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BIOLOGY OF *PIERIS BRASSICAE* (LINN.) ON DIFFERENT *BRASSICA* SPECIES IN THE PLAINS OF PUNJAB

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Abstract: Biology of *Pieris brassicae* reared on *Brassica napus, B. juncea, B. rapa,* and *B. carinata* was studied. Adult butterflies, collected in November from the plains of Punjab, India, were sexed, paired, and released onto the four *Brassica* spp. in a greenhouse. In a multigeneration study (Parental, F1, and F2), the effect of the four *Brassica* spp. on the egg laying, incubation period and hatching percentage was assessed in a greenhouse study. Hatched larvae were collected, reared on fresh leaves of respective *Brassica* spp, in laboratory conditions. Data collected on larval stadia, pre-pupal and pupal durations, adult longevity, and sex ratio were assessed to understand the effects of these four species. Of the four species, *B. carinata*, with a shorter incubation period, higher hatching percentage, and shorter developmental periods was most susceptible. In this study, *B. rapa* was the most resistant species and may be recommended for further breeding programs in order to reduce the economic damage caused by *P. brassicae*.

Key words: biology, *Brassica* species, multigeneration, *Pieris brassicae,* resistant species

INTRODUCTION

Rapeseed mustard (*Brassica* spp.) is an important and highly diversified group of crops grown worldwide (Arora 1999). Grown in more than 120 countries and cultivated from subtropical to temperate climates, rapeseed mustard responds well to low inputs and can withstand harsh conditions. The variety of uses of *Brassica* seeds and their adaptability to the growing system reflects the importance and impact of rapeseed mustard particularly for people in the Indian subcontinent. In India, rapeseed mustard, *Brassica* spp. is attacked by more than 40 insect pests. *Pieris brassicae* (Lepidoptera: Pieridae) is commonly known as the large white butterfly. It is a destructive cosmopolitan and the most widely distributed lepidopteran pest of crucifers (Ansari *et al*. 2012). Besides mustard, *P. brassicae* readily feeds on five main plant families, including Brassicaceae, Tropaeolaceae, Capparaceae, Resedaceae and Papilinoaceae (Feltwell 1982). A 92 percent yield loss in *Brassica* spp. and a 19.76 per cent yield loss exclusively on *Brassica carinata* PC-5 (Anonymous 1996) was due to this pest infestation. Since mechanical control is cheap, environmentally safe, and easy to adopt, it may become an important component of integrated pest management. Moreover, damage caused by *P. brassicae* could be reduced by growing resistant plants. It could be recommended that resistant plants be incorporated in breeding programmes to reduce losses. Past studies on biology/bionomics revealed that *P. brassicae* lays eggs in clusters and that first instar larvae feed gregariously (Rataul 1959; Chandel *et al*. 1998 and Thakur *et al*. 1998). However a complete study assessing the effects of *Brassica* spp. on the biology of *P. Brassicae* needs attention. For this reason, the study of the biology of *P. brassicae* on four *Brassica* spp. was done, on the plains of Punjab. The aim was to find effective management of the *P. brassicae* pest on the *Brassica* family. *P. brassicae* is attaining important pest status on the *Brassica* family. This research addresses the need for finding effective options for managing *P. brassicae* in the face of the declining availability and popularity of conventional chemical insecticides.

MATERIALS AND METHODS

Maintenance of test plants

Seeds of four *Brassica* spp. [(*Brassica napus* (GSL-2), *B. juncea* (PBR-91), *B. rapa* (TL-15) and *B. carinata* (PC-5)] were obtained from the Division of oil seed, Department of Plant Breeding and Genetics, Punjab Agricultural University (PAU), Ludhiana. Seeds were sown in earthen pots at the Entomological Research farm. Each pot was filled with a mixture of soil and FYM (1:1); the soil was filled up to 2 cm from the upper edge. The pots were watered when required. 30 days old plants were used in the experiments.

Maintenance of *P. brassicae* colonies

Light yellow eggs and larvae of the butterfly were collected from the rapeseed and mustard crop adjoining the University. These larvae were reared on the mustard

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leaves under controlled conditions. Every day the larvae were transferred to fresh leaves. A camel hair brush was used to transfer the larvae. Action solution (2% ethyl alcohol) was used to disinfect the camel hair brush and to avoid spreading infections (viral or fungal) to the immature stages of *P. brassicae.*

Experimental procedure

Adults of *P. brassicae* were collected from the field in November, sexed, paired, and released on the four *Brassica* spp. Five plants (sample) of each *Brassica* spp. represented one replication. The experimental design had four replications of four *Brassica* spp. totalling 80 pots placed in a completely randomized manner. Data were collected daily on number of eggs laid and percent viable (hatching percentage), starting from the release of adults till the mortality of all the butterflies. The experiments were carried out for up to three generations [Parental (P), F1, and F2] during the crop season (December to March).

In a different experiment conducted under controlled conditions, the leaves with eggs from the greenhouse experiments were placed on wet sponge/foam sheets in glass jars (20x10 cm). This technique was used to avoid drying. On hatching, the larvae were provided with fresh leaves of the same *Brassica* spp. (from the leaves of which these were collected) to study the biology of *P. brassicae* for up to three generations (P, F1, and F2). Ten larvae (sample) fed on each *Brassica* spp., represented one replication. The experimental design had five replications of four *Brassica* spp. totalling 200 larvae placed in test tubes and arranged in a completely randomized manner. Daily data were collected on larval stadia, pre-pupal and pupal durations, adult longevity, and sex ratio. Fresh leaves were replaced every 12 hours till the larvae entered the pre-pupal stage.

Data analysis

The data from the greenhouse and laboratory experiments were assumed to be normal. Necessary transformations were made only when the normality assumptions were not met. The analysis was conducted using CPCS version 1 and significance of p-value < 0.05 was reported as the critical difference (CD).

RESULTS

Plants have a wide spectra of action; the same plant may affect insects through a completely different action mechanism. The results of the effects of *Brassica* spp. on the biology of *P. brassicae* are presented in tables 1–3.

Egg laying

The data (Tables 1–3) revealed that the significantly highest number of eggs per plant were recorded on *B. carinata* (19.86±2.91; 16.67±2.58 and 30.17±1.40) during the P, F1, and F2 generations, and lowest on *B. juncea* (3.39±0.81 and 27.6±1.86) during the P generation and *B. napus* (7.34±1.10 and 5.95±0.79) during F1, and F2 generations. The present study is in agreement with Chandel *et al*. (1998) who reported 17–35 eggs per plant, Thapa (1987) 36.11 eggs per plant, and Thakur *et al*. (1998) 29.66 eggs per plant. However, our study differs from Gupta's (1984) 2–195 eggs/plant, probably due to different environmental conditions prevailing at that time.

Incubation period

In all the generations (P, F1, and F2), the significantly longest incubation period (Tables 1–3) was observed on *B. rapa* (20.00±2.45; 19.50±1.62 and 16.25±1.53 days), and the shortest on *B. carinata* (17.56±2.56; 15.83±1.49 and 12.34±2.71 days). Gupta (1984) and Thakur *et al.* (1998) reported the similar results of 18 days and 22.66 days for the incubation period, respectively. On the other hand, Sood and Bhalla (1996), Chandel *et al.* (1998) and Thapa (1987) reported 7 days, 4.75–11.90 days, and 3.1 days, respectively. Their results were probably due to different hosts and prevailing environmental conditions.

Hatching Percentage

Hatching percentage was significantly higher in all generations (Tables 1–3) on *B. carinata* (99.01±0.22; 98.28±0.13 and 98.06±0.16) and lower on *B. rapa* (96.92±0.27) (Table 1), *B. napus* (96.54±0.24) (Table 2) and *B.juncea* (94.06±0.49) (Table 3) during the P, F1, and F2 generations, respectively. The results were in agreement with findings of Thapa (1987) where a 98.06 per cent hatching was reported.

Larval period

First Instar

The growth period of the 1st larval instar (Tables 1–3) was significantly maximum on *B. juncea* (4.22±0.11; 6.87±0.27 and 6.03±0.27 days) while minimum on *B. carinata* (3.62±0.06; 6.42±0.12 and 4.63±0.09 days) during the P, F1, and F2 generations. Similar results were reported as 3-6 days (Sood and Bhalla 1996), 9–12 days (Chandel *et al.* 1998), 3–4 days (Gupta 1984), and 2–3 days (Thapa 1987).

Second Instar

The growth period of the second instar (Table 1) was significantly maximum on *B. juncea* (4.79±0.19 days), while minimum on *B. carinata* (4.18±0.06 days) during the P generation. During the F1, and F2 generations (Table 2, 3), the second instar growth period was significantly maximum on *B. napus* (4.33±0.18 and 3.07±0.63 days) while minimum on *B. carinata* (4.04±0.12 and 2.06±0.04 days), respectively. Our study was inagreement with the reports of Sood and Bhalla (1996) for 2.3–4.7 days, Chandel *et al.* (1998) 4.7–6.87 days, Gupta (1984) 2.5–3.5 days, and Thapa (1987) 2–3 days of the second instar period.

Third Instar

The data (Table 1) revealed that the maximum third instar growth period on *B. rapa* (4.13±0.29 days) was during the P generation. During the F1, and F2 generations (Table 2, and 3), the maximum third instar period was on *B. juncea* (5.08±0.14 and 3.08±0.14 days). It was minimum on *B. rapa* (4.20±0.13 days) and *B. carinata* (2.94±0.03 days) during the F1, and F2 generations. Our study confirms the findings of Sood and Bhalla (1996) who reported 2.2–4.9 days, Chandel et al. (1998) 4.82–6.87 days, Gupta (1984) 2.5–3.5 days, and Thapa (1987) 2.5–3.5 days.

Table 1. Biology of *P. brassicae* on different *Brassica* species during the Parent (P) generation

Stage/Period		Mean* duration (days)				CD (p = 0.05)
		B. napus	*B. juncea*	*B. rapa*	*B. carinata*	
Egg	eggs/plant	5.71±1.06 (2.56)	3.39±0.81 (2.07)	6.24±1.56 (2.69)	19.86±2.91 (4.53)	(0.80)
	incubation period	17.79±2.22 (4.33)	19.00±1.91 (4.47)	20.00±2.45 (4.58)	17.56±2.56 (4.30)	(0.09)
	hatching [%]	97.4±0.15 [80.79]	97.02±0.27 [80.11]	96.92±0.52 [80.22]	99.01±0.22 [84.25]	(0.29)
Larva	I	4.09±0.87 (2.25)	4.22±0.11 (2.29)	4.20±0.15 (2.28)	3.62±0.06 (2.15)	(0.12)
	II	4.29±0.12 (2.30)	4.79±0.19 (2.41)	4.32±0.22 (2.30)	4.18±0.06 (2.27)	(0.10)
	III	3.77±0.14 (2.18)	3.77±0.13 (2.18)	4.13±0.29 (2.26)	3.77±0.19 (2.18)	ns
	IV	3.60±0.30 (2.14)	3.70±0.17 (2.17)	3.80±0.20 (2.19)	3.58±0.18 (2.14)	(0.05)
	V	3.41±0.15 (2.10)	3.21±0.26 (2.05)	3.22±0.18 (2.05)	2.98±0.08 (1.99)	ns
Total larval period		19.16± 0.59 (4.49)	19.69±1.13 (4.55)	19.67±0.88 (4.55)	18.13±0.41 (4.37)	(0.23)
Pre-pupal period		2.15±0.05 (1.77)	2.08±0.09 (1.75)	2.12±0.06 (1.76)	1.98±0.06 (1.72)	ns
Pupal period		20.95±0.68 (4.68)	19.19±0.44 (4.49)	20.98±0.53 (4.68)	18.45±0.74 (4.41)	(0.20)
Adult period		7.17±0.22 (2.86)	7.51±0.47 (2.91)	7.15±0.32 (2.85)	5.83±0.25 (2.61)	(0.17)
Total development period		67.22±0.86 (8.26)	67.47±0.52 (8.28)	69.92±0.61 (8.41)	61.95±0.45 (7.93)	(0.14)
Male: female		1.02±0.24 [5.66]	1.27±0.68 [5.98]	1.26±0.44 [5.98]	1.14±0.22 [5.73]	[0.23]

*mean of four replications based on five plants; ( ) – parentheses are √ (n+1) transformation; [ ] – parentheses are arc sine transformation; ns – not significant; CD – critical difference

Table 2. Biology of *P. brassicae* on different *Brassica* species during the F1 generation

Stage/Period		Mean* duration (days)				CD (p = 0.05)
		B. napus	*B. juncea*	*B. rapa*	*B. carinata*	
Egg	eggs/plant	7.34±1.10 (2.87)	7.97±2.13 (2.98)	9.19±3.03 (3.14)	16.67±2.58 (4.16)	(0.82)
	incubation period	17.62±2.05 (4.31)	18.17±2.60 (4.37)	19.50±1.62 (4.52)	15.83±1.49 (4.10)	(0.21)
	hatching [%]	96.69±0.24 [79.41]	97.69±0.22 [81.31]	96.54±0.37 [79.75]	98.28±0.13 [82.79]	[0.26]
Larva	I	6.63±0.15 (2.76)	6.87±0.27 (2.80)	6.65±0.45 (2.76)	6.42±0.12 (2.72)	ns
	II	4.33±0.18 (2.31)	4.10±0.24 (2.26)	4.10±0.14 (2.26)	4.04±0.12 (2.24)	ns
	III	4.72±0.13 (2.39)	5.08±0.14 (2.46)	4.20±0.13 (2.28)	4.64±0.18 (2.37)	(0.09)
	IV	4.00±0.13 (2.23)	4.13±0.29 (2.26)	4.33±0.25 (2.30)	3.47±0.19 (2.11)	(0.12)
	V	4.73±0.19 (2.39)	4.80±0.28 (2.40)	4.56±0.14 (2.35)	4.46±0.13 (2.33)	(0.04)
Total larval period		24.41±0.35 (5.04)	24.98 ± 0.85 (5.10)	23.84±0.46 (4.98)	23.03±0.61 (4.90)	(0.08)
Pre-pupal period		2.38±0.20 (1.83)	2.41±0.10 (1.84)	2.35±0.12 (1.83)	1.98±0.09 (1.72)	(0.09)
Pupal period		9.56±0.30 (3.35)	9.34±0.73 (3.21)	10.23±0.14 (3.25)	8.55±0.09 (3.09)	(0.11)
Adult period		5.49±0.13 (2.54)	5.12±0.18 (2.47)	6.02±0.23 (2.65)	4.77±0.24 (2.40)	(0.12)
Total development period		59.46±0.52 (7.82)	60.02±0.69 (7.81)	61.94±0.48 (7.89)	54.16±0.38 (7.42)	(0.07)
Male: female		1.53±0.43 [6.85]	1.46±0.66 [6.39]	1.80±0.54 [7.56]	1.23±0.24 [5.90]	[0.32]

*mean of four replications based on five plants; ( ) – parentheses are √ (n+1) transformation; [ ] – parentheses are arc sine transformation; ns – not significant; CD – critical difference

Table 3. Biology of *P. brassicae* on different *Brassica* species during the F2 generation

Stage/Period		Mean* duration (days)				CD (p = 0.05)
		B. napus	*B. juncea*	*B. rapa*	*B. carinata*	
Egg	eggs/plant	5.95±0.79 (2.62)	27.6±1.86 (5.34)	7.71±2.14 (3.97)	30.17±1.40 (5.54)	(0.88)
	incubation period	14.43±1.36 (3.92)	16.03±1.98 (4.12)	16.25±1.53 (4.15)	12.34±2.71 (3.65)	(0.17)
	hatching [%]	95.25±0.13 [77.41]	97.85±0.49 [75.86]	94.06±0.31 [81.37]	98.06±0.16 [82.13]	(2.56)
Larva	I	5.33±0.07 (2.51)	6.03±0.20 (2.65)	5.34±0.10 (2.52)	4.63±0.09 (2.37)	(0.07)
	II	3.07±0.63 (2.01)	2.78±0.08 (1.94)	2.48±0.08 (1.86)	2.06±0.04 (1.75)	(0.12)
	III	2.97±0.07 (1.99)	3.08±0.14 (2.02)	2.99±0.06 (1.99)	2.94±0.03 (1.98)	NS
	IV	2.60±0.11 (1.89)	3.09±0.28 (2.13)	3.54±0.14 (2.02)	2.34±0.10 (1.82)	(0.12)
	V	2.63±0.10 (1.90)	2.97±0.21 (1.99)	2.72±0.19 (1.93)	2.23±0.10 (1.79)	(0.12)
Total larval period		16.60±0.35 (4.20)	18.40±0.85 (4.40)	16.62±0.46 (4.20)	14.20±0.61 (3.90)	(0.11)
Pre-pupal period		1.93±0.04 (1.71)	1.88±0.14 (1.76)	2.11±0.08 (1.69)	1.79±0.03 (1.67)	ns
Pupal period		9.50±0.25 (3.24)	9.60±0.45 (3.38)	10.47±0.18 (3.25)	8.52±0.25 (3.08)	(0.14)
Adult period		5.69±0.21 (2.58)	6.06±0.38 (2.65)	5.96±0.28 (2.64)	5.53±0.37 (2.55)	ns
Total development period		48.15±0.87 (7.01)	51.52±0.63 (7.35)	51.86±0.85 (7.16)	42.38±0.29 (6.58)	(0.15)
Male: female		2.10±0.60 [8.06]	1.71±0.75 [6.93]	2.03±0.80 [7.88]	1.90±0.60 [7.76]	[0.42]

*mean of four replications based on five plants; ( ) – parentheses are √ (n+1) transformation; [ ] – parentheses are arc sine transformation; ns – not significant; CD – critical difference

Fourth Instar

Development of the fourth instar was significantly maximum (Table 1–3) on *B. rapa* (3.80±0.20; 4.33±0.25 and 3.54±0.14 days) while minimum on *B. carinata* (3.58±0.18; 3.47±0.19 and 2.34±0.10 days) during the P, F1, and F2 generations, respectively. The study was in agreement with Sood and Bhalla (1996) who reported 2.9–5.9 days, Gupta (1984) 3.5–5 days, and Thapa (1987) 2–3 days, but differed from Chandel et al. (1998) who reported 5.82–10.13 days.

Fifth Instar

The data (Table 1) revealed that the growth period of the fifth instar was significantly maximum on *B. napus* (3.41±0.15 days) during the P generation. and on *B. juncea* (4.80±0.28 and 2.97±0.21 days) (Table 2 and 3) during the F1, and F2 generations, respectively. Growth of this instar was significantly minimum (Tables 1, 2, and 3) on *B. carinata* (2.98±0.08, 4.46±0.13 and 2.23±0.10 days) during the P, F1, and F2 generations, respectively. Sood and Bhalla (1996) reported 5.9–9.9 days, Chandel *et al.* (1998) 7.81–23.60 days, Gupta (1984) 4–6 days, while Thapa (1987) reported 5–7 days of the fifth instar period.

Total larval period

The total larval period (Table 1–3) was significantly maximum on *B. juncea* (19.69±1.13, 24.98±0.85 and 18.40±0.85 days) while minimum on *B. carinata* (18.13±0.41, 23.03±0.61 and 14.20±0.61 days) during the P, F1, and F2 generations, respectively.

Pre-pupal period

The data (Table 1) revealed that the pre-pupal period was significantly maximum on *B. napus* (2.15±0.05 days), *B. juncea* (2.41±0.10 days) (Table 2), and *B. rapa* (2.11±0.08days) (Table 3) while minimum (Tables 1, 2, and 3) on *B. carinata* (1.98±0.06, 1.98±0.13 and 1.79±0.03 days) during the P, F1, and F2 generations, respectively.

Pupal period

The pupal period (Tables 1–3) was significantly maximum on *B. rapa* (20.98±0.53, 10.23±0.14 and 10.47±0.45 days) while minimum on *B. carinata* (18.45±0.74, 8.55±0.09 and 8.52±0.25 days) during the P, F1, and F2 generations, respectively.

Adult longevity

The data (Table 1) revealed that the adult period was significantly maximum on *B. napus* (7.51±0.47 days), *B. rapa* (6.02±0.23 days) (Table 2), and *B. juncea* (6.06±0.38 days) (Table 3) while minimum on *B. carinata* (5.83±0.25, 4.77±0.24 and 5.53±0.37 days) (Tables 1–3) during the P, F1, and F2 generations, respectively. Our study is in agreement with Thapa (1987) 1–7 days, but differs from Sood and Bhalla (1996) who reported18 days, Chandel *et al.* (1998) 3.15–4 days and Chandra and Lal (1976) 4–5 days, which may be due to different host and environmental conditions.

Sex ratio

The data in Table 1 reveal that the sex ratio (Male: Female) was significantly maximum on *B. juncea* (1.27±0.68) while minimum on *B. napus* (1.02±0.24) during the P generation. During the F1 generation (Table 2), the sex ratio was significantly maximum on *B. rapa* (1.80±0.54) while minimum on *B. carinata* (1.23±0.24). During the F2 generation (Table 3), the ratio was highest on *B. napus* (2.10±0.60) while minimum on *B. juncea* (1.71±0.75 days). Thapa (1987) reported a 1.22:1 (Male: Female) ratio on Chinese sarson which may be due to different host and prevailing environmental conditions.

Total developmental period

The total development period (Tables 1–3) was significantly maximum on *B. rapa* (69.92±0.61, 61.94±0.48 and 51.86±0.85 days) while minimum on *B. carinata* (61.95±0.45, 54.16±0.38 and 42.38±0.29 days) during the P, F1, and F2 generations, respectively. Our study is agreement with Sood and Bhalla (1996) who reported the total development period ranging from 32–64 days in three generations.

DISCUSSION

Our experiments demonstrated that the *B. rapa* probably has an antixenosis effect on the life cycle of *P. brassicae*, which caused a longer development period. The data also showed that *B. rapa* delayed the hatching of eggs of *P. brassicae* by elongating the incubation period during parental, F1, and F2 generations. Moreover, it also elongated the pupal period during the three generations under study. It could probably be due to the absence of sinigrin or related glucosinolates in this species (Fahey *et al.* 2001). On the other hand, *B. carinata, B. juncea,* and *B. napus* possess the attractant, sinigrin. However, the authors are not certain whether antibiosis also plays a significant role on the life cycle of *P. brassicae.*

B. carinata had the highest total number of eggs laid per plant and eggs per cluster suggesting the preference of *P. brassicae* over other species, and this is in agreement with Behan M. and Schoonhoven L.M (1978) and Mitchell N.D. (1977). The incubation period was shortest on *B. carinata* during all three generations in this study. On the other hand, higher hatching percentage of eggs, faster growth of insect during different larval instars, pre-pupal, pupal and adult stages were observed in this species. The total development period during all three generations was shortest on *B. carinata*. Jindal *et al.* (2010) also reported that *P. brassicae* preferred *B. carinata* for egg laying rather than *B. rapa, B. juncea* and *B. napus.*

Based on the results from this study and the above conclusions, damage caused by *P. brassicae* could be reduced by growing resistant *Brassica* sp (*B. rapa*) and using a recommend breeding programs on this species to reduce the yield loss.

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