the aphid parasitoid *Diacretiella rapae* has an innate response to the volatile isothiocyanates but not to the other volatile glucosinolate derivatives. Thus different glucosinolates have different species-specific effects. Therefore, significant variation in the predator preferences on the preys ingesting different glucosinolate compositions is highly likely. In the light of this, our findings that *C. carnea*, *C. septempunctata*, *P. paykulli* and *W. fidelis* are hardly perturbed by their prey's hostplant's glucosinolate arsenal are highly important for the pest management programs of cole crops.

It is notable that most of the pests detoxify glucosinolates and isothiocyanates they ingest from their hosts (Schramm *et al.*, 2012; Sun *et al.*, 2019; Wittstock *et al.*, 2003), Obviously, detoxification dilutes of the detrimental effects of these plant defenses before reaching the natural enemies that prey upon these pests. Instead of detoxifying, *L. erysimi* sequesters these plant defenses and repurposes them against its natural enemies. This is like amplifying their effect and actually, acting itself as a mustard oil bomb. Such an adaptation is highly likely to deter predators. On the other hand, In the view of these facts, the consistency shown by the predators has been promising. It also suggests that these predators also have counteradapted against the glucosinolate-myrosinase defense system. It will be a useful future study to reveal their counteradaptation mechanisms. All of these are generalist predators and are known to prey upon a vast diversity of herbivores including lepidopteran larvae, aphids, thrips, bugs, etc. Thus, these predators can be used for the management of wide range of pests. Moreover, these predators have been recorded from diverse habitats like cold hilly regions and warmer plains. They are capable of adapting to diverse environmental conditions; therefore, they can be used in the different geoclimatic zones of India.

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6. References

- Andreasson E, Jorgensen LB (2003) Localization of plant myrosinases and glucosinolates. *Recent Adv Phytochem* 37:79-99
- Bhandari SR, Jo JS, Lee JG (2015) Comparison of glucosinolate profiles in different tissues of nine *Brassica* crops. *Molecules* 20:15827-15841
- Bridges M *et al.*, (2002) Spatial organization of the glucosinolate-myrosinase system in brassica specialist aphids is similar to that of the host plant. *Proceedings of the Royal Society of London Series B: Biological Sciences* 269:187-191
- Bruce TJA (2014) Glucosinolates in oilseed rape: secondary metabolites that influence interactions with herbivores and their natural enemies. *Ann Appl Biol* 164:348-353 doi:Doi 10.1111/Aab.12128
- Carlson DG, Daxenbichler M, VanEtten C, Kwolek W, Williams P (1987) Glucosinolates in crucifer vegetables: broccoli, Brussels sprouts, cauliflower, collards, kale, mustard greens, and kohlrabi. *Journal of American Society of Horticultural Science* 112(1):173-178. *Journal of American Society of Horticultural Science* 112(1):173-178.
- Cartea ME, Velasco P, Obregón S, Padilla G, de Haro A (2008) Seasonal variation in glucosinolate content in *Brassica oleracea* crops grown in northwestern. *Spain Phytochemistry* 69:403-410
- Ciska E, Honke J, Kozłowska H (2008) Effect of light conditions on the contents of glucosinolates in germinating seeds of white mustard, red radish, white radish, and rapeseed. *Journal of agricultural and food chemistry* 56:9087-9093
- Colon B, Toor G (2016) A review of uptake and translocation of pharmaceuticals and personal care products by food crops irrigated with treated wastewater. In: *Advances in agronomy*, vol 140. Elsevier, pp 75-100
- Force LE, O'Hare TJ, Wong LS, Irving DE (2007) Impact of cold storage on glucosinolate levels in seed-sprouts of broccoli, rocket, white radish and kohlrabi. *Postharvest biology and technology* 44:175-178
- Gols R, Bukovinszky T, van Dam NM, Dicke M, Bullock JM, Harvey JA (2008a) Performance of generalist and specialist herbivores and their endoparasitoids differs on cultivated and wild *Brassica* populations. *Journal of Chemical Ecology* 34:132-143 doi:DOI 10.1007/s10886-008-9429-z

- Gols R, Wagenaar R, Bukovinszky T, van Dam NM, Dicke M, Bullock JM, Harvey JA (2008b) Genetic variation in defense chemistry in wild cabbages affects herbivores and their endoparasitoids. *Ecology* 89:1616-1626 doi:10.1890/07-0873.1
- Gols R, Witjes LMA, van Loon JJA, Posthumus MA, Dicke M, Harvey JA (2008c) The effect of direct and indirect defenses in two wild brassicaceous plant species on a specialist herbivore and its gregarious endoparasitoid. *Entomologia Experimentalis Et Applicata* 128:99-108 doi:10.1111/j.1570-7458.2008.00681.x
- Guarena M (2006) Cole crops and other Brassicas: Organic production. ATTRA National Sustainable Agriculture Information Service, Fayetteville, AR, USA.
- Halkier BA, Gershenzon J (2006) Biology and biochemistry of glucosinolates. *Annual Review of Plant Biology* 57:303-333 doi:10.1146/annurev.arplant.57.032905.105228
- Jonsson M, Anderson P (2007) Emission of oilseed rape volatiles after pollen beetle infestation; behavioural and electrophysiological responses in the parasitoid *Phradis morionellus*. *Chemoecology* 17:201-207 doi:10.1007/s00049-007-0379-7
- Kawakishi S, Kaneko T (1987) Interactions of proteins with allyl isothiocyanate. *Journal of Agricultural and Food Chemistry* 35:85-88 doi:10.1021/Jf00073a020
- Kim JK *et al.*, 2010 Variation of glucosinolates in vegetable crops of *Brassica rapa* L. ssp. *pekinensis* *Food chemistry* 119:423-428
- Koroleva OA, Davies A, Deeken R, Thorpe MR, Tomos AD, Hedrich R (2000) Identification of a new glucosinolate-rich cell type in *Arabidopsis* flower stalk. *Plant Physiology* 124:599-608 doi:10.1104/Pp.124.2.599
- Kushad MM, Brown AF, Kurilich AC, Juvik JA, Klein BP, Wallig MA, Jeffery EH (1999) Variation of Glucosinolates in Vegetable Crops of *Brassica oleracea*. *Journal of agricultural and food chemistry* 47:1541-1548
- Mishra V, Singh N (2019) Evaluation of glucosinolate in different varieties/lines against mustard aphid, *Lipaphis erysimi* (Kalt.) on mustard crop. *Journal of Applied Zoological Researches* 30:64-68
- Newton EL, Bullock JM, Hodgson DJ (2009) Glucosinolate polymorphism in wild cabbage (*Brassica oleracea*) influences the structure of herbivore communities. *Oecologia* 160:63-76
- Park WT *et al.* (2012) Metabolic profiling of glucosinolates, anthocyanins, carotenoids, and other secondary metabolites in kohlrabi (*Brassica oleracea* var. *gongylodes*). *Journal of agricultural and food chemistry* 60:8111-8116
- Parween T, Jan S, Mahmooduzzafar S, Fatma T, Siddiqui ZH (2016) Selective effect of pesticides on plant—A review, *Critical reviews in food science and nutrition* 56:160-179
- Pope TW, Kissen R, Grant M, Pickett JA, Rossiter JT, Powell G (2008) Comparative innate responses of the aphid parasitoid *Diaeretiella rapae* to alkenyl glucosinolate derived isothiocyanates, nitriles, and epithionitriles, *Journal of Chemical Ecology* 34:1302-1310 doi:10.1007/s10886-008-9531-2
- Reichelt M *et al.* (2002) Benzoic acid glucosinolate esters and other glucosinolates from *Arabidopsis thaliana*. *Phytochemistry* 59:663-671 doi:10.1016/S0031-9422(02)00014-6 Doi 10.1016/S0031-9422(02)00014-6
- Rungapamestry V, Duncan AJ, Fuller Z, Ratcliffe B (2006) Changes in glucosinolate concentrations, myrosinase activity, and production of metabolites of glucosinolates in cabbage (*Brassica oleracea* var. *capitata*) cooked for different durations. *Journal of Agricultural and Food Chemistry* 54:7628-7634
- Schramm K, Vassao DG, Reichelt M, Gershenzon J, Wittstock U (2012) Metabolism of glucosinolate-derived isothiocyanates to glutathione conjugates in generalist lepidopteran herbivores *Insect Biochemistry and Molecular biology*: DOI 10.1016/j.ibmb.2011.12.002
- Singh B Vegetable production in cold desert of India: a success story on solar greenhouses. In: *International Conference and British-Israeli Workshop on Greenhouse Techniques towards the 3rd Millennium* 534, 2000. pp 205-212
- Smid HM, van Loon JJA, Posthumus MA, Vet LEM (2002) GC-EAG-analysis of volatiles from Brussels sprouts plants damaged by two species of *Pieris* caterpillars: olfactory receptive range of a specialist and a generalist parasitoid wasp species *Chemoecology* 12:169-176 doi:10.1007/Pl00012665
- Spaldon S, Masoodi T, Namgial D, Angmo T, Yangdol D (2018) Performance of knolkhol in chinese type polyhouse during peak winter in cold arid Ladakh *Indian Journal of Agricultural Research* 52:330-332

- Sun R, Jiang X, Reichelt M, Gershenzon J, Pandit SS, Vassão DG (2019) Tritrophic metabolism of plant chemical defenses and its effects on herbivore and predator performance *eLife* 8
- Thakur NA, Firake D, Behere G, Firake P, Saikia K (2012) Biodiversity of agriculturally important insects in north eastern Himalaya: an overview *Indian Journal of Hill Farming* 25:37-40
- Van Poecke RMP, Posthumus MA, Dicke M (2001) Herbivore-induced volatile production by *Arabidopsis thaliana* leads to attraction of the parasitoid *Cotesia rubecula*: Chemical, behavioral, and gene-expression analysis *Journal of Chemical Ecology* 27:1911-1928 doi:DOI 10.1023/A:1012213116515
- Zhao ZX, Zhang W, Stanley BA, Assmann SM (2008) Functional proteomics of *Arabidopsis thaliana* guard cells uncovers new stomatal signaling pathways *Plant Cell* 20:3210-3226 doi:DOI 10.1105/tpc.108.063263