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RESEARCH

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Economical factitious diet for mass rearing of greater wax moth, *Galleria mellonella* (Lepidoptera: pyralidae), a promising host for entomopathogenic nematodes

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Abstract

Background Greater wax moth (*Galleria mellonella* L.) (Lepidoptera: Pyralidae) is most commonly used for mass multiplication of entomopathogenic nematodes. An investigation on economical modification and comparative analysis of factitious diets of *G. mellonella* was carried out under laboratory conditions. Different diets were assessed in comparison with standard diet.

Results Modifications based on quantity change of dietary components (wheat flour, maize flour, dry milk, dry yeast, honey and glycerin), addition of vitamin E and vitamin-B complexes, elimination of dry milk and replacement of glycerin with sorbitol showed reduction in the cost of production and increase in food conversion efficiency in *G. mellonella*. The weight of larva in diet III (303.40 mg) was highest as compared to the larval weight in control diet (280.00mg). The conversion of ingested and digested food into biomass of *G. mellonella* was the highest for factitious diet III. Also, the highest number of *Steinernema abbasi* was produced from *G. mellonella* larvae reared on diet III (25418.43IJs) followed by diet I (21805.31IJs). The economics of the diets also showed that the percent cost reduction in factitious diet III and diet I was 48.86 and 28.85%, respectively. Overall, the results suggested that factitious diet III was superior to diet I followed by diet II.

Conclusion Addition of supplements of vitamin E and B-complexes enhances the biological activity of the insect, thereby increasing its food conversion efficiencies. Factitious diet III can be successfully used for mass multiplication of the *G. mellonella*.

Keywords Factitious diet, *Galleria mellonella*, Nutritional indices, Mass production, Economics, *Steinernema abbasi*

Background

The greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae), is an important pest of honeybees, but the pest is very susceptible to various biological control agents. Laboratory studies on potential host are required to determine their effective management in the field. Also, due to its high susceptibility this pest is considered as an important factitious host for laboratory mass rearing techniques of many biocontrol agents including entomopathogenic nematodes (Shapiro-Ilan et al. 2003). The first pre-requisite of successful mass rearing of biocontrol agents is the adequate, healthy and hygienic supply of the host. Different artificial diets of *G. mellonella* have been studied in the past (Ellis et al. 2013). Studies on individual components of some artificial diets of *G. mellonella* revealed that dry milk and powdered yeast contributed better growth than dried meat and also that beeswax used in diet component was superfluous (Sohail

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et al. 2020). Previous studies have also concluded that use of beeswaxes as a food source only acts as a source of their metabolic water in diets with low water content (Hickin et al. 2021). Wax moth reared on artificial diets developed faster with increased females moth ratio and higher fecundity than those reared on the brood comb as natural food.

The goal of the present investigation is to reduce the cost economics of artificial diet for laboratory mass rearing of *G. mellonella.* Besides the cost reduction, it is also important to maintain the suitability of the modified factitious diet composition on hosts without compromising their growth and development. The present reports on economical factitious diet for mass rearing of *G. mellonella* were carried out on different growth parameters for five generations.

Methods

Collection and laboratory rearing of greater wax moth, *Galleria mellonella*

The larvae of the wax moth were taken from Honeybee Research and Training Center, Pantnagar, Uttarakhand (India). The culture was further maintained in Bee Disease Diagnostic laboratory under Department of Entomology, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, on natural beeswaxes. Culture maintenance and experiments were carried out in controlled temperature at 27 ± 2 °C, 75 ± 5% relative humidity and a 16:8 h L: D photoperiod laboratory conditions.

Experimental factitious diet composition

Three experimental factitious diets (I, II, and III) were formulated for the rearing of wax moth larvae by modifying a recommended artificial diet used as standard/control for comparison of parameters (Figs. 1 and 2). In all the factitious diets glycerin was replaced with sorbitol, wheat bran was completely removed, vitamin E (diet III) and vitamin B (diet I) complexes were added and quantity modifications in dietary components. The specific compositions of the factitious diets are presented in Table 1.

Methodology of factitious diet preparation

For diet preparation, firstly sterilizing the wheat flour, maize flour and wheat bran in pressure cooker at 100 °C for 45 min and carefully measuring of dietary ingredients and evenly mixing in plastic jars and store in refrigeration for long term use, vitamin E and vitamin complexes are used in semi-liquid tablet form. In small plastic containers (6 × 5 cm), 10 g of diet (each factitious diet in separate jar) was placed (Fig. 1). Freshly laid eggs were collected from stock culture, counted under stereomicroscope and

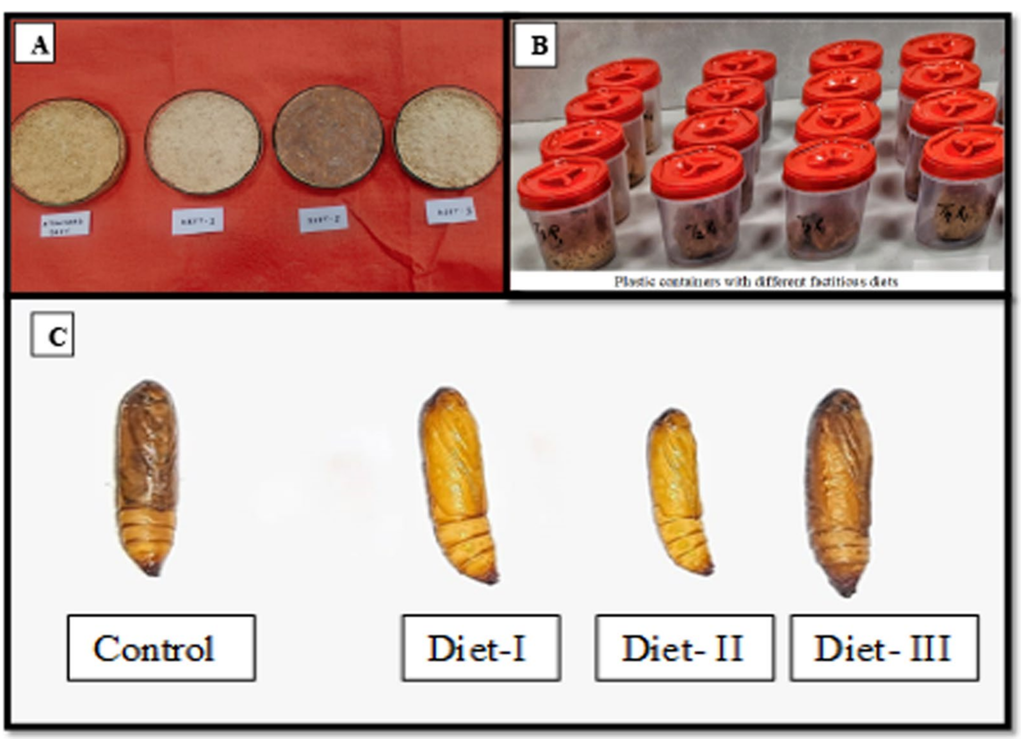

Fig. 1 **A** Different factitious diets; **B** experimental containers with different treatment diets; **C** Pupa developed from different factitious diets

Fig. 2 *Galleria mellonella* larvae reared on different factitious diets

divided into batches of 500 eggs. In each container one egg batch was placed.

Experimental assays

Effect of different factitious diets on development, survival, and reproduction of greater wax moth, Galleria mellonella

Each diet treatment consists of 500 egg of *G. mellonella* with three replications which sums up with the total of 2000 eggs including control. Daily observations were done to record egg hatchability and sex ratio at same time. After 20th day of egg hatching each larva/diet was placed individually to record the observations on development and mortality of larval until pupation. Larval and pupae weight were recorded on electric balance (0.0001g). As newly formed pupae were very delicate, therefore their weight was recorded third day after pupation. Newly emerged adults (< 24 h) were allowed to mate before transferring into oviposition boxes lined with black paper as oviposition substrate (1 pair/cup) to record the observation on daily fecundity. Papers were checked daily for eggs laid and were replaced every third day. Twenty pairs of freshly emerged male and female adults were kept separately in a plastic cup of 500 ml (1 adult/cup) until death to observe adult longevity. Data on adult emergence were also recorded.

Effect of different factitious diets on nutritional consumption and energy utilization of greater wax moth, Galleria mellonella

For nutritional observations, 20-day-old larvae were used in ten replications for all four compositions sets including control diet throughout the experimental period. Consumption observations and utilization of factitious diet were proposed by (Waldbauer 1968). These were:

$$\text{Consumption index(CI)} = \frac{\text{weight of food ingested}}{\text{duration of feeding period (days)} \times \text{mean weight of larva during feeding period}}$$

$$\text{Growth rate(GR)} = \frac{\text{weight gain of larva during feeding period}}{\text{duration of feeding period (days)} \times \text{mean weight of larva during feeding period}}$$

$$\text{Approximate digestibility(AD)} = \frac{\text{weight of food ingested} - \text{weight of faeces excreted}}{\text{weight of food ingested}} \times 100$$

$$\text{Efficiency conversion of ingested food(ECI)} = \frac{\text{weight gain of larva during feeding period}}{\text{weight of food ingested during feeding period}} \times 100$$

Table 1 Compositions of factitious diets of greater wax moth, *Galleria mellonella*

Factitious Diets	Wheat flour (g)	Wheat bran	Maize flour (g)	Dry milk	Dry yeast (g)	Honey (ml)	Sorbitol	Glycerin	Vitamin E	Vitamin B
I	250	–	250	50 g	70	150	100 ml	–	–	2 N°
II	350	–	250	100 g	70	100	100 ml	–	–	–
III	450	–	250	–	70	50	100ml	–	2 N°	–
Control	100	100 g	200	100 g	50	175	–	175 ml	–	–

$$\text{Efficiency conversion of digested food(ECD)} = \frac{\text{weight gain of larva during feeding period}}{\text{weight of food ingested} - \text{weight of faeces excreted}} \times 100$$

Cost analysis of different factitious diets of greater wax moth, Galleria mellonella

The cost of all dietary components used in diet preparations was calculated, and the cost of per Kg diet was estimated.

Effect of factitious diet on entomopathogenic nematode production

Efficiency of factitious diets in production of entomopathogenic nematode was tested using Pantnagar, Uttarakhand strains; *Steinernema abbasi*- PN1. Culture of *S. abbasi* was collected from insect pathology laboratory, Department of Entomology, GBPUAT, Pantnagar, Uttarakhand, India. Ten larvae from each diet were infected with *S. abbasi* (PN 1). Dead larvae were placed on white traps and nematode production recorded using counting Petri dishes.

Statistical analysis

To assess the effect of different factitious diets on the development and reproductive parameters, one-way analysis of variance (ANOVA) was used, followed by Tukey–Kramer post hoc tests to compare treatment means for finding any possible differences. The data collected were initially transformed to square root transformation before subjecting to ANOVA. Sex ratios were analyzed by Chi-squared test. Analysis was performed using SPSS 16.0 statistics with significance level of $p<0.05$.

Results

The present study was conducted to explore the possibility of developing economically viable and cost-effective factitious diet for *G. mellonella*, which acts as fictitious host for mass multiplication of entomopathogenic nematodes. An investigation on detailed biology, nutrients' consumption and utilization for growth and development was necessary to evaluate the acceptability of modified factitious diets.

Biological parameters of *G. mellonella* on different factitious diets

The data on biological parameters of *G. mellonella* reared on three different factitious diets are presented in Tables 2 and 3. Results showed that the longest larval period was observed in factitious diet III (49.12 ± 0.75 days) with minimum larval days in artificial diet II (29.40 ± 0.93 days). Larval longevity in tested diet I (39.50 ± 1.41 days) was at par with those in popular diet (39.60 ± 1.57 days). The highest mean larval weight was recorded on factitious diet III (303.40 ± 6.45 mg), followed

Table 2 Developmental parameters of *Galleria mellonella* on different factitious diet (Mean ± SE, n = 10)

Factitious diets	Larval weight (mg)	Larval period (days)	Larval mortality (%)	Pupal period (days)	Fecundity (eggs/female/day)	Egg hatching (%)
Diet I	283.00 ± 2.55b	39.50 ± 1.41b	8.12 a	9.06 ± 0.46a	1178.8 ± 42.70ab	83.24 bc
Diet II	217.20 ± 9.13a	29.40 ± 0.93a	10.80 b	10.36 ± 0.47a	1034.4 ± 61.54a	45.60 a
Diet III	303.40 ± 6.45c	49.12 ± 0.75bc	6.32 a	9.12 ± 0.47a	1270 ± 23.66bc	91.40 c
Popular diet (control)	280.00 ± 8.37b	39.60 ± 1.57b	9.54 ab	9.18 ± 0.22a	1038.40 ± 39.15a	75.20 b

Means followed by different letters in the same columns are significantly different ($p<0.05$)

Table 3 Developmental parameters of *Galleria mellonella* on different factitious diet (Mean ± SE, n = 10)

Factitious diets	Adult emergence (%)	Adult longevity (days)		Sex ratio (Male: Female)	Adult weight (mg)		Total developmental time (days)	
		Male	Female		Male	Female	Male	Female
Diet I	83.20 a	13.02 ± 0.24ab	10.60 ± 0.26c	3:2	185.20 ± 1.16b	195.20 ± 0.97b	70.46 ± 0.72ab	64.60 ± 1.03ab
Diet II	72.80 ab	11.80 ± 0.37a	7.80 ± 0.37a	1.2:1	161.20 ± 1.96c	188.60 ± 4.34bc	72.80 ± 1.07b	63.20 ± 1.12a
Diet III	84.08 a	16.80 ± 0.25bc	12.28 ± 0.58b	3:1	201.20 ± 3.09a	220.20 ± 4.22a	82.48 ± 0.67c	72.80 ± 0.37c
Popular Diet (control)	83.20 a	12.18 ± 0.31ab	10.80 ± 1.34c	2.5:1	185.20 ± 1.46b	199.20 ± 2.44b	71.42 ± 1.22b	68.64 ± 1.91b

Means followed by different letters in the same columns are significantly different ($p<0.05$)

by diet I (283.00 ± 2.55 mg). The lowest weight of matured larvae was recorded in factitious diet II (217.20 ± 9.13 mg). In contrary, larval mortality was observed the highest in diet II (10.80%) and the lowest in diet III (6.32%). Larval mortality of diet I (8.12%) was at par with popular diet (9.54%). The highest mean fecundity was recorded in factitious diet III (1270 ± 23.66 eggs/female) which was at par with diet I (1178 ± 42.70 eggs/female) and the lowest in factitious diet II (1034.4 ± 61.54 eggs/female) in comparison with control diet (1038.40 ± 39.15 eggs/female). The percent of adult emergence of diet I (83.20%) and diet III (84.08%) was at par with control diet (83.20%). Highest pupal period was observed in diet II (10.36 ± 0.47 days).

Adult longevity of male was higher than that of female adult. The highest male and female longevity was recorded in diet III (16.80 ± 0.25 days) and (12.28 ± 0.58 days), respectively. Also adult weight of male and female was recorded the highest in diet III (201.20 ± 3.09 mg and 220.20 ± 4.22 mg). Females are slightly bigger and heavier than males. The male: female sex ratio of factitious diet III (3:1) was at par with the control diet (2.5:1). The sex ratios for all the diets showed a major male biasness and there were fewer female adults; thus, these differences were statistically non-significant (Table 3).

Also, the total developmental time of adult male was comparatively longer than female adult of *G. mellonella*. In factitious diet III total developmental period of adult male (82.48 ± 0.67 days) and adult female (72.80 ± 0.37 days) was the highest of all diets. Developmental time of adults of diet I, II and popular (control) diet was at par with each other (Table 3).

Nutrients consumption and energy utilization of greater wax moth, *Galleria mellonella*

Nutritional indices of *G. mellonella* larvae were significantly different on all three factitious diets (Table 4). In the mass production process, CI showed how a weight of food ingested is converted into a weight of larvae. The larvae fed on factitious diet-II had the highest CI values (1.40 ± 0.06 g/g/d). However, the lowest value of CI (1.24 ± 0.07 g/g/d) was observed on factitious diet-I. The ECI (48.24%) and ECD (55.46%) were the highest in factitious diet-III, suggesting that larvae were more efficient in conversion of ingested and digested food to insect biomass with an increase in larval weight and full body growth and development.

The highest (88.50%) and lowest (85.92%) AD values of *G. mellonella* larvae were recorded on factitious diet III and II, respectively.

Even though the CI of diet II was the highest of all factitious diets, but its low ECI%, ECD% and approximate digestibility suggested that much of the energy of *G. mellonella* was eliminated without being digested through the fecal matter. Characteristically, when the quantity of food ingested decreases, the duration of development is extended and the insect becomes smaller and lighter as in factitious diet II.

Table 5 Cost analysis of different factitious diets of *Galleria mellonella*

Factitious diets	Cost of total diet (in INR) per Kg	Percent cost reduction per Kg (%)
Factitious diet I	148.00	28.85
Factitious diet II	156.30	22.00
Factitious diet III	128.10	48.86
Popular diet (control)	190.70	–

Cost analysis

Relative costs of factitious diets I, II and III in reference to the popular (control) diet were 148.00 ₹/kg, 156.30 ₹/kg and 128.10 ₹/kg, respectively, over control cost 190.70 ₹/kg (Table 5). Components of all factitious diets were similar to those of standard diet with slight modifications. Modifications in quantity change of dietary components resulted in the highest percent cost reduction was in factitious diet III (48.86%), followed by diet I (28.85%) over control diet. Apart from change in quantity of dietary components, modifications including addition

Table 4 Consumption and utilization of energy utilization by *Galleria mellonella* larvae grown on different diets (Mean ± SE, n = 10)

Factitious diets	AD %	GR	CI	ECI %	ECD %	Nematode production (*Steinernema abbasi*)
Diet I	86.97^{ab} ± 0.74	0.58^{b} ± 0.07	1.24^{b} ± 0.05	47.27^{ab} ± 0.75	53.44^{ab} ± 1.11	21,805.31^{a} ± 2832.19
Diet II	85.92^{a} ± 1.03	0.52^{a} ± 0.05	1.40^{a} ± 0.06	37.02^{c} ± 1.14	43.07^{c} ± 0.55	18,380.04^{b} ± 1384.72
Diet III	88.50c ± 1.04	0.63^{c} ± 0.05	1.25^{b} ± 0.01	48.24^{a} ± 0.66	55.46^{a} ± 0.70	25,418.43^{a} ± 2803.91
Popular Diet (control)	88.00^{c} ± 1.53	0.59^{b} ± 0.03	1.29^{b} ± 0.04	45.95^{b} ± 0.88	52.33^{b} ± 0.67	28,546.94^{a} ± 3220.00

Means followed by different letters in the same columns are significantly different ($p < 0.05$). AD = approximate digestibility, GR = growth rate, ECI = efficiency of conversion of ingested food, ECD = efficiency of conversion of digested food, CI = consumption index of vitamin B complexes in factitious diet I, substitution of glycerin with sorbitol (20% of the glycerin cost) in all the factitious diets and complete elimination of dry milk and addition of vitamin-E in diet III helped in decreasing the cost of the formulated factitious diets.

Production of entomopathogenic nematode

The nematode production of these larvae ranged 28,546.94 ± 3220.00 to 18,380.04 ± 1384.72 infective juveniles (IJs)/larva (Table 4). Although *S. abbasi* production from all the factitious diet was non-significant, factitious diet III and diet I were statistically at par with control diet. This means that apart from being economically sound with high food conversion efficiency and increased consumption rate factitious diet III and I were also effective in production of entomopathogenic nematode, *S. abbasi*.

Discussion

Due to high vulnerability to diseases *G. mellonella* is a suitable host for variety of biological control agents and also it is easy to mass produce in comparison with other hosts under laboratory conditions. The study revealed that the quantities of consumed diets are crucial for the gain in body weight. Also, a major effect of factitious diet on larval durations, larval weight, fecundity and adult weight was found. These findings are in agreement with (Lee et al. 2008) who found a linear relationship existed between the dry weight of food eaten and the weight gain in dry biomass. The present investigation also revealed that *G. mellonella* larvae were capable of converting diverse foods into their biomass with variations in larval growth rate on different factitious diets, which was directly proportional to differences in their consumption. These findings are in agreement with (Sohail et al. 2020) who stated that food quality influences the feeding rate and growth and development of *G. mellonella*.

Efficiency of conversion of ingested food (ECI) is a food efficiency metric that links body growth to the quantity of food consumed on a dry weight basis, whereas the efficiency of nutrients is used for growth and development is measured by efficiency of conversion of digested food (ECD). When *G. mellonella* was raised on factitious diets, the nutritional indices, especially the ECI and ECD values, showed significant differences, indicating that different dietary components with different nutritional values had different impact on growth and development of *G. mellonella*. It is a known fact that the mass of the insect and its nutritional needs will always positively correlate (Rho and Lee 2022). The characteristics of *G. mellonella* food consumption and utilization can be studied viz. feeding behavior, food digestion and nutrients assimilation efficiency. This sequence showed how food is transformed into the insect biomass.

Body weight is a crucial fitness metric for analyzing the insect populations (Liu et al. 2004). The larval weight among all the factitious diet was the highest on diet III and lowest on diet II. The highest ECI and ECD values were on factitious diet III, suggesting that the larvae were more efficient at conversion of ingested and digested food to body biomass with an increase in larval weight. Although factitious diet II having the highest CI value, it also had the lowest values of ECI% and ECD% indicating that larvae feeding on this diet were less effective in converting ingested and digested food to biomass. It is well known that the degree of food utilization depends on the digestibility of food, and the efficiency with which digested food is converted into biomass (Batista Pereira et al. 2002).

Analysis of nutritional indices can lead to understanding of the behavioral and physiological basis of insect response to different dietary ingredients (Lazarevic and Peric-Mataruga 2003). Fecundity is significantly affected by diet composition. Growth improvement by fatty acids indicates that fatty materials facilitate the absorption and utilization of other nutrients more efficiently. Vitamin E (α-tocopherol) functions as protective covering of cell membranes by reducing toxic peroxidation derivatives of unsaturated fatty acids and also plays synergistic role in fecundity of insects. In the present study, the number of eggs per female was the highest when larvae were reared on factitious diet III containing vitamin E as a dietary component. Evidently in majority of insects vitamin-E plays a significant role in reproduction. Therefore including vitamin E adds extra advantage in insect factitious diets. Also, yeast was reported as a very important component in *G. mellonella* diets (Gross et al. 1996). However, the nutritional efficiencies of all factitious diets were roughly close to the control diet which proves that the prepared factitious diets were able to provide almost same kind and type of nutrition to the *G. mellonella* as per the popular diet.

Natural diets are restrained by many limiting factors and can easily deteriorate disturbing the mass production cycle. Artificial diets are therefore served greater advantage over natural diet due to their low cost, preservation and long-term use. Also, the best and appropriate diet for rearing *G. mellonella* depends upon the subsequent use of the insects. For physiological or pathological studies, uniformity and vigor of *G. mellonella* are important factors to consider and therefore in such type of diet cost matters little. Cost is an important factor when insects are mass reared for egg or larval production. Observing the cost analysis ratio, the cost of all the factitious diet was significantly less than the popular diets. The highest percent of cost reduction was recorded on diet III. Also, the biological parameters and nutritional indices of diet III were found to be more economically suitable and viable without compromising any nutritional value as well as biological health and vigor of *G. mellonella* which can be used further as a host in mass production of various biocontrol agents majorly entomopathogenic nematodes. These findings are in agreement with (Sohail et al. 2020).

Conclusion

Analyzing the biological and nutritional parameters of greater wax moth after consumption of diet, factitious diet with addition of vitamin-E was superior to all the diets in the experiment. Also, production of entomopathogenic nematodes was high in vitamin-E supplemented diet. Therefore, such diet which are half the costs of production and double in energy conversion efficiency should be further studied for more complex biological and physiological behavior before widely acceptance.

Abbreviations

EPNs	Entomopathogenic nematodes
IJs	Infective juveniles
ECI	Efficiency of conversion of ingested food
ECD	Efficiency of conversion of digested food
AD	Approximate digestibility
GR	Growth rate
CI	Consumption index
SPSS	Statistical product and service solutions

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Author contributions

Renu Pandey designed and conceptualized the research and contributed data tools. Parul Suyal carried out the research under the supervision of Renu Pandey, collected the data and performed analysis. The first version of the manuscript was drafted by Parul Suyal. Renu Pandey revised and finalized the manuscript.

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Not applicable

Declarations

Ethics approval and consent to participate

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Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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