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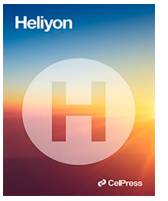

Nature to Nurture: Chitosan nanopowder a natural carbohydrate polymer choice of egg parasitoid, *Trichogramma Japonicum* Ashmead

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ABSTRACT

Chitosan is a naturally occurring linear biopolymer made of partially deacetylated acetyl and N-acetyl glucosamine. Its biocompatible physiochemical and biochemical properties are unmatched. Chitosan is transformed to nanopowder for use in agriculture and associated industries as nanocarriers for existing agrochemicals, ensuring the delayed release of chemicals with better solubility. Chitosan nanopowder applied to leaves or soil can activate a plant's natural defences against insects and pathogens. These studies were carried out because there is a potential for toxicological risk linked with products created utilizing nanotechnology, such as chitosan nanopowder, and therefore researchers felt the need to investigate this. The egg parasitoides *Trichogramma Japonicum* Ashmead was used as a low-cost biomarker to determine the potential toxicity of chitosan nanopowder. This study looked into the possibility that the adult stage of the egg parasitoids, *Trichogramma Japonicum* Ashmead might be negatively impacted by chitosan nanopowder (80–100 nm). Unpaired *t*-test statistical analysis has been carried out. According to the statistical analysis, host eggs exposed to chitosan nanopowder showed noticeably greater parasitization than the control group. As a natural supply of carbohydrate polymers chitosan nanopowder promotes the parasitization of *T. Japonicum*. The findings showed that *T. Japonicum* favoured chitosan nanopowder. Through Y dual choice, eight-arm multiple choice, and no-choice olfactometer experiments, as well as images from a stereozoom microscope and a scanning electron microscope (SEM), the data was thoroughly supported. Future agricultural applications of chitosan nanopowder will benefit from a deeper understanding of our findings.

Key Message

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- The unintended consequences of utilizing chitosan nanopowder in agriculture are the main topic of this study.
- The study was conducted to fill the information gap between the investigation of chitosan nanopowder and its impacts on bioindicators.
- Because of the positive response from the subject, more chitosan nanopowder can be used in agricultural practices.

1. Introduction

The utilization of nanotechnology in various fields has been a significant technological advancement in the 21st century [1]. Several regulatory bodies across the globe, including Canada [2], Australia [3], the European Union [4], Switzerland, Iran, Taiwan, and India [5], have formulated policies and regulations to ensure the safe and responsible use of nanoproducts in agriculture [6]. These policies and regulations have been developed to overcome the barriers associated with the technology readiness of nanoproducts. This paper aims to explore the current state of nanomaterials impact on the natural enemies in agriculture to support the regulatory frameworks in place to ensure its safe and responsible utilization. Nanotechnology has emerged as a promising field with the potential to revolutionize various sectors, including agriculture and food systems. The application of nanotechnology in agriculture has been explored extensively in recent years, with a focus on developing nano-fertilizers [7–11] and nano-enhanced seed treatments [12]. Additionally, the development of food packaging nanosystems [13], nanozymes [14], and bioanalytical nanosensors [15] has shown great potential in improving food safety and quality. Furthermore, the use of nanotechnology in plant genetic engineering [16] and nanopore-based technologies beyond DNA sequencing [17] has opened up new avenues for research and development. Moreover, the reduction and conversion of agricultural materials into valuable products through nanotechnology can contribute to environmental protection [18]. In recent years, the development of novel nanocatalysts has garnered significant attention in the field of sustainable energy and chemical production. One such application involves the conversion of vegetable oils into biobased fuels and biodegradable industrial solvents. In a recent study by Ref. [19] Hilman et al. (2023), the synthesis and characterization of these nanocatalysts were reported. This research represents a promising advancement in the pursuit of environmentally friendly and economically viable alternatives to traditional fossil fuel-based products. The use of nanopesticide formulations [20] has been proposed as a potential solution for managing insect pests in agriculture. These formulations have been shown to enhance water solubility and bioavailability, while also providing environmental protection. As reported by Su et al. (2022) [21], the application of nanopesticides may offer a promising approach for improving pest management strategies in the agricultural sector. The use of nanocarriers as an encapsulating agent has emerged as a novel approach to agricultural pest management. This technique involves the use of nanocarriers to deliver active ingredients to the target site, thereby enhancing the efficacy of the treatment. The encapsulation of the active ingredients within the nanocarriers also provides protection against degradation and increases their stability, thereby prolonging their shelf life [20]. The field of nanotechnology has garnered significant attention in recent years due to its potential to revolutionize various industries. One area where nanotechnology has shown promise is in improving regulated ecological life support systems. This has been highlighted in several recent studies, including those conducted by Zannat R et al. (2021) [22], [23], and [24]. These studies have demonstrated the potential of nanotechnology to enhance the efficiency and effectiveness of ecological life support systems, thereby contributing to the sustainable development of our planet. The potential of nanotechnology to contribute to the achievement of Sustainable Development Goals (SDGs) by 2030 has been explored in recent research. Specifically, Aithal and Aithal (2021) [25] have identified that thirteen out of the seventeen SDGs could be realized through the application of nanotechnology. This finding highlights the significant role that nanotechnology could play in addressing global sustainability challenges and underscores the importance of continued research and development in this area. The utilization of nanotechnology in the development of chitosan products has been found to hold significant promise in the achievement of the United Nations Sustainable Development Goals (SDG 3), as reported by Amiri et al. in 2023 [26].

The polysaccharide known as chitosan has garnered significant attention in both the medical and agricultural fields due to its promising potential [27–29] which is obtained from waste of fishing industry [30] and insect sources [31,32]. The utilization of nanomaterials has been a subject of interest in various fields, including food packaging, delivery systems, and biomedical applications. Among these nanomaterials, chitosan nanopowder has shown great potential in enhancing the functional properties of biocomposites. As reported by Garavand et al. (2022) [33], chitosan nanopowder has been identified as a promising candidate for improving the utilitarian properties of biocomposites for various applications. In recent years, there has been a growing interest in chitosan nanopowder as a potential polymeric and bio-based material. This promising nanopowder has garnered significant attention due to its unique properties and potential applications [34]. The utilization of chitosan nanopowder in agriculture has gained significant attention due to its superior performance compared to its bulk form, chitosan [35].

The use of chitosan nanopowder in modern agriculture production has been identified as a potential solution for promoting agricultural ecosystem sustainability. According to Meng et al. (2010) [36], chitosan nanopowder has been found to completely inhibit spore germination, elongation of germtube, and mycelial growth of fungi and also acts as antimicrobial [37]. This suggests that chitosan nanopowder could play a key role in enhancing agricultural productivity. The antibiotic activity of a certain substance has been observed against bacteria, with reports indicating its ability to disrupt their plasma membranes [38]. In recent years, the use of chitosan nanopowder has gained significant attention in the field of agriculture due to its potential as a natural and eco-friendly. One area of particular interest is its effectiveness against rice plant hoppers [39], which have been a major source of distress for farmers worldwide. In a recent study conducted by Xie et al. (2022) [40], chitosan nanopowder was found to be highly efficient in controlling rice plant hoppers, highlighting its potential as a promising solution for pest management in agriculture.

The utilization of biopolymers in the field of nanotechnology has gained significant attention in recent years. Biopolymers such as

polynucleotides, cellulose, silk fibroins, collagen, albumin, and chitosan-based nanomaterials have been found to provide nanoparticles with desirable properties such as biocompatibility, biodegradability, and low toxicity. In a study conducted by Rizaq et al. (2019) [41], it was observed that the incorporation of chitosan nanopowder in nanomaterials resulted in enhanced biocompatibility and reduced toxicity. It is approved for nutritional applications in countries such as Japan, Italy and Finland and it has been approved by the FDA for use in wound dressings [42]. The potential biological activities of nanopowdered chitosan have been investigated in recent studies. Kim et al. (2014) [43] reported that this material exhibits a blood cholesterol lowering effect and antidiabetic activity. The hypolipidemic effect of chitosan nanopowder in comparison to ordinary chitosan in rats, as reported by Zhang et al. in 2013 [44]. The study found that chitosan nanopowder exhibited superior hypolipidemic effects in rats when compared to ordinary chitosan. The removal of heavy metals from water bodies is a major concern due to their toxicity and potential harm to the environment and human health. In recent years, magnetic chitosan nanopowder has emerged as a promising biosorbent for this purpose. This is due to its strong metal chelating capability, which is attributed to the presence of amine and hydroxyl groups in the chitosan backbone. Previous studies have demonstrated the effectiveness of magnetic chitosan nanopowder in removing heavy metals from water bodies [45].

Trichogramma Japonicum Ashmead (*T. Japonicum*) has gained significant attention as a biological control agent in agricultural practices, particularly for the management of lepidopteran pests [46]. The release of *T. Japonicum* has been reported worldwide as a method for managing major pests of order Lepidoptera that infest crops such as maize corn borer [47,48], diamondback moth [49], cotton bollworms [50], rice striped stem borer [51], rice yellow stem borer [52] and leaf folder [53]. The gustatory response of *T. Japonicum* to polysaccharides, such as glucose and fructose, has been observed to have a significant impact on its fecundity and longevity, as reported by Jun-Ce Tian et al. in 2016 [54].

The multifaceted role of chitosan nanopowder in the field of agriculture has led to an increased interest in its non-targeted impact on biocontrol agents. As such, this area of study has become a vital prospect for researchers. The present study focuses on the utilization of chitosan nanopowder with a size range of 80–100 nm. The selection of this particular size range is based on the previous research conducted by Milusheva et al. (2019) [55], where chitosan nanopowder with a size range of 20–100 nm was isolated from Bombyx mori Linnaeus insect. The research findings suggested a significant enhancement in the immune response of the living system. Therefore, the current study aims to investigate the potential applications of chitosan nanopowder 80-100 nm. The present study focuses on the impact of chitosan nanopowder (size 80-100 nm) on the egg parasitoid *T. Japonicum*. This paper aims to investigate the potential of chitosan nanopowder as a tool for nanomaterials in the parasitization of *T. Japonicum*, a low cost bioindicator. The study seeks to explore the role of nanomaterials in this process and evaluate the effectiveness of chitosan nanopowder in this application. Through this research, we hope to contribute to the understanding of the use of chitosan nanopowder in the field of nanomaterials and its potential impact on the environment.

2. Materials and methods

The present study was conducted to investigate the impact of chitosan nanopowder on the parasitizing efficacy of egg parasitoid

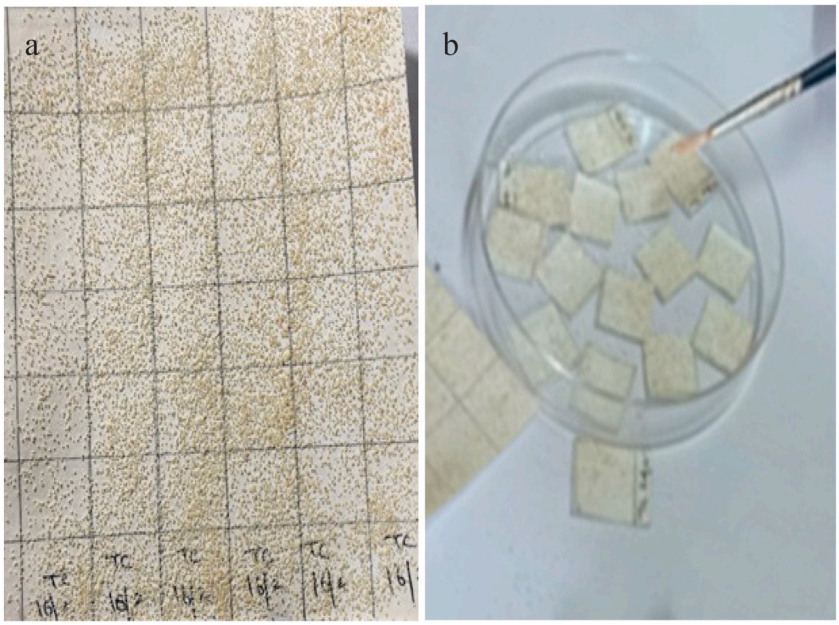

Fig. 1. a The experiment involved the use of UV sterilized *C. cephalonica* eggs that were sprinkled onto paper card strips measuring referred to as egg cards **b** a treatment egg card was prepared by sprinkling chitosan nanopowder (0.2 mg per card) onto an egg card using a brush of zero size (Faber Castell).

T. Japonicum. The experiments were carried out under laboratory conditions of 27 ± 1 °C and relative humidity (RH) of 70 ± 10 %. The chitosan nanopowder was obtained from a commercial source to maintain uniformity. The egg parasitoids were reared in the laboratory. The *Corycra Cephalonica* Stantoin host eggs were exposed with the chitosan nanopowder. The parasitizing efficacy of *T. Japonicum* was evaluated by exposing the parasitoids to the treated and untreated eggs of the host eggs. The percentage of parasitism was calculated by counting the number of parasitized eggs. The data obtained were subjected to statistical analysis using GraphPad Dotmatics software.

3. Materials and methods

The chitosan nanopowder was procured from a commercial source to ensure uniformity. Specifically, for this study, we obtained the chitosan nanopowder from Sisco Research Laboratories Pvt. Ltd. (Mumbai, India). The nanopowder exhibited a size range of 80–100 nm and a purity level of 99 % as determined by assay. It is worth noting that chitosan, derived from crustaceans, serves as the fundamental material for producing the chitosan nanopowder used in this product.

3.1. Insect culture

The egg parasitoids *T. Japonicum* cultures were sustained in the laboratory by supplying them with the eggs of *Corcyra cephalonica* Stainton (*C. cephalonica*). Two-day-old adult population were utilized in the experimental procedures.

Fresh eggs of *C. cephalonica* were utilized for the experiments.

The experiment involved the use of UV sterilized *C. cephalonica* eggs that were sprinkled onto paper card strips measuring 1.5 cm ×

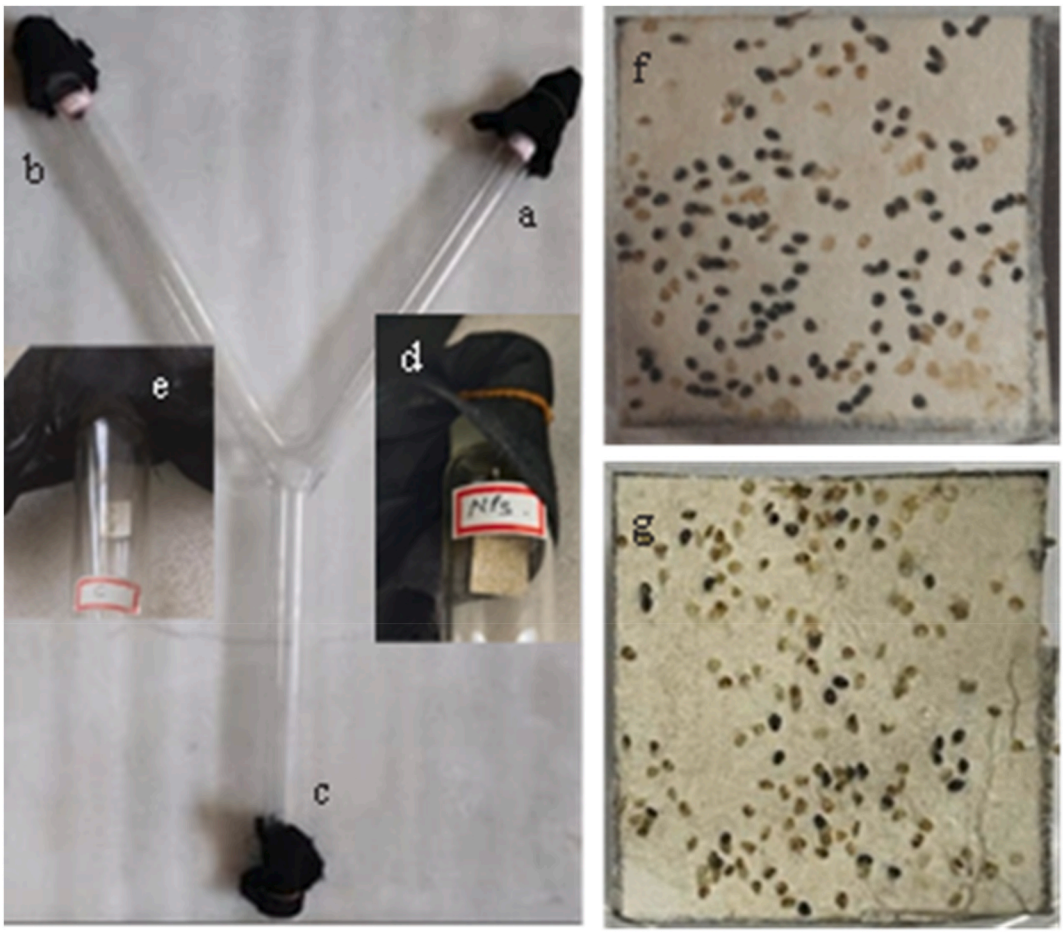

Fig. 2. Experiment 1 which was conducted in Y dual choice olfactometer; **a** treatment egg card sprinkled with chitosan nanopowder (0.2 mg per egg card) placed in the treatment arm,
b Control (untreated) egg card placed in control arm, **c** *T. Japonicum* was released through culture release arm, **d** inset picture shows treatment egg card sprinkled with chitosan nanopowder (0.2 mg per egg card) in the treatment arm, **e** inset picture shows control (untreated) egg card in control arm, **f** Treatment egg card sprinkled with chitosan nanopowder 0.2 mg Replication 1 (R1) parasitized egg cards after five days, **g** Control, Replication 1 (R1) parasitized egg cards after five days.

1.5 cm (L x B), referred to as egg cards (Fig. 1a).

In this study, a treatment egg card was prepared by sprinkling chitosan nanopowder (0.2 mg per card) onto an egg card using a brush of zero size (Faber Castell), as shown in Fig. 1b.

We have made a significant discovery concerning the development of non-UV treated/non-sterilized eggs of *Corcyra cephalonica*. These eggs naturally progress through developmental stages and transform into larvae within a span of five days. However, our observations have brought to light a crucial fact: *Trichogramma* adults do not emerge from these untreated eggs. This points to their inadequacy in generating the desired parasitoid population essential for our study's objectives.

The application of UV radiation has yielded substantial results, particularly in terms of increasing the emergence rates of adult *Trichogramma Japonicum*. This radiation has proven highly beneficial in facilitating the successful parasitization of *Corcyra cephalonica* eggs by *Trichogramma Japonicum*, eliminating potential hurdles. Notably, in instances where the eggs manage to evade the parasitoid, the UV radiation effectively inflicts damage upon the host eggs. Although UV treatment disrupts the egg's physiological processes, halting further development, the nutrients inherent within these eggs hold the potential to support the rearing of *Trichogramma Japonicum*.

Trichogramma species commonly encounter challenges when attempting to parasitize eggs under regular circumstances, mainly due to the host's evolved natural resistance mechanisms. However, the introduction of stressors such as UV radiation has demonstrated a remarkable capability to enhance the emergence percentage, effectively addressing this issue. The utilization of these UV-treated *Corcyra cephalonica* eggs holds promise in successfully rearing *T. Japonicum*, serving as a well-established biocontrol agent as supported by existing literature.

Building upon these significant findings, we wish to underscore the critical necessity of incorporating sterilization or UV treatment protocols for *Corcyra cephalonica* eggs before embarking on any biological control strategies involving *Trichogramma* species. Our research primarily centers around assessing the impact of chitosan nanopowder (sized between 80 and 100 nm) on the egg parasitoid *T. Japonicum*. Consequently, the utilization of UV-treated or sterilized *Corcyra cephalonica* host eggs emerges as an indispensable and pivotal step. In presenting this information, we wish to highlight the paramount importance of employing proper UV treatment/sterilization methodologies to significantly enhance the success of our research endeavors.

3.2. Olfactometers

1. The experimental setup utilized a Y dual choice olfactometer, as depicted in Fig. 2, results mentioned in Table 1. The olfactometer consisted of three arms, with one arm designated as the treatment arm (Fig. 2a), one as the control arm (Fig. 2b), and the remaining arm as the culture release arm (Fig. 2c). Each arm was constructed from transparent, non-absorbent, non-odorant glass, with dimensions of 31.5 cm × 2.5 cm (length x diameter). The inset picture **2d** shows treatment egg card sprinkled with chitosan nanopowder (0.2 mg per egg card) in the treatment arm and **2e** shows control (untreated) egg card in control arm.
2. In this study, an eight arm multiple choice olfactometer Fig. 3 was utilized for experimentation, results mentioned in Table 2. The olfactometer was constructed using transparent, non-absorbent, and non-odorant glass, with each arm measuring 31.5 cm × 2.5 cm (L × D). The centre round aperture were designed with a diameter of 9.3 cm, with four arms being allocated for treatment (Fig. 3 a, c, e, g), while the remaining four arms were used for control (Fig. 3 b, d, f, h). The central round aperture (Fig. 3 i) was utilized for releasing culture.

A no choice olfactometer was constructed using the Transparent, non-absorbent, non-odorant polyacrylic sheet with dimensions 19.5 cm × 110 cm (L × D). The olfactometer was designed in accordance with the specifications shown in Fig. 4. The experimental setup utilized in this study is a no-choice olfactometer, which comprises two tunnels. One of the tunnels was designated as the treatment group (Fig. 4a), while the other served as the control group (Fig. 4b). The results mentioned in Table 3.

3.3. Equipments

The research equipment utilized in this study was the Weswox Stereo Zoom Microscope, model SZM-100, manufactured by The Western Electric & Scientific Works (Weswox) in India. The microscope is equipped with an achromatic lens and has a zoom ratio of 6:1. The calibration of the microscope was performed utilizing a microscope stage micrometer calibration slide. A scale bar was

Table 1
Statistical Analysis: Unpaired *t*-test was done for data obtained from Experiment 1 which was conducted in Y-Dual choice olfactometer.

Experiment 1	Replications							Statistical Analysis		
	R1	R2	R3	R4	R5	R6	Mean	S. D.	S. E.M	N
Treatment (% of Parasitized Eggs)	40.94	67.07	47.36	47.19	46.55	44	48.85	9.25	3.7763	6
Control (% of Parasitized Eggs)	21.84	17 .7	37.37	37.68	36.66	37.66	31.50	9.14	3.7314	6
	95 % confidence interval of difference							t	Significance p-value	
	Lower value			Upper value						
	5.5212			29.1788				3.2681	0.0085	

Note: In this study, mean values were calculated and reported along with their corresponding Standard Deviation (S.D) and Standard Error Mean (S.E. M). Additionally, t value and p value were calculated and reported.

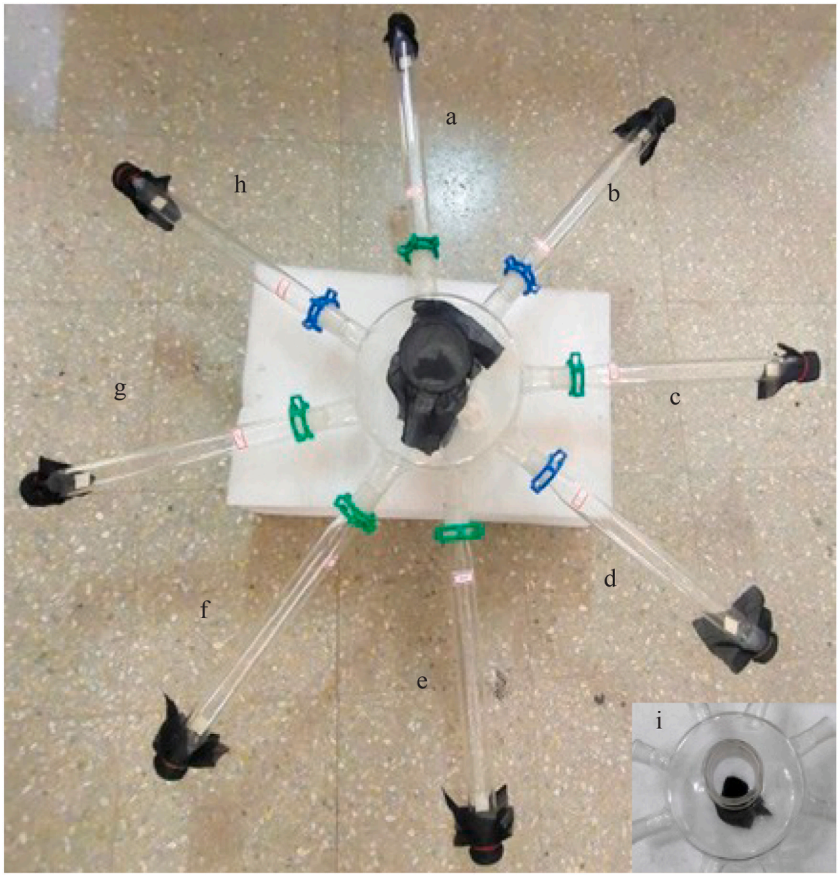

Fig. 3. a, c, e, g. Experiment 2 which was conducted in Eight arm multiple choice olfactometer; treatment egg card sprinkled with chitosan nanopowder (0.2 mg per egg card) placed in the treatment arm, **b, d, f, h.** control (untreated) egg card placed in control arm**, i.** Inset of culture release centre from where *T. Japonicum* was released.

Table 2
Statistical Analysis:Unpaired t-test was done for data obtained from Experiment 2 which was conducted in Eight arm multiple choice olfactometer.

Experiment 2	Replications				Statistical Analysis			
	R1	R2	R3	R4	Mean	S.D	S.E.M	N
Treatment (% of Parasitized Eggs)	32.20	44.44	47.61	35.24	39.87	7.32	3.66	4
Control (% of Parasitized Eggs)	26.47	27.61	32.33	29.63	29.01	2.57	1.28	4
	95 % Confidence interval of difference			t		Significance p-value		
	Lower		Upper					
	0.3684		19.3516	2.5419		0.0440		

Note: In this study, mean values were calculated and reported along with their corresponding Standard Deviation (S.D) and Standard Error Mean (S.E.M). Additionally, t value and p value were calculated and reported.

utilized for measuring the dimensions of the insect eggs. The photomicrograph of insect eggs was obtained utilizing the MagCam DC-5 CMOS 5 MP camera, accompanied by software.

The equipment utilized in this study was a Hitachi Ltd, Japan-manufactured TM3030Plus low-vacuum Scanning Electron Microscope. The experimental setup employed a Hitachi TM3030 plus table top model. The specimens were affixed onto the aluminum mounts utilizing double-sided copper adhesive tape. The imaging was conducted using a Back Scattered Electron Detector and Scattered Electron Detector mixture at a voltage of 15 KV. The specimens were devoid of any metal coating or dehydration treatment. The images were saved in Tagged Image File Format (TIFF) with an embedded measurement scale provided by the imaging system.

4. Methods

In this study, three olfactometers were utilized to conduct experiments. These olfactometers included the Y dual choice

Fig. 4. Experiment 3 which was conducted in No choice olfactometer **a** treatment egg card sprinkled with chitosan nanopowder (0.2 mg per egg card) placed in the treatment wind tunnel at one end whereas *T. Japonicum* was released from the other end of the tunnel **b** control (untreated) egg card placed in one end of the control wind tunnel whereas *T.Japonicum* was released from the other end of the tunnel.

Table 3
Statistical Analysis:Unpaired t-test was done for data obtained from Experiment 3 which was conducted in No choice olfactometer.

Experiment 1	Replications							Statistical Analysis		
	R1	R2	R3	R4	R5	R6	Mean	S. D.	S. E.M	N
Treatment (% of Parasitized Eggs)	50.81	68.75	55.55	60.86	63.15	58.57	59.62	6.20	2.56	6
Control (% of Parasitized Eggs)	46.42	45.16	39.39	31.11	40.84	39.62	40.42	5.42	2.21	6
	95 % confidence interval of difference							t	Significance p-value	
	Lower value			Upper value						
	11.6542			26.7458				5.6694	0.0002	

Note: In this study, mean values were calculated and reported along with their corresponding Standard Deviation (S.D) and Standard Error Mean (S.E. M). Additionally, t value and p value were calculated and reported.

olfactometer, Eight arm olfactometer, and No choice olfactometer. The methodology employed in this research involved conducting a series of experiments. The details of these experiments are presented in this section.

In this study, Experiment 1 was conducted using a Y Dual choice olfactometer.

The experiment involved the placement of treatment egg cards in the treatment arm, as depicted in Fig. 2a. The egg cards were sprinkled with chitosan nanopowder at a concentration of 0.2 mg per card. In this study, untreated egg cards were utilized as the control group. The egg cards were placed in the control arm, as depicted in Fig. 2b, and were sealed appropriately with black cloth. The experiment involved exposing a set of egg cards to around one hundred adult participants in olfactometers for a duration of 10 h. The parasitized egg cards were subjected to standard laboratory conditions, which included a temperature of 27 $\pm$ 1 °C and a relative humidity of 70 $\pm$ 10 %. The cards were kept under these conditions for a period of five days after being removed from the olfactometer. The experiment was conducted using a Y-dual choice olfactometer, and six replications were maintained for Experiment 1. After five days of parasitization, the parasitized (Black colour) eggs were counted. Treatment egg card sprinkled with chitosan nanopowder (0.2 mg per card) and control (untreated) egg card were compared and analyzed. The results were documented in Fig. 2 f and Fig. 2 g, respectively. The Parastization Percentage was determined using Formula 1, as per the research methodology.

$$\% \text{ of Parasitized Eggs} = (\text{Number Parasitized (Black) Eggs} / \text{ Total number of eggs}) \times 100$$

The second experiment was conducted using an Eight Arm Multiple Choice olfactometer.

The experiment involved the use of treatment egg cards that were sprinkled with chitosan nanopowder at a concentration of 0.2 mg per card. These treated egg cards were placed in the treatment arm, as depicted in Fig. 3a, c, e, and g. Control egg cards, which were not treated, were placed in the control arm, as shown in Fig. 3b, d, f, and h. The study involved the release of approximately one hundred adults from a culture release central aperture, as depicted in Fig. 3i. The Eight Arm Multiple Choice olfactometer was utilized in Experiment 2 to investigate the effects of treatment and control on eight distinct pathways. The experiment maintained a total of four replications to ensure accuracy and consistency of results. The study was conducted under controlled laboratory conditions. The temperature was maintained at 27 ± 1 °C, while the relative humidity was kept at 70 ± 10 %. The experiment was carried out for a period of 10 h. The study involved the counting of parasitized eggs cards from the olfactometer, after period of five days. To determine the percentage of parasitized eggs, Formula 1 was utilized based on the observations made.

In this study, Experiment 3 was conducted using a No choice olfactometer. The experimental method employed in this study involved the use of a No Choice Olfactometer, which consisted of two tunnels. One of the tunnels was designated as the treatment group, while the other served as the control group. The study utilized a treatment egg card that was sprinkled with chitosan nanopowder at a concentration of 0.2 mg per card. The treatment egg cards were placed in a wind tunnel for treatment, as depicted in Fig. 4a. The wind tunnel was used to release *T. Japonicum* from the opposite end of the tunnel. Control egg cards, which were not treated, were placed in a separate wind tunnel for control purposes, as shown in Fig. 4b. The study exposed cards to a population of approximately one hundred adults in each wind tunnel. The experiment involved subjecting the cards to standard laboratory conditions, which were maintained at a temperature of 27 ± 1 °C and a relative humidity of 70 ± 10 %. The cards were kept undisturbed for a period of five days following the 10-h exposure period. Experiment 3 was conducted in a No-choice olfactometer and was replicated six times. The method used in this study to determine the percentage of parasitized eggs involved the application of Formula 1 to the observations made.

5. Results

T. Japonicum has been demonstrated to be a highly efficacious biocontrol agent for managing diverse agricultural pests. Here, we had used *T. Japonicum* as inexpensive bioindicator. The present study investigates the potential non-targeted effects of chitosan nanopowder, a commonly utilized nanocarrier of agrochemicals in agriculture, on *T. Japonicum*.

The present study reports the results of three experiments conducted to investigate preferences in various olfactometers. Experiment 1 utilized the Y dual choice olfactometer, Experiment 2 utilized the Eight arm multiple choice olfactometer, and Experiment 3 utilized the No choice olfactometer. The obtained experimental data was subjected to statistical analysis using GraphPad Dotmatics software.

In Experiment 1, the choice orientation of the parasitoid *T. Japonicum* was evaluated in the Y dual choice olfactometer. The probability of two choices, namely treatment egg cards sprinkled with chitosan nanopowder (0.2 mg per card) and control (untreated) egg cards, was determined. The results of the study indicate that *T. Japonicum* exhibited a preference for egg cards treated with chitosan nanopowder at a concentration of 0.2 mg per card, as compared to the control group consisting of untreated eggs. The results of Experiment 1, conducted in a Y dual choice olfactometer, indicate that the application of chitosan nanopowder (0.2 mg per card) to egg cards resulted in a relatively higher parasitization rate compared to untreated egg cards. The mean value of parasitization in the treatment group was 48.85, while the mean value in the control group was 31.50. These findings suggest that the use of chitosan nanopowder may be a promising approach with not deleterious effect rather enhancing parasitization. The results presented in Table 1

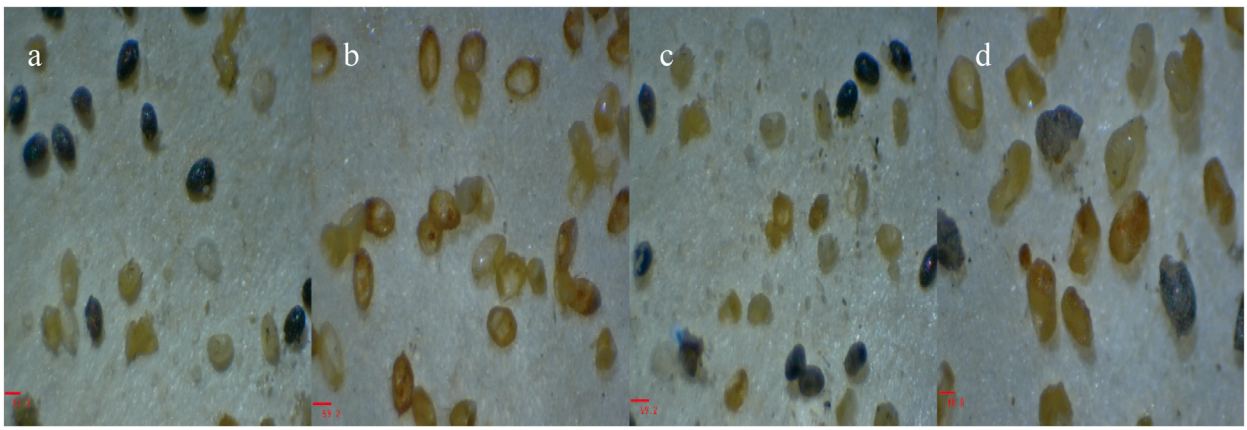

Fig. 5. Stereozoom images of Experiment 1 egg card after five days of the **a** and **b** (Replication 1) which was conducted in Y dual choice olfactometer where more parasitization can be observed in treatment egg card sprinkled with chitosan nanopowder (0.2 mg per egg card) as shown in **a** in comparison to **b** control (untreated) egg card with less parasitization. **c** and **d** (shows Replication 2 of Experiment 1) where more parasitization can be observed in treatment egg card sprinkled with chitosan nanopowder (0.2 mg per card) as shown in **c** in comparison to **d** control (untreated) egg card with less parasitization.

demonstrate the outcomes of an unpaired *t*-test conducted on data obtained from Y dual choice olfactometer. The statistical analysis revealed a significantly higher rate of parasitization in egg cards treated with chitosan nanopowder (0.2 mg per card) compared to the control (untreated) egg cards. The mean value of parasitization for the treated egg cards was 48.85, while that of the control was 31.50. This difference was found to be statistically significant with a p-value of 0.0085. These findings were obtained from six replications of Experiment 1 conducted in Y dual choice olfactometer. The images after five days of the egg cards were captured utilizing a stereozoom microscope. The results of the stereozoom microscope imaging are presented in Fig. 5, depicting images (a-d). In this study, we present the results of Replication 1 of Experiment 1, which was conducted using a Y dual choice olfactometer. The findings are depicted in Fig. 5a and b. The results of the study indicate that the application of chitosan nanopowder (0.2 mg per card) on egg cards led to a relatively higher level of parasitization, as observed in Fig. 5a, compared to the control group (Fig. 5b) which remained untreated. The results depicted in Fig. 5c and d correspond to the second replication of Experiment 1, which was carried out using a Y dual choice olfactometer. The results of the study indicate that the application of chitosan nanopowder (0.2 mg per card) on egg cards led to a higher level of parasitization, as observed in Fig. 5c, compared to the control group (Fig. 5d), which did not receive any treatment. The results of Experiment 1, conducted in a Y dual choice olfactometer, were analyzed through Scanning Electron Microscope images (Fig. 8 a,b,c). The images, taken after five days, revealed that eggs from the treatment egg card, which was sprinkled with chitosan nanopowder at a concentration of 0.2 mg per card, exhibited similar levels of health as the eggs obtained from the control (untreated) egg card. The emergence of an adult from the treatment egg card, which was sprinkled with chitosan nanopowder at a concentration of 0.2 mg per card, is depicted in Fig. 8b. This observation serves as evidence that the egg subjected to this treatment is in a healthy state, as detailed in Supplementary Information 1.

In Experiment 2, an Eight Arm Multiple Choice Olfactometer was utilized to investigate the impact of treatment and control on choice orientation. The olfactometer featured eight distinct pathways, which were alternated between treatment and control conditions. As a result, four distinct outcomes were possible, and the choice orientation was subsequently determined. The results of Experiment 2 indicate that the application of chitosan nanopowder (0.2 mg per card) to egg cards led to a higher rate of parasitization by *T. Japonicum*. Specifically, the mean value of parasitization in the treatment group was 39.87, which was significantly higher than the mean value of 29.01 observed in the control group. These findings suggest that *T. Japonicum* may exhibit a preference for egg cards treated with chitosan nanopowder, as evidenced by the higher rate of parasitization observed in the treatment group. Table 2 presents the results of a statistical analysis using an unpaired *t*-test on data obtained from an Eight arm multiple choice olfactometer. The findings indicate that egg cards treated with chitosan nanopowder (0.2 mg per card) exhibited a higher rate of parasitization compared to the control (untreated) egg cards. Specifically, the mean value for the treated egg cards was 39.87, while the mean value for the control group was 29.01. This difference was statistically significant, with a p-value of 0.0440. The experiment was conducted four times (Experiment 2) and the results were consistent across all replications. Following a five-day experimental period, stereozoom microscope imaging was conducted on the egg cards. The stereozoom microscope images, depicted in Fig. 6a, b, 6c, and 6d, were analyzed and evaluated. In this study, we present the results of Replication 1 of Experiment 2, which was carried out using an Eight Arm Multiple Choice Olfactometer. The findings are illustrated in Fig. 6a and b. The results of the experiment indicate that the application of chitosan nanopowder at a concentration of 0.2 mg per egg card led to a significant increase in the parasitization, as compared to the untreated control group. Specifically, the parasitization were found to be relatively higher in the treatment group (Fig. 6a) where chitosan nanopowder was applied, as compared to the control group (Fig. 6b). In this study, we present the results of Replication 2 of Experiment 2, which was conducted using an Eight Arm Multiple Choice Olfactometer. The findings are depicted in Fig. 6 c,d. The results of the study indicate that the application of chitosan nanopowder at a concentration of 0.2 mg per egg card led to a significant increase in parasitization compared to the untreated control group. Specifically, the *parasitization* were found to be higher

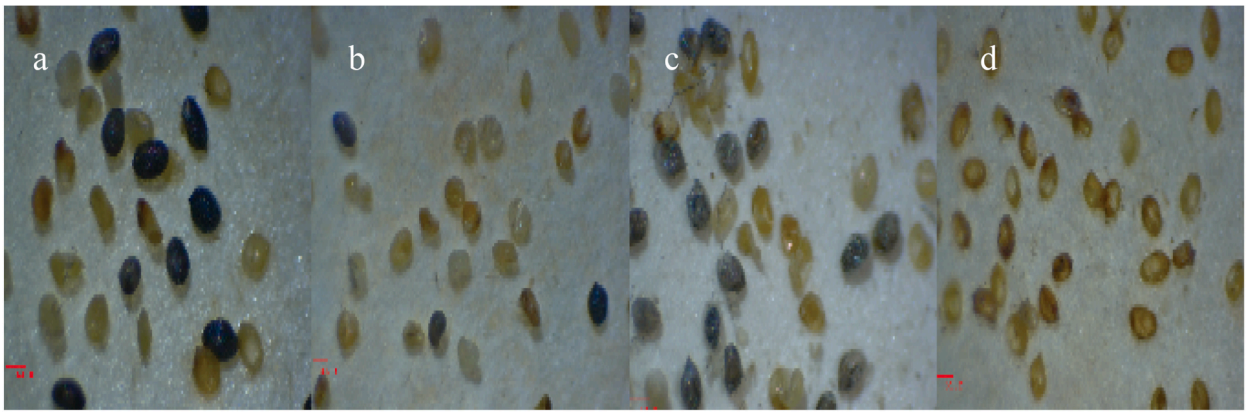

Fig. 6. Stereozoom images of Experiment 2 egg card after five days of the **a** and **b** (Replication 1) which was conducted in eight arm multiple choice olfactometer where more parasitization can be observed in treatment egg card sprinkled with chitosan nanopowder (0.2 mg per egg card) as shown in **a** in comparison to **b** control (untreated) egg card with less parasitization. **c** and **d** (shows Replication 2 of Experiment 2) where more parasitization can be observed in treatment egg card sprinkled with chitosan nanopowder (0.2 mg per card) as shown in **c** in comparison to **d** control (untreated) egg card with less parasitization.

in the treatment group where the egg card was sprinkled with chitosan nanopowder, as evidenced by the data presented in Fig. 6c. In contrast, the parasitization in the control group (Fig. 6d) were lower, indicating that the absence of chitosan nanopowder may have contributed to the reduced parasitization observed in this group. The results of Experiment 2, conducted in an Eight Arm Multiple Choice Olfactometer, were analyzed using Scanning Electron Microscope images (Fig. 8 d,e,f). After a period of five days, it was observed that eggs from the treatment egg card, which was sprinkled with chitosan nanopowder at a concentration of 0.2 mg per card, exhibited a level of health comparable to that of eggs obtained from the control (untreated) egg card. The results of the experiment indicate that the application of chitosan nanopowder (0.2 mg per card) to the treatment egg of *T. Japonicum* resulted in the emergence of an empty shell, as observed in Fig. 8 f (SI 1).

In Experiment 3, a No Choice Olfactometer was utilized to investigate the effects of chitosan nanopowder on egg cards. The treatment group received egg cards sprinkled with 0.2 mg of chitosan nanopowder per card, while the control group received untreated egg cards. No alternative was provided between the two groups. The present study reports a significant increase in parasitization levels in eggs treated with chitosan nanopowder (0.2 mg per card). The mean parasitization rate was found to be 59.62 for six replicates, indicating a relatively higher level of parasitization in the treatment group.

The results of six replications of Experiment 3, conducted in a No Choice Olfactometer, were compared to a control group (untreated egg card). The mean value of the experimental group was found to be 40.42. Table 3 displays the results of a statistical analysis using an unpaired *t*-test on data obtained from the No choice olfactometer. The findings indicate that treatment egg cards sprinkled with chitosan nanopowder (0.2 mg per card) had a higher rate of parasitization compared to the control (untreated) egg cards. The mean value of the treatment group was 59.62 for six replications of Experiment 3, while the mean value of the control group was 40.42 for six replications of Experiment 3. The observed p-value of 0.0002 was statistically significant. Following a five-day experimental period, images of the egg cards were captured utilizing a stereozoom microscope. The results of the stereozoom microscope imaging are presented in Fig. 7, which includes images (a), (b), (c), and (d). In this study, we present the results of Replication 1 of Experiment 3, which was carried out using a No Choice Olfactometer. The findings are depicted in Fig. 7 a, b. The results of the study indicate that the application of chitosan nanopowder at a concentration of 0.2 mg per egg card led to a significantly higher level of parasitization in comparison to the untreated control group. This finding is supported by the data presented in Fig. 7, where a relatively higher amount of parasitization was observed in the treatment group (Fig. 7a) as compared to the control group (Fig. 7b). In this study, we present the results of Replication 2 of Experiment 3, which was carried out using a No Choice olfactometer. The findings are depicted in Fig. 7c and d. The results of the experiment indicate that the application of chitosan nanopowder at a concentration of 0.2 mg per egg card led to a significant increase in parasitization compared to the untreated control group. This finding is supported by the data presented in Fig. 7c, which shows a higher level of parasitization in the treatment group compared to the control group (Fig. 7d). The results of Experiment 3, conducted in a No Choice Olfactometer, indicate that eggs treated with chitosan nanopowder (0.2 mg per card) were as healthy as untreated eggs. This conclusion is supported by the Scanning Electron Microscope images (Fig. 8 g,h) taken after five days of the experiment. The results of the study indicate that the emergence of adult *T. Japonicum* from eggs treated with chitosan nanopowder (0.2 mg per card) resulted in the observation of an empty shell, as depicted in Fig. 8h of Supplementary Information 1.

The present study reports the results of three experiments conducted to investigate the preference of *T. Japonicum* towards egg cards treated with chitosan nanopowder. Experiment 1 was conducted in a Y dual choice olfactometer, Experiment 2 was conducted in an Eight arm multiple choice olfactometer, and Experiment 3 was conducted in a No choice olfactometer. The results obtained from all three experiments consistently demonstrate that *T. Japonicum* exhibits a significant preference for egg cards treated with chitosan nanopowder (0.2 mg per card) over untreated control egg cards. This preference was found to be statistically significant, thus providing strong evidence to support the hypothesis that *T. Japonicum* prefers chitosan nanopowder-treated egg cards.

The present study has yielded results that are supported by empirical evidence. Specifically, Experiment 1, which utilized a Y dual

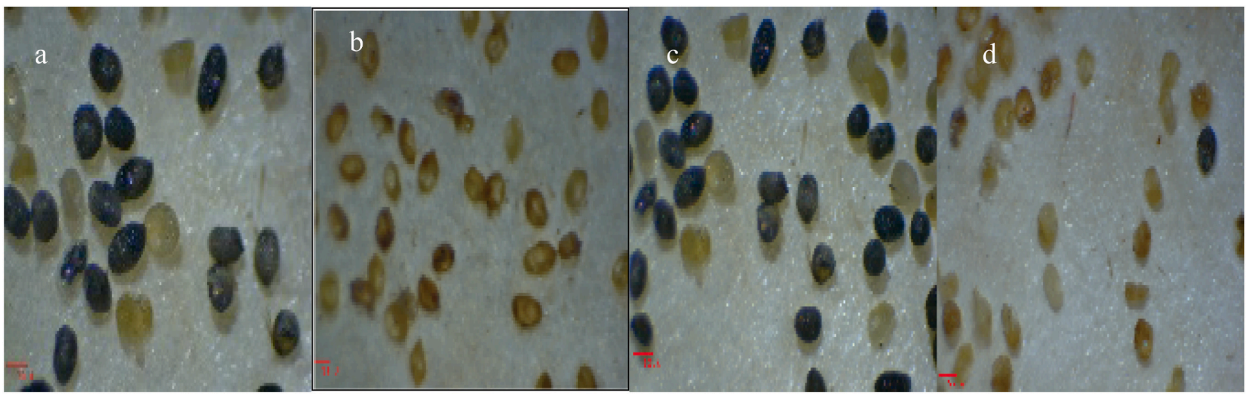

Fig. 7. Stereozoom images of Experiment 3 egg card after five days of the **a** and **b a** and **b** (Replication 1) which was conducted in No choice olfactometer where more parasitization can be observed in treatment egg card sprinkled with chitosan nanopowder (0.2 mg per egg card) as shown in a in comparison to b control (untreated) egg card with less parasitization. **c** and **d** (show Replication 2 of Experiment 2) where more parasitization can be observed in treatment egg card sprinkled with chitosan nanopowder (0.2 mg per card) as shown in **c** in comparison to **d** control (untreated) egg card with less parasitization.

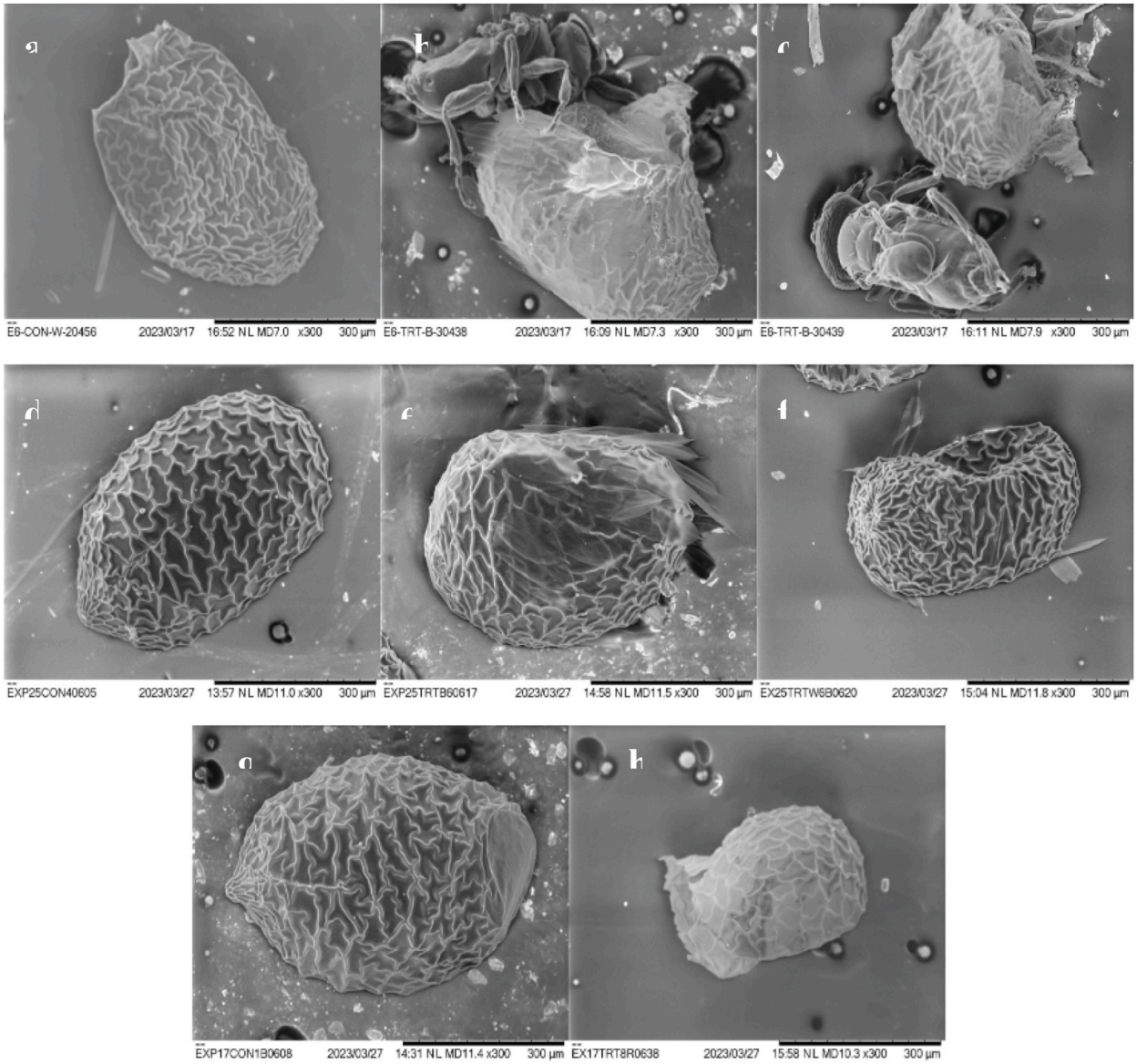

Fig. 8. Scanning Electron Microscope images of Experiment 1 **a,b,c** which was conducted in Y dual choice olfactometer: **a** shows healthy control (untreated) egg **b** shows adult *T. Japonicum* emergence from the egg taken from treatment egg card sprinkled with chitosan nanopowder (0.2 mg per card) **c** a healthy adult emerging from the treatment egg sprinkled with chitosan nanopowder (0.2 mg per card). Experiment 2 **d,e,f** which was conducted in Eight arm multiple choice olfactometer: **d** shows a healthy control (untreated) egg **e** shows a healthy egg after treatment sprinkled with chitosan nanopowder (0.2 mg per card) **f** represents empty egg shell after adult emergence after treatment sprinkled with chitosan nanopowder (0.2 mg per card). Experiment 3 **g,h** which was conducted in No choice olfactometer: **g** shows a healthy control (untreated) egg without any deformation **h** empty egg shell after emergence of adult from egg taken from treatment egg card sprinkled with chitosan nanopowder (0.2 mg per card).

choice olfactometer, and Experiment 2, which employed an Eight arm multiple choice olfactometer, both produced findings that are consistent with those obtained from Experiment 3, which utilized a no choice olfactometer. The results of our study indicate that the application of Chitosan nanopowder significantly enhances parasitization efficacy. Our robust methodology supports this conclusion.

6. Discussion

The present study investigated the effect of chitosan nanopowder on the efficiency of parasitization of *T. Japonicum*. The results revealed a significant increase in the parasitization efficiency upon treatment with egg card sprinkled with chitosan nanopowder at a concentration of 0.2 mg. These findings are in agreement with previous studies that have reported the beneficial effects of chitosan nanopowder on various biological processes the efficiency may be attributed to the antimicrobial [37] and immunostimulatory

properties of chitosan [56], which could enhance the host's immune response and reduce the risk of infection. The chitosan's efficacy in increasing parasitization efficiency can be attributed to its unique dual attributes. Chitosan possesses inherent antimicrobial properties, which make it effective at reducing infection risks. Simultaneously, it has the ability to stimulate the immune system. This dual action works synergistically to bolster the host's defense response, ultimately lowering the host's susceptibility to infection. It's important to note that while parasitoids typically suppress the host's immune system to complete parasitization, chitosan's immunostimulatory effects serve a different purpose. Chitosan enhances the immune response in the host, not the parasitoid, thereby creating an environment conducive to amplifying parasitization activity. This observed increase in the percentage of parasitization of *Trichogramma Japonicum* in the presence of chitosan nanopowder underscores its efficacy in promoting health attributes, particularly in terms of its immunostimulatory effects. Moreover, chitosan's immunostimulatory characteristics have been extensively explored (55), revealing its ability to fortify the host's immune response. This, in turn, creates an environment conducive to amplifying parasitization activity. The observed increase in the percentage of parasitization of *Trichogramma Japonicum* in the presence of chitosan nanopowder underscores its efficacy in promoting health attributes, particularly in terms of its immunostimulatory effects on host eggs. The present study discusses the role of chitosan nanopowder as a potential carbohydrate source for parasitoids. It is noteworthy that chitosan nanopowder is a chemically classified polysaccharide, which is known to exhibit carbohydrate-like properties. The findings of this study suggest that the parasitoid is able to utilize chitosan nanopowder as a carbohydrate source, owing to its chemical composition. Therefore, it can be inferred that chitosan nanopowder may serve as a promising alternative carbohydrate source for parasitoids. The present study discusses the factors that have been found to influence oviposition in *T. Japonicum*. According to Li et al. (2019) [14], host egg size, age, density, and environmental conditions such as temperature and relative humidity have been identified as key factors that affect oviposition in this species. These findings are consistent with previous research on the topic [14]. The authors propose that the obtained results provide support for the idea that *T. Japonicum* populations could serve as a cost-effective bioindicator in studies related to nanomaterials exposure. The maintenance of constant factors is a crucial aspect of experimental design aimed at reducing the occurrence of variability. In this particular study, the researchers ensured that these factors were kept constant to minimize the potential for confounding variables to influence the results. By doing so, the study was able to achieve a higher degree of internal validity, which is essential for drawing accurate conclusions from experimental data. The use of constant factors is a common practice in scientific research, as it allows for greater control over the experimental conditions and helps to ensure that the results are reliable and reproducible. The present study investigated the impact of chitosan nanopowder application on the host's parasitization efficacy. The results revealed that the application of chitosan nanopowder was the only variable in play, and it significantly increased the parasitization efficacy. The results of the study suggest that the observed increase in parasitization can be attributed to the presence of chitosan nanopowder. It can be inferred that other factors did not play a significant role in the observed outcome. These findings has demonstrated the effectiveness of chitosan nanopowder in promoting parasitization and strengthing the use of nano form of chitosan. Overall, the results of this study provide further support for the potential use of chitosan nanopowder. The present study reports the absence of any discernible morphological anomalies in the parasitization and subsequent emergence of adult *T. Japonicum*. The absence of such deformities suggests that *T. Japonicum* is well adapted to its host exposed with chitosan nanopowder.

6.1. Future prospects

The potential contribution of the present study lies in the utilization of *T. Japonicum* as an expensive bioindicator in the investigation of nanomaterials toxicity, thereby advancing the field of nanotoxicology. Our findings will contribute to a better understanding for future agricultural uses of chitosan nanopowder and broaden its utilization in agricultural and allied fields. The findings presented in this study are expected to have a significant impact on the deliberate application of nanomaterials, specifically chitosan nanopowder, in the field of agriculture. This research has the potential to contribute significantly to the development of guidelines for the safe and responsible use of nanomaterials in agriculture. The findings of this study may help to minimize any negative impacts on non-target organisms, thereby enhancing the sustainability of agricultural practices.

Data availability

All data is available as supplementary information.

Ethics approval

Not applicable Consent to participate: Not applicable.

Ethics declaration

In adherence to the highest ethical standards, we hereby affirm that our activities and endeavors have been conducted without any involvement, experimentation, or exploitation of animals or humans. Our commitment to upholding these principles underscores our dedication to the responsible and conscientious pursuit of knowledge and progress. Through our unwavering commitment to ethical practices, we strive to ensure that our work remains aligned with the values of compassion, integrity, and the well-being of all living beings.

CRediT authorship contribution statement

Deepa Bhagat: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Aamina Manzoor:** Formal analysis, Visualization. **Akanksha Mahajan:** Formal analysis, Validation. **Umesh Kumar Sanjeev:** Resources. **B.C. Sharma:** Formal analysis. **Paramanandham Krishnamoorthy:** Formal analysis. **Duleep Kumar Samuel:** Formal analysis, Investigation. **S.N. Sushil:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e20724.

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