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Colour preference and percent parasitization of *Trichogramma chilonis* on of *Corcyra* eggs obtained from *Corcyra cephalonica* reared on different diets

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Abstract

The current investigations were conducted between 2021 and 2023 in the Biological Control laboratory, Department of Entomology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya (IGKV), Raipur, Chhattisgarh. Studies on the egg laying preference and emergence pattern of *Trichogramma chilonis* on different coloured sentinel cards under laboratory conditions revealed that the species revealed varied behavioural pattern of egg laying (parasitization) and emergence on the three distinct colour cards examined under multiple-choice conditions: yellow, pink, and blue. The overall mean parasitization percentage of *T. chilonis* during the entire experimental period was found highest on yellow card *i.e.*, 80.07% and most preferred card for adult emergence was pink cards with 184.4%. Thus, it can be concluded that *T. chilonis* preferred yellow cards for maximum parasitization and pink cards for maximum adult emergence.

Keywords: Colour preference, *Trichogramma* spp., sentinel cards, colour cards

Introduction

Biological control of agricultural pests is a safe, cost-effective, and ecologically friendly strategy within integrated pest management systems. Recent study emphasises the need of bulk raising of biocontrol organisms to permit augmentation and field releases. Among the significant biocontrol agents, include egg parasitoids, such as *Trichogramma* spp., and egg-larval parasitoids, including *Chelonus* spp, which are reared using *Corcyra cephalonica* as host insect (Kumar and Murthy, 2000) [1]. According to Jalali and Singh (1992) [6], the rice moth is used in a variety of biocontrol research, development, and extension units in India to produce a large number of natural enemies. This cosmopolitan pest is commonly found in stored grains and infests rice, millets, and other cereal grains. It shows a preference for broken grains and flour rather than whole grains. Due to its ability to be mass-produced year-round, it serves as an ideal host insect for the large-scale rearing of many parasitoids and predators.

The first *Trichogramma* species in North America was described by Charles V. Riley in 1871. *T. minutum* was the name he gave to the small wasps that emerged from viceroy butterfly eggs. *Trichogramma* species have been found in India, with *T. chilonis*, *T. japonicum*, and *T. achaeae* being particularly important in biological control. *Trichogramma* (Hym.: Trichogrammatidae) egg parasitoids are essential natural enemies of a variety of insect pests and are effectively used in biological control efforts across the world. (Smith, 1996) [11].

Trichogramma genera is receiving attention because they assist in reducing pest damage to crops such maize, cole, rice, sugarcane, vegetables, fruit trees, and stored grains (Li 1994; Smith 1996; Gagnon *et al.*, 2017) [8, 11, 5]. Research indicates that our nation experiences an annual agricultural loss of approximately 30 percent due to a variety of pests, resulting in an estimated economic impact of around Rs. 1.40 lakh crore (Businessline, 2007) [4].

The present investigation was conducted to investigate the egg laying responses of female *Trichogrammatid* to various coloured cards, with the purpose of stimulating the parasitization to deposit more eggs on the egg cards containing its host, hence increasing the frequency of parasitization. The colour of trichocards influences the parasitic effectiveness of *Trichogramma* spp.

Materials and Methods

The current research was conducted in the Biological Control Laboratory, Department of Entomology, College of Agriculture, IGKV, Raipur (C.G.).

In multi-choice situations, the parasitization response of *Trichogramma chilonis* to different coloured sentinel cards was studied. 4 × 3.5cm cards in pink, yellow, and blue were utilised for these three coloured cards. The coloured sentinel cards were made by glueing 100 newly deposited UV irradiated *C. cephalonica* eggs on corresponding coloured cards with 10% acacia gum. As a multichoice experiment, these cards were kept equidistantly inside a petridish plate 4 x 3.5cm, and at the centre of the container, a small already parasitized egg card of respective species containing 10 numbers of parasitized eggs (likely to emerge) was placed on the top of petridish with the help of a piece of two way tape, and this cloth was turned upside down inside basin and covered with parafilm tape. Thus, three different coloured sentinel cards were exposed to previously parasitized cards that were expected to develop as adults of (*T. japonicum*) and kept in separate petridishes. Following that, the container was covered to prevent mature parasitoids from escaping. For 24 hours, the wasps were permitted to parasitize the new egg cards. The parasitized eggs became black after about 4 days. The percentage of parasitization was determined by examining the egg hole, and adult emergence was determined following parasitization. (Singh *et al.*, 2017)^[9]. Each trial was repeated five times for conformity.

Statistical analysis

Black coloured eggs were considered as the parasitized eggs, on the basis of which, percent parasitization was determined by following formula given by Janghel *et al.*, 2019^[7].

$$\text{Percent parasitisation} = \frac{\text{Number of parasitised eggs in trichocards}}{\text{Total number of eggs in trichocard}} \times 100$$

Out of total parasitized eggs, the number of parasitoids emerged on the basis of holes on parasitized eggs, were counted and percent adult emergence was determined by given by Janghel *et al.*, 2019^[7].

$$\text{Adult Emergence Percentage} = \frac{\text{Number of adults emerged from trichocards}}{\text{Total number of parasitized eggs in trichocards}} \times 100$$

Results and Discussion

Mean percent parasitization of *Trichogramma chilonis*

In the case of *T. chilonis*, highest parasitization was found on yellow cards, followed by pink cards.

In the 1st set of experiment, conducted during (05/10/21), yellow cards showed highest parasitization rate, 83.6%, which was considerably higher than cards in blue and pink. Table 1 from the data, it is also clear that blue cards were significantly least preferred, with 67.9%. In the next year (06/10/2022), highest percentage of parasitization was again recorded on yellow cards with 84.1%, which was much higher than blue and pink cards. Table 2 showed that the blue cards were the least preferred, with 68.81%, which was far higher than the blue and pink cards.

In the 2nd set (11/11/21), yellow cards had the greatest parasitization rate, 81.75%, which was much higher than all other colour cards. Pink cards had the lowest parasitization rate, 67.3%, which is much lower than yellow and blue cards, as seen in Table 1. The next year (12/11/22), it was discovered that yellow cards had the greatest parasitization rate of 82.68%, which was much higher than any other colour

cards. As shown in Table 2, the pink card had the lowest parasitization rate of 68.5%, which was much lower than the yellow and blue cards.

In the 3rd set (09/12/21) the most favoured cards for parasitization by *T. chilonis* were yellow cards (77.3%), which were substantially superior to other cards, however blue cards were on par. Pink cards were shown to be the least desired for parasitization, with a 60.49% preference, which was much lower than yellow and blue cards, as seen in Table 1. Similarly, in the following year (10/12/2022), yellow cards were the most preferred by *T. chilonis*, with 78.45% being significantly superior to other cards, but blue cards were at par significant for parasitization and were significantly superior to other cards but on par with blue cards. The least preferred cards for percent parasitization was pink cards *i.e.*, 61.51%, which was significantly lower than yellow and blue cards as Table 2.

Highest percentage of parasitization was observed on yellow cards in the 4th set of experiment (10/01/22) with 78.55%, which was significantly superior than blue and pink cards. (Table 1) revealed that the blue cards had the lowest parasitization rate, with 52.32%, which was significantly lower than other cards. During (11/01/23), the maximum percentage of parasitization was recorded on yellow cards, 79.59%, which was substantially higher than blue and pink cards, while the lowest percentage of parasitization was found on blue cards, 47.63%, as shown in Table 2.

In 5th set (04/02/22) maximum percent parasitization was recorded on pink cards *i.e.*, 78.35% which was significantly higher than blue except yellow cards which is at par. The minimum percent parasitization was found on blue cards with 67.42% which is significantly lower as given in Table 1. During (05/02/23) it was observed that the maximum percent parasitization was recorded on pink cards *i.e.*, 79.45% which is significantly higher than blue except yellow cards which is at par. The minimum percent parasitization was found on blue cards with 68.45% which is significantly lower as given in Table 2.

Table 3 clearly showed that in both years, the mean percent parasitization was considerably greater in yellow cards in the months of October, November, December, January, and February, *i.e.*, 83.6%, 81.75%, 77.3%, and 78.55%, respectively. In February, the egg laying percentage on pink colour cards was high, at 79.45%.

Thus, the Over All parasitization percent during the entire experimental period was maximum on yellow cards *i.e.*, 80.07%. Hence based on the above data it can be concluded that yellow colour cards were most preferred by *T. chilonis* followed by pink cards *i.e.*, 70.95%.

The current findings agree with Vishla *et al.*, (2007)^[12], who mentioned that *T. chilonis* parasitized maximum on yellow cards (81.37%), but do not agree with Bhattacharya *et al.*, (2003)^[3], Who discovered the most parasitism on mint green coloured egg cards (64.7%) and the least parasitism on chass is grey coloured egg cards (11.50%).

The present findings, are in agreement with Singh *et al.* (2017)^[9] corroborated these findings by noting peak parasitism rates on yellow cards, reaching 85.00% and 86.00% after 8 days in 2014–2015, respectively. Conversely, white-colored cards demonstrated the least parasitization, recording rates of 75.25% and 77.40%, respectively. A contrasting outcome was reported by Singh and Singh (2001)^[10], who observed higher parasitization on pink tricho-cards, with a notable 80.50 eggs per female after 6 days.

Table 1: Mean percent parasitization of *Trichogramma chilonis* during the year 2021-22

Colour card	05-10-2021	11-11-2021	09-12-2021	10-01-2022	04-02-2022	Overall mean
Yellow	83.6	81.75	77.3	78.55	76.87	79.614
	(67.83)	(66.81)	(62.67)	(63.6)	(61.4)	
Pink	78.9	67.3	60.49	67.45	78.35	70.498
	(64.15)	(55.7)	(51.11)	(55.1)	(63.2)	
Blue	67.9	76.59	74.57	52.32	67.42	67.76
	(55.99)	(62.09)	(60.21)	(46.6)	(54.68)	
S.Em±	3.17	3.43	2.89	2.63	2.14	
CD at 5%	9.19	10.05	8.4	7.67	6.15	

(Figures in parentheses arc sin transformed values)

Table 2: Mean percent parasitization of *Trichogramma chilonis* during the year 2022-23

Colour card	06-10-2022	12-11-2022	10-12-2022	11-01-2023	05-02-2023	Overall mean
Yellow	84.1	82.68	78.45	79.59	77.9	80.544
	(68.85)	(67.84)	(63.69)	(64.9)	(62.03)	
Pink	79.11	68.5	61.51	68.52	79.45	71.418
	(65.16)	(56.81)	(52.23)	(57.31)	(64.27)	
Blue	68.81	77.61	75.63	54.61	68.45	69.022
	(56.05)	(63.16)	(61.15)	(47.63)	(55.48)	
S.Em±	3.2	3.45	2.91	2.64	2.15	
CD at 5%	9.2	10.07	8.51	7.7	6.21	

(Figures in parentheses arc sin transformed values)

Table 3: Effect of coloured cards on percent parasitization of *T. chilonis* at during the year 2021-22, 2022-23

Colour card	Mean percent parasitization		Overall parasitisation
	2021-22	2022-23	
Yellow	79.614	80.544	80.079
Pink	70.498	71.418	70.958
Blue	67.76	69.022	68.391

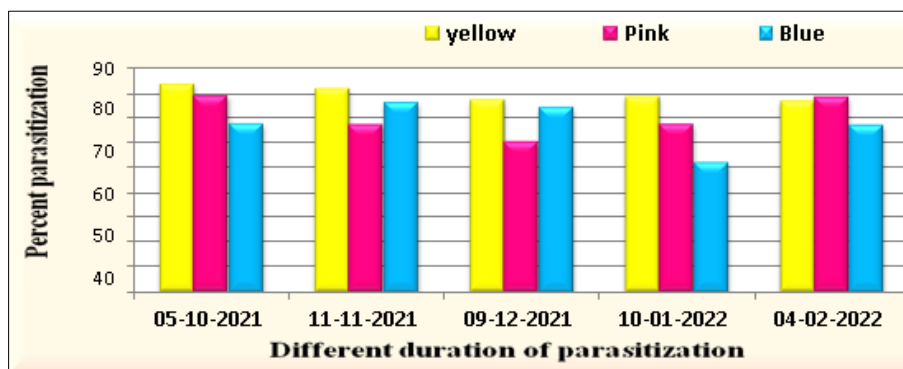


Fig 1: Mean percent parasitization of *T. chilonis* during the year 2021-22

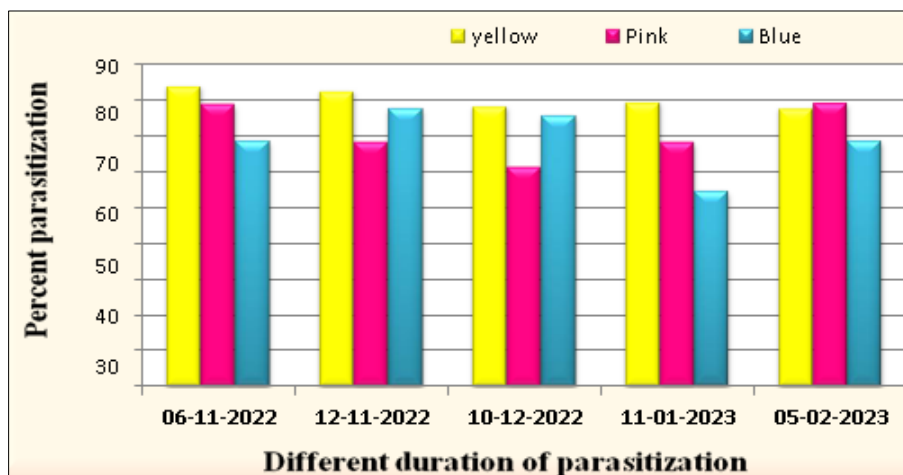


Fig 2: Mean percent parasitization of *T. chilonis* during the year 2022-23

Mean percentage of adult emergence of *Trichogramma chilonis*

In 2021, within the 1st set of observations, the highest percentage of adult emergence was noted on yellow cards, reaching 203%, a statistically significant difference when compared to all other color cards. Conversely, the lowest adult emergence percentage was recorded on blue cards, measuring 181%. This value was notably lower than that of the pink cards, as indicated in Table 4. Similarly, in 2022, the trend persisted, with the maximum adult emergence percentage occurring once again on yellow cards at 205%,

significantly surpassing all other color cards. Conversely, the minimum adult emergence percentage was observed on blue cards at 182%, which was notably lower than the emergence rate observed with pink color cards (191%), as detailed in Table 5.

In the 2nd set during (11/11/21), highest number of adult emergence percentage was found on pink cards, 198%, and the on yellow cards, 131%, which showed significant differed with pink cards except for blue colour cards, which was on par, as shown in Table4. On 12/11/2021, the investigation revealed that the greatest percentage of adult emergence

occurred on pink cards, reaching 199%. In contrast, the lowest emergence percentage was observed on yellow cards, amounting to 132%. Notably, this difference was statistically significant when compared to the emergence on pink cards, while the emergence on yellow cards was similar to that on blue cards, as detailed in Table 5.

In the 3rd set of experiment (09/12/21) maximum adult emergence percentage was found on pink *i.e.*, 198% which was significantly higher than other and lowest was found on yellow card *i.e.*, 126% which was on par with blue card (129%) is given in Table 4. Repetition of the experiment in the next year (10/12/22) again revealed that maximum adult emergence percentage was found on pink *i.e.*, 198% which was significantly superior than other and lowest was adult was found on yellow card *i.e.*, 127% which was on par with blue card (130%) as given in Table 5.

In the 4th set of observations (10/01/22), the highest percentage of adult emergence was recorded on pink cards, reaching 171%. This value was significantly higher than the emergence on other cards. Conversely, the lowest emergence percentage was observed on yellow cards, amounting to 96%, a value significantly lower than that on blue cards (108%), as outlined in Table 4. Similarly, in the subsequent year (11/01/23), the maximum percentage of adult emergence was significantly found on pink cards at 172%. In contrast, the minimum emergence percentage occurred on yellow cards, measuring 97%, and this was notably lower than the emergence on blue cards (110%), as presented in Table 5.

In the 5th set of observations (04/02/22), the highest percentage of adult emergence was documented on pink cards, amounting to 166%. This value was notably higher than the emergence percentages observed on yellow and blue cards. Conversely, the lowest emergence percentage was recorded on yellow cards, measuring 78%, a result comparable to the emergence on blue cards (84%), as outlined in Table 4. Continuing into the following year (05/02/23), the maximum adult emergence percentage was once again recorded on pink cards, reaching 168%, mirroring the previous year's findings. This value remained significantly higher than the emergence percentages observed on yellow

and blue cards. Table 5 indicated that the minimum adult emergence percentage was noted on yellow cards (80%), aligning with the emergence rate on blue cards (85%).

Looking to the monthly observations, maximum percentage of mean adult emergence percentage (205%) were found on yellow cards in October followed by 199% on pink cards in November, 198% on pink cards in February. However, over all mean depicted maximum adult emergence percent in 185.6% in pink cards and lowest in blue cards that is 127.7%. Thus, it can be concluded highest adult emergence percentage were found on pink cards when temperature was comparatively lower followed by blue colour cards as given in Table 6.

Similar findings were reported by Singh *et al.* (2017) [9], who noted that maximum emergence on pink-colored egg cards was observed in the years 2014 and 2015, respectively, to the amount of 87.57 and 86.85 percent. On white coloured egg cards, the lowest percentage of emergence was seen on the 12th day in both years, to the amount of 76.35 and 77.40 adults, respectively. This finding is in accordance with Bhattacharya *et al.*, (2003) [3] who observed minimum adult emergence on white (87.76%).

Table 4: Mean percentage of adult emergence of *T. chilonis* during the year 2021-22

Colour card	09-10-2021	15-11-2021	13-12-2021	14-01-2022	08-02-2022	Overall mean
	203*	131*	126*	96*	78*	126.8
Yellow	2.03	1.31	1.26	0.96	0.78	
	(8.1)	(6.54)	(6.4)	(5.54)	(4.8)	
	189*	198*	198*	171*	166*	184.4
Pink	1.89	1.98	1.98	1.71	1.66	
	(7.79)	(7.98)	(7.98)	(7.22)	(7.19)	
	181*	133*	129*	108*	84*	127
Blue	1.81	1.33	1.29	1.08	0.84	
	(7.64)	(6.51)	(6.42)	(5.65)	(4.9)	
S.Em±	0.38	0.41	0.44	0.62	0.7	
CD at 5%	1.12	1.92	1.28	1.83	2.05	

(Figures in parentheses arc sin transformed values)

(Value with * mark are the percent emergence after multiplication with 100)

Table 5: Mean percentage of adult emergence of *T. chilonis* during the year 2022-23

Colour card	10-10-2022	16-11-2022	14-12-2022	15-01-2023	09-02-2023	Overall mean
	205*	132*	127*	97*	80*	128.2
Yellow	2.05	1.32	1.27	0.97	0.8	
	(8.3)	(6.55)	(6.5)	(5.55)	(4.96)	
	191*	199*	198*	172*	168*	185.6
Pink	1.91	1.99	1.98	1.72	1.68	
	(7.8)	(7.99)	(7.98)	(7.23)	(7.2)	
	182*	135*	130*	110*	85*	128.4
Blue	1.82	1.35	1.3	1.1	0.85	
	(7.65)	(6.52)	(6.43)	(5.67)	(4.91)	
S.Em±	0.4	0.42	0.45	0.63	0.85	
CD at 5%	1.14	1.93	1.29	1.84	2.15	

(Figures in parentheses arc sin transformed values)

(Value with * mark are the percent emergence after multiplication with 100)

Table 6: Effect of coloured cards on percentage of adult emergence of *T. chilonis* at during year 2021-22, 2022-23

Colour card	Mean emergence		Overall Adult Emergence
	2021-22	2022-23	
Yellow	126.8	128.8	127.8
Pink	184.4	185.6	185.0
Blue	127	128.4	127.7

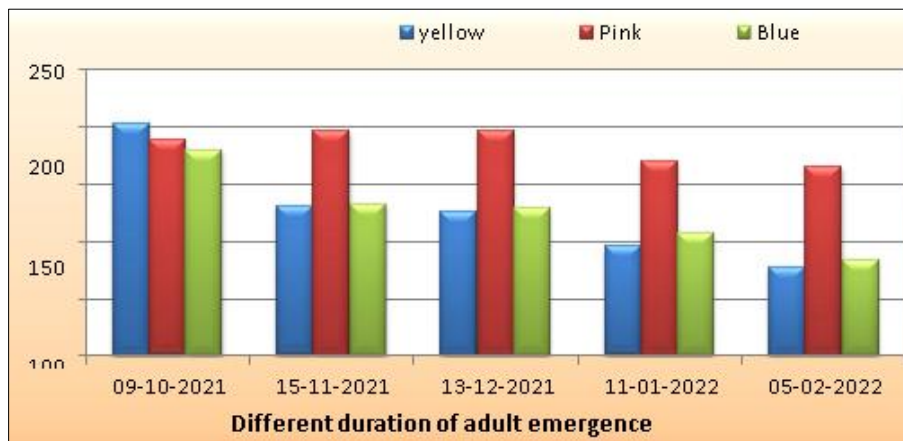


Fig 3: Mean percentage of adult emergence of *T. chilonis* during the year 2021-23

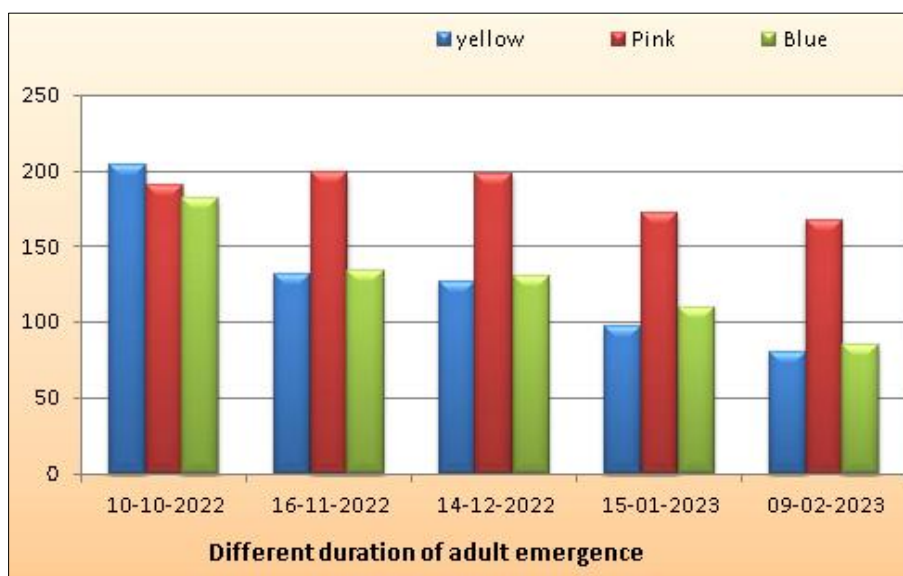


Fig 4: Mean percentage of adult emergence of *T. chilonis* during the year 2022-23

Conclusions

From the above studies it can be concluded that yellow cards were the most preferred colour for *T. chilonis* for egg laying, followed by pink cards. However, the percentage of *T. chilonis* emerging from eggs was greater in pink cards. Blue sentinel cards were not selected for egg laying and did not exhibit a good emergence pattern also. To conclude among the four coloured sentinel cards yellow and pink cards can be used for mass production of *T. chilonis*.

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