HOST PLANTS ALTER THE REPRODUCTIVE BEHAVIOR OF PIERIS BRASSICAE (LEPIDOPTERA: PIERIDAE) AND ITS SOLITARY LARVAL ENDO-PARASITOID, HYPOSOTER EBENINUS (HYMENOPTERA: ICHNEUMONIDAE) IN A CRUCIFEROUS ECOSYSTEM

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ABSTRACT

The behavior of most destructive pest of cabbage, Pieris brassicae and that of its potential parasitoid, Hyposoter ebeninus, were studied under the influence of 4 common Brassica host plants species, cabbage, broccoli, cauliflower and knol-kohl. These host plant species were found to have considerable influence on egg distribution and leaf surface preference for oviposition and pupation. The number of egg masses was highest on knol-kohl; however, the number of eggs per mass was highest on cabbage. Similarly, larval incidence was also highest on cabbage throughout the season, indicating that cabbage is the most preferred host. Natural parasitism on Pieris brassicae larvae by Hyposoter ebeninus was higher on knol-khol and cabbage. The weight of the third instar parasitized caterpillars was the highest on cabbage, suggesting that cabbage is the most favorable of these 4 hosts of Pieris brassicae for mass rearing of Hyposoter ebeninus. The development time of Hyposoter ebeninus was also shortest for cabbage-reared larvae. Also the cocoon weight of the parasitoid was significantly higher when its host larvae were reared on cabbage. Overall from this study it can be concluded that, of the host plants evaluated, cabbage was preferred for oviposition by Pieris brassicae and its parasitoid. In addition, cabbage was found to be the best host plant for producing excellent quality Hyposoter ebeninus parasitoids.

Key Words: oviposition, leaf surface preference, field parasitism, cocoon parameters, koinobiont parasitoid

RESUMEN

Se estudió un aspecto del comportamiento de la plaga más destructiva del repollo, Pieris brassicae y su parasitoide potencial, Hyposoter ebeninus, bajo la influencia de 4 especies de plantas huéspedes del género Brassica, el repollo, el brócoli, la coliflor y el knol de kohl (=kohlrabi). Se encontró que estas especies de plantas hospedadoras tenían una influencia considerable en cuanto a la distribución de huevos y la preferencia de superficie de las hojas para la oviposición y pupación. Además, se encontró el número de masas de huevos más alto sobre knol de kohl, sin embargo la cantidad de huevos por masa fue la más alta sobre repollo. Del mismo modo, la incidencia de larvas también fue el más alto sobre el repollo durante toda la temporada, lo que indica que el repollo es el hospedero preferido. El parasitismo natural de larvas de Pieris brassicae por Hyposoter ebeninus fue mayor en el knol-khol y repollo, lo que implica que la planta huésped tiene un papel significativo en la interacción de los tres niveles tróficos. El peso del tercer estadio de las orugas parasitadas fue el más alto en el repollo, lo que sugiere que es el más favorable de los 4 hospederos de Pieris brassicae para la cria masiva de Hyposoter ebeninus. También, el periodo de desarrollo de Hyposoter ebeninus fue la más corta sobre larvas criadas sobre el repollo. El peso del capullo del parasitoide fue significativamente mayor cuando las larvas hospederas fueron criadas sobre el repollo. En general para este estudio, se puede concluir que las diferentes plantas hospederas tienen efectos significativamente diferentes en cuanto a la preferencia de oviposición de un herbívoro y sobre el comportamiento de sus
parasitoides. Además, se encontró que el repollo fue la mejor planta hospedera para *P. brassicae* para producir parasitoides del *H. ebeninus* de excelente calidad.

Palabras Clave: oviposición, preferencia de superficie de las hojas, parasitismo de campo, parámetros de capullo, parasitoides koinobiontes

Cole crops are a major component of vegetables in human diets. The greater cabbage white butterfly, *Pieris brassicae* (L.) (Lepidoptera: Pieridae) is one of the most serious pests of cabbage (*Brassica oleracea* var. *capitata* L.), cauliflower (*Brassica oleracea* var. *botrytis* L.) and many crucifers found along temperate, tropical and subtropical regions of the eastern hemisphere (Jainlabdeen & Prasad 2004; Lal & Ram 2004; Younas et al. 2004). The young caterpillars feed gregariously on leaves, defoliating the plants and making insecticidal applications necessary for the cultivation of cole crops. Protective measures using different chemicals can cause undesirable side effects to human health, as the cole crops are being used as fresh vegetables in human diet. These chemicals are costly and cause hazardous health related issues in animals including humans (Bam 2008; Dasgupta et al. 2007). Moreover, uncontrolled use of synthetic chemicals cause hazardous health related issues in animals including humans (Bam 2008; Dasgupta et al. 2007). In order to avoid these hazardous effects, biological control is now, emerging as an important component of pest management (Balevski et al. 2007). Biological control of cruciferous pests, including cabbage butterflies, has traditionally relied on microbial pesticides, predators and parasitoids (Harcourt 1966; Biever & Wilkinson 1978; Peters & Coaker 1993; Van Driesche et al. 2003). Many workers have recommended the use of parasitoids for the management of *P. brassicae* (Harvey 2004; Patriche 2006; Bhat & Bhagat 2009). Among the parasitoids attacking this pest, *Cotesia glomerata* (L.) (Hymenoptera: Braconidae) and *Hyposoter ebeninus* (Gravenhorst) (Hymenoptera: Ichneumonidae) are the 2 most important endo-larval parasitoids, with both being widely distributed across the world (Lozan et al., 2008; Harvey et al. 2010). Unlike *C. glomerata*, *H. ebeninus* (Supplementary Fig. 1) develops inside second and third instar larvae of *Pieris* spp. as a solitary endoparasite with the distinctive habit of forming a cocoon within the shrunken larval body (Supplementary Fig. 2) (Moiseeva 1976; Gauld 1988). Besides the generally recognized excellence of *C. glomerata* as a natural enemy, excellent bio-control potential of *H. ebeninus* has been reported from different parts of the world (Thakur & Deka 1997; Bhat & Bhagat 2009; Harvey et al. 2010; Razmi et al. 2011). Although, *C. glomerata* has been extensively studied around the world, *H. ebeninus*, is often ignored. Surprisingly, even basic biological studies on this species were not available until recent report of Harvey et al. (2010).

In terms of fitness of the pest, selection of a good oviposition site is critical (Janz 2002). Ovipositional preferences of *Pieris* spp. are affected by several factors, including thickness of the plant’s wax layer, physiological age and its biochemical composition (Bernays & Chapman 1994; Schoonhoven 1972; Schoonhoven et al. 1998), plant size (Reudler et al. 2008) and color (Radcliffe & Chapman 1966). Similarly, different host plants have considerable effects on host preference of *P. brassicae*; thus the latter’s pattern and incidence of laying of eggs also varies on different hosts, even when these are grown together at same place under same physical conditions (Lytan & Firake 2012).

Tritrophic interactions (host plant-herbivore-parasitoid) play a crucial role in biological control...
of insect pests in every crop ecosystem. Many reports have suggested that female parasitoids usually detect their host by some important inherent cues (Fatouros et al. 2005), probably derived from the host plant in combination with herbivore (Geervliet et al. 1996). Parasitism by endoparasitoids can also induce physiological alterations in the herbivore (Gu et al. 2003), and the herbivore species consumes more food due to enhanced digestibility (Parker & Pinnell 1973; Sato et al. 1986; Schof & Steinberger 1997; Nakamatsu et al. 2001) and grow faster than unparasitized larvae (Coleman et al. 1999). The characteristics of the host's growth, however, depend on the parasitoid species and the nature of the parasitism. In general, the herbivore eats more when parasitized by a gregarious species than by a solitary one.

The cabbage butterfly, *P. brassicae*, is one of the most widely studied insects. Although, extensive work has been done on several ecological aspects of this species (Ansari et al. 2012; Rather & Azaim 2009; Metspalu et al. 2003; David & Gardiner 1961); some important behavioral aspects have been overlooked or underestimated. Mass rearing and utilization of high quality bio-control agents may also be essential in successful bio-control programs. Moreover, different host plants of the herbivore have considerable effect on the behavior of their natural enemies, especially koinobiont species. Accordingly, the host plant has significant influence on field parasitism, and growth and development of the parasitoid. A few studies on *Cotesia* have revealed improved biological parameters, when parasitoids developed inside herbivores reared on a highly favorable host plant (Hasan et al. 2011). Unlike *Cotesia*, the parasitoid *H. ebeninus* comparatively prefer early instar larvae for oviposition and develop into fully grown pupae in third instar *P. brassicae* caterpillars. Therefore, weights of second and third instar parasitized larvae and cocoon parameters of *H. ebeninus* may vary on different host plants. There is a direct relationship between weight of the parasitoid and fecundity (Takagi 1985); hence a higher quality host plant of *P. brassicae* may produce a more efficient parasitoid for use in bio-control programs.

Additionally, tritrophic communication also results in significant changes in the behavior of both the herbivore and its natural enemies. Globally, most of the bio-control attempts against crop pests have been unsuccessful, and such lack of success is frequently the result of too little knowledge of parasitoid biology and behavior, especially as related to bio-control potential (Beirne 1963; Peter 1993; Loura et al. 1997; Myers 2000; Hokkanen 2002). Therefore, the main objective of this study was to ascertain host plant-mediated effects on behavioral and biological aspects of the herbivore, *P. brassicae* and on its potential parasitoid, *H. ebeninus*. In this study attempts were also undertaken to determine the level of quality of host plant of the *P. brassicae* for mass rearing of *H. ebeninus*, so that highly efficient parasitoid could be produced.

**Materials and Methods**

All experiments were carried out at an experimental field of the Division of Crop Improvement (Entomology), ICAR Research Complex for the NEH Region, Umiam, India during 2010-11. These experimental fields were situated at N 25° 41' 01.91' E 91° 54' 46.24'.

About 1-month old seedlings of 4 cruciferous crops, i.e., broccoli (*Brassica oleracea* L. var. *italica* Plenc; Capparales: Brassicaceae) (*Pushpa*), cabbage (*Brassica oleracea* L. var. capitata L., 'Wonder ball'), cauliflower (*Brassica oleracea* var. botrytis L., 'Him Kiran') and knol-khol (*Brassica oleracea* var. *gongylodes* L., 'Sultan's knol-khol' and 'Early White Vienna') were transplanted in the experimental field of the Entomology unit at Umiam. Experimental plot size was 3 × 3 m and each plot was separated the next by 1 m on all sides. All the necessary horticultural practices (i.e., irrigation, weeding and other intercultural operations) were followed for healthy growth of the crop. Butterflies were allowed to lay eggs on different host plants, and observations on number of eggs masses per plant and number of eggs per mass were taken at weekly intervals during the peak infestation period (Feb-Mar 2011). Similarly, leaf surface preference for oviposition and pupation was also studied by noting the site of egg laying and pupation (abaxial/adaxial surface) and the percent oviposition/pupation on the lower leaf (abaxial) surface was calculated. Observations on number of larvae per plant were also taken at weekly intervals during the peak infestation period of *P. brassicae*.

Natural larval parasitism by *H. ebeninus* was studied on the 4 above mentioned host plants of *P. brassicae* under field conditions. Observations were taken at weekly intervals during peak season of larval parasitism (Azad 1994; Lytan 2012; Firake, unpublished). The weight (g) of second and third instar parasitized caterpillars, developmental period (days), and adult emergence of *H. ebeninus* were recorded. Similarly, various cocoon parameters of *H ebeninus*, i.e., weight (g), length (mm) and diam (mm) were also noted. Larval parasitism throughout the season was considered for calculation of per cent parasitism. Parasitized caterpillars and parasitoid cocoon were weighed on digital electronic balance (Mettler Toledo® AB analytical balance, Model AB104-S). Diam of thorax (greatest width of cocoon) was measured with the help of 'Vernier Caliper' (Mitutoyo, Japan).

All the experiments were carried out in a Randomized Block Design (RBD) and each treatment
was replicated 3 times. The complete experiment was repeated 3 times each in a different field. Differences between treatments were analyzed using ANOVA at a significance level of 0.05 and Tukey’s HSD (Tukey’s Honestly Significant Difference) test was used to find out the significant differences between mean values. Statistical software SPSS 13.0 for windows (SPSS, Inc., 2004) was used for overall statistical analysis.

Behavioral Response of *Pieris brassicae* to the Different Host Plants

Host plants of the *P. brassicae* significantly influenced its reproductive behavior. Numbers of egg masses of *P. brassicae* (L.) were significantly higher ($F = 11.54, df = 11, P = 0.03$) on knol-khol than other host plants (Fig. 1). Interestingly, the number of eggs per mass was also variable on different host plants, there being significantly more eggs per mass ($F = 57.67, df = 11, P < 0.001$) on cabbage followed by on cauliflower and knol-khol; while lowest on broccoli (Fig. 2).

Mean larval infestation was also highest ($F = 13.44, df = 11, P = 0.002$) on cabbage followed by cauliflower and knol-khol, and the least on broccoli (Fig. 3). Similarly, leaf surface preferences for oviposition and pupation were found to vary on different host plants (Fig. 4). Furthermore, preference for oviposition (Supplementary Fig. 3) and pupation on abaxial surface of leaves was significantly higher on cabbage ($F = 31.20, df = 11, P = 0.000$ and $F = 6.56, df = 11, P = 0.002$, respectively) followed by broccoli, knol-khol and cauliflower.

Effect of Different Host Plants of *Pieris brassicae* on Biological Parameters of Larval Parasitoid, *Hyposoter ebeninus*

Under field conditions, significantly higher larval parasitism was observed in knol-khol ($F = 4.57, df = 11, P = 0.017$) followed by cabbage and cauliflower and broccoli (Table 1). Effect of larvae reared on different hosts plants and subsequent parasitoid emergence from them was found to be non-significant ($F = 1.08, df = 11, P = 0.39$). However, it was higher on cabbage than on other crops.

Effect of different hosts on the weight of the second instar parasitized caterpillar (SIPC) was also found to be non-significant ($F = 1.08, df = 11, P = 0.39$). Though, it was comparatively higher on cabbage than other crops. In contrast, the weight of the third instar parasitized caterpillar (TIPC) was significantly higher ($F = 4.57, df = 11, P = 0.017$) on cabbage followed in decreasing by cauliflower, broccoli and knol-khol (Table 1).

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Influence of Host Plants on Developmental Period of *H. ebeninus*

Development period was found to be lower on cabbage followed by cauliflower and broccoli; while it was extended on knol-khol (Table 1).

**Discussion**

Several factors affect egg laying behavior of the female insect and her choice has considerable influence on the life history of her progeny. In the present study, *P. brassicae* laid significantly more eggs on the knol-khol plant followed in descending order by cauliflower, cabbage and broccoli. This behavior might be attributed to the host plant phenology and tenderness of leaves as found in knol-khol and cauliflower. However, the number of eggs per mass was found to be higher...
on cabbage, followed in descending order by cauliflower, knol-khol and broccoli. This behavior suggests that, although number of egg masses was few on cabbage the butterfly preferred to lay more eggs per mass on it. Therefore, the egg laying preference of *P. brassicae* may depend on the host size, stage and phenology. There are several factors that affect the oviposition behavior of butterflies. The thickness of wax layer, physiological age (Bernays & Chapman 1994), size (Reudler et al. 2008), color (Radcliffe & Chapman 1966) and biochemical composition (Schoonhoven et al. 1998) of the plant are among the most significant factors during selection of oviposition site by butterfly.

Some *Pieris* species tend to lay eggs in large masses when locating large-sized hosts with abundant leaves and such display of egg laying preference associated with host size has also been found for *P. brassicae* (Stamp 1980; Le Masurier 1994). Many reports also suggested that *Pieris* butterfly’s flight and egg laying patterns are influenced by factors such as plant size, phenology, species, humidity content, nutrients, leaf color and plant chemistry (Jones 1977; Latheef & Irwin 1979; Myers 1985; Andow et al. 1986; Hern et al. 1996; Hooks & Johnson 2001). In several cases, insect egg laying behavior depended on various factors which included minimizing parasitic and predatory risk, selecting the most nutritious host, avoiding intra-specific competition for food and maximizing egg laying (Myers 1985; Ohsaki & Sato 1999). For that, the insect internally weighs the various stimuli and inhibitors perceived through visual, chemical, and mechanical signals (Thompson & Pellmyr 1991; Hern et al. 1996).

In our study, preference of lower leaf surface for oviposition as well as pupation was significantly higher in cabbage as compared to other cole crops. This behavior might be attributed to host phenology and also to avoid the risk of parasitism and predation of the early instar larvae. Earlier findings (Kobayashi 1965; Tagawa et al. 2008) revealed that, *P. rapae crucivora* eggs were generally found on the lower or abaxial surface of leaves in cabbage under field conditions. The strong bias towards the lower surface preference might be due to the egg-laying posture of females, which generally land on the leaf margins from above and bend their abdomens to lay eggs (Kobayashi 1963; Tagawa et al. 2008). Besides this, oviposition and pupation on the lower surface could avoid the increased risk of parasitism and predation on upper surface (Tagawa et al. 2008). In general, it is easier for the parasitoids to locate their host on the exposed leaf surface (Zago et al. 2010); so chances of parasitization of *Pieris* larvae are higher on upper leaf surface (Supplementary Fig. 4). Moreover, the phenology of the cabbage plant is completely different from the other 3 crops and especially cauliflower. The lower surface of the newly formed cabbage leaf
is slightly concave (Supplementary Fig. 5), which is analogous to that of the upper leaf surface of cauliflower (Supplementary Fig. 6). Therefore, it is easier for the female butterfly to sit on such a concave leaf surface to lay eggs (Supplementary Fig. 7). As a result, maximum egg masses might be observed on lower leaf surface of cabbage.

Natural parasitism of *P. brassicae* by the larval parasitoid, *H. ebeninus* was highest on knol-khol followed by cabbage and it was comparatively less on cauliflower and broccoli. Tritrophic interaction has a noteworthy role in the biological control of crop pests. Host plants invite natural enemies to reduce herbivore pressure and in several cases the female parasitoid depends on smell of a host plant in combination with the herbivore for this purpose (Geervliet et al. 1996). The female can also detect its host on different plants, such as red and white cabbage, brussels sprouts (*Brassica oleracea* L. var. *gemmifera* Zenker) and nasturtium (*Nasturtium* sp.; Capparales: Brassicaceae); but the parasitoid seems to have a preference for host larvae in particular crop (Kaushal & Vats 1983; Geervliet et al. 1996). Therefore, the chemical composition of knol-khol might be different than other host plants, and it may be responsible for the attraction of parasitoids. Besides, the shapes of knol-khol and cabbage are also responsible for retaining the excreta and larval frass of *P. brassicae* on nearby leaves, which is most favorable for female parasitoids to locate their host larvae.

In the present findings, the weight of the second instar parasitized caterpillars was not affected by the different host plants. However, the weight of the third instar parasitized caterpillars was higher on cabbage followed by cauliflower and lower still on knol-khol. This effect on weight of the third instar parasitized caterpillars might be attributed to host plant phenology, because in the initial growth phase, the weight of the caterpillar is usually greater on the most suitable or preferred host plant, i.e., cabbage as observed by Talaei (2009). Furthermore, the plant population on which the caterpillars were reared differentially affects herbivore performance. The successful development of a parasitoid requires that the resources available in its hosts should satisfy their minimal nutritional requirements. Therefore, host nutrition can have profound effects on the ability of a parasitoid to develop optimally (Hawkins 1994; Abrahamson & Weis 1997). Parasitoid host relations can be altered by the food plants of their respective hosts in 2 ways. Plants may affect the host selection activities of parasitoids (Mueller 1983) or the nutritional quality of the host’s food can influence parasitoid growth and survival (House & Barlow 1961; DeMoras & Escher 1990).

There is a positive correlation between host plant suitability of the herbivore and its parasitoids. For instance, vigorous herbivores feed more and therefore produce more body mass on which the parasitoids can feed (Karowe & Schoonhoven 1992; Mueller 1983). Body size of the wasp is affected by the plant species on which its host caterpillar feeds (Price 1986; Guillot & Vinson 1973; Zohdy & Zohdy 1976; Beckage & Riddiford 1983; Mackauer 1986; Slansky 1986), where development of the wasps is synchronized with the host. Plant species may also change herbivore suitability and parasitoid development time (Pierce & Holloway 1912).

<table>
<thead>
<tr>
<th>Host plants of <em>P. brassicae</em></th>
<th>Weight of cocoon (g), n = 612</th>
<th>Length of cocoon (mm), n = 612</th>
<th>Diameter of cocoon (mm), n = 612</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cabbage</td>
<td>0.028 ± 0.0004 <strong>b</strong></td>
<td>9.6 ± 0.51 <strong>a</strong></td>
<td>2.68 ± 0.18 <strong>a</strong></td>
</tr>
<tr>
<td>Cauliflower</td>
<td>0.023 ± 0.001 <strong>a</strong></td>
<td>9.1 ± 0.29 <strong>a</strong></td>
<td>2.66 ± 0.09 <strong>a</strong></td>
</tr>
<tr>
<td>Knol-khol</td>
<td>0.028 ± 0.0006 <strong>b</strong></td>
<td>9.0 ± 0.22 <strong>a</strong></td>
<td>2.84 ± 0.14 <strong>a</strong></td>
</tr>
<tr>
<td>Broccoli</td>
<td>0.022 ± 0.0002 <strong>a</strong></td>
<td>9.1 ± 0.24 <strong>a</strong></td>
<td>2.34 ± 0.14 <strong>a</strong></td>
</tr>
<tr>
<td>F Value</td>
<td>12.34</td>
<td>0.645</td>
<td>2.161</td>
</tr>
<tr>
<td>F test (p ≤ 0.05)</td>
<td>&lt;0.001*</td>
<td>0.597</td>
<td>0.133</td>
</tr>
</tbody>
</table>

Note: Different small letter after mean values indicate significant differences among host plants (Tukey’s HSD, *P* ≤ 0.05).
Overall from this study it can be concluded that different host plants have significantly different effects on the ovipositional preference of an herbivore and on the behavior of its parasitoids. The female butterfly discriminates the leaf surface for oviposition/pupation and her preference depends on host plant phenology. Her preference may also express an avoidance of increased risk of predation/parasitism and intra specific competition. Host plants also invite natural enemies to reduce herbivore pressure; consequently natural field parasitism varies per host plant species. In addition, cabbage is found to be the best host plant of *P. brassicae* for producing excellent quality *H. ebeninus* parasitoids.

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