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Role of glucosinolates in *Brassica juncea* on the Incidence and Development of *Lipaphis erysimi* and its Parasitoid *Diaeretiella rapae*

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Abstract:
Brassica juncea, the Indian mustard suffers from Lipaphis erysimi infestation to a great extent. The role of glucosinolates and its breakdown plant volatiles, time of sowing etc. seems to be related to the incidence and development of this aphid. The studies indicated that the parasitoid co-evolved along with the aphids to utilize the products of glucosinolates for the identification of the habitat of the host. These studies clearly specified the role of allyl isothiocyanate and hydroxyl indolyl isothiocyanate as kairomone. Under field conditions, aphid infestation and extent of parasitism was significantly higher at flowering stage than that on the vegetative stage. The qualitative and quantitative differences in the content of volatile aglucones of the cultivar and growth stages indicated that changes affected the extent of parasitism. It was observed that response of the parasitoid was higher towards the volatiles from the reproductive stage where the concentration of allyl isothiocyanate and hydroxyl indolyl isothiocyanatewas less than in the vegetative stage. The information generated during the investigations can be utilized to exploit the potential of biological control of the aphid when the population is still low so that the glucosinolates are not digested into aglucones by myrosinase, as these breakdown products play a very important role in attracting the aphids and the parasitoids towards the cultivars. It is also proposed that if the infestation of the aphid still increases then by parasitization of it by D.rapae can decrease its attack.

Keywords: *Lipaphis erysimi, allyl isothiocyanate, hydroxyl indolyl isothiocyanate, myrosinase, parasitization*

1. Introduction

A number of important vegetables, oil seeds, fodder crops, green manure and condiments belong to genus Brassica of Cruciferae. *B. juncea* (Indian, oriental or brown mustard) occupies the largest acreage among all oleiferous brassicae grown in India with high oleic, moderate linoleic and low linolenic acid contents in the oil, making it beneficial for human consumption [Kaushik and Agnihotri, 2003]. It has enhanced seedling vigour, blackleg resistance, shatter resistance, plus high tolerance to drought and high temperature stresses[Kirk and Oram, 1981].This species contain a group of secondary metabolites called glucosinolates [Jwanny et al., 1995] which upon mechanical damage, infection, pest attack or cellular breakdown gets exposed to degradative enzymes called myrosinase or β-thioglucoside glucohydrolase. Glucosinolates are produced in the cytoplasm and stored in the vacuole until the cells are disrupted to give breakdown products. To prevent damage to the plant itself, the myrosinase and glucosinolates are stored in separate compartments of the cell and come together only under conditions of stress or injury [Bones and Rossiter, 1996; Bridges et al., 2001]. These breakdown products are known as 'mustard oils' and they give mustard its flavor [Olsen and Sorensen, 1981; Fahey et al, 2001]. The degradation of glucosinolate in the said products affects the value of glucosinolate containing plants when used as food for humans or for feeding animals [Croft, 1979; Chew, 1988]. In a review by Raybould and Moyes, 2001, they pointed out that although studies indicate that herbivory can impact plant population dynamics but the level to which variation in glucosinolate phenotypes alters herbivory is still not known. The host range of insects attacking crucifers is restricted to a few closely related plant families which contain glucosinolates. These compounds act as semiochemicals for these specialist feeders.The mustard aphid, *Lipaphis erysimi*, Kaltenbach (Hemiptera: Aphididae) is undoubtedly the most destructive specialist pest in India and other tropical and sub-tropical parts of the world [Pandey and Singh, 2008]. The yield loss of mustard crop caused by *L. erysimi* is very high in India [Hossain et al., 2006]. The mustard aphid has overcome the barrier of glucosinolates being involved in defense against insects by feeding on the phloem [Nault and Styer, 1972; Agarwala, 2007] as well as sequestering these compounds and retaining them in the body [Bridges et al., 2002]. Aphid infestations cause decrease in yield by way of decreasing number of pods per plant, number of grains per pod and oil content of the grains. In addition to this, *Diaeretiella rapae*, M'Intosh (Hymenoptera: Braconidae) is a key parasitoid of *L. erysimi* throughout the country [Blande et al., 2004]. *D. rapae*is a promising bioagent against *L. erysimi*was reported by [Ohiman and Kunar, 1986; Shukla and Tripathi, 1993]. The shape and pattern does not play a significant role in the detections of plants or aphids by *D. rapae* but colour preferences are an important feature for plant recognition in *D. rapae*. Once in the selected habitat, discovery of the hosts occur by random searching[Liu and Sengoneo, 1994]. The enemies of herbivores may use the same or similar chemicals to find the plant first and then the herbivore on the plant [Read et al., 1970].By studying the role of glucosinolates found in *Brassica juncea* in attracting its parasite *Lipaphis erysimi* and the behavioral response of its parasitoid *Diaeretiella rapae*

towards the plant volatiles,an attempt was made to restrict the recurring menace of the specialist parasite, *L. erysimi* by using minimum and judicious bio rational methods.

2. Materials and Methods

The plantswere raised in a plot of randomized block design with three dates of sowing at an interval of 15 days each in subplots between November to December with three replications each.The seeds were also sown in ten earthen pots for raising aphids for bioassay using staggered sowing at weekly interval. To observe the migration of mustard aphids, *L. erysimi* from the other hosts, yellow traps were installed in the plots of first sowing. These yellow traps were replaced with new traps after two days and the observations were recorded from the last week of February to the second week of March.Parasitoid *D. rapae* started appearing in the third week of February. The twigs having mummified aphids were collected from the mustard field and as suggested byDeshraj and Lakhanpal, 1998, these twigs were surface sterilized using 1000 ppm silver nitrate solution for 10 minutes. The leaves having mummified aphids containing *D. rapae* were removed from the plant and stored in petri dishes until parasitoid emergence[Bueno et al., 1992]. Adult females were mated at emergence and then held for 24 hours in glass tubeswhere they were supplied with a dilute honey solution (80%) but no aphids or plants. The parasitoids used for all experiments were 24-48 hours old [Desneux et al., 2005].On each potted plant having the aphid clones, two pairs of these mated adults of *D. rapae* were released and allowed to multiply. By this method, stock culture of *D. rapae*was prepared on the aphid host.To study the moving behaviour of the parasitoid, olfactometers were fabricated [Elst et al., 1991].Response of the parasitoid *D. rapae* to the test solutions was calculated for the index of attractiveness (IOA) as follows:

$$\text{IOA} = \text{No. of parasitoid responded to treatment} - \frac{\text{No. of parasitoid respondent to control}}{\text{Total no of parasitoids}}$$

IOA = Index of Attractiveness

To collect samples of plant volatiles from whole plant, three-four plants of *B. juncea* were uprooted from the field and after washing and drying with blotting papers their roots were clipped-off and remaining plant was finely chopped and mixed properly. Then a representative sample of 50g was taken for steam distillation. This flask was incubated on a water bath at 30°C for 85 minutes for the conversion of glucosinolates into their volatile aglucones. The volatiles were extracted by steam distillation. The volatiles were partitioned using diethyl ether. The ether was evaporated under reduced pressure at 30°C and the residue was dissolved in n-pentene.One ml was preserved in a glass ampoule for chemical analysis and from remaining 5 ml, five serial dilutions were prepared further with n-pentene.

Volatiles were also collected from the head spaces of the vegetative and reproductive stages of *B. juncea*. To collect these, a suction funnel was placed just above the canopy of plants. Air was passed through scrubber containing 5 ml of methanol and on one side of the scrubber inlet aMillipore suction pump was fixed whose suction was maintained at a negative pressure of 600 mm Hg with the help of a pinch cock. Outlet of the suction pump helps in passing out the air sucked in whereasthe volatiles get collected in methanol. The whole apparatus was run for 30 minutes and collections were emptied into the glass vials separately maintaining a final volume of 5 ml. Again one ml of the head space volatiles was preserved in a glass ampoule for chemical analysis and with the remaining 4 ml; three serial dilutions were prepared with methanol for bioassays.Standardized method for the analysis of aglucones in the samples was adopted to carry out the chemical analysis [Dilawari et al., 1998].

3. Results and Discussion

The role of glucosinolates in *Brassica juncea*on the incidence and development of its parasite *Lipaphis erysimi*and the parasitoid *Diaeretiella rapae* was assessed from experimental data collected for two years.The observations revealed that the aphid population build-up in *B. juncea*and the parasitism of the aphids started in the fourth week of February (Table 1).

Stages (S)	No. of parasitoids per cm. of the aphid population							
	Dates of observations(O)							
	First year				Second year			
	24th Feb. (O_1)	3rd March (O_2)	10th March (O_3)	Mean (S)	23rd Feb. (O_1)	1st March (O_2)	8th March (O_3)	Mean (S)
S_1 (Vegetative)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
S_2 (Bolting)	1.00 (1.42)	0.00 (0.00)	0.00 (0.00)	0.33 (0.47)	1.00 (1.38)	0.00 (0.00)	0.00 (0.00)	0.33 (0.46)
S_3 (Flowering)	1.66 (1.82)	4.00 **(5.14)**	0.00 (0.00)	1.83 (2.32)	1.56 (1.78)	4.00 **(5.20)**	0.00 (0.00)	1.85 (1.99)
S_4 (Podding)	0.00 (0.00)	3.00 (3.59)	**8.33** (0.85)	3.77 (1.48)	0.00 (0.00)	3.00 (3.50)	**8.37** (0.84)	3.79 (1.44)
Mean (O)	0.66 (0.81)	1.75 (2.18)	2.08 (0.21)	1.49 (1.06)	0.64 (0.79)	1.75 (2.17)	2.09 (0.21)	1.49 (1.06)
	F-Test	S.Ed.(±)	C.D. at 5%		F-Test	S.Ed.(±)	C.D. at 5%	
Stages (S)	S	0.15	0.32		S	0.11	0.23	
Date of Observation(O)	S	0.13	0.28		S	0.10	0.20	
Interaction (S x O)	S	0.27	0.55		S	0.19	0.40	

Table 1: Parasitism of mustard aphid and its parasitoid on different stages of B. juncea

* Values in parenthesis pertain to the aphid population in centimeters

When the four stages i.e. vegetative, bolting, flowering and podding present in the field at the same time were studied, it was observed that the aphid and parasitoid population were reported to be on peak during flowering and pod stages respectively [Sekhon et al., 1996; Hossain et al., 2006] indicating that the inherent plant factors including the characteristic volatiles of glucosinolates could be playing a role in the selective behaviour of the parasitoid.

As mentioned in Table 2, the bioassays carried out in the selected olfactometer using n-pentene as control showed that the parasitoid *D. rapae* responds negatively to the first three dilutions collected from the vegetative stages as well as flowering or reproductive stage but showed attraction towards the volatiles at 1:100 dilution. The parasitoid did not show any positive oriented response to higher dilutions of plant volatiles.

No. of parasitoids responding to the treatment						
Vegetative Stages				Reproductive Stages		
First year				First year		
Dilution	Treatment	Control	IOA*	Treatment	Control	IOA*
1:0	0	15 (100)	-1.00	0	15 (100)	-1.00
1:1	0	15 (100)	-1.00	0	15 (100)	-1.00
1:10	3 (20)	12 (80)	-0.60	1 (6.66)	14 (93.33)	-0.86
1:100	11 (73.2)	4 (26.80)	0.46	14 (93.33)	1 (6.66)	0.86
1:1000	No oriented movement	No oriented movement	No oriented movement	No oriented movement	No oriented movement	No oriented movement
Second year						
Dilution	Treatment	Control	IOA*	Treatment	Control	IOA*
1:0	0	15 (100)	-1.00	0	15 (100)	-1.00
1:1	0	15 (100)	-1.00	0	15 (100)	-1.00
1:10	2 (13.33)	13 (86.66)	-0.73	1 (6.66)	14 (93.33)	-0.86
1:100	13 (86.66)	2 (13.33)	0.46	13 (86.66)	2 (13.33)	0.73
1:1000	No oriented movement	No oriented movement	No oriented movement	No oriented movement	No oriented movement	No oriented movement

Table 2: Behavioural response of D. rapae towards plant volatiles collected from the vegetative stage and reproductive stages

Figures in parenthesis are percentage values
Mean of 15 replications

Number of parasitoids responding to treatment			
Concentration	Treatments	Control	IOA*
0.1 µm/ml	7(46.66)	8(53.33)	-0.06
0.01 µm/ml	10(66.66)	5(33.33)	0.33
0.001 µm/ml	14(93.33)	1(6.66)	0.86

Table 3: Behavioural response of D. rapae towards the standard allyl isothiocyanate concentrations
Mean of 15 replications. Control: n-pentene

In a test with standard allylisothiocyanate compound, the parasitoid showed the maximum response to 0.001 µm/ml concentration (Table 3). Chemical analysis of steam distilled volatiles from the vegetative stages and reproductive stages of*B.juncea*(Table4) showed that onlyallyl isothiocyanate and hydroxyl indolyl isothiocyanate were present in the test cultivar. It can be reasoned that as no breakdown plant volatiles were present during the vegetative stages and the concentration of allyl isothiocyanate along with hydroxy-indolyl isothiocyanate was also high thus *the* aphid *L. erysimi* did not infest the plants in the field during the vegetative stages (Table 1)and in the laboratory showed positive response at 1:100 dilution only.

Stages/Parts of plant	Relative retention time	Identification	Concentration(µ m/ml)	
			First year	Second year
Vegetative stages of Whole plant	1.00	Allyl isothiocyanate	0.88	0.80
	1.29	Hydroxy-indolyl isothiocyanate	1.02	1.07
Reproductive stages of Whole plant	1.00	Allyl isothiocyanate	0.27	0.28
	1.30	Hydroxy-indolyl isothiocyanate	0.06	0.05
Head space volatiles from Vegetative stages	1.00	Allyl isothiocyanate	0.32	0.34
Head space volatiles from Reproductive stages	1.00	Allyl isothiocyanate	0.18	0.19

Table 4: Chemical analysis of the steam distilled aglucones of glucosinolates from different stages/parts of B.juncea

Chemical analysis of head space volatiles from the vegetative stages and reproductive stages of the test cultivar showed that 0.32 µm/ml and 0.34 µm/ml allyl isothiocyanate was present in the vegetative stages and 0.18 µ m/ml and 0.19 µ m/ml in the reproductive stages in the two years respectively (Table 4). Hydroxy indolyl isothiocyanate was not reported in both the samples.

No. of parasitoids responding to the treatment						
Vegetative Stages				Reproductive Stages		
First year						
Dilutions	Treatment	Control	IOA*	Treatment	Control	IOA*
1:0	12(80)	3(20)	0.60	7(46.66)	8(53.33)	-0.06
1:1	11(73.33)	4(26.66)	0.46	12(80.00)	3(20.00)	0.60
1:10	No oriented movement	No oriented movement	No oriented movement	No oriented movement	No oriented movement	No oriented movement
Second year						
Dilutions	Treatment	Control	IOA*	Treatment	Control	IOA*
1:0	13(86.66)	2(13.33)	0.73	6(40.00)	9(60.00)	-0.20
1:1	11(73.33)	4(26.66)	0.46	13(86.66)	2(13.33)	0.73
1:10	No oriented movement	No oriented movement	No oriented movement	No oriented movement	No oriented movement	No oriented movement

Table 5: Behavioural response of D. rapae to head space volatiles from vegetative stages and reproductive stages

Mean of 15 replications

Figures in parenthesis are percentage value

Head space volatiles were also collected from the vegetative and reproductive stages of the test cultivar both the years in methanol for bioassays. The parasitoid showed an oriented movement to the first two dilutions of volatiles collected from vegetative stage. Likewise the parasitoid showed an oriented negative response to the first dilution (1:0) of the head space volatiles collected from the reproductive stage but were attracted towards 1:1 dilution (Table 4). It did not show any oriented movement to higher dilution in both the stages. The response of the parasitoid to the standard compounds i.e. allyl isothiocyanate at 0.001 µ m/ml (Table 3) was comparable to the response of the parasitoid towards head space volatiles from reproductive stages at 1:1 dilution(Table 5).

In the present study, it has however been proven that volatiles of the host plant help in finding the habitat of host of parasitoid and subsequently the host of the insect. These results were in accordance with the findings of [Read et al., 1995], who reported that *D. rapae* response shows as innate odour preference for crucifer feeding aphids.The chemical precursors of glucosinolates like isothiocyanates had shown biological activity against insect-pests of mustard [Issacs and Hardie, 1993]. Similarly in the present investigations also, as the mustard aphids attack the cultivars causing mechanical damage to the plant cells, the cellular breakdown exposes stored glucosinolates to degradative enzymes which lead to the cleavage of the thioglucoside linkage yielding D-glucose and an unstable thiohydroximate-o-sulphonate which spontaneously rearranges to form thiocyanates, isothiocyanates or nitriles [Bones and Rossiter, 1996]. These volatiles were found to attract the parasitoid *D. rapae* towards the host plant of the aphids in the flowering stage.It is noteworthy that here the hydroxyl indolyl isothiocyanate concentration had also decreased. The studies beyond doubt clearly specified the role of glucosinolates in mustard aphid,*Lipaphis erysimi* and its parasitoid *Diaeretiella rapae* interactions, therefore, it can be said that if the attack of aphids can be decreased initially, then the breakdown products of glucosinolates will not attract the aphids and parasitoids further to increase the infestation. It is studied that the glucosinolate profile changes with the growth of plants which affects the apparency of the plants to the aphids [Mithen, 1992] and possibly to the parasitoid also.

4. Summary and Conclusion

Brassica oilseed species occupy a prominent place in world's agrarian economy as vegetables, oilseeds, feed and fodder, green manure & condiment. Since no information was available on the role of volatiles from different stages of crop growth of *B. juncea* on the incidence and development of the mustardaphid L. *erysimi* and its interaction with its parasitoid*D. rapae*, it became essential to study this relationship.Under field conditions, aphid infestation and extent of parasitism was significantly higher at flowering stage than that on the vegetative stage. The qualitative and quantitative differences in the content of volatile aglucones of the cultivar and growth stages indicated that changes affected the extent of parasitism. In order to explain the adaptation of the parasitoid to aphids and host plants, the steam distilled head space volatile aglucones of test cultivar was made available to the parasitoid in an olfactometer. It was observed that response of the parasitoid was higher towards the volatiles from the reproductive stage where the concentration of allyl isothiocyanate was slightly more than in the vegetative stage at 1:1 dilution.It was also observed that as the infestation of D. rapae increased, the aphid population decreased.The studies indicated that the parasitoid has co-evolved along with the aphids to utilize the products of glucosinolates for the identification of the habitat of the host.

The information generated during the investigations can be utilized to exploit the potential of biological control of the aphid when the population is still low so that the glucosinolates are not digested into aglucones by myrosinase as these breakdown products play a very important role in attracting the aphids and the parasitoids towards the cultivars .It is also proposed based on the studies that if the infestation of the aphid still increases then by parasitization of it by *D.rapae* can decrease the infestation and the loss in biochemical nutritive of plant foliage which degrades the quality of products made of from different parts of mustard crops[Singh et al., 2011]can be reduced or removed.

5. References

i. Agarwala, B.K., (2007), "Phenotypic plasticity in aphids (Homoptera: Insecta): Components of variation and causative factors". Current Science, 93 (3): 308-313.
ii. Blande, J.D.; Pickett, J.A. and Poppy, G.M., (2004), "Attack rate and success of the parasitoid Diaeretiella rapae on specialist and generalist feeding aphids". J. Chem. Ecol., 30 (9): 1781-1795.
iii. Bones, A.M. and Rossiter, J.J., (1996), "The myrosinase-glucosinolate system-an innate defense system in plants". Physiol. Plantarum, 97 (1): 194-208.
iv. Bridges, M.; Jones, A.M.E.; Bones, A.M.; Hodgson, C.; Cole, R.; Bartlet, E.; Wallsgrove, R.; Karapapa, V.K.; Watts, N. and Rossiter, J.T., (2001), "Spatial organization of the glucosinolate-myrosinase system in Brassica specialist aphids is similar to that of the host plant". Proc. R. Soc. Lond. 1861:01.
v. Bridges, M.; Jones, A.M.E.; Bones, A.M.; Hodgson, C.; Cole, R.; Bartlet, E.; Wallsgrove, R.; Karapapa, V.K.; Watts, N. and Rossiter, J.T., (2002), "Spatial organization of the glucosinolate-myrosinase system in Brassica specialist aphids is similar to that of the host plant". Proceedings: Biological Sciences, 269 (1487): 187-191.
vi. Bueno, V.H.P.; Souza, B.M.de and De Souza, B.M., (1992), "Ethology and life span of Diaertiella rapae M'Intosh (Hymenoptera: Aphidiidae)". Revista de Agricultura Piracicaba, 67: 49-54.
vii. Chew, F.S., (1988), "Biological effects of glucosinolates". In Biologically active natural products (ed. H. Cutler), pp 155-181, Washington, D.C. American Chemical Society Symposium.
viii. Croft, A.G., (1979), "The determination of total glucosinolates in rapeseed meal by titration of enzyme liberated acid and the identification of individual glucosinolates". J. Sci. Food Agric., 30: 417-423.
ix. Deshraj and Lakhanpal, G.C., (1998), "Efficiency of parasitoid Diaeretiella rapae (M'Intosh) on aphid complex infesting rapeseed in mid hill zone of Himachal Pradesh". J. Ent. Res., 22: 245-51.
x. Desneux, N.; Fauvergue, X.; Moncharmont, F.X.D.; Kerhoas, L.; Ballanger, Y. and Kaiser, L., (2005), "Diaeretiella rapae limits Myzus persicae populations after applications of deltamethrin in oilseed rapa". J. Econ. Entomol., 98 (1): 9-17.
xi. Dilawari, V.K.; Kumari, A. and Dhaliwal, G.S., (1998), "Mechanism of host specificity of mustard aphid". Ecological, Agriculture and Sustainable Development, Punjab Agri. Univ., Ludhiana, India, 1: 457-62.
xii. Elst, I.F.A.M.; Vernede, R. and Pak, G.A., (1991), "Response of the parasitoid wasp Cotesia glomerata to odour cues from the host-habitat complex". Pro. Exper. And Appl. Entomol., 2: 109-114.
xiii. Fahey, J.W.; Zalcmann, A.T. and Talalay, P., (2001), "The chemical diversity and distribution of glucosinolates and isothiocyanates among plants". Phytochemistry, 56: 5-51.
xiv. Hossain, M.A.; Maiti, M.K.; Basu, A.; Sen, S.; Ghosh, A.K. and Sen S.K., (2006), "Transgenic expression of Onion leaf lectin gene in Indian mustard offers protection against aphid colonization". Crop Sci., 46: 2022-2032.
xv. Isaacs,R.; Hardie, J; Hick, A.J.; Pve, B.J.; Smart, L.E.; Wadhams, L.J. and Woodcock, C.M., (1993), "Behavioural responses of Aphis fabae to isothiocyanates in the laboratory and field". Pestic. Sci.,39(4): 349-55.
xvi. Jwanny, E.W.; El-Sayed, S.T.; Rashad, M.M.; Mahmoud, A.E. and Abdallah, N.M., (1995), "Myrosinase from roots of Raphanus sativus". Phytochem., 39: 1301-1303.
xvii. Kaushik, N. and Agnihotri, A., (2003), "Fatty acid variability in Indian mustard (B. juncea) lines generated through inter-and/or intra-specific hybridization". Proceedings of the Eleventh International Rapeseed Congress, Vol.-I: 274-276.
xviii. Kirk, J.T.O. and Oram, R.N., (1981), "Isolation of erucic acid-free lines of Brassica juncea: Indian mustard now a potential oilseed crop in Australia". Journal of the Australian Institute of Agricultural Science, 47: 51-52.
xix. Liu, B. and Sengonca, C., (1994), "Development of 8-armed airflow olfactometers for measuring olfactory responses of insect predators". Anz. Schädlingskde., Pflanzenschutz, Umweltschutz, 67: 30 34.
xx. Mithen, R.F., (1992), "Leaf glucosinolates profile and their relationship to pest and disease resistance in oilseed rape". Euphytica, 63: 71-83.
xxi. Nault, L.R. and Styer, W.E., (1972), "Effects of sinigrin on host selection by aphids". Entomol. Exp. Appl., 15: 423-429.
xxii. Ohiman, S.C. and Kunar, V., (1986), "Biological control of mustard aphid, Lipaphis erysimi by using the parastoid D. rapae: Influence of parasitoid on aphids". J. Environ. Entomol., 16 (3): 219-222.
xxiii. Olsen, O. and Sorensen, H., (1981), "Recent advances in the analysis of glucosinolates". J. Am. Oil Chem. Soc., 58: 857-65.
xxiv. Pandey, R. and Singh, N.N., (2008), "Effectiveness of bio-control based IPM modules against Lipaphis erysimi Kaltenbach (Hemiptera: Aphididae)". Journal of Plant Protection Research, 48 (1).
xxv. Raybould, A.F. and Moyes, C.L., (2001), "The ecological genetics of aliphatic glucosinolates". Heredity, 87: 383-391.
xxvi. Read, D.P.; Feeny, P.P. and Root, R.B., (1970), "Habitat selection by the aphid parasite Diaeretiella rapae (Hymenoptera:Braconidae) and hyperparasite Charips brassica (Hymenoptera:Cynipidae)".Can.Ent.102:1567-78.
xxvii. Read, H.C.; Tan, S.H.; Haapanen, K.; Killmon, M.; Reed, D.K. and Elliott, N.C., (1995), "Olfactory responses of the parasitoid Diaeretiella rapae (Hymenoptera:Aphidiidae) to odour of plants, aphids and plant – aphid complexes". Journal of Chemical Ecology, 21 (4): 407-418.
xxviii. Sekhon, B. S. ; Bakhetia, D.R.C. and Arora, R., (1996), "Yield losses due to mustard aphid, Lipaphis erysimi, Kalt. in some Brassica species in Punjab". J. Aphidology. 3: 112-115
xxix. Shukla, A.N. and Tripathi, C.P.M., (1993), "Effect of food plants on the off-spring sex ratio of Diaeretiella rapae (Hymenoptera:Aphidiidae), a parasitoid of Lipaphis erysimi Kalt. (Hemiptera:Aphidadae)". Biol. Agric. and Hort., 9 (2): 137-146.
xxx. Singh, Pushpa;Sinhal,Vijay Kumar, (2011), "Effect of aphid infestation on the biochemical constituents of mustard (Brassica juncea) plant".Journal of Pathology,3(8); 28-33.