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Mortality of Tribolium castaneum and quality changes in Oryza sativa by indirect exposure to Non-Thermal Plasma

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Abstract: The management of stored product pests is a serious concern as it contributes to postharvest product losses. This study investigated the influence of NTP on the control of *Tribolium castaneum* adults and the quality of *Oryza sativa* and compared with phosphine fumigation. The experiments were performed at 100 - 200 V of NTP and 100 - 200 ppm of phosphine for the exposure periods of 8, 16, and 24 h. Mortality of 100 % and 86.67 % was obtained at 24 h of exposure for plasma treatment (200 V) and phosphine (200 ppm), respectively. The quality changes in rice during the mortality studies were also evaluated for treated samples. The cooking properties, texture, hydration behavior, and pasting profile along with color and moisture content were investigated. The statistical analysis did not report any significant quality changes for plasma and phosphine treated rice compared to the untreated samples. The microstructural changes in the rice was also examined by scanning electron microscope. The results suggest that NTP treatment can be used as a useful tool for the control of *T. castaneum* without affecting the properties of rice. However, large scale studies have to be explored for practical usage of NTP in management of stored product pests.

Keywords: Non-Thermal Plasma, Phosphine, Mortality, Tribolium castaneum, Quality changes, Oryza sativa.

1. Introduction

Disinfestation of stored products is an always evolving field. The demand for greener and efficient technologies are increasing in postharvest insect control because the existing fumigants are facing regulatory actions due to environmental and occupational hazards. Phosphine is a fumigant used globally, which is very effective in controlling all stages of insect population and doesn't cause any quality effects in treated commodities. Conversely, the unceasing phosphine fumigations from the mid-1950s and improper fumigation practices led to the development of phosphine resistance by stored product pests [1-4]. Moreover, the phasing out of chemicals like methyl bromide upsurge the need for better alternatives. Novel techniques that are efficient, economical, and environment-friendly should be explored and used in the integrated pest management.

One of the emerging technologies for the control of stored product pests is the cold or non-thermal plasma treatment. Plasma is an ionized gas, having unique properties with highly reactive species like electrons, photons, ions, free radicals, and UV light present in it. NTP is often regarded as a nonchemical environment-friendly method [5], which doesn't leave any harmful residues in food [6]. The application of plasma in insect disinfestation has been studied mainly in horticultural pests and was found to be effective in complete control of insect pests [7-10]. However, the works reported on stored product pests are very few namely, larvae and pupae of Indian meal moth, *Plodia interpunctella* using APPJ pulses [11], *T. castaneum* in wheat grains using Argon plasma [12] and on T. *castaneum* using low-pressure DBD plasma [13,14]. Although these studies have put forward the potential of NTP as a pest management tool, ensuing almost 100% mortality in stored products, more research has to be done to expand the plasma applications.

The efficiency of NTP treatments mainly depends on the principle of plasma generation, the process gas (gas used for plasma generation), the geometry of the apparatus and the experimental parameters like voltage, frequency, the distance between electrodes and time of exposure. Further, the influence of plasma on insects, microbes, and treated commodities significantly varies for direct, semi-direct, and indirect plasma exposures [5, 15]. For direct plasma treatments, the glow discharge of plasma falls directly on the treated commodity. On the other hand, in indirect plasma treatments, NTP is not exposed directly to the commodities but generated remotely and transferred to the treatment location, such as external chambers.

Investigations have been performed on the influence of NTP on brown rice (*Oryza sativa* L.) through direct DBD discharge, where significant physicochemical changes were reported. The plasma treatment has reported modifying the microstructure of rice resulting in decreased cooking time, increased water absorption, and a softer texture when cooked. Besides, increased whiteness of brown rice and starch modifications have also been reported at higher power and exposure times [16,17]. In this study, the efficacy of indirect NTP treatment on the mortality of *T. castaneum* was evaluated as well as on the quality parameters of treated brown rice. The present investigation aims to suggest an alternative to existing chemical disinfestation methods without altering the quality of treated commodities. A comparison is made with phosphine fumigation as it is a method commonly used in integrated pest management. Though the treatments are not technically equivalent, a comparative study in terms of mortality of insects is essential in assessing the adequacy of indirect plasma exposure under similar fumigation conditions. Further, indirect exposures of plasma to stored commodities would be feasible and eliminates direct exposure, making it more practical in terms of commercial applications similar to other gaseous fumigations.

2. Materials and Methods

2.1 Insects

Tribolium castaneum (red flour beetle) adults were collected from the culture maintained at primary processing and storage laboratory, Indian Institute of Food Processing Technology (IIFPT), India. Thirty insects were randomly selected to oviposit on pre-sieved uninfested wheat flour. After two days, these adults were removed, and the insect culture was incubated for 45 days at 28-30°C temperature and 70 % relative humidity [14] The newly emerged adults of 1–2-month-old were separated using a sieve and were selected for the insect disinfestation studies.

2.2 Commodity

Paddy (ADT 43, F1 seed variety, medium slender grains; 6 months after the harvest) was collected from Soil and Water Management Research Institute (SWMRI), Thanjavur, India. The paddy was dehusked and cleaned from other impurities. The tests were performed using brown rice, with 10.6 ± 0.2 % moisture content. The samples were checked free from prior infestations manually by visual inspection and stored in airtight containers before the treatments.

2.3 Treatments

The mortality of *T. castaneum* adults, upon exposure to NTP treatment and phosphine gas, was studied in model fumigation chambers. The treatments were performed individually in two different chambers at the same time. Indirect exposure was given here to assess the efficacy of plasma and phosphine, as direct plasma exposures are known to alter the quality of the treated commodities. The fumigation studies were performed in airtight containers of 6 cm diameter and 28 cm height (791 cm^3) made of Poly Vinyl Chloride (PVC). For each trial, 30 insects were added into 60 g (100 cm^3) of rice in a 1:2 proportion as in [14].

The experimental set up for atmospheric pressure indirect NTP treatment includes a plasma generating gadget, developed at IIFPT, Thanjavur, working based on the Dielectric Barrier Discharge (DBD) method. Previous studies have reported significant changes in brown rice after plasma treatment at high voltages for direct plasma exposures [16,17]. Therefore, lower voltages in a range of 100 - 200 V with 0.05 - 0.1 A and power of 7.5 - 20 W respectively were used in this study. Atmospheric air with 70 % relative humidity was used as the process gas for the mortality studies, which was given into the plasma generator by an air pump at a controlled flow rate of 10 liters per hour (lph). An AC step-up transformer was connected between the voltage regulator and the plasma generator. The ionized gas from the generator was given into the treatment chamber filled with rice and insects via a 25 cm long connecting tube with an inner diameter of 0.6 cm, and the mortality assessments were performed at 8, 16, and 24 h exposure.

For phosphine treatment, Aluminium phosphide tablets were kept in a separate compartment (14 L) which reacts with the moisture present in the air to produce phosphine gas, based on the given chemical reaction.

$$AlP + 3H_2O \rightarrow PH_3 + Al(OH)_3 \quad \textit{Eqn. 1}$$

The phosphine gas was then passed on to the fumigation chamber at a controlled flow rate (2 lph) by connecting a tube of 25 cm length and 0.6 cm in diameter. The gas concentrations were measured using a phosphine sensor (FUMON, C-DAC, Kolkata). The data obtained from the sensor indicated that the concentration was gradually increased to a peak point and it was maintained for some time and later the concentration was slowly dropped down, similar to industrial fumigation practices [18, 19]. Different amounts of AlP tablets ranging from 0.25 - 0.5 g were taken and the peak concentrations were noted as the treatment concentrations ranging from 100 - 200 ppm. Insect mortality studies were performed for 8, 16, and 24 h of phosphine exposure. All sets of treatments (concentration-time combinations) were repeated thrice for NTP and phosphine concurrently in separate chambers. Untreated rice samples with insects were kept as control, and the corrected mortality was determined by Abbot's formula. [20] The block diagram of the NTP and phosphine treatment system used for the study is depicted in Figure 1.

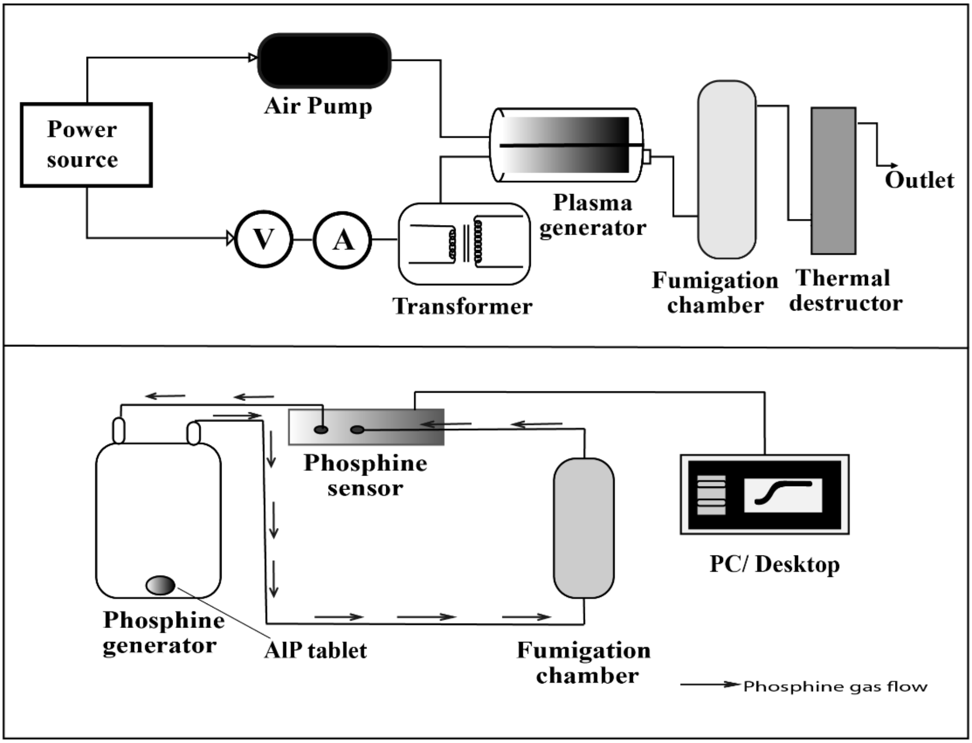

Figure 1. **NTP (top) and phosphine (bottom) treatment system.**

2.4. Effects on rice after treatments

2.4.1. Surface morphology

The surface topography of the brown rice samples was evaluated using a Scanning Electron Microscope (SEM: Tescan Vega 3 at IIFPT). The microstructure of the NTP and phosphine treated samples, along with the untreated control, was observed under SEM to scrutinize the alterations.

2.4.2. Moisture content

The moisture content (% wb) of the treated rice samples and control were analyzed using the oven drying method (AOAC, 2010).

2.4.3. Cooking properties

The optimal cooking time and water uptake ratio of rice samples were studied by taking 2 g of brown rice in 20 ml distilled water and placing the test tubes in a boiling water bath. The cooking time was determined by pressing the rice kernels between two glass slides at frequent intervals of time until there is no white core left. The Water Uptake Ratio (WUR) was calculated, as the difference between the weight of cooked and uncooked rice, to determine the amount of water absorbed during cooking. Initially, 2 g of rice was weighed and cooked till the optimal cooking time, excess water was drained out, and superficial water was removed from the cooked rice samples before weighing [21]. The cooking properties of each set of the sample were examined thrice for experimental replications.

2.4.4. Hydration behavior

The water absorption study was conducted by immersing 1 g of rice in 5 ml distilled water. The rice was soaked, removed from the water, and weighed to determine the moisture content at definite intervals of time (5 min). The soaked rice was properly blotted before weighing until there is no glistening appearance left [22].

2.4.5. Pasting properties

Pasting properties are a measure to assess the quality of the rice flour, and it is largely affected by components like starch, protein, and lipids [23]. The properties were measured by Rheoplus/32 (V3.62) software using Modular Compact Rheometer (MCR52, Anton Paar, Austria). Rice flour (3 g) of uniform sieve size (0.16 mm) was suspended in 25 ml distilled water, and then pasting profile was obtained through a heating and cooling cycle. The suspension was stirred at a constant rotational speed of 160 rpm in a steel container. Initially, the suspension was equilibrated at 50°C for 1 min and then heated at the rate of 6°C/min to 95 °C and was held for 5 min. It was again cooled back to 50 °C at the same rate and held for 2 min [24].

2.4.6. Textural profile

The textural profile was analyzed using Exponent Connect software in TA.XT, Texture analyzer (Stable Micro Systems, Surrey, UK), which was calibrated with a 30 kg load cell. The cooked rice grains were arranged as a single layer on the base plate, and a cylindrical probe of a 35 mm radius (P/35) was used. The compression cycle was repeated twice with a test speed of 1 mm/s, and the probe was descended 2 mm into the sample with a 50 % strain. Each sample had three replicate aliquots for texture analysis [17].

2.4.7. Color and Whitening index

Color measurements were performed on treated rice samples using Hunter lab colorimeter (Colourflex EZ model: 45/0 LAV). L (lightness ranging from 0 to 100 indicating black to white), a (- greenness: + redness), b (- blueness: + yellowness) values were recorded, and ΔE was calculated [5].

$$\Delta E = \sqrt{(L_C - L_T)^2 + (a_C - a_T)^2 + (b_C - b_T)^2}$$

Where L_C, a_C and b_C are for control samples and L_T, a_T, and b_T represents treated samples. The whitening index (WI) was also determined for the rice samples using the equation [16].

$$WI = 100 - [(100 - L)^2 + a^2 + b^2]^{1/2}$$

2.5. Statistical analysis

The statistical analysis was performed using univariate ANOVA (Analysis of Variance) for the mortality studies and the influence of plasma and phosphine treatments on rice quality in SPSS V20.0 software (IBM Corp., Armonk, NY, USA). To compare the observed means, post hoc tests were performed between treatment inputs (plasma – voltage and phosphine – concentration) and time intervals with Least Significant Difference (LSD) at a 95 % confidence level ($P \leq 0.05$) [25].

3. Results

3.1. Effect on Tribolium castaneum

The mortality percentage of *T. castaneum* has recorded in Table 1 and the mortality rate was less for both NTP and phosphine for 8 h treatment, but as the exposure time was increased to 16 and 24 h, there was a significant difference in the percent mortality rates ($P < 0.05$). The analysis indicated that the phosphine exposure was more effective in controlling insects at lower concentrations and longer exposure times. At 8 h of exposure, there was no substantial difference between treatment conditions of 100 V and 100 ppm statistically, but as the exposure time was further increased to 16 and 24 h, a significant difference was witnessed in the mortality values.

On the other hand, at voltages greater than 150 V, plasma treatment improved mortality of *T. castaneum* i.e. when 200 ppm phosphine was used, the mortality rate of *T. castaneum* was found to be 86.67 % after 24 h, while NTP requires only 150 V input to achieve an equivalent mortality rate of 83.33 % (no significant difference at $P \geq 0.05$) for that given time. In NTP treatment the highest mortality was found to be 100 % at 200 V which is 13.33 % higher than the phosphine gas treatment at 200 ppm. Nevertheless, the level of phosphine resistance of the particular strain of insects may also affect the efficacy of the mortality study, but it was beyond the scope of this study, as this work focussed on finding the efficacy of indirect NTP exposure on treated commodities.

Table 1. Mortality of Tribolium castaneum during NTP and phosphine exposures

Treatment	**Input**	**Insect mortality (%) at different time intervals**		
		8 h	**16 h**	**24 h**
Plasma	100 V	$5.55 \pm 3.85^{a,1}$	$14.44 \pm 1.93^{a,2}$	$35.56 \pm 1.93^{a,3}$
	125 V	$16.66 \pm 5.77^{b,1}$	$36.67 \pm 6.67^{b,2}$	$55.56 \pm 5.09^{b,3}$
	150 V	$32.22 \pm 5.09^{c,1}$	$58.89 \pm 3.85^{c,2}$	$83.33 \pm 3.34^{c,3}$
	175 V	$37.78 \pm 1.92^{c,d,1}$	$61.11 \pm 5.09^{c,2}$	$93.33 \pm 5.77^{d,3}$
	200 V	$45.56 \pm 5.09^{e,1}$	$70.00 \pm 3.33^{d,2}$	$100.00 \pm 0.00^{e,3}$
Phosphine	100 ppm	$7.78 \pm 1.92^{a,1}$	$24.44 \pm 1.93^{e,2}$	$47.78 \pm 1.92^{f,3}$
	125 ppm	$20.00 \pm 3.33^{b,1}$	$42.22 \pm 1.92^{b,2}$	$58.89 \pm 1.92^{b,3}$
	150 ppm	$28.89 \pm 1.92^{c,1}$	$50.00 \pm 5.77^{f,2}$	$70.00 \pm 5.77^{g,3}$
	175 ppm	$33.33 \pm 3.34^{c,1}$	$55.56 \pm 1.93^{c,f,2}$	$74.44 \pm 1.93^{g,3}$
	200 ppm	$43.33 \pm 3.34^{d,e,1}$	$63.33 \pm 6.67^{c,d,2}$	$86.67 \pm 5.77^{c,3}$

The mortality values are expressed as mean ± standard deviations.

Values followed by different superscripts letters $^{(a\text{-}g)}$ within the same column are significantly different ($p \leq 0.05$) at different treatment levels for the same time interval.

Values followed by different superscript numbers $^{(1\text{-}3)}$ within the same row are significantly different ($p \leq 0.05$) at different time intervals for the same treatment level.

3.2 Effects on rice

The samples treated at the highest voltage (200 V) and concentration (200 ppm) with an exposure time of 24 h were used for investigating the alterations in the quality of rice. The SEM images (Figure 2) of the control, NTP treated, and phosphine treated samples were used to scrutinize the surface morphology. The SEM analysis did not display any significant dissimilarities between the treated and control rice samples. Yet, from the detailed examination, it was observed that the indirect NTP had produced minute pores on the superficial bran layer. The fissures and roughness on the grain surface, usually occurring on grains with direct plasma exposure was not seen in the indirect treatment.

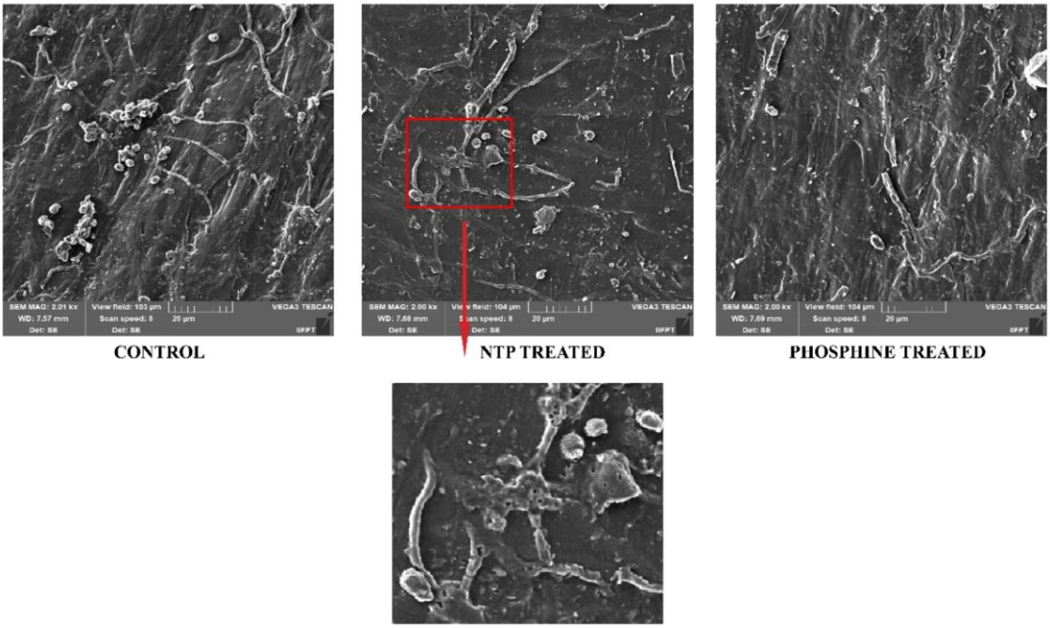
CONTROL NTP TREATED PHOSPHINE TREATED

Fig 2. Microstructure of control, NTP and phosphine treated rice samples at 2 kx magnification

The mean values of moisture content, cooking, textural, and pasting properties are tabulated in Table 2. There was a slight increase in the moisture content of the treated samples, but the results were statistically insignificant. The cooking properties, both cooking time and water uptake ratio, also exhibited no difference between the treatments. The properties like hardness, stickiness, springiness, and adhesiveness were determined from the textural profile of the rice, and ANOVA results at a 95% confidence level showed no significant changes for the treated and control samples. The hydration behavior of the brown rice samples during soaking is depicted in Figure 3, and the graph shows that the soaking characteristics were similar for the rice irrespective of the treatments. The results were contradictory to the previous direct NTP treatment studies, where a substantial increase in water absorption was observed during soaking [16,22].

The pasting profile of the NTP and phosphine treated rice and control samples is shown in Figure 4. The properties like pasting viscosity, pasting temperature, peak, setback, breakdown, and final viscosity data were obtained during the rheological measurements and compared to that of the control in Table 2. Although there were no significant varietal effects for pasting parameters of rice samples, slight variations in the viscosities of the treated samples were discerned from the control. The peak viscosity of NTP treated rice was upraised from 0.63 ± 0.26 Pa.s to 0.89 ± 0.08 Pa.s and the final viscosity was lower for NTP treated rice compared to raw rice.

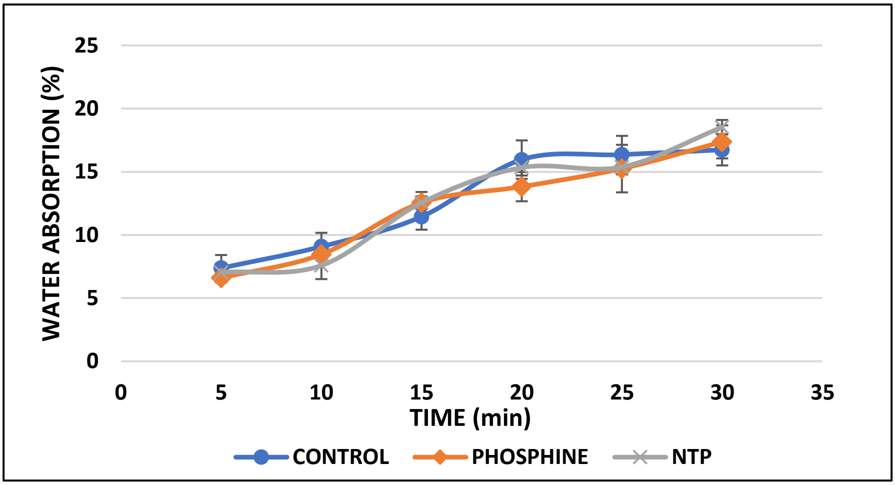

Fig 3. Soaking characteristics of rice samples

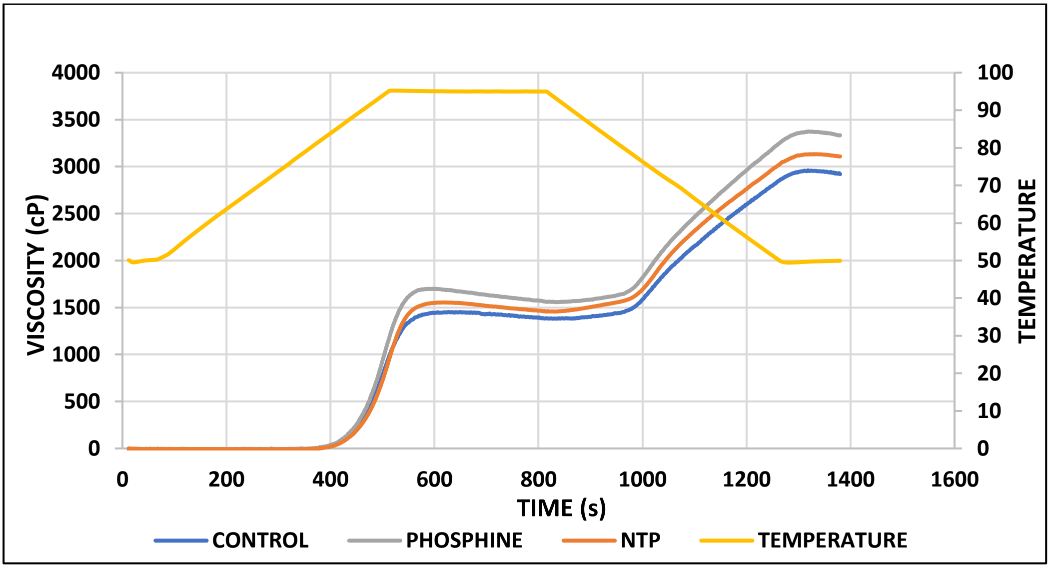

Fig 4. Pasting profile of rice samples

The color of the rice samples was measured and is provided in Table 3. The whitening index of the brown rice ranged from 56.75 ± 0.24 to 57.46 ± 0.24 and showed no significant difference compared to the untreated control. The ΔE value for the treated rice samples were less than 2 for both, indicating no change after the treatments.

Table 3. Colour properties of treated and untreated rice samples

Parameters	Control	NTP	Phosphine
L	64.09 ± 0.43	65.55 ± 0.47	64.55 ± 0.46
a	4.81 ± 0.02	4.81 ± 0.07	4.66 ± 0.09
b	23.61 ± 0.49	24.47 ± 0.34	22.97 ± 0.19
ΔE	-	1.69 ± 0.24	1.69 ± 0.37
Whitening Index	56.75 ± 0.24	57.46 ± 0.24	57.13 ± 0.69

4. Discussions

The resistance developed by major stored pests to phosphine and hazards due to improper handling of the chemical is a major concern in phosphine fumigation for the postharvest disinfestation. The dosage requirement for maintaining effective phosphine fumigation has increased to 710 ppm for 14 d [26] or 1000 ppm for 7 d [27]. making fumigation practices cumbersome under commercial storage conditions. The mode of action of phosphine on insects was reported as the disruption of major respiratory enzymes like cytochrome oxidase and dihydrolipoamide dehydrogenase and thereby affecting ATP synthesis [28-30]. A study on *Drosophila melanogaster* revealed that phosphine lowered the aerobic respiration rates with higher levels of hydrogen peroxide and lipid peroxidation [31].

On the other hand, the reactive oxygen and nitrogen species and free radicals present in plasma cause oxidative stress to the insects by affecting catalase, lipid peroxide, and glutathione reductase enzymes and affects the innate immunity system [11]. The charged particles damage the insect surfaces by electrostatic disruption, especially on irregular surfaces of the cell membrane [32]. Oxidation of biological components and alteration of DNA also lead to insect death [33]. The synergistic effect of different mechanisms of insect-killing makes NTP treatment more acceptable and reduces the fear of resistance developed by insects. In a study conducted by [11] on *P. interpunctella* larvae, 86.7 % mortality was obtained with 20 plasma jet pulses with 11 cm electrode distance. [14] also studied the mortality of *T. castaneum* using DBD discharge direct NTP treatment in wheat flour with voltage, time and electrode distance as the process variables. The conditions were optimized at 2500 V, 3.7 cm electrode distance for a 15 min exposure to give 100 % mortality of *T. castaneum*. These studies suggest the influence of plasma exposure, the geometry of apparatus and type of plasma generation like DBD or plasma jet on insect control.

The previous studies have reported the potential of plasma in the removal of insects in a short span of time, but the type of exposure being indirect augmented the time required for the control of *Tribolium* to 24 h. The UV radiations and short-lived plasma components were less effective in these indirect treatments. In a study conducted by [5] for assessing the antibacterial efficacy of plasma in highly perishable fresh produce, the direct, semi-direct, and indirect exposures were compared. It was reported that indirect and semi-direct treatments were more suitable for non-destructive sanitation of corn salad. [34] also revealed that indirect plasma treatment was effective in the decontamination of fresh strawberries achieving up to 95 % reduction in surface yeasts and molds, without significantly affecting its quality.

Regarding the effect of plasma treatment on rice, [16] studied the influence of low-pressure NTP treatment on brown rice at a high voltage of 1-3 kV for 30 min, and significant changes were observed in the microstructure of rice grains with fissures on the surface. According to [17], cooking time was reduced, and water uptake was increased with an increase in plasma power and time of exposure. Cooking or gruel loss was also reported in plasma-treated rice samples due to leaching out of starch components. However, [16] observed no cooking loss in plasma-treated brown rice and claimed that the grain surface was not severely degraded by NTP treatment. The study also revealed an upsurge in the whitening index and lightness of the treated rice samples, possibly because of the free radical reactions and bleaching effect of ozone present in the plasma [35]. On the other hand, in our study, charged particles could not reach the chamber with full potential, which resulted in surface etching or fissures only on the outer rice bran layer, as evident from the SEM images. Hence, cooking, water absorption, or textural properties are not changed in the treated rice samples. The color of the rice samples was also maintained. Moreover, the pasting properties confirmed that the starch modifications did not occur in the indirect NTP treatment.

Even though the time taken to achieve 100 % mortality is more in indirect plasma treatments, it is benefited by the factor that the quality parameters of the treated rice remain unchanged. This type of system could be feasible for commercial applications similar to other gaseous fumigations like phosphine and ozone, which are prevalent techniques in the field of disinfestation [36,37]. The indirect NTP treatment has an added benefit of the presence of free radicals and other charged components. Hence, the efficacy of indirect NTP can also be explored for the disinfestation in other cereals, pulses, millets, and processed products. NTP treatment being a greener, residue-free, and efficient method [6], can be considered to be an alternative in integrated pest management.

5. Conclusion

The effectiveness of indirect NTP treatment on the mortality of *T. castaneum* adults in brown rice was performed, and the results were compared with phosphine. The mortality obtained for plasma treatment was 100 % and 86.6% for phosphine fumigation after 24 h of exposure at 200 V and 200 ppm respectively. Further, the quality of treated rice samples was not affected after the treatments. Hence, the application of indirect NTP in the field of disinfestation is promising. The fear of resistance can also be avoided, due to the combined action of different plasma components in pest control. The indirect plasma treatments are technically similar to existing fumigation practices and can be used in bulk storages like silos, making the commercial applications much more manageable. Although the time taken to achieve complete mortality is more, the quality of treated commodities is retained in these treatments. Therefore, further research should be performed on the potential of NTP for the control of other major stored product pests in different produces.

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