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Anjali Manhar
Department of Entomology,
College of Agriculture, Indira
Gandhi Krishi Vishwavidyalaya,
Raipur, Chhattisgarh, India

RN Ganguli
Department of Entomology,
College of Agriculture, Indira
Gandhi Krishi Vishwavidyalaya,
Raipur, Chhattisgarh, India

Jayalaxmi Ganguli
Department of Entomology,
College of Agriculture, Indira
Gandhi Krishi Vishwavidyalaya,
Raipur, Chhattisgarh, India

Rohit Kumar
Department of Genetics and
Plant Breeding, Indira Gandhi
Krishi Vishwavidyalaya, Raipur,
Chhattisgarh, India

Corresponding Author:
Anjali Manhar
Department of Entomology,
College of Agriculture, Indira
Gandhi Krishi Vishwavidyalaya,
Raipur, Chhattisgarh, India

Colour preference for egg laying and emergence of *Trichogramma japonicum* (Ashmead) using sentinel cards under laboratory condition

Anjali Manhar, RN Ganguli, Jayalaxmi Ganguli and Rohit Kumar

Abstract

The present investigation on colour preference for egg laying and emergence of *Trichogramma japonicum* was conducted at the Biocontrol laboratory, Department of Entomology, IGKV, Raipur (C.G.) during the year 2021-2022. The results revealed that *Trichogramma* spp. showed different behavioural pattern of egg laying on different colour cards viz., yellow, pink, blue and white under multi-choice conditions. Over all mean percent parasitization revealed that *T. japonicum* laid more number of eggs on pink card from December to April (mean 58.13), however, when the temperature was low (i.e., during December, January and February) yellow cards were preferred for egg laying and maximum adult emergence percentage was observed in yellow cards i.e., 152% where as white colour cards were recorded with minimum adult emergence, i.e., 104%.

Keywords: Egg laying and emergence, *Trichogramma japonicum*, sentinel cards

Introduction

Trichogrammatid egg parasitoids (Hymenoptera: Trichogrammatidae) are the most widely used natural enemies in biological control strategies worldwide. They are effective biocontrol agents for suppression and control of lepidopterous pests on many economic crops. The genera *Trichogramma* has gained an attention as they are capable of reducing pest damage in corn, cole, rice, sugarcane, vegetables, fruit trees, and stored grains (Li 1994; Smith 1996; Gagnon *et al.*, 2017) [4, 3, 5]. Due to their worldwide success, these minute polyphagous endoparasitoids are being commercially produced. *Trichogramma* wasps belong to the family Trichogrammatidae of order hymenoptera these are tiny (0.5 mm long) parasitoid that attack the eggs of over 200 species (mostly lepidoptera). The tiny females drill into the host egg to lay their own egg within. They occur naturally but in most crops production systems the number of caterpillar eggs destroyed by the native population of *Trichogramma* is not sufficient to prevent pest population from damaging level. *Trichogramma* wasps are reared and released in the field. The present studies were undertaken to investigate the egg laying responses of female Trichogrammatid to different coloured cards with an objective that suitable colour of the egg card may stimulate the parasitization to deposit more eggs on the egg cards containing its host and thus increasing the extent of parasitization. Colour of trichocards plays an important role in parasitizing efficiency of *Trichogramma japonicum* (Singh *et al.*, 20017) [1].

Material and Methods

Investigation of the parasitization response of *Trichogramma japonicum* to different coloured egg cards were under taken by performing multi choice test. For this four coloured cards viz., yellow, pink, blue and white coloured cards (4 x 3.5 cm) were used replicated seven times. The temperature and relative humidity were maintained in the laboratory at 27±2 °C and 65±5%, respectively. The life cycle of *Trichogramma* ranges from 8-10 days in summer and 9-12 days in winter. The coloured sentinel cards were prepared by glueing 100 number of freshly laid UV irradiated eggs of *C. cephalonica* on respective coloured cards with the help of 10% acacia gum. These cards were kept equidistantly on the bottom of a plastic basin of rim size 27 cm and 9 cm depth as a multichoice experiment and at the centre of container, a small already parasitoid egg card respective species containing 20 numbers of parasitoids eggs (likely to emerge) was placed on the top of muslin cloth (with the egg side facing the inside of basin) with the help of a pieces of two way tape, and this cloth was turned inside basin and covered

by the help of rubber band (Plate 1, 2, 3, 4 & 5). Thus, four different coloured sentinel cards were exposed to already parasitized card which were likely to emerge as adults of *T. japonicum* were kept in separate plastic basins. Then the container was covered in order to prevent the possible escape of adult parasitoids. The wasps were allowed to parasitize fresh egg cards for 24 hrs. After around 4 days the parasitized eggs turned black. The percent parasitization was recorded by observing the egg hole and adult emergence was recorded on after parasitization (Singh *et al.*, 2017) [1]. This method was repeated at 15 days interval. In this way 10 repeated sets were experimented for conformity.



Plate 1: Materials required for making coloured sentinel cards



Plate 2: Materials required for experiment



Plate 3: Four different coloured sentinel cards placed in the plastic tubs



Plate 4: Trichocards placed under muslin cloth inside plastic tubs covered with white muslin cloth



Plate 5: Parasitized eggs of *C. cephalonica* eggs turned black after 5 days

Result and Discussion

Mean percent parasitization of *T. japonicum*

In case of *T. japonicum* the highest number of parasitization were recorded in yellow coloured cards followed by pink coloured cards. The results are presented in Table 1 and Fig 1. In 1st set (01-12-21), highest percent parasitization was recorded in yellow cards *i.e.*, 81.42% which was significantly superior over the other coloured cards, except pink cards, where it was 71.71%. The least preferred colour was white *i.e.*, 59.71% which was significantly lower than other colours. In the 2nd set (17-12-21), highest percent parasitization was recorded in yellow cards *i.e.*, 78% followed by pink cards *i.e.*, 70.85% which was non significant. The lowest percent parasitization was recorded on white cards *i.e.*, 59.28% which was significantly lower than yellow cards.

In the 3rd set (02-01-22), maximum percent parasitization was seen on blue cards *i.e.*, 78.14% followed by yellow cards *i.e.*, 71.71% which were non significant to each other and the lowest percent parasitization was recorded on white cards *i.e.*, 61.71% which was significantly lower than blue cards.

In the 4th set (18-01-22), maximum percent parasitization were recorded on yellow cards and blue cards *i.e.*, 77.71 in both and the lowest percent parasitization is recorded on white *i.e.*, 61.85% which showed non significant differences from each other.

In the 5th set (01-02-22), highest parasitization percentage was recorded on blue cards *i.e.*, 61.42% which was significantly superior over other colour cards and lowest parasitization

percentage recorded on white cards with 45% which was significantly lower than yellow cards and non significant with respect to blue and pink colour cards.

In the 6th set (19-02-22), maximum parasitization percent was recorded on pink cards *i.e.*, 60.85% which was significantly superior to all other cards except yellow one. The colour cards which showed lowest parasitization percentage was white cards *i.e.*, 45.28% and exhibited non significant differences between yellow and blue cards.

In the 7th set (04-03-22), the highest percent parasitization was shown on pink cards *i.e.*, 57.57% and least percent parasitization recorded on white cards *i.e.*, 45.85%, with non significantly differences from each other.

In the 8th set (14-03-22), maximum percent parasitization recorded on pink cards *i.e.*, 49.71% which significantly higher than yellow and white cards, whereas it was on par with blue cards with 49.28% parasitization. The lowest percent parasitization was recorded on yellow cards *i.e.*, 34.28 % which was non significantly different from that of white cards.

In the 9th set (05-04-22), highest percent parasitization showed on pink cards *i.e.*, 49.42% which significantly superior than yellow and white and the lowest percent parasitization is recorded on white *i.e.*, 36.85% which was non significantly different than yellow cards.

In the 10th set (16-04-22), maximum percent parasitization recorded on pink cards *i.e.*, 40.14 % which was significantly superior than yellow and white cards except blue cards. The lowest percent parasitization was recorded on white cards *i.e.*, 12.42% which significantly inferior than blue and pink cards except yellow cards.

The monthly average and over all mean percent parasitization of different coloured sentinel cards by *T. japonicum* is given in Table 2 and Fig 2.

Perusal of Table 2 and Fig 2, clearly indicates that during the months of December and January the mean egg laying percentage were comparatively higher in yellow cards *i.e.*, 79.71%, 74.71% and 48.42%, respectively. In the later months, the egg laying percentage was higher in pink colour cards *i.e.*, 53.21%, 53.64% and 44.78% during the month of February, march and April, respectively. Therefore, from the overall mean parasitization data, it can be concluded that *T. japonicum* laid more number of eggs on pink cards from December to April (mean 58.13), however, when the temperature was low (*i.e.*, during December and January) yellow cards were preferred for egg laying as it is clear from the data shown in Table 2 and Fig 2.

These results are in accordance with the findings of earlier studies done by Vishla *et al.* (2017) ^[2] who also recorded maximum parasitization of *T. japonicum* on red (52.12%).

Table 1: Mean percent parasitization of *T. japonicum* during the year 2021-22

Colour cards	01-12-21	17-12-21	02-01-22	18-01-22	01-02-22	19-02-22	04-03-22	14-03-22	05-04-22	16-04-22
Yellow	81.42 (65.02)	78.00 (62.31)	71.71 (57.57)	77.71 (58.76)	48.28 (43.99)	48.57 (44.13)	46.71 (43.66)	34.28 (35.71)	38.28 (38.05)	13.42 (20.97)
Blue	64.42 (64.42)	64.28 (53.6)	78.14 (63.08)	71.71 (57.57)	61.42 (51.75)	45.42 (42.31)	48.57 (44.13)	49.28 (44.7)	48.71 (44.15)	37.42 (37.68)
Pink	71.71 (71.71)	70.85 (58.35)	65.57 (54.3)	70.00 (57.89)	45.57 (42.4)	60.85 (51.43)	57.57 (48.14)	49.71 (44.81)	49.42 (44.66)	40.14 (39.2)
White	59.71 (50.64)	59.28 (50.38)	61.71 (51.84)	61.85 (52.15)	45.00 (42.06)	45.28 (42.23)	45.85 (43.46)	36.14 (36.79)	36.85 (37.3)	12.42 (20.1)
SE(m)±	2.47	2.90	2.75	3.22	2.20	2.50	2.41	2.58	2.14	1.86
CD at 5%	7.26	8.51	8.1	NS	6.48	7.36	NS	7.57	6.3	5.47

(Figures in parentheses arc sin transformed values)

Table 2: Effect of coloured cards on percent parasitization of *T. japonicum* at different months during the year 2021-22

S. No	Colour cards	Mean percent parasitization of <i>Trichogramma japonicum</i>					Over all mean
		December	January	February	March	April	
1	Yellow	79.71	74.71	48.42	40.49	25.85	53.83
2	Blue	64.35	74.92	53.42	48.92	43.06	56.93
3	pink	71.28	67.78	53.21	53.64	44.78	58.13
4	White	59.49	61.78	45.14	40.99	24.63	46.40

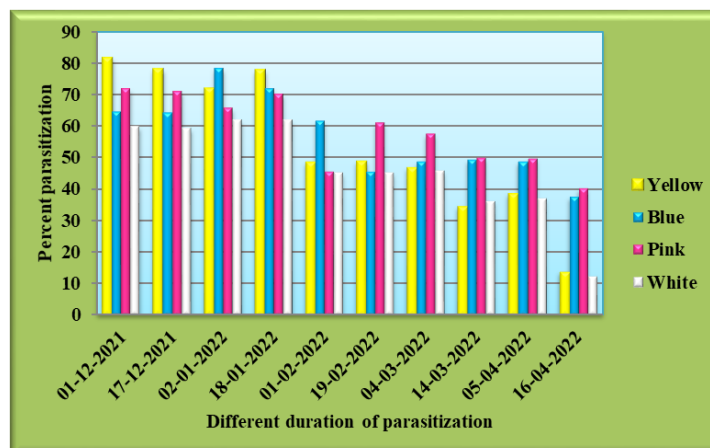


Fig 1: Mean percent parasitization of *T. japonicum* during the year 2021-22

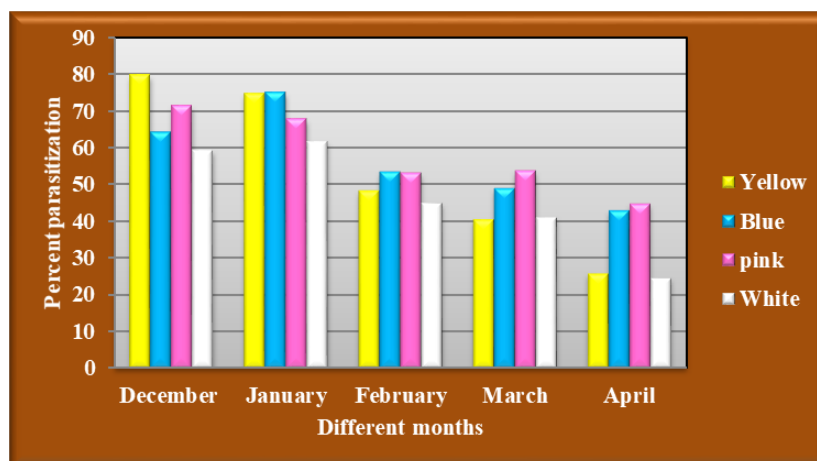


Fig 2: Effect of coloured cards on percent parasitization of *T. japonicum* at different months during the year 2021-22

Mean adult emergence percentage of *T. japonicum*

Polyembryony: In case of the study of emergence of *Trichogramma* adult from parasitized eggs, in most of the cases from a single parasitized eggs more than one *Trichogramma* adult emerged due to the character of polyembryony in *Trichogramma* spp.

Since, the adult emergence percentage of *Trichogramma* were more than 100 percent in many cases due to polyembryony, therefore the Angular transformation software was not supporting the data. As suggested by the Statistician, all the emergence data were calculated without multiplying by 100 and while interpretation of the data have been mentioned after multiplying with 100. The results are presented in Table 3 and Fig 3.

In 1st set, maximum percent emergence was observed on yellow cards *i.e.*, 199% and minimum percent emergence is observed on white cards *i.e.*, 149% which were non significantly different with each other.

In 2nd set, highest percent emergence was observed on yellow cards *i.e.*, 197% which were significantly higher than all colour cards except pink cards and lowest emergence percent was found on white cards *i.e.*, 138% which were significantly lower than all colour cards except pink cards.

In 3rd set, highest emergence percentage found on yellow cards (197%) and was significantly higher than all colour cards except blue and white colour cards which showed lowest emergence percent which is 137% that was significantly lower than all colour cards except blue cards.

In 4th set, maximum emergence percentage was found on yellow cards *i.e.*, 193% and was significantly higher than white cards except blue and pink cards were on par. The minimum emergence percentage found on white cards *i.e.*, 130% which is significantly lower than all colour cards except pink cards.

In 5th set, the most preferred card for emergence percent was yellow cards *i.e.*, 190% which were significantly higher than white cards except blue and pink cards which were on par. The least preferred card for emergence was white cards with 130% which is significantly lower than yellow and blue cards except pink cards which was on par with them.

In 6th set, maximum emergence percentage was found on blue cards *i.e.*, 190% which was significantly higher than all colour

cards except yellow cards and the minimum emergence percentage was found on which 123% which is significantly lower than all except pink cards is at par.

In 7th set, highest emergence percentage was found on blue cards *i.e.*, 182% which was significantly higher than white except yellow and pink cards and the lowest emergence was found on white cards with 118% which is significantly lower than blue cards.

In 8th set, highest emergence percentage was found on blue cards *i.e.*, 174% which were significantly higher than all colour cards but pink cards was on par with blue cards. The lowest emergence percentage were found on white cards *i.e.*, 113% which were significantly lower than blue cards except yellow and pink cards.

In 9th set, maximum emergence percentage was found on blue cards *i.e.*, 171% which were significantly higher than all colour cards except yellow cards and minimum emergence percentage was recorded on white cards *i.e.*, 108% which is significantly lower than blue cards.

In 10th set, maximum emergence was recorded on blue cards *i.e.*, 162% which were significantly higher than all colour cards except yellow cards and the minimum emergence was recorded on white cards 102% which were significantly lower than all colour cards except pink cards which was on par non significant.

In Table 4 and Fig 4, monthly observation and over all mean of percent parasitization of different coloured cards were observed. The mean adult emergence percentage were 198% and 195% on yellow cards in December and January, respectively which were higher than other colour cards but during later months 187%, 188%, 166% emergence were recorded on blue cards in February, March and April, respectively.

As we can conclude the over all mean date that maximum adult emergence percentage was seen in yellow cards *i.e.*, 152% from where as white colour cards showed minimum emergence *i.e.*, 104%.

The present studies contradicts with Vishla *et al.* (2017) [2] who found that the percent adult emergence of *T. japonicum* on different coloured egg cards showed non significant differences from each other.

Table 3: Mean adult emergence percentage of *T. japonicum* during the year 2021-22

Colour cards	05-12-21	22-12-21	07-01-22	23-01-22	06-02-22	24-02-22	09-03-22	19-03-22	10-04-22	21-04-22
Yellow	199* 1.99 (8.06)	197* 1.97 (8.03)	197* 1.97 (8.03)	193* 1.93 (7.95)	190* 1.90 (7.89)	183* 1.83 (7.74)	154* 1.54 (7.08)	119* 1.19 (6.23)	149* 1.49 (6.96)	140* 1.40 (6.72)
Blue	154* 1.54 (7.11)	148* 1.48 (6.96)	148* 1.48 (6.95)	186* 1.86 (7.80)	185* 1.85 (7.78)	190* 1.90 (7.89)	182* 1.82 (7.70)	174* 1.74 (7.53)	171* 1.71 (7.46)	162* 1.62 (7.27)
Pink	195* 1.95 (7.98)	189* 1.89 (7.86)	188* 1.88 (7.84)	147* 1.47 (6.93)	143* 1.43 (6.85)	140* 1.40 (6.76)	144* 1.44 (6.87)	150* 1.50 (6.97)	113* 1.13 (6.05)	111* 1.11 (6.04)
White	149* 1.49 (6.91)	138* 1.38 (6.66)	137* 1.37 (6.64)	130* 1.30 (6.44)	130* 1.30 (6.42)	123* 1.23 (6.28)	118* 1.18 (6.14)	113* 1.13 (6.01)	108* 1.08 (5.85)	102* 1.02 (5.69)
SE(m)±	0.37	0.36	0.35	0.36	0.36	0.36	0.36	0.37	0.38	0.37
CD at 5%	NS	1.06	1.05	1.06	1.08	1.06	1.07	1.09	1.13	1.11

(Figures in parentheses arc sin transformed values)

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

Table 4: Effect of coloured cards on adult emergence percentage of *T. japonicum* at different months during the year 2021-22

S. No	Colour cards	Mean emergence percent of <i>Trichogramma japonicum</i>					Over all mean
		December	January	February	March	April	
1	Yellow	198	195	186	136	144	152
2	blue	151	167	187	188	166	147
3	pink	192	167	126	147	112	114
4	White	144	89	126	115	105	104

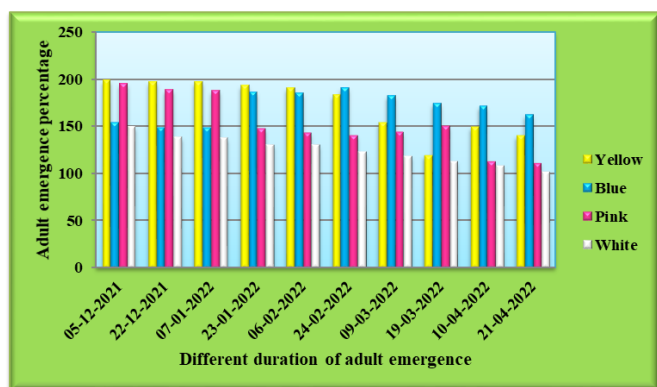


Fig 3: Mean adult emergence percentage of *T. japonicum* during the year 2021-22

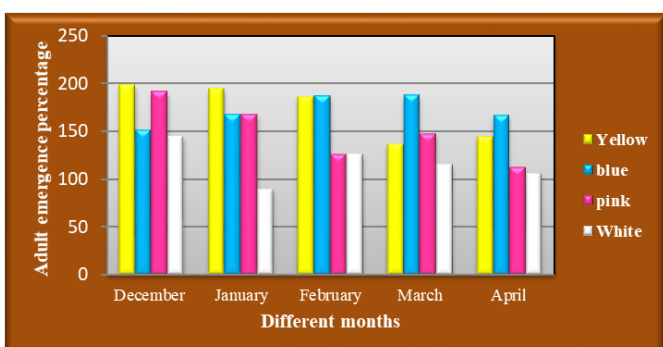


Fig 4: Effect of coloured cards on adult emergence percentage by *T. japonicum* at different months during the year 2021-22

Conclusions

The most preferred coloured cards for egg laying of *T. japonicum* was pink cards followed by yellow cards. However, the emergence percentage of *T. japonicum* from eggs was better in yellow cards. The white sentinel cards were neither preferred for egg laying nor it showed good

emergence pattern. To conclude among the four coloured sentinel cards pink or yellow cards can be used for mass production of *T. japonicum*.

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